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

BY

DALE J. TOMASIEWICZ

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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University of Manitoba
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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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LIST OF ABBREVIATIONS

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,

compared to that of leaf P_t. This poor performance of the shoot P_t test at and beyond the heading stage may account for the limited success with plant analysis for P in the past. Critical nutrient concentrations (CNC's) were determined for each test and stage, from 10 to 55 days after emergence (DAE). CNC's were generally similar to or higher than those suggested in other sources.

Shoot P_t, leaf P_t, and leaf P_i can each be used to predict if P deficiency will limit grain yield for spring wheat, using the time/stage-specific interpretive criteria presented. Sampling at the tillering to boot stage was optimal.

3.2 Introduction

Analysis of the nutrient content of plant tissue to assess the sufficiency of plant nutrition has been conducted extensively for several decades. It is premised on the existence of meaningful or at least somewhat consistent relationships between tissue nutrient concentrations and plant growth. A complete quantitative understanding of the physiological and biochemical relationships would form the ideal basis of the practice, but the complexity of the system precludes that. However, testing can be useful as long as there is a relationship between nutrient concentration and growth response (e.g. yield) which has been characterized (Smith 1962). Plant nutrient concentration reflects the plant's own integration of all the factors influencing the state of its nutrition, and therefore is a more direct and definitive method of assessing the adequacy of nutrition than soil testing.

Many variables influence the minimum tissue nutrient concentration which is required for maximum growth, i.e. the CNC (Bates 1971). These variables include the plant species (and even variety), the plant part(s) or tissue sampled, the stage of growth, the growing conditions and

growth rate, the nutrient fraction determined (i.e. total elemental concentration vs. concentration of some specific fraction of the element), and the concentrations of other nutrients. For wheat, Elliott et al. (1997c) determined that cultivar and N supply had only minor effects on CNC values for interpretation of plant P tests. Time of day can influence the CNC, especially for tests determining only certain fractions of the nutrients. Furthermore, the particular combination of these variables which provides the most accurate diagnostic test for one nutrient may not be optimal for another.

For plant tests for use in commercial crop production, several practical considerations as well as the diagnostic accuracy of the tests, will influence their suitability and adoption. The difficulty, time required for, and cost of sampling, sample processing, and analysis; the range of growth stages over which the test can be used; the range of nutrients which can be tested with acceptable diagnostic value on the same sample and the same extract or digest of that sample; the suitability of the procedure for a wide range of crops; and the existence of interpretive criteria for other nutrients and crops are all considerations.

The physiological role and behavior of a nutrient in the plant provides some direction as to how plant analysis should be carried out for it. Phosphorus is taken up actively by plant roots as the orthophosphate anions $H_2PO_4^-$ and HPO_4^{2-} (Barber 1980). It can be rapidly incorporated into organic compounds, and is readily transported both upward and downward in the vascular system (Mengel and Kirkby 1982, Pate 1976), as well as from xylem to phloem tissue (Bieleski 1973). The youngest leaves and meristematic tissues are generally avoided in testing for such mobile nutrients, since they can receive nutrients translocated from older tissues (Marschner 1986). Sampling whole shoots is also not generally recommended (Jones 1985); different tissues vary in their elemental compositions, and the proportions of the tissues change as growth progresses. Despite this, the entire above-ground plant is most commonly selected for analysis of small

grains (Hanway and Olson 1980). Most available compilations of critical nutrient concentrations for small grains cite values for total P in whole-shoot samples, and many also provide criteria for selected leaf samples. The preponderance of whole-shoot data may have arisen in part out of the need for this analysis to determine nutrient accumulation in many research trials. The leaf, being the principle site of metabolism for most nutrients, is generally the favored tissue for testing (Bould 1984, Marschner 1986). This would seem particularly relevant to P because of its unique role in energy transfer (energy associated with the pyrophosphate bond) required in almost all metabolic processes. The youngest fully expanded leaves are often recommended for testing for annual crops because their composition and function is relatively stable (Smith 1962). To be useful diagnostically, the plant part tested should show a wide range in nutrient concentrations between deficient and adequate nutritional status (Tisdale et al. 1985). Partly on this basis, Knowles et al. (1990) preferred the basal stem tissue of durum wheat over leaf tissue for analysis of extractable phosphate. They also found much higher levels of phosphate in the flag leaf than in lower leaves.

Determination of only the inorganic fraction of nutrients in plants is sometimes referred to as *tissue testing*, as distinct from *plant* or *total analysis* which can imply total elemental analysis (Tisdale et al. 1985). Tissue testing is widely used for horticultural crops, but should also be as valid for small grains, even though it is rarely used for cereals at present. In tissue testing for P, the fraction used is extractable P_i. Inorganic P varies more widely with plant P nutritional status than any other P fraction in the plant (Chapin and Bieleski 1982). Cytoplasmic P_i concentrations are maintained within narrow limits, while the concentration of P_i in the vacuole may vary 30-fold or more in response to varying P nutrition (Bieleski 1973, Mimura et al. 1990). The vacuole represents most of the volume of many plant cells, including leaf mesophyll cells, so whole-tissue P_i concentrations reflect primarily vacuolar concentrations. The P_i test should be very sensitive, since it detects only the plant P fraction that varies most widely with P nutrition.

Also, it can be adapted for use as a quick-test in the field, since the laboratory tissue digestion procedure and tissue drying are not required. As indicators of plant P sufficiency, P_t and P_i were similarly effective for wheat (Elliott et al. 1997b,d) and potatoes (Maier 1989), while P_i was slightly better for pecan (Sparks 1988).

Concentrations of most nutrients in whole shoots usually decline with increasing plant age or development stage. This has been shown specifically for P in field-grown spring wheat (Bauer et. al 1987, Boatwright and Haas 1961, Elliott et al. 1997b,d, Racz et al. 1965). Tissues that are less metabolically active and lower in mineral nutrient concentrations (e.g. structural and energy storage tissues) increase in proportion as the plant develops. Critical nutrient concentrations also decline for the same reasons, though not necessarily in direct proportion to the changes in the nutrient concentrations themselves, since the degree of P stress may vary over the season and plants may take up and store P in excess of their metabolic needs in times of good P supply. Regardless of the crop stage at testing, it is important that interpretive criteria be specific to the stage, especially if whole-shoot samples are used.

Deficiency of P may restrict plant growth at the earliest stages, when the "pop-up" effect of seed-placed P fertilizer on spring cereals can be observed. The P content of the wheat seed itself can affect early growth, including shoot weight and the number and size of leaves (DeMarco 1990). Slow growth and reduced tillering, the most common visual symptoms of P deficiency (Elliott et al. 1997a, Hoffer and Krantz 1949), are evident early in the growth of cereals, the former leading to delayed heading and maturity later. Goos et al. (1994) reported that fertilization of spring wheat with P in the seedrow caused substantial improvements in main stem development stage, and in shoot dry matter and P accumulation, at both the 2 to 3-leaf and 6 to 7-leaf stages of growth. In their study, fertilization with P also strongly enhanced development of tillers, especially the T1 and T2 tillers.

In studies of P nutrition at various growth stages, early season P deficiency has generally reduced yield; e.g. P deficiency during the first few days of growth for lettuce (Avnimelech and Scherzer 1971), and for spring wheat during the first week (Claassens 1990) or two weeks (Boatwright and Viets 1966, Elliott et al. 1997a) of growth. In contrast, wheat grain P content, but not yield, was increased by improving the P status of P-deficient plants four days prior to anthesis (Batten and Wardlaw 1987). Deficiency of P at early growth stages promotes greater P accumulation ("luxury consumption") in wheat at later stages if the nutrient becomes more available (Boatwright and Viets 1966, Claassens 1990). For these reasons, plant analysis conducted at early growth stages should be most likely to correctly diagnose yield-limiting P deficiency. However, plant analysis of cereal crops is often conducted at or near the time of heading. The common range in shoot P concentrations at that stage is rather narrow, and it is unclear how well the test levels are related to yield limitations.

Plant analysis is usually carried out to assist the grower in optimizing economic returns, so all factors potentially influencing returns should ideally be considered in correlation and calibration of the test. However, field calibration of P tests for cereals only with respect to their relationship with grain yield at maturity is acceptable for several reasons: wheat straw is of low value; grain P concentration does not affect its market value at the farm level; other factors which do affect grain market value are not strongly influenced by P nutrition; and possible indirect effects of P nutrition on yield (e.g. resistance to pests) are taken into account in trials that are carried out under field conditions. It is difficult to assign an economic cost to the often-cited delay in maturity associated with P deficiency, but this delay is likely most important in the P nutrition range where deficiency would cause loss due to reduced yield as well (Hanway and Olson 1980).

Fertility management of small grains on the Canadian Prairies could benefit from increased use of plant tissue nutrient testing. This is particularly true for P, because deficiency of P is

widespread, yet visual symptoms of the problem are not very obvious or diagnostic. However, it is necessary to optimize the procedures for detecting deficiency, and to develop test interpretive criteria specific to our varieties and conditions. This study was conducted to evaluate and calibrate various plant tests and times of testing, for assessing yield limiting P stress in field-grown spring wheat.

3.3 Materials and Methods

Field trials were conducted at five sites in 1990 and three in 1991 (Table 3.1). Conventional (disk and sweep) tillage practices had been used at all sites for many years. Small grains had been grown in the preceding year, except at the Osborne site, where flax (*Linum usitatissimum*) was grown.

The treatments varied in amount and placement of granular monoammonium phosphate fertilizer (MAP; 12-51-0). Both broadcast-incorporated and seed-placed P treatments were included, to create as wide a range of P-supply conditions as possible:

1) no applied P (0-0)
2) 5 kg ha^{-1} of P placed in the seedrow (5-0)
3) 20 kg ha^{-1} of P placed in the seedrow (20-0; 1991 only)
4) 20 kg ha^{-1} of P broadcast (0-20)
5) 20 kg ha^{-1} of P placed in the seedrow, plus either 50 kg ha^{-1} of P broadcast (20-50; 1990 only) or 100 kg ha^{-1} of P broadcast (20-100; 1991 only)

Broadcast P treatments, plus all other fertilizers applied to meet or exceed crop requirements for other nutrients based on soil testing at each site were broadcast and incorporated in the spring. Urea to bring the total applied N to 120 kg ha^{-1} in all treatments. Fertilizers were incorporated by

Table 3.1 Soil characteristics for field experimental sites.[z]

Site (soil series)	Soil sub-group	Taxonomy sub-group	Legal location	Texture	Clay	pH	Org. C	CO_3-C	$NaHCO_3$-P
1990 Sites					%		g kg^{-1}	g kg^{-1}	mg kg^{-1}
A Rignold	Gleyed Orthic Black	Aquic Haploboroll	NW25-5-5 W1	FSL[x]	14	7.1	19	0	14
B Elm River90	Cumulic Regosol	Typic Udifluvent	Wood Lot #300[y]	SiL	26	7.6	27	16	5
C Willowcrest	Gleyed Orthic Black	Aquic Haploboroll	NE10-9-8-W1	FS	4	7.9	8	0	18
D Plum Ridge	Gleyed Rego Black	Aeric Calciaquoll	NE23-19-3-E1	FSL	13	8.0	20	11	9
E Lakeland	Gleyed Rego Black	Aeric Calciaquoll	SE2-19-3-E1	SiC	43	7.9	34	14	30
1991 Sites									
F Reinfeld	Orthic Black	Udic Haploboroll	SW36-5-5-W1	FSL	14	7.3	18	0	9
G Elm River91	Cumulic Regosol	Typic Udifluvent	Wood Lot #300[y]	SiCL	32	7.7	26	11	3
H Osborne	Rego Humic Gleysol	Vertic Cryaquoll	NW12-6-3-W1	HvC	70	7.8	31	18	5

[z] Analytical results are for the 0-15 cm depth. Procedures used: texture and clay content by pipette method after carbonate and organic matter removal, pH in 0.01M $CaCl_2$, organic C by wet oxidation with dichromate (Yeomans and Bremner 1988), carbonate-C by titrimetry (Bundy and Bremner 1972), and $NaHCO_3$-P by method of Olsen and Sommers (1982).

[y] Parish of Portage la Prairie; Elm River90 and Elm River91 sites were approximately 0.5 km apart.

[x] FSL - fine sandy loam, SiL - silt loam, FS – fine sand, SiC - silty clay, SiCL - silty clay loam, HvC - heavy clay.

harrowing twice at the Willowcrest site, cultivating twice at the Plum Ridge site (light duty cultivator, 7 cm deep), harrowing once at the Lakeland site (which had been cultivated just prior to broadcasting of the fertilizer), and rotovating to a depth of 8 to 10 cm at all other sites. Spring wheat (*Triticum aestivum*, cv. Katepwa) was seeded at 135 kg ha^{-1} with a double-disc press drill at all sites. Seedrow spacing was 17.8 cm.

Plots were laid out in a randomized complete block design, with five blocks at each site. Plots were eight rows wide by 15 or 16 m long, to permit sampling of undisturbed areas several times during the season. All samples were taken from the centre four rows.

Plants and soils were sampled 7 to 10 d after crop emergence, and at intervals of approximately 10 d (ranging from 7 to 14) thereafter, to a total of six sampling times at each site. The fifth sampling usually coincided approximately with the heading stage of the crop. Plant samples were taken from a four-row by 1-m area within each plot; the sampling location within the plots was the same for all treatments within a block, but randomized among sampling times, blocks, and sites. Thirty to 80 of the youngest fully emerged and expanded leaf blades within each sampling area were taken as the "leaf" sample. Then all remaining above-ground growth was harvested as the "shoot" sample. Separate leaf samples were not taken at the first sampling time at any site, as well as at the second sampling time at the Willowcrest, Lakeland, and Osborne sites, due to the small amount and early stage of the above-ground growth. In those cases, the shoot samples were almost entirely leaf material, so were considered both "shoot" and "leaf" for data analysis purposes. Large shoot samples taken later in the season were weighed and subsampled in the field.

Fresh tissue samples were kept cool until they could be processed, usually the day after sampling. Shoot samples were cut up and mixed, and a subsample was oven-dried at 60°C; weights were recorded for gravimetric moisture calculation. Leaf samples were similarly processed, except

that extraction for soluble P_i was carried out on a subsample immediately after mixing the cut-up sample.

Extractable P_i was determined for all of the fresh leaf samples. Either 2.5 or 3.0 g of tissue, an equal weight of acid-washed 30-40 mesh silica sand, and 25 to 30 mL of 2% acetic acid (HOAc; 20 mL L^{-1} of glacial acetic acid) were placed in a 275-mL porcelain crucible. The tissue was ground by hand with a pestle until thoroughly macerated, which required almost one minute of vigorous grinding. Additional 2% HOAc was added to bring the total liquid volume to 75 mL (for 2.5 g of tissue) or 90 mL (3.0 g tissue), including the estimated liquid volume of the leaf sample (approximately 1.75 to 2.50 mL). The suspension was mixed very briefly with the pestle, then for 30 s with a hand-held household-type immersion blender, and filtered by gravity through Whatman #5 filter paper. Inorganic P in the filtrates was determined colorimetrically on the day of extraction as a phosphomolybdate complex by the procedure of Murphy and Riley (1962).

Extractable P_i was also determined on the dried leaf samples ("P_{id}") for all of the 1991 samples and a representative cross section (about 20%) of the 1990 samples. The oven-dried tissue was extracted with 2% HOAc at a 1:150 tissue:extractant ratio. The suspensions were shaken in bottles on a reciprocating shaker for one hour and filtered through Whatman #5 filter paper; P_i in the extract was determined as described above.

Total P was determined on all shoot and leaf samples after oven-drying. Sub-samples were ignited with $Mg(NO_3)_2$, the ash was dissolved in dilute HCl (modification of method 3.095, A.O.A.C. 1984), and P in the digest was determined by the method of Murphy and Riley (1962).

At crop maturity, all above-ground plant material was taken from a 1.5 m^2 (1990) or 2.0 m^2 (1991) area, air-dried, and threshed for grain and straw yield determination. Total P was determined in the straw by methods described above, and in the grain after wet digestion in a 2:1 nitric:perchloric acid mixture.

3.4 Results and Discussion

3.4.1 Growing Conditions and Yields

The 1990 growing season was wet at all sites to the heading stage and dry thereafter, whereas 1991 was dry to approximately the boot stage and moist thereafter. Rainfall during the first 30 d after crop emergence averaged 14 cm at the 1990 sites, but only 6 cm at the 1991 sites. Mean rainfall during the subsequent 40-day period was 5 cm in 1990 and 17 cm in 1991. Yield (Table 3.2) was likely limited by delayed germination, soil blowing, and damage by grasshoppers at the Willowcrest site, and by leaf diseases (tan spot and septoria) at the Reinfeld site.

Grain yield was increased by P fertilization at all five sites with $NaHCO_3$-P concentrations lower than 10 mg kg^{-1}; P deficiency was severe at the three sites testing less than 6 mg kg^{-1} (Tables 3.1, 3.2). The large loss in yield due to P deficiency in the 20-0 treatment at the Osborne site is notable, because that treatment is in the range of recommended phosphate fertilizer rates for

Table 3.2 Yield of spring wheat grain at eight sites, as affected by application rate and placement of phosphate fertilizer.

P Applied (Seedrow-broadcast)	Grain yield							
	Rignold	Elm River90	Willowcrest	Plum Ridge	Lakeland	Reinfeld	Elm River91	Osborne
kg ha^{-1}	t ha^{-1}							
0-0	3.61	2.97*	2.46	3.01*	3.47	2.41*	1.02*	1.17*
5-0	3.61	3.23*	2.60	3.11*	3.52	2.66	1.57*	1.34*
20-0	--	--	--	--	--	2.72	2.37*	1.95*
0-20	3.69	3.47*	2.53	3.21	3.64	2.69	2.51*	1.70*
20-50	3.60	3.98	2.39	3.31	3.43	--	--	--
20-100	--	--	--	--	--	2.80	3.44	3.33
LSD (0.05)	ns	0.46	ns	0.19	ns	0.25	0.39	0.26

* yield significantly ($p<0.05$) lower than for the 20-50 or 20-100 treatment at the same site.

wheat on low-P soils of the Canadian prairies. Grain yields significantly lower than those of the corresponding 20-50 or 20-100 treatment at each site, which correspond to all relative yields of <95% in this study, indicate grain yield-limiting P deficiency.

The time from the average date of emergence to the average heading date was 47 d at the Plum Ridge and Lakeland sites, and 42 to 45 d at all other sites. The typical Zadoks growth stage (Zadoks et al. 1974) of the crop was 13 at 10 DAE, 20 at 15 DAE, 22 at 20 DAE, 30 at 25 DAE, 31 at 30 DAE, 46 at 40 DAE, and 64 at 50 DAE.

3.4.2 Tissue P Concentrations

The pattern of changes in tissue test levels through the sampling period differed among the three tests (Figure 3.1). Whole-shoot P_t concentrations generally declined throughout the season, except for the severely P-deficient treatments at the Osborne site where there was little change with time. General declines in shoot P_t concentrations with time in field-grown spring wheat were also shown by Bauer et al. (1987), Elliott et al. (1997b,d), and Racz et al. (1965). Shoot P_t concentrations also converged among sites and treatments through the growing season; the proportional as well as absolute increase in shoot P_t concentration due to P fertilization declined with time.

Total P concentrations in the leaf samples were similar to, or slightly lower than, those in the whole-shoot samples to approximately the beginning of stem extension, about 30 DAE (Figure 3.1; "leaf" P_t levels for the first one or two sampling times are actually whole-shoot values, since leaves were not sampled separately when there was little other shoot growth present). After this stage, leaf P_t concentrations were relatively stable in most cases, though they also continued to converge among sites and treatments. Bauer et al. (1987) and Frank et al. (1989) also reported

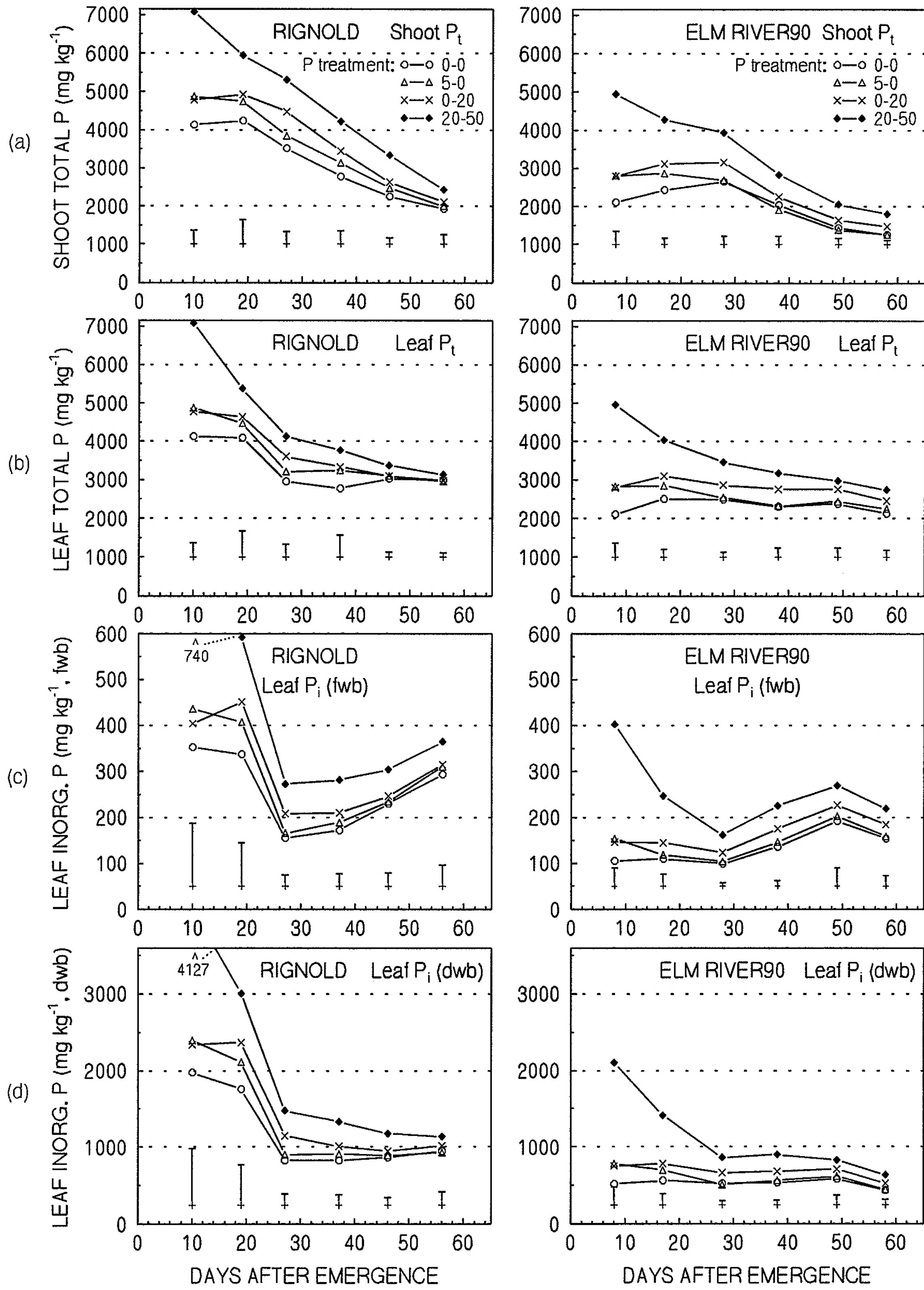
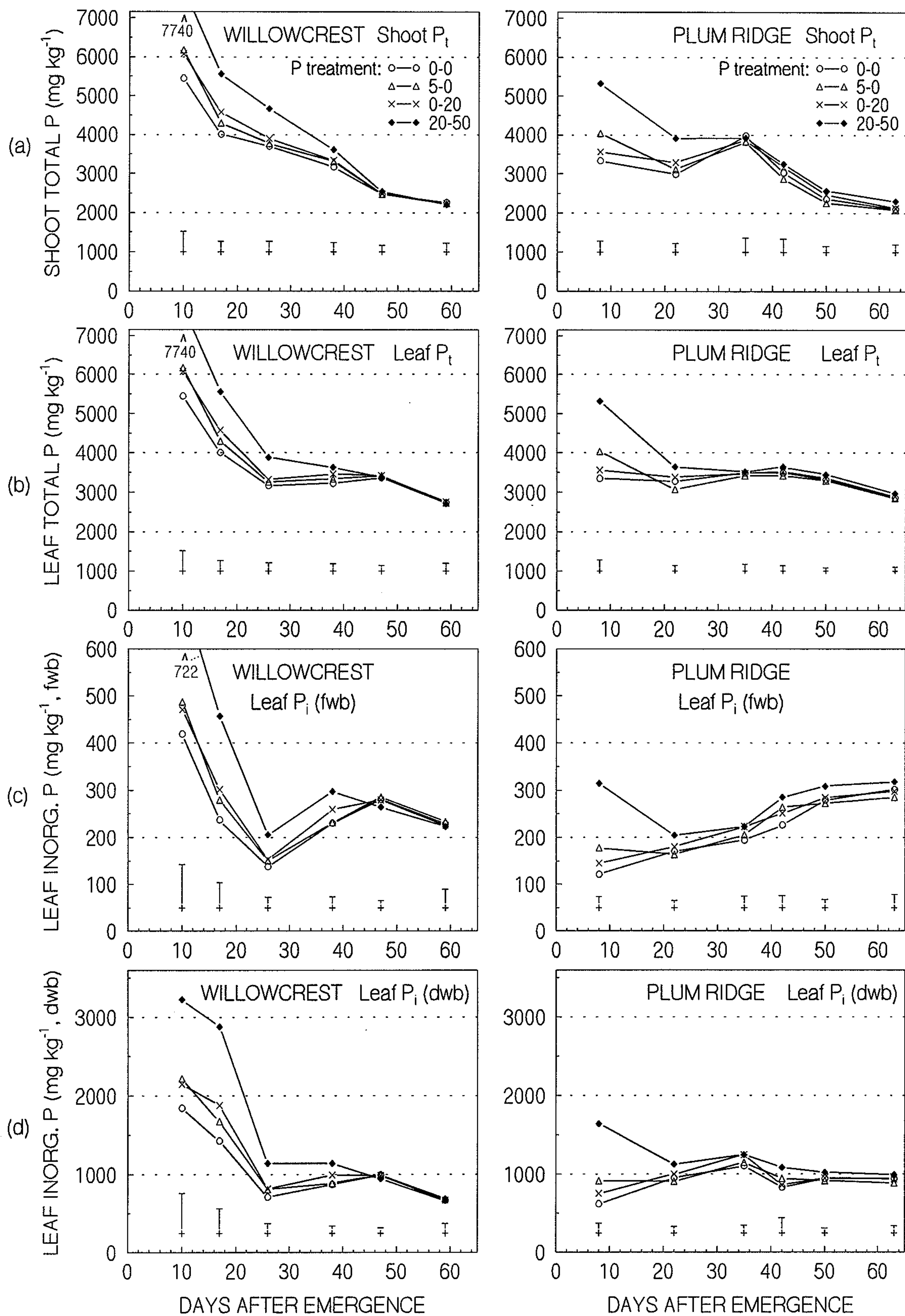


Figure 3.1 Concentrations of total P (P_t, dwb) in (a) shoot and (b) leaf, and of inorganic P (P_i) extractable from the fresh leaf tissue [(c)fwb and (d)dwb], for all sampling times, sites, and treatments. Error bars indicate LSD (P<0.05). cont.



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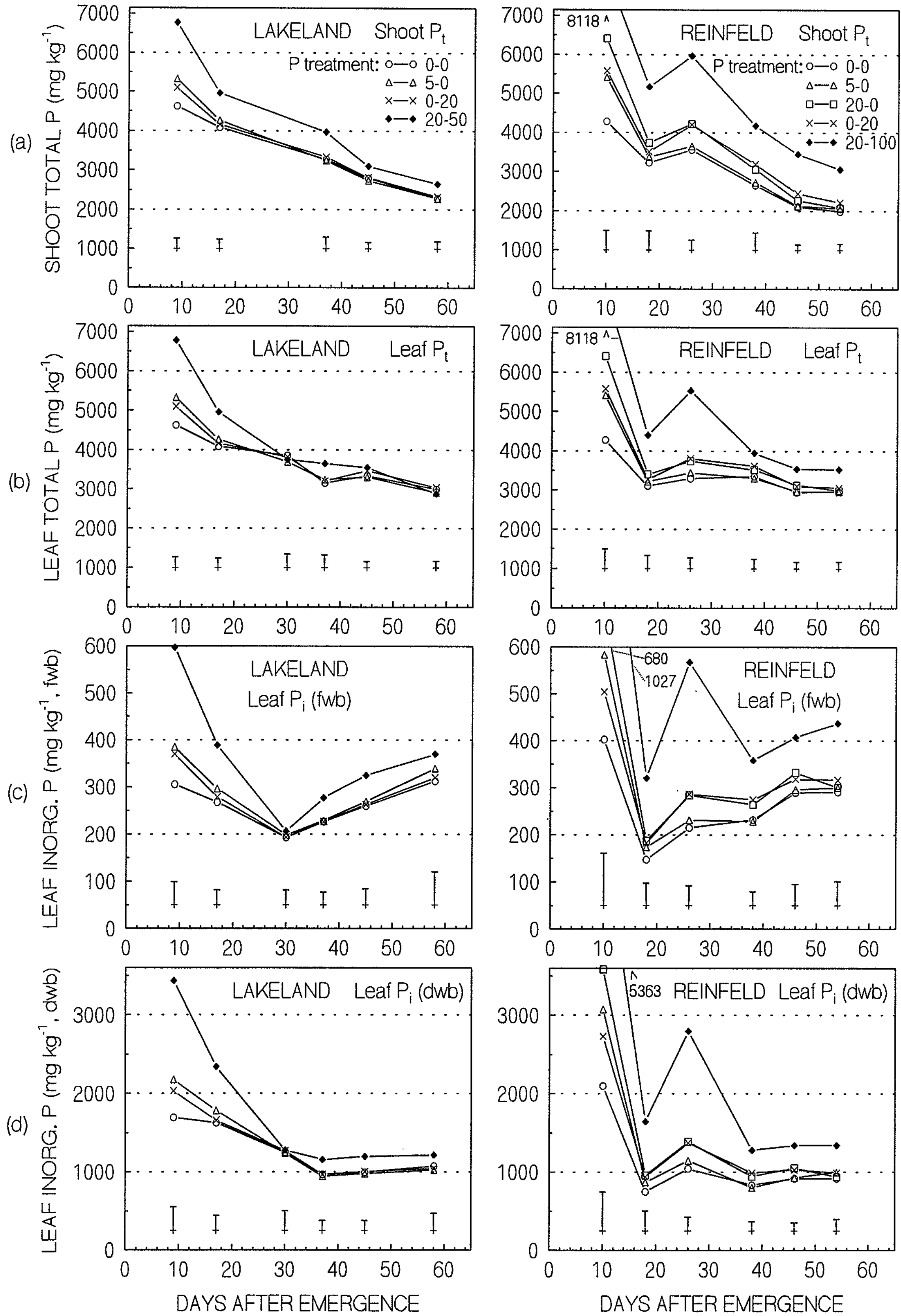

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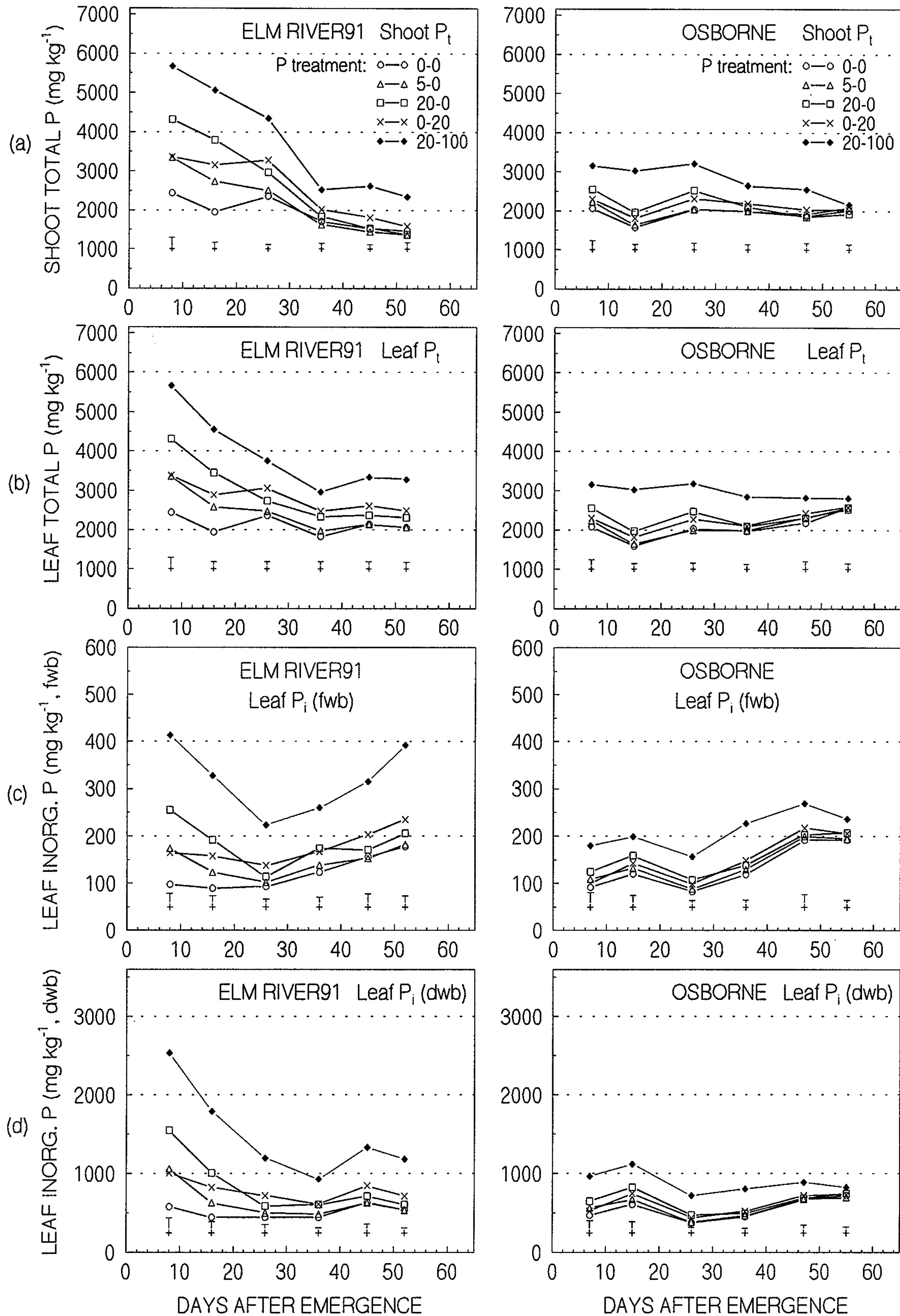


Figure 3.1. cont.

higher P concentrations in stems than in leaves of spring wheat, and leveling off of leaf P concentrations, at approximately the same growth stages as observed in our study.

Plant P concentrations for all tissue test methods generally increased with both soil extractable P and fertilizer P applied. Other variables, such as other soil characteristics or growing season, did not appear to have a consistent effect on plant P in these trials. Some such effects (e.g. of moisture and texture) might have been apparent with more site-years of data.

Effects of P fertilizer treatments on shoot and leaf P_t levels are very apparent early in the growing season. However, after mid-season, only the highest P treatment resulted in substantially higher tissue P_t concentrations than in the check treatments. Even for the 20 kg ha^{-1} broadcast P treatment (0-20), which was not expected to provide good P nutrition early in the growing season, tissue P_t concentrations are increased over those in the checks to the greatest extent early in the growing season. The differences in tissue P concentrations are greatest at the earliest sampling time, but sampling at that time did not always best reflect the influence of fertilizer P on grain yield at maturity. For example, tissue P concentrations by all tests at the first sampling were similar to higher in the 5 kg ha^{-1} seed-placed P treatment (5-0), as compared to the 20 kg ha^{-1} broadcast P treatment (0-20) within sites. However, the latter treatment had higher P concentrations by the second or third sampling time and produced higher grain yields. In effect, use of tissue P concentrations very early in the season (one to two weeks after emergence) may lead to an exaggerated indication of the value of very small amounts of seed-placed P vs. broadcast P in increasing yield. Goos et al. (1994) reported that seedrow placement of P fertilizer strongly increased growth and P uptake at the 2 to 3-leaf stage of spring wheat, but these benefits did not always result in improved grain yield at maturity.

Concentrations of P_i extracted from the fresh leaf tissues were expressed on both the fresh and dry weight bases (Figure 3.1c and d). The latter permits direct comparison with P_t. However, the

greatest potential use of this test procedure is as a field quick-test; the moisture content of the sample, required to convert results from the fresh to the dry weight basis, would not normally be known in that situation. Therefore, evaluation of the test without conversion of concentrations to the dry-weight basis is desirable.

Concentrations of extractable P_i in the leaves differed among sites, treatments, and times in a manner similar to those of leaf P_t, when both were expressed on the same moisture basis (dwb). However, P_i concentrations were usually less than half of those of P_t, and had greater proportional variation. Inorganic P concentrations in the plant are much more sensitive to P supply than are P_t or organic P ("P_o") concentrations (Barr and Ulrich 1963, Bouma and Dowling 1982, Chapin and Bieleski 1982); Bieleski (1973) states that concentrations of P_i change up to 50-fold with P nutrition, while those of the major P_o forms vary only five-fold. For this reason, P_i levels may better reflect plant P nutritional status than those of P_t. The wider range of P_i concentrations may not only result in a more sensitive test of plant P status, but may also allow the test to be useful where analytical methods or conditions are not ideal (reducing accuracy), such as in field quick-test situations.

The relationship between P_i (extractable from fresh leaf tissue) and P_t for the same samples shows the increasing proportion of the P_t which is extractable as P_i as the P nutrition of the plant increases (Figure 3.2). Site and sampling time had little obvious effect on that relationship. Bouma and Dowling (1982) obtained very similar results with P in clover leaves, as did Elliott et al. (1997d) in wheat leaves, and Chapin and Bieleski (1982) in barley shoots.

A concentration of a nutrient in tissue dry matter is a ratio of the amount of nutrient to the total amount of tissue. Plant tissue dry matter is mostly C, H, and O, of which C is a relatively large and constant fraction. Therefore, the concentration of P is equivalent to a P:C ratio. The physiological role of C changes during growth, with increasing fractions serving structural (and

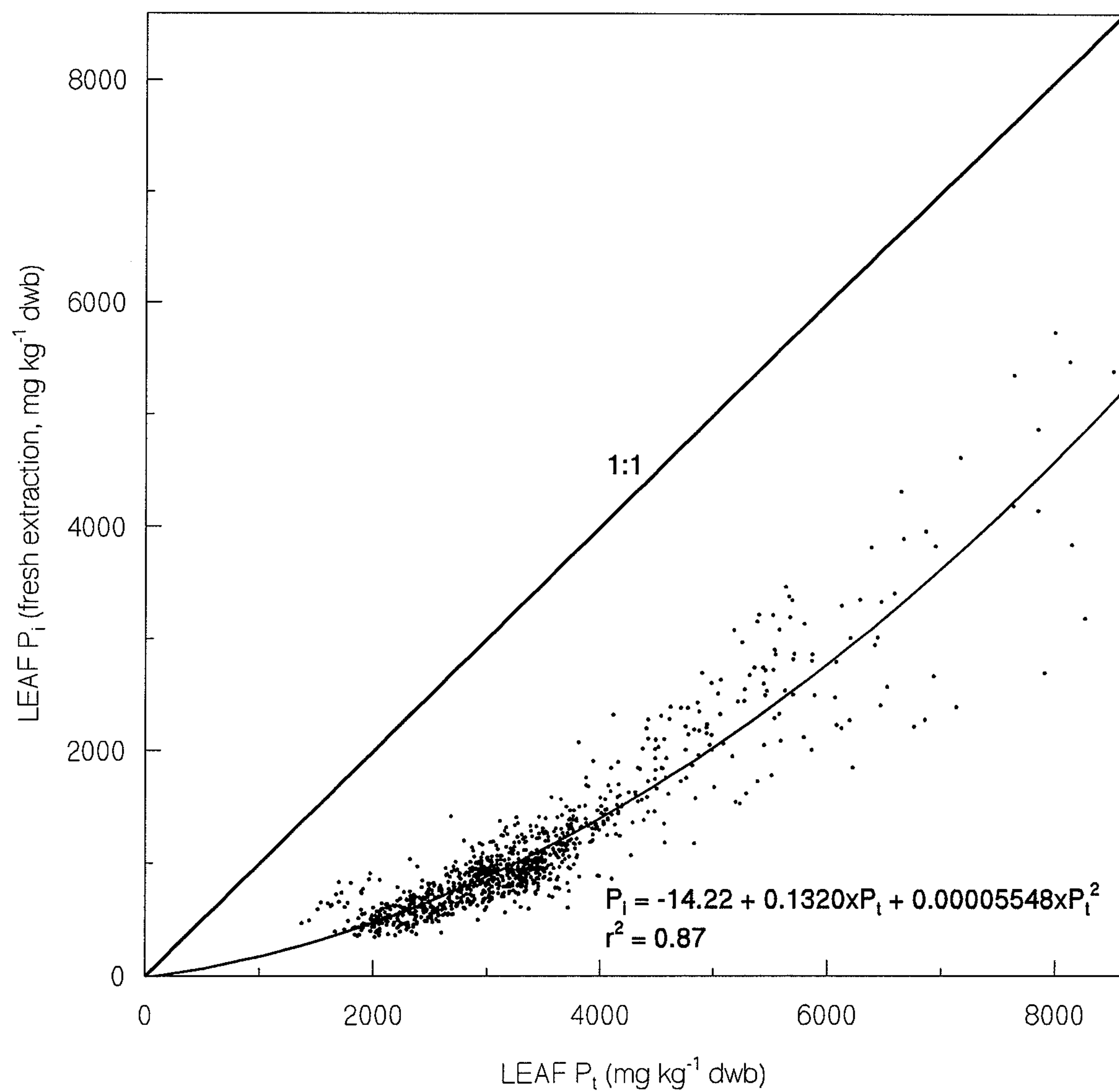


Figure 3.2. Relationship between leaf P_i extracted from fresh tissue and leaf P_t, for all sites, treatments, and sampling times.

later storage) functions, and decreasing fractions involved in growth and metabolism. Structural tissue requires lower concentrations of many nutrients, including P, so critical levels of these nutrients tend to decline as growth progresses. This decline reduces the sensitivity of plant analysis using whole-shoot samples for detecting deficiencies, since critical levels must be accurately adjusted for growth stage. Selection of tissue of constant physiological function (e.g. the youngest fully expanded leaf blades in this study) attempts to minimize this problem. Interpretive criteria under some systems use nutrient concentrations expressed as ratios with concentrations of nutrients other than C (or dry matter), such as Beaufill's Diagnosis and Recommendation Integrated System (Walworth and Sumner 1988). Such criteria tend to change less during growth, since most other nutrients are being similarly diluted by the increasing amounts of structural C.

Other nutrient concentrations were not routinely determined in this study. However the concentration of P_o may serve as a better reference level than that of C, for expressing nutrient concentrations in assessment of plant nutritional status. Unlike C, P_o would not normally be accumulating in less metabolically active pools during vegetative growth (Bieleski 1973). The difference between P_t and P_i, when both are expressed on the same tissue moisture basis, provides an estimate of tissue P_o in this work. Leaf P concentrations were therefore expressed as P_i/P_o ratios as an alternative means of expression for evaluation (Figure 3.3). In this context P_o concentration serves as an index of tissue metabolic activity and hence of demand for P_i. The P_i/P_o ratios followed patterns with site, treatment, and time that were similar to those of P_i (dwb; Figure 3.1d). Determination of the ratios required two separate tissue tests rather than one, and resulting ratio values had greater proportional error. Therefore, there was no advantage indicated for the use of P_i/P_o ratios in preference to P_i or P_t. Elliott et al. (1997b) observed a critical labile P:total P ratio for wheat leaves of 0.3 in South Australia; that critical ratio did not vary with growth stage, but was not regarded as superior to other methods for routine plant P diagnosis.

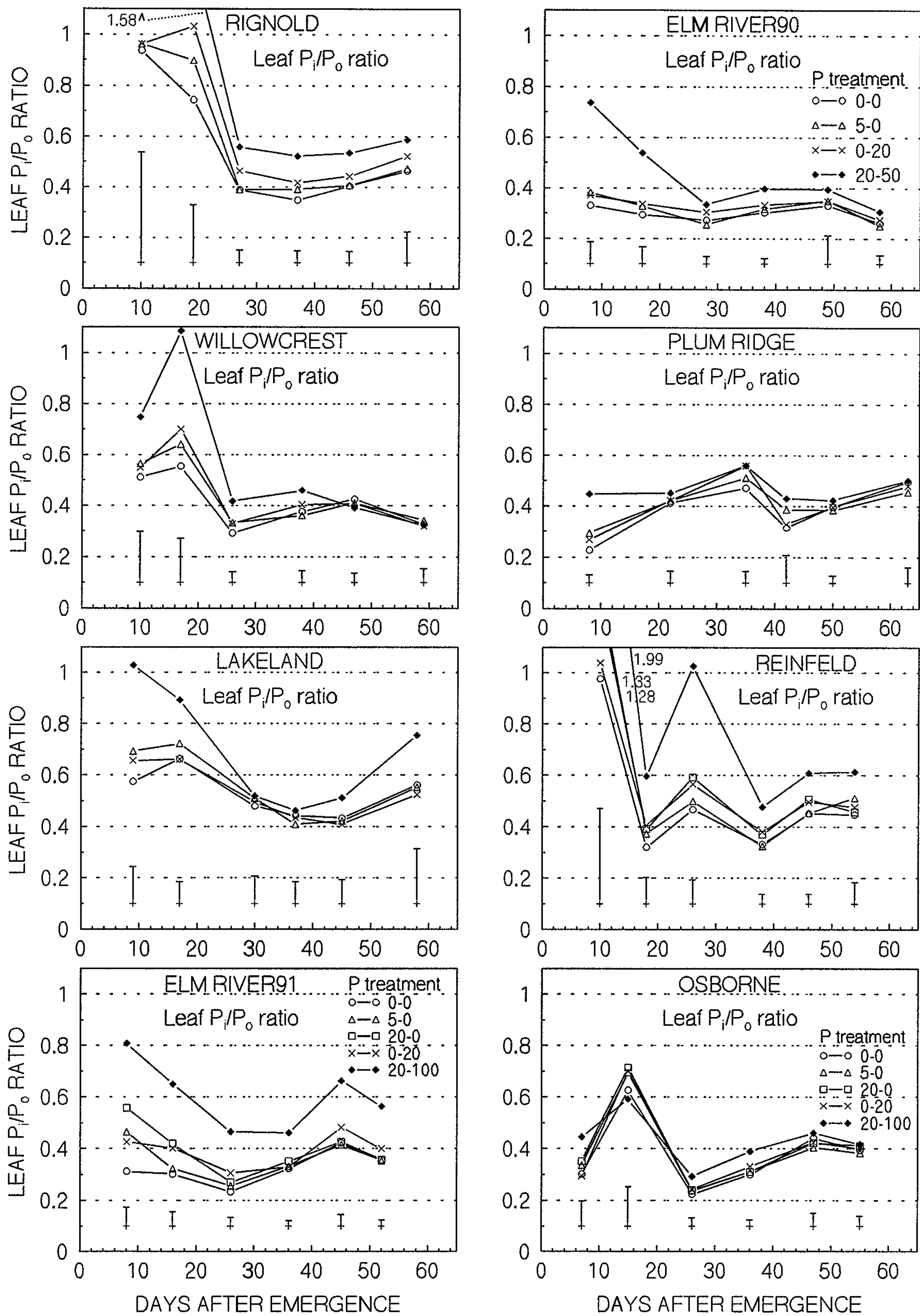


Figure 3.3 Ratios of extractable leaf P_i to P not extractable as P_i (P_o), for all sites, treatments, and sampling times. Error bars indicate LSD among P treatments within sites (P<0.05).

3.4.3 Tissue P Tests as Indicators of Yield-Limiting P Deficiency

The diagnostic value of a tissue test is determined by the nature and reliability of the relationship between the test values and yield or quality reductions caused by nutrient deficiencies and toxicities. That relationship should be constant across a reasonably broad range of conditions (moisture, temperature, soil type, daylength, cultivar) for the test to be sufficiently robust.

The eight sites in our study could not be sampled at identical growth stages or times after emergence, but it is necessary to compare data across sites for specific times. Phosphorus concentrations on non-sampling days were estimated by linear interpolation between the values for the sampling days. Relative yields (RY) of grain at maturity from all sites were then related to tissue test levels for each of the tests used, for each of ten times after emergence. Relative yield was the treatment yield expressed as a percentage of the yield of the highest P treatment within the same site. The highest P treatment was assumed sufficient in P for maximum growth at all times, though tissue P concentrations suggested that this may not have been true for the Osborne site. Since the highest P treatment produced the highest grain yield at each site, or a yield not significantly lower than the highest yield, toxicity effects of the higher P rates on grain yield were assumed to be negligible. The RY vs. test value relationships were evaluated by regression using a linear-plateau model (SAS Institute Inc. 1989). Results are tabulated (Table 3.3), and those for 10, 25, 40, and 55 DAE are presented graphically (Figure 3.4). All calculated plateau values were at RY levels within the range of 98 to 102%.

Relative yields were closely related ($r^2>0.8$) to tissue P concentrations from about the mid-tillering to the flag leaf stages (19 to 38 DAE), for all three of the tests used (Figure 3.5). Prior to 13 to 19 DAE, the P test levels were not as closely correlated with grain yield. Both leaf tests remained good predictors of RY to at least a week after heading (50 DAE), while the correlation of RY with shoot P_t declined sharply with time after the early boot stage. This was primarily due

Table 3.3 Relationships between wheat grain RY at maturity and tissue P concentrations at 10 to 55 DAE for all sites.

Tissue and P test	DAE	RY =[z]	break point[z]	r^2
	d	%	mg kg^{-1}	
Leaf P_t	10	3.8 + 0.02267x	4255	0.71
	15	-3.7 + 0.02702x	3874	0.84
	20	-30.8 + 0.03736x	3554	0.90
	25	-60.8 + 0.04844x	3323	0.88
	30	-91.3 + 0.06346x	3005	0.91
	35	-91.7 + 0.06752	2830	0.94
	40	-110.4 + 0.07494x	2801	0.92
	45	-139.7 + 0.08472x	2822	0.90
	50	-129.9 + 0.07982x	2873	0.84
	55	-77.5 + 0.05909x	2992	0.67
Shoot P_t	10	10.4 + 0.02013x	4501	0.70
	15	3.2 + 0.02421x	4040	0.80
	20	-24.0 + 0.03432x	3652	0.84
	25	-45.1 + 0.04072x	3586	0.88
	30	-86.4 + 0.06086x	3054	0.93
	35	-75.0 + 0.06434x	2712	0.86
	40	-36.0 + 0.05057x	2695	0.76
	45	-19.5 + 0.04681x	2567	0.66
	50	-3.4 + 0.04223x	2469	0.51
	55	12.9 + 0.03603x	2438	0.36
Leaf P_i	10	-11.8 + 0.5816x	190	0.66
	15	-23.3 + 0.6482x	188	0.68
	20	-23.0 + 0.6740x	184	0.84
	25	-45.3 + 0.9647x	150	0.87
	30	-38.8 + 0.8286x	167	0.82
	35	-57.0 + 0.8458x	175	0.82
	40	-86.7 + 0.9158x	203	0.82
	45	-67.5 + 0.7014x	238	0.76
	50	-80.4 + 0.7027x	256	0.82
	55	-63.4 + 0.6434x	251	0.60

[z] x and break point in mg of P kg^{-1} of tissue (dwb for P_t, fwb for P_I). Equations describe RY as a function of P concentration for the linear-increasing portion of the relationship only, to the break point.

to shoot P_t concentrations at the Osborne site, which did not decline with time as at other sites. McLachlan (1982) also determined highly significant linear correlations (r = 0.83 to 0.92) between wheat shoot P_t concentrations and grain yield at maturity for shoots sampled prior to

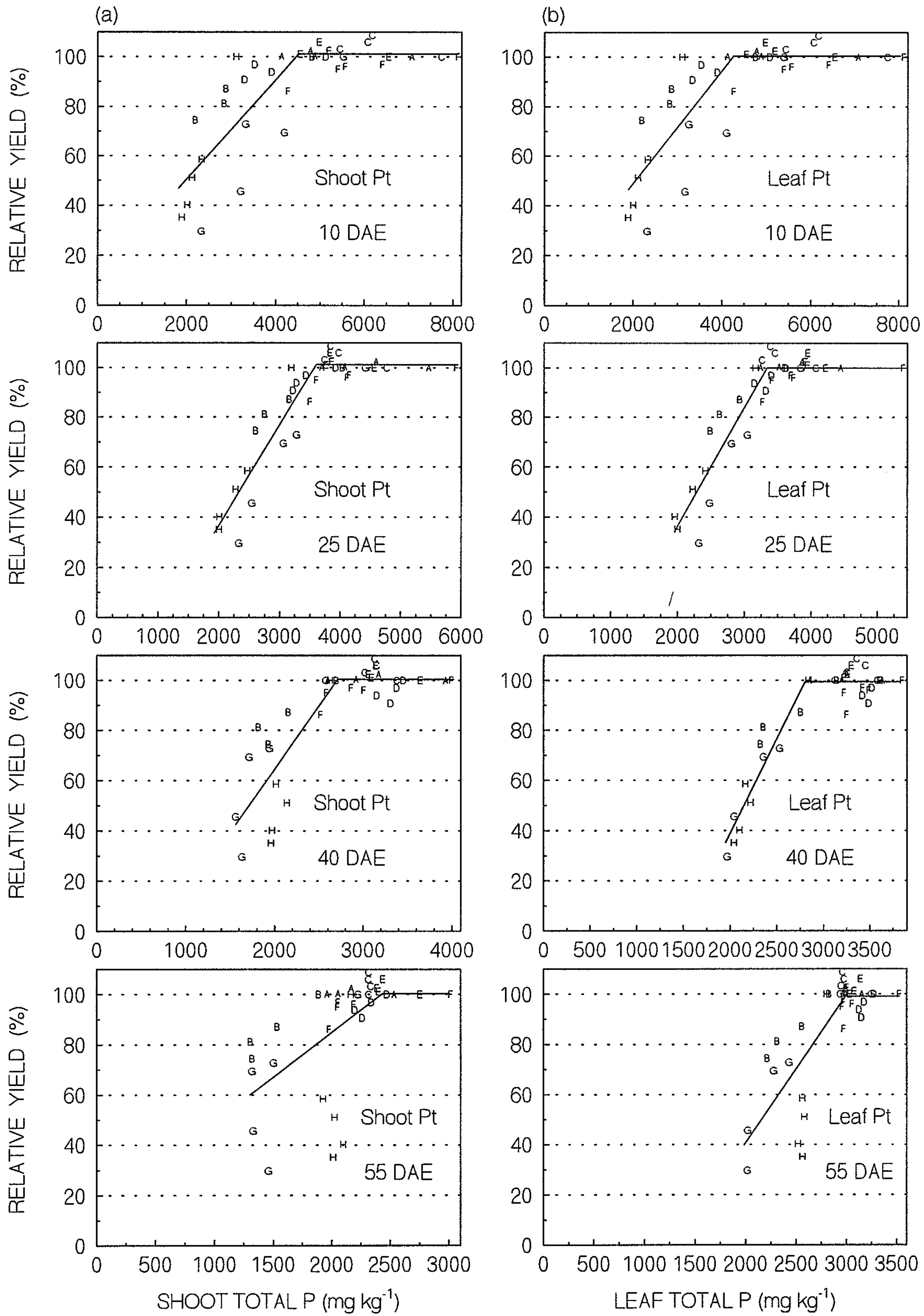


Figure 3.4 Relationships between relative yield of grain at maturity and (a) shoot P_t (dwb), (b) leaf P_t (dwb), and (c) leaf P_i (fwb), at 10, 25, 40, and 55 DAE. Note X-axis scales differ among all graphs. Point markers indicate site as listed in Table 3.1. cont.

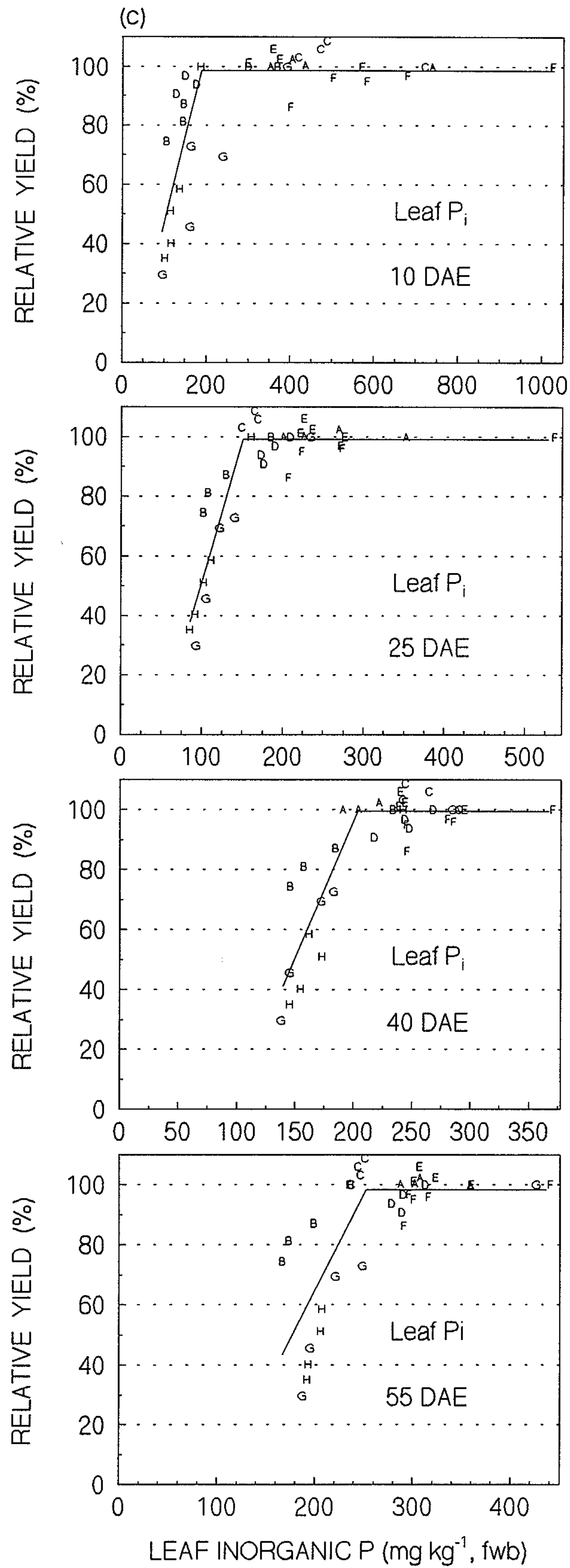


Figure 3.4. cont.

booting, but not during or after booting ($r<0.25$). Mallarino (1996) determined that P concentration in young corn plants, or in their ear-leaf blades at silking, much better reflected the plant P status for yield production than did P in the stalk or grain at maturity. In an Australian study very similar to the work reported here, Elliott et al. (1997b,c,d) concluded that leaf total and labile P, and shoot total P, provided good indices of wheat P status, and recommended sampling at the tillering stage.

It is desirable to have widely differing test values among plants differing in P nutritional status, rather than most values grouped closely around the CNC, for routine diagnostic purposes. A wide range of test values reduces the number of test results which will be in a marginal range close to the CNC, where diagnosis is unsure, and also reduces the need for as high a degree of analytical accuracy. Variation among samples in P concentration, on a relative as well as absolute basis, declined through the growing season for all tests (Figure 3.6). The P_i concentrations were much more widely spread than the P_t concentrations until about 35 DAE; the wider range in values could help to compensate for the lower analytical accuracy that would likely be achievable in a field quick-test situation with the P_i test. Elliott et al. (1997b,d) also recognized the narrowing in range of observed plant P concentrations for all P tests as a factor favouring early season plant analysis.

Critical nutrient concentrations for all three P tests declined throughout the early part of the growing season (Figure 3.7; each CNC is taken as the break point in the corresponding relationship between P test level and RY in Figure 3.4 and Table 3.3). The CNC for shoot P_t continued to decline for the rest of the analysis period. This may be due to increasing proportions of structural materials which are not metabolically active (e.g. cellulose in the stem) making up the plant's dry weight. Critical levels of leaf P_t tended to level out at about 2800 to 3000 mg kg^{-1} of P after about 30 DAE (beginning of stem extension). Critical nutrient

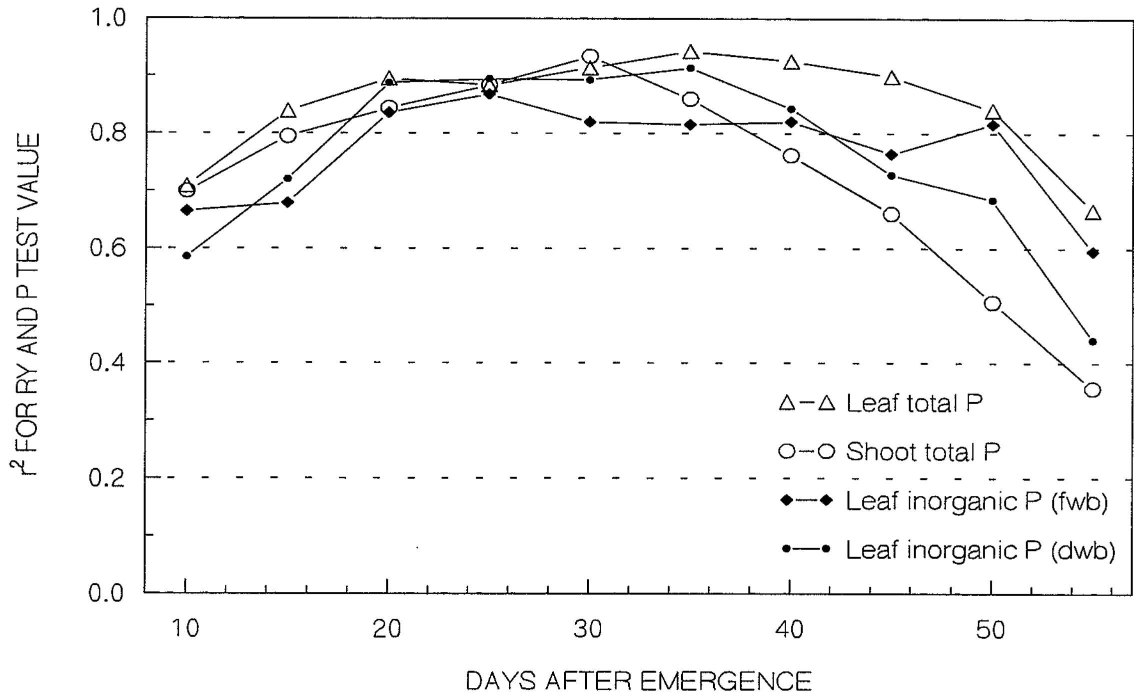


Figure 3.5. Coefficients of determination (r^2) for the regressions of relative grain yield on P test levels, for four measures of tissue P at 10 to 55 DAE.

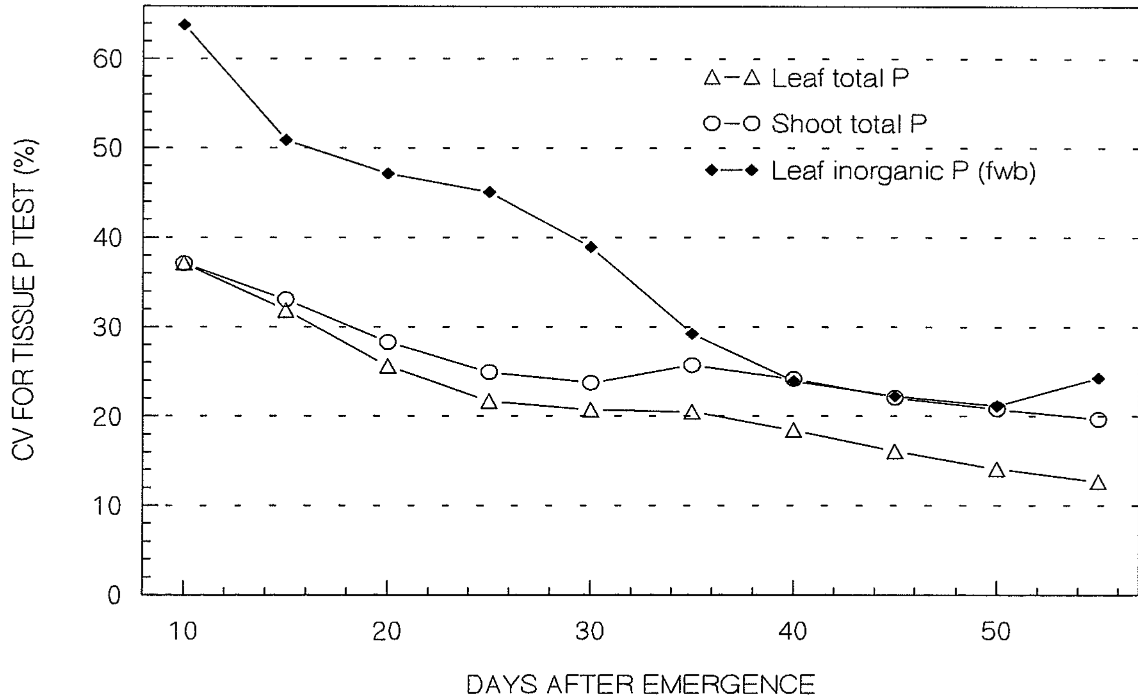


Figure 3.6 Variation in observed P concentrations (site x treatment x time means) for three measures of tissue P at 10 to 50 DAE, expressed as coefficients of variation.

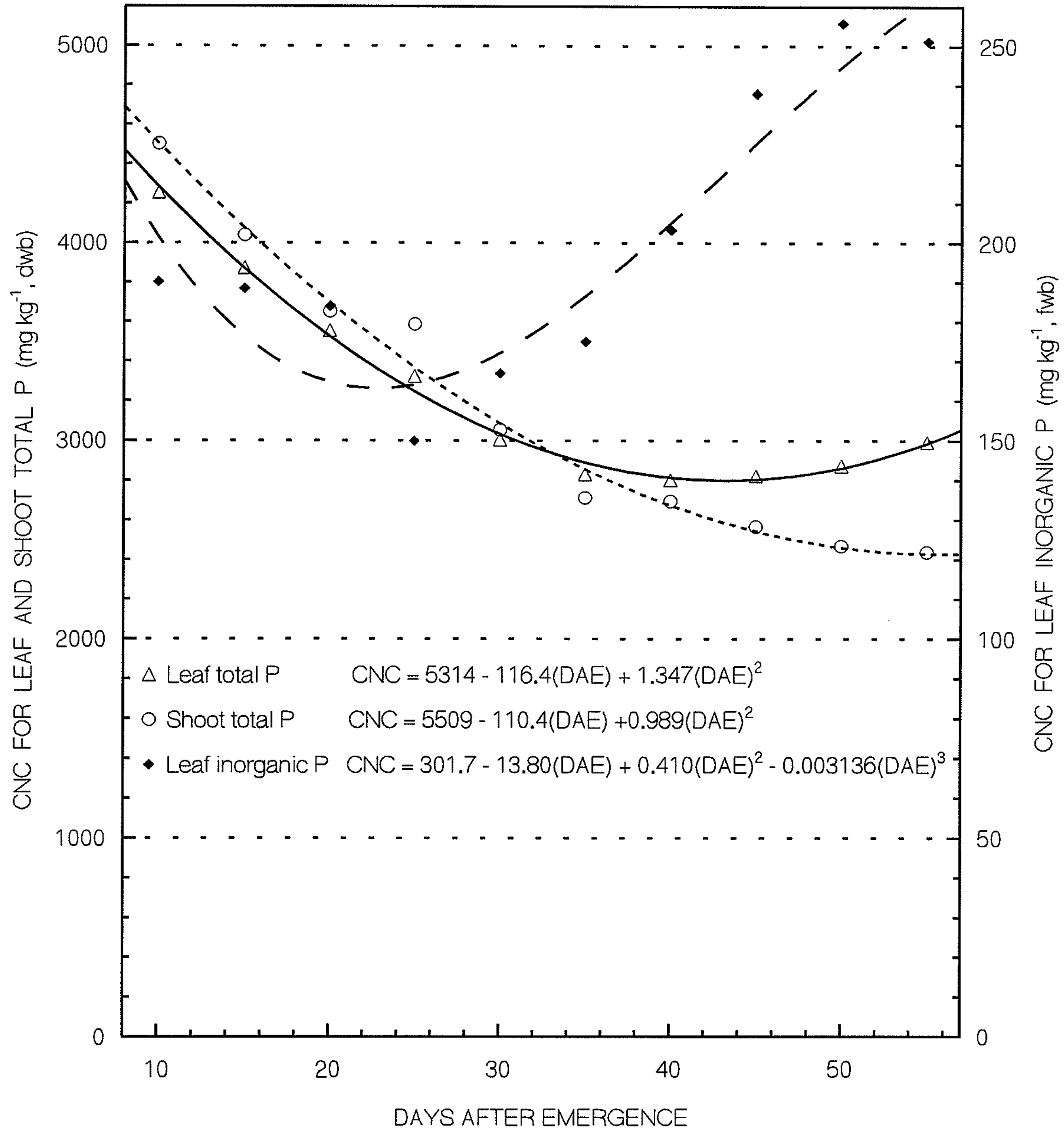


Figure 3.7. Critical nutrient concentrations for wheat tissue P through the growing season for three tissue tests.

concentrations for leaf P_i generally increased after 25 DAE. However, P_i concentrations in Figure 3.7 are expressed on the fresh weight basis, rather than the dry weight basis as used for P_t. Leaf moisture content declined after 25 DAE (data to be presented later - Figures 4.2 and 4.3). When extractable P_i concentrations were expressed on a leaf dry weight basis (Figure 3.1d), and CNC's were determined in the same manner as those above, the CNC's throughout the 10 to 55 DAE period were all in the range of 715 to 984 mg kg^{-1} of P (data not presented).

Rapid changes with time in CNC around the time of tissue sampling are undesirable for diagnostic purposes, since such changes necessitate that the plant stage be very accurately assessed at sampling to allow the correct interpretive criteria to be applied. Based on this consideration, and the close relationship between leaf P_t and RY of grain, the leaf P_t test is the preferred test for the 30 to 50 DAE interval.

The CNC's for each of the three tests at 10 to 55 DAE as determined in this study (Figure 3.7) are in general agreement with, to substantially higher than, many of the tabulated criteria in use for spring wheat. The interpretive criteria approved for Manitoba (Anonymous 1991) classified 0.15-0.24% P_t in the "whole above ground plant prior to filling" as "marginal" for cereals. Sampling was recommended "just prior to heading" by McGill (1981). The upper end of the "marginal" range corresponds approximately to the CNC determined in this study at heading or just after. However, the lower half of the range corresponds to shoot P_t levels that were associated with some substantial yield reductions. Jones et al. (1991) indicated the lower end of the sufficiency range is 0.20% P_t for the whole shoot of spring wheat at heading, compared to 0.25% in this study. Elliott et al. (1997d) developed comprehensive criteria for wheat in South Australia. Their shoot and leaf CNC's were higher at the tillering stage (>0.48% P), and similar to lower by the heading stage, as compared to those developed in this study for Manitoba.

The concentrations of P_i extractable from the dried leaf tissues (P_{id}) were compared with leaf P_t and leaf P_i (fresh tissue extraction) by regression, instead of being related directly to grain yields, since the dry tissue extractions were not carried out on all samples. All concentrations were converted to the dry leaf tissue weight basis prior to regression.

As for P_i extractable from the fresh tissue, P_{id} accounted for increasing fractions of P_t as tissue P concentrations increased:

$$P_{id} = 339.8 + 0.1472P_t + 0.00009058P_t^2 \qquad [1]$$

$$(r^2 = 0.859; n = 535)$$

The fraction of P_t present as P_{id} was little affected by sampling time or site.

The relationships involving P concentrations should be assumed to have validity only over the 10 to 55 DAE interval, and only within the ranges of most of the measured values, which were about 1700 to 6500 mg kg^{-1} for leaf P_t, 400 to 4200 mg kg^{-1} for P_i, and 700 to 5000 mg kg^{-1} for P_{id}.

Drying the leaf tissue substantially increased the fraction of its P which was determined as extractable orthophosphate. Barr and Ulrich (1963) found that drying induced increases in extractable P_i for lima bean, and determined a concomitant loss of soluble organic P on drying. Concentrations of P extractable by the fresh vs. dry methods in this study were more similar during the latter part of the sampling period (after about a week after the beginning of stem extension) than at earlier stages. For samples taken at 7 to 35 DAE:

$$P_i = -509.5 + 0.7706P_{id} \quad (r^2 = 0.949; n = 266) \qquad [2]$$

whereas, for samples taken 36 to 63 DAE:

$$P_i = 185.9 + 0.4919P_{id} \quad (r^2 = 0.615; n = 269) \qquad [3]$$

Substituting P_{id} values for P_t values (using Eq. 1) into the equation relating CNC by the leaf P_t test to time after emergence (Figure 3.7), yields an estimate of CNC for dried leaf tissue

extractable P_i (i.e for P_{id}) as a function of sampling time, approximately described by:

$$CNC(P_{id}) = 3402 - 91.38(DAE) + 1.0688(DAE)^2 \quad [4]$$

The various tissue P tests used in this study could provide reasonably good bases for diagnosing grain yield limiting P deficiency in wheat. Sampling at the tillering to boot stage is recommended, though the leaf tests were still effective to at least a week after heading. It is critical that interpretive criteria be specific for the plant part sampled, the time/stage of sampling, and the analytical procedure used.

The CNC's were determined empirically in this analysis in a manner that should provide the best criteria for the prediction of the occurrence of grain yield limiting P stress, regardless of when the plant is tested. A CNC so determined may not necessarily be equal to the critical concentration required for optimum growth *at the time of sampling*. For example, P deficiency in the field may limit plant growth and development only at early growth stages, so the CNC's established in this study for later stages may be higher than the concentrations actually required for optimum growth at those times. However, these CNC's provide the best criteria for separating field-grown crops that have sufficient P for optimum grain yield, from those that will produce less grain due to P deficiency at some point in their growth.

3.4.4 Olsen Soil Test P as an Indicator of Yield-Limiting P Deficiency

The field data on phosphate fertilizer response generated in this study support the current use and general interpretation of the Olsen $NaHCO_3$ soil test for field crops in Manitoba. There was no response to fertilizer phosphate at the three sites testing 14 to 30 mg kg^{-1} in Olsen P (0-15 cm depth; Figure 3.8). The five sites testing 3 to 9 mg kg^{-1} showed a roughly diminishing response with increasing test level. At a given soil test level, greater yield reduction due to phosphate deficiency occurred in 1991, probably due to the drier conditions in 1991 limiting P uptake at the

critical early stages of crop growth (Section 3.4.1). Black (1982) reported a critical range for the Olsen P test of 10 to 15 mg kg^{-1}, which is supported by our results.

Although soil test calibration was not a major objective of this study, the success of the Olsen P test in predicting crop response to fertilizer P in this work is significant in relation subsequent studies. Possible limitations of the Olsen test are investigated in Sections 5 to 7. However, the Olsen test has generally been effective for diagnosing soil P status for field crop production under Manitoba conditions.

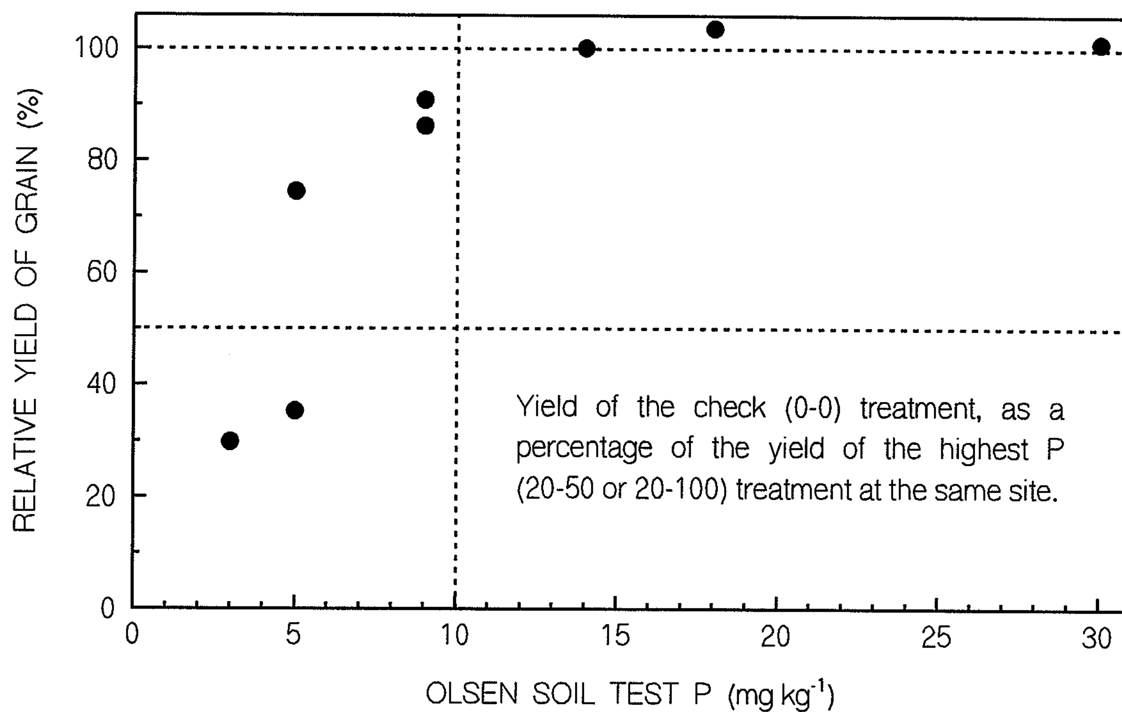


Figure 3.8 Relationship between Olsen soil test P level and relative yield of wheat grain in check treatments at all field sites.

3.5 Conclusions

For field-grown spring wheat, whole-shoot P_t concentrations and the corresponding CNC's declined through the growing season. Leaf P_t and P_i concentrations and CNC's initially declined and then stabilized by the stem extension stage of growth, when expressed on the tissue dry weight basis. Tissue P test levels converged as the season progressed, reducing the differences among sites and fertilizer treatments which were pronounced during early growth stages.

Generally less than half of the leaf P_t was extractable from the fresh tissue as P_i. The P_i concentrations showed much greater proportional variation than P_t; P_i was much more sensitive to P supply. This wider spread in test values would reduce the analytical accuracy needed for reliable diagnosis, which is desirable under "quick-test" conditions.

The percentage of wheat grain yield lost due to P deficiency 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 (shoot P_t, and leaf P_t and P_i), across all sites and P treatments. The correlation of shoot P_t with RY declined much more sharply with time after the early boot stage, compared to that of leaf P_t. Critical nutrient concentrations were determined for each test and stage, from 10 to 55 DAE. In general, CNC's were similar to or higher than those suggested in other sources.

Shoot P_t, leaf P_t and leaf P_i can each be used to predict if P deficiency will limit grain yield for spring wheat, using the time/stage-specific interpretive criteria presented. Sampling at the tillering to boot stage was optimal. The shoot P_t test was less effective than the leaf tests at and beyond the heading stage. The leaf P_i test appears to be adaptable for use as an in-field quick-test. Use of the Olsen soil test for P was effective for predicting crop P deficiency.

4. THE EFFECT OF PHOSPORUS DEFICIENCY ON GROWTH OF SPRING WHEAT IN THE FIELD

4.1 Abstract

The effects of P deficiency on field-grown spring wheat were quantitatively characterized by monitoring selected growth parameters under a range of P-supply and growing conditions in Manitoba.

In treatments deficient in P for maximum grain yield, shoot growth was restricted as early as one to two weeks from crop emergence, even where phosphate fertilizer was placed with the seed. Visual observation and plant tissue P concentrations also had indicated very early stress. Phosphorus deficiency reduced shoot relative growth rates, in comparison to those for P-sufficient treatments, during the first two to five weeks from emergence only. Deficiency of P also resulted in increased dry matter percentages in the tissue, more so for whole shoots than for leaves.

Shoot P accumulations at maturity were 4 to 19 kg ha^{-1}, of which 68 to 91% was contained in the grain. Components of grain yield were determined in one year of the study (three of the eight site-years). Phosphorus fertilization increased number of heads, number of kernels per head, and kernel weight, at two very P-deficient sites, but only number of heads at the slightly deficient site.

4.2. Introduction

Plants respond to stresses, including nutrient deficiencies, with a wide range of biochemical, physiological, morphological, and other reactions. Many such responses help the plant to avoid or better cope with the stress, and some are visually apparent as deficiency symptoms. Knowledge of how crops respond to stresses under actual conditions in the field is essential for crop diagnosis, growth modeling, and devising of management practices that minimize the stress or its undesirable consequences at minimal agronomic and environmental cost.

The general effects of P deficiency on growth are qualitatively well-known, as are the quantitative effects on grain yield for many situations. Phosphorus is involved in almost all metabolic and growth processes, due to the importance of the pyrophosphate bond in energy transfer, and also as a component of nucleic acids, phospholipids (cell membranes), and many intermediary compounds of metabolism (Glass et al. 1980). Thus almost every function could be inhibited by P deficiency. The process first affected may be the transport of photosynthate out of the chloroplast. Photosynthate leaves the chloroplast as triose-phosphates, in an obligatory counter exchange for P_i across the chloroplast envelope (Leegood 1996). Other processes throughout the plant are then starved for energy, including synthesis of nucleic acids and protein, N_2 fixation in legumes, nutrient absorption and transport, and growth.

In small grain species, P deficiency reduces growth in general, but especially growth of the shoot; reduces tillering, which has the major effect on grain yield; and delays plant development. Foliage color remains dark, but the older leaves of wheat may die back from the tip (Elliott et al. 1997a, Hoffer and Krantz 1949). However, visual symptoms of P deficiency are not distinctive enough to be very diagnostic.

In this study several selected growth parameters were monitored for field-grown spring wheat in Manitoba, under a wide range of P-supply and growing conditions. Tissue P concentrations and

their relationships with grain yield were presented in Section 3. Effects on shoot dry matter and P accumulation, tissue moisture levels, grain and straw P concentrations and P off-take, and harvest index follow. Little quantitative documentation exists of the effects of P deficiency on these characteristics for field-grown small grains under western Canadian conditions.

4.3. Materials and Methods

Shoot growth, and leaf and whole-shoot P concentrations, were monitored during the growing season at five field sites in 1990 and three in 1991 (Table 3.1). Four or five P treatments, varying in both fertilizer rate and placement, were used at each site to create a wide range of P supply conditions. Site characteristics, treatments, field methods, and most laboratory methods were reported previously (section 3.2).

Components of yield (number of heads, mean number of kernels per head, and mean kernel weight) were determined for the whole-shoot samples taken at maturity from the three 1991 sites. The heads, including those with no seed, were counted in each sample. After threshing, a 200-seed portion of each grain sample was weighed to determine mean kernel weight.

4.4. Results and Discussion

Growth, in terms of changes in accumulated above-ground dry matter with time, followed the typical S-shaped curve. Data is presented in Table 4.1 for the highest P fertilizer treatment only at each site. For all other treatments, shoot dry matter accumulation at each sampling time is presented as a fraction of the accumulation in the highest P fertilizer treatment at the same site and time (i.e. relative yield - RY; Figure 4.1).

Table 4.1 Shoot dry matter accumulation through the growing season and at maturity for the highest P fertilizer treatment at each site.

DAE	Shoot dry matter accumulation		DAE	Shoot dry matter accumulation	
d	kg ha^{-1}	%^{z}	d	kg ha^{-1}	%^{z}
Rignold			**Elm River90**		
10	128	1.31	8	78	0.72
19	435	4.4	17	299	2.8
27	1328	13.5	28	1488	13.8
37	3423	34.8	38	4042	37.4
46	5642	57.4	49	7533	69.7
56	7900	80.3	58	9298	86.0
mat.	9833	100.0	mat.	10,808	100.0
Willowcrest			**Plum Ridge**		
10	39	0.57	8	41	0.48
17	147	2.2	22	386	4.5
26	663	9.8	35	1696	19.6
38	2296	33.8	42	3048	35.2
47	4619	68.0	50	5241	60.6
59	4910	72.3	63	7609	88.0
mat.	6790	100.0	mat.	8652	100.0
Lakeland			**Reinfeld**		
9	71	0.68	10	113	1.46
17	259	2.5	18	519	6.7
37	3162	30.1	26	1290	16.6
45	4784	45.6	38	3488	44.9
58	7149	68.1	46	5065	65.3
mat.	10,498	100.0	54	6468	82.9
			mat.	7761	100.0
Elm River91			**Osborne**		
8	83	0.94	7	68	0.79
16	387	4.4	15	193	2.2
26	1342	15.2	26	737	8.6
36	3046	34.4	36	2461	28.6
45	4875	55.1	47	4604	53.5
52	7022	79.3	55	6507	75.6
Mat.	8855	100.0	mat.	8612	100.0

^{z} percentage of dry matter accumulation at maturity

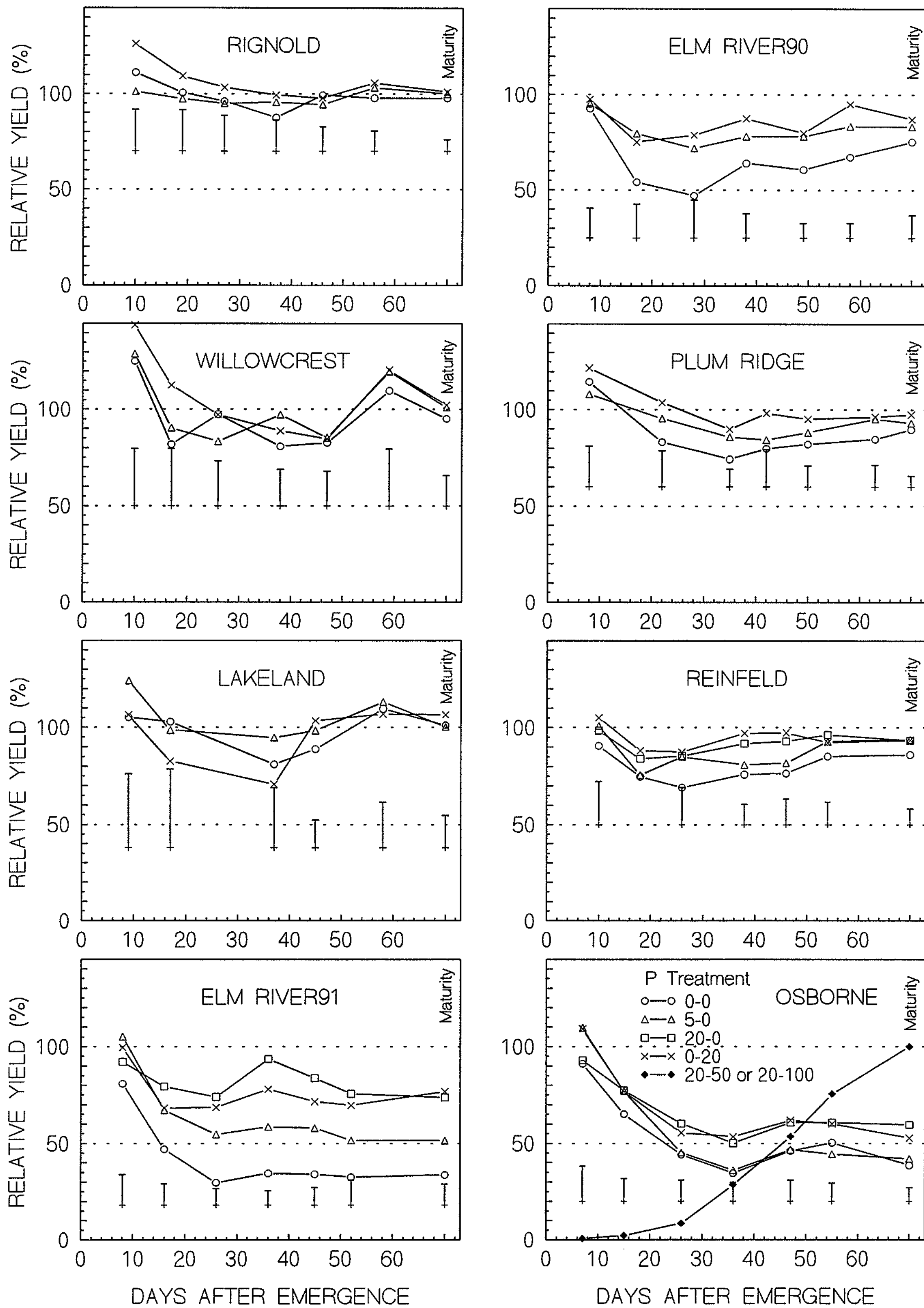


Figure 4.1 Shoot dry matter yield as a percentage of the dry matter yield of the highest P treatment at the same site and time (i.e. relative yield), for all sites and sampling times. Error bars indicate LSD (P<0.05) among P treatments within site and time. The Osborne site legend applies to all sites.

Phosphorus deficiency significantly reduced shoot growth to the first sampling time (7 to 10 DAE) only at the Elm River91 site (Figure 4.1). Seed P can provide a substantial portion of the seedling P requirements to this stage (DeMarco 1990). Assuming a typical concentration of 3700 mg kg^{-1} of P in the seed, the 0.5 kg ha^{-1} of P in the seed was greater than the shoot P content at the first sampling time for most situations (Table 4.2). There were, however, strong effects of P treatment on tissue P concentrations at all sites at 7 to 10 DAE (section 3.3). Similarly, for perennial ryegrass seedlings grown in solution culture at various phosphate concentrations, plant P concentration was affected by P treatment several days before dry weight was influenced (Breeze et al. 1984). In our study some suppression of seedling growth due to toxicity of seed-placed P fertilizer was suggested by the trend toward lower shoot yields with increasing seed-placed P at the earliest sampling time at some of the coarser-textured sites (Figure 4.1, table 3.1). Shoot dry matter accumulation for the P-deficient treatments (i.e. for the treatments deficient in P as defined by grain yield at maturity) were already much less than accumulations in the highest P treatment, at 17 to 22 DAE. This effect agrees with the visual observation that topgrowth response to P became apparent at one to two weeks after emergence at low P sites ("pop-up" effect). Similarly, Elliott et al. (1997a) observed very early visual and shoot growth effects of P deficiency on wheat. Goos et al. (1994) showed that P fertilization in the seedrow caused substantial improvements in growth and development of spring wheat at the 2 to 3-leaf stage.

Relative, rather than absolute, shoot dry matter accumulations (yields) are shown for all P treatments except the highest P rate at each site (Figure 4.1; if shown, relative yield for the highest P treatment would be along the 100% line). Consequently, for the intervals between sampling times, the slopes of the lines joining the graphed points in Figure 4.1 are proportional to the relative growth rates in the various treatments, with the slope of the highest P treatment set to zero (i.e. along the 100% relative yield line). The reduction in relative growth due to P deficiency tended to occur for only the first two to five weeks after emergence, depending on site

Table 4.2 Shoot P accumulation through the growing season and at maturity at all field sites.

Site and P treatment		Shoot P accumulation												
		---------- kg ha^{-1} ----------							% of P accumulation at maturity					
Rignold	**DAE:**	**10**	**19**	**27**	**37**	**46**	**56**	**mat.**	**10**	**19**	**27**	**37**	**46**	**56**
0-0		0.59	1.88	4.48	8.3	12.6	14.9	14.7	4.0	12.8	31	57	86	102
5-0		0.63	2.05	4.91	10.5	13.2	16.4	15.3	4.1	13.4	32	68	86	107
0-20		0.77	2.39	6.17	11.8	14.7	17.7	16.1	4.8	14.9	38	73	91	110
20-50		0.91	2.58	7.01	14.3	18.9	19.1	18.8	4.8	13.7	37	76	100	102
	LSD (0.05):	0.16	0.72	1.39	2.3	2.6	2.0	1.6						
Elm River90	**DAE:**	**8**	**17**	**28**	**38**	**49**	**58**	**mat.**	**8**	**17**	**28**	**38**	**49**	**58**
0-0		0.15	0.39	1.52	5.3	6.6	7.9	8.8	1.7	4.5	17	60	75	89
5-0		0.21	0.68	2.89	6.0	8.2	9.7	9.9	2.1	6.9	29	60	83	98
0-20		0.21	0.70	3.71	8.0	9.8	13.0	12.1	1.8	5.8	31	66	81	108
20-50		0.39	1.28	5.87	11.5	15.5	16.7	16.7	2.3	7.7	35	69	93	100
	LSD (0.05):	0.07	0.19	1.03	1.3	1.2	1.2	1.8						
Willowcrest	**DAE:**	**10**	**17**	**26**	**38**	**47**	**59**	**mat.**	**10**	**17**	**26**	**38**	**47**	**59**
0-0		0.27	0.48	2.42	5.8	9.4	12.0	11.2	2.4	4.3	22	52	84	107
5-0		0.31	0.58	2.11	7.4	9.8	13.0	11.5	2.7	5.1	18	64	85	113
0-20		0.35	0.77	2.58	6.6	9.7	13.1	11.7	3.0	6.6	22	57	83	112
20-50		0.30	0.83	3.13	8.3	11.7	10.6	11.4	2.6	7.3	27	73	102	93
	LSD (0.05):	0.08	0.18	0.60	1.5	2.2	2.6	1.4						
Plum Ridge	**DAE:**	**8**	**22**	**35**	**42**	**50**	**63**	**mat.**	**8**	**22**	**35**	**42**	**50**	**63**
0-0		0.16	0.96	5.03	7.4	10.1	13.5	13.5	1.2	7.1	37	55	75	100
5-0		0.18	1.15	5.61	7.4	10.5	15.1	13.7	1.3	8.4	41	54	76	110
0-20		0.18	1.34	5.89	9.4	12.3	15.6	14.6	1.2	9.1	40	64	84	107
20-50		0.22	1.51	6.65	9.9	13.5	17.4	16.1	1.4	9.4	41	61	84	108
	LSD (0.05):	0.03	0.31	0.53	1.5	1.2	1.5	1.4						
Lakeland	**DAE:**	**9**	**17**		**37**	**45**	**58**	**mat.**	**9**	**17**		**37**	**45**	**58**
0-0		0.34	1.10		8.7	12.1	17.7	17.9	1.9	6.2		49	68	99
5-0		0.48	1.11		10.2	13.0	18.4	17.8	2.7	6.3		57	73	104
0-20		0.39	0.90		8.1	14.1	17.6	18.7	2.1	4.8		43	75	94
20-50		0.48	1.27		12.7	14.9	18.9	18.2	2.6	7.0		70	82	104
	LSD (0.05):	0.15	0.44		3.4	1.7	4.0	2.1						
Reinfeld	**DAE:**	**10**	**18**	**26**	**38**	**46**	**54**	**mat.**	**10**	**18**	**26**	**38**	**46**	**54**
0-0		0.44	1.21	3.17	7.0	8.1	11.0	10.5	4.2	11.5	30	67	77	104
5-0		0.62	1.30	4.01	7.7	8.9	12.3	11.4	5.4	11.4	35	67	77	108
20-0		0.71	1.60	4.64	9.8	10.7	12.9	11.9	6.0	13.4	39	83	90	109
0-20		0.66	1.61	4.73	10.8	12.1	13.2	12.4	5.3	12.9	38	87	97	106
20-100		0.92	2.89	7.67	14.7	17.5	19.8	15.9	5.8	18.2	48	92	110	124
	LSD (0.05):	0.15		1.22	2.1	1.9	2.3	1.7						
Elm River91	**DAE:**	**8**	**16**	**26**	**36**	**45**	**52**	**mat.**	**8**	**16**	**26**	**36**	**45**	**52**
0-0		0.16	0.36	0.94	1.8	2.5	3.4	4.0	4.1	8.9	24	45	63	84
5-0		0.29	0.71	1.84	2.9	4.1	4.9	6.4	4.6	11.1	29	46	64	77
20-0		0.33	1.17	2.94	5.1	6.3	7.4	9.3	3.6	12.5	31	54	67	79
0-20		0.28	0.83	3.03	4.8	6.4	7.9	10.0	2.8	8.3	30	48	64	79
20-100		0.47	1.95	5.82	7.9	12.9	16.5	17.1	2.8	11.4	34	46	75	96
	LSD (0.05):	0.05	0.17	0.40	0.5	1.4	1.6	1.7						
Osborne	**DAE:**	**7**	**15**	**26**	**36**	**47**	**55**	**mat.**	**7**	**15**	**26**	**36**	**47**	**55**
0-0		0.13	0.20	0.67	1.7	4.0	6.6	5.5	2.3	3.6	12	31	73	119
5-0		0.16	0.24	0.69	1.8	4.1	6.1	6.3	2.6	3.9	11	28	66	97
20-0		0.16	0.30	1.11	2.6	5.1	7.6	9.0	1.8	3.3	12	29	57	85
0-20		0.17	0.27	0.95	2.9	5.8	8.0	7.6	2.2	3.6	12	38	76	105
20-100		0.22	0.59	2.37	6.5	11.8	14.1	14.9	1.4	3.9	16	44	79	95
	LSD (0.05):	0.04	0.06	0.24	0.6	1.3	1.0	1.5						

and P treatment. After that time, to maturity, the relative growth rates within sites were either similar among treatments (relative yield curves flat, e.g. Elm River91), or else the lower P treatments had higher relative growth (e.g. Elm River90). The relative yields observed indicate that the greatest effect of P deficiency on growth occurred during the first few weeks of growth, and that the relative yield patterns established early in the season carried through to a large extent to maturity. However, the relative yields do not clearly indicate the degree of P stress that the plants were under later in the season. Growth chamber and solution culture studies have shown that if small grains are stressed by P deficiency at early growth stages, an improvement in P nutrition at the heading stage or later has little effect on grain yield at maturity (Batten and Wardlaw 1987, Boatwright and Viets 1966). Perennial ryegrass required higher solution P concentrations for maximum growth for the first 29 d from sowing, than for the 29 to 43-day period (Breeze et al. 1985).

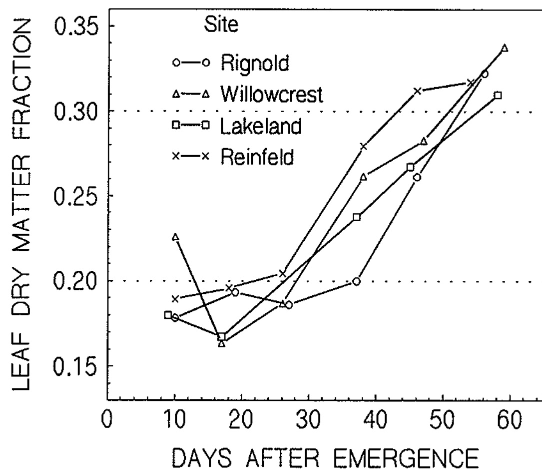
The method of placement of P fertilizer had only a small influence on the timing of the growth reduction due to P deficiency. By the second sampling time, 15 to 22 DAE, growth was clearly limited at low-P sites, even in treatments receiving 5 or 20 kg ha^{-1} of P placed in the seedrow at planting (Figure 4.1). Total P accumulation in the shoot to that point for the treatments receiving P with the seed was only 0.24 to 1.60 kg ha^{-1} (Table 4.2). Clearly, uptake of fertilizer P applied at normal application rates, even if seed-placed, is insufficient to alleviate early season P stress in very low-P soils; the major stress occurs early regardless of P placement. Plant P test levels indicated that placing small amounts of fertilizer P with the seed improved plant P nutrition for the first 1 to 2 wk after emergence, but this did not carry through to result in much grain yield advantage (Section 3.4.2).

Tissue nutrient concentrations have usually been expressed on the dry tissue weight basis, even though nutrient activities in the cell fluids should have a more direct effect on growth, at least for

mobile nutrients (Cassidy 1966). Leaf and whole-shoot moisture levels were determined in this study to permit comparisons among nutrient concentrations and CNC's determined in fresh and dried tissue (Section 3.4). Leaf dry matter concentrations increased gradually after about two to four weeks after emergence (Figure 4.2). Whole-shoot dry matter concentrations, on the other hand, were relatively stable until about five weeks after emergence, and then increased sharply.

Tissue moisture levels were lower in P-deficient treatments than where P was sufficient. The degree of that effect generally increased with P deficiency of the site (little to no effect at non-responsive sites), and it was more pronounced for the whole-shoot than leaf samples. Tissue dry matter fractions are presented for the Elm River91 site (Figure 4.3). Other workers (Batten and Wardlaw 1987) also observed that the developing grain of wheat grown in sand culture also contained less water when stressed by low P. Asher and Loneragan (1967) harvested eight temperate annual pasture species five to six weeks after seeding; in every case both root and shoot tissue moisture levels were much reduced in P-deficient treatments. Hylton et al. (1965) noted lower moisture contents associated with P deficiency in Italian ryegrass. Eaton (1949) related the lower tissue moisture in P-stressed sunflower to higher carbohydrate contents, whereas Avnimelech and Scherzer (1971) suggested that P stress may have reduced moisture levels in setaria through an effect on auxin metabolism. Reducing tissue moisture levels would tend to concentrate soluble P in the water remaining. Reduced water content may not mean that drought stress has been induced; leaf water potential was not affected by P nutrition of wheat in the study of McLachlan (1984).

Shoot P accumulation is a product of shoot dry matter accumulation (discussed above) and the concentration of P in that dry matter (section 3.4). Accumulation is presented as both absolute amounts on an areal basis and as percentage of the accumulation at mature harvest (Table 4.3). The accumulated amounts and changes with time are in general agreement with results of other



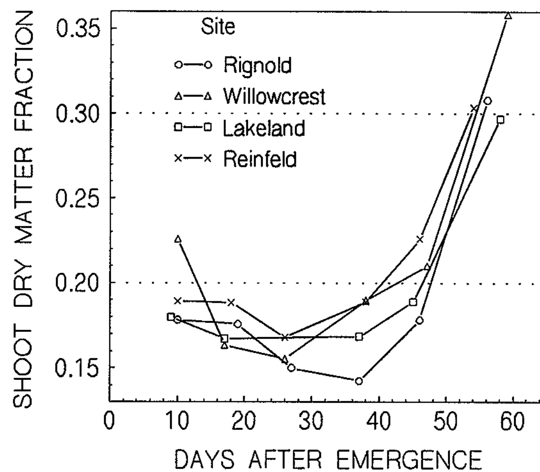
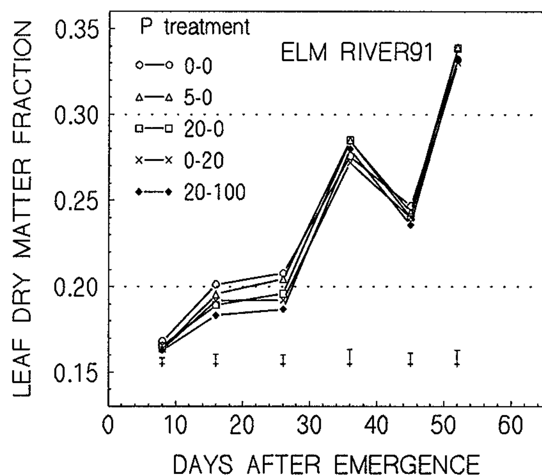


Figure 4.2. Leaf and shoot dry matter fractions through the growing season for four sites sufficient to slightly deficient in P. Plotted points are the means for all treatments.



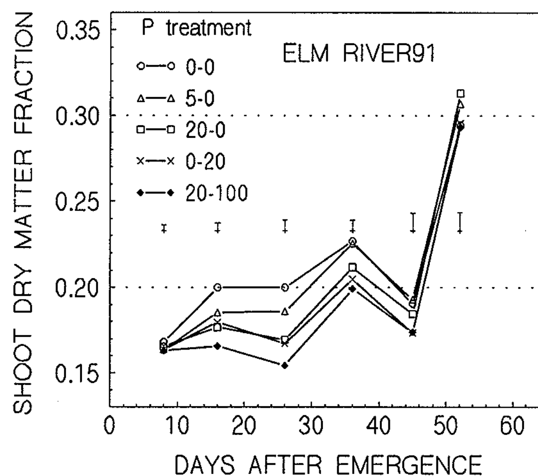


Figure 4.3. Leaf and shoot dry matter fractions through the growing season for individual treatments at the Elm River91 site, which was severely deficient in P. Error bars indicate LSD ($P<0.05$) among P treatments for each sampling time.

Table 4.3 Shoot P concentrations and distribution at maturity for all field sites.

Site and P Treatment	Relative yield of grain	P concentration		Harvest index	
		Grain	Straw	for dry matter	For P
	%	------ mg kg^{-1} --------		---------------- % ----------------	
Rignold					
0-0	100 a[z]	3547 b	314 b	37.3 a	86.9 a
5-0	100 a	3620 b	360 b	36.7 a	85.3 a
0-20	102 a	3720 b	374 b	37.1 a	85.4 a
20-50	100 a	4143 a	620 a	36.6 a	79.7 b
LSD (0.05):	8	172	103	1.1	2.8
Elm River90					
0-0	75 c	2715 c	146 c	36.4 a	91.4 a
5-0	81 bc	2776 c	164 bc	36.0 a	90.5 a
0-20	87 b	3160 b	189 b	36.9 a	90.7 a
20-50	100 a	3696 a	291 a	36.8 a	88.1 b
LSD (0.05):	12	122	34	1.1	1.4
Willowcrest					
0-0	103 a	4025 a	322 b	38.4 a	88.5 a
5-0	109 a	3909 a	336 b	38.0 ab	87.7 a
0-20	106 a	3996 a	372 ab	36.4 bc	86.0 a
20-50	100 a	4115 a	459 a	35.4 c	83.2 b
LSD (0.05):	19	302	92	1.8	2.8
Plum Ridge					
0-0	91 c	3917 ab	367 b	38.8 a	87.1 a
5-0	94 bc	3868 b	348 b	38.6 ab	87.5 a
0-20	97 ab	3967 ab	369 b	38.0 b	86.9 ab
20-50	100 a	4150 a	445 a	38.2 ab	85.2 b
LSD (0.05):	6	239	48	0.6	1.7
Lakeland					
0-0	101 a	3834 b	545 b	32.9 a	77.7 a
5-0	103 a	3838 b	631 ab	33.3 a	75.3 ab
0-20	107 a	3763 b	608 b	32.8 a	75.2 ab
20-50	100 a	4117 a	743 a	32.9 a	73.2 b
LSD (0.05):	17	229	126	1.3	4.1
Reinfeld					
0-0	86 b	3645 a	412 b	36.1 b	83.3 a
5-0	95 a	3586 a	406 b	36.8 ab	83.6 a
20-0	97 a	3586 a	448 b	37.2 a	82.6 a
0-20	96 a	3668 a	513 b	37.2 a	80.8 a
20-100	100 a	3752 a	1006 a	36.4 ab	68.3 b
LSD (0.05):	9	394	200	0.9	5.0
Elm River91					
0-0	30 d	3205 b	363 b	34.2 c	82.0 b
5-0	46 c	3382 b	351 b	34.6 c	83.5 ab
20-0	69 b	3349 b	319 b	36.6 b	85.7 a
0-20	73 b	3330 b	379 b	36.7 b	83.5 ab
20-100	100 a	3886 a	681 a	38.9 a	78.5 c
LSD (0.05):	11	337	64	1.1	2.7
Osborne					
0-0	35 c	3721 a	551 a	35.1 c	78.5 b
5-0	40 c	3836 a	486 ab	37.0 b	82.2 a
20-0	59 b	3861 a	429 b	38.0 ab	84.6 a
0-20	51 b	3644 a	485 ab	37.6 ab	81.9 a
20-100	100 a	3622 a	529 ab	38.7 a	81.2 ab
LSD (0.05):	8	325	99	1.3	3.2

[z] Means followed by the same letter within a group are not significantly different by Duncan's multiple range test (P= 0.05).

studies of field-grown spring wheat (Black 1970, Boatwright and Haas 1961, Elliott 1997a, Racz et al. 1965), considering the likely effects of differing conditions among the studies, such as soil P status, seeding date, and yield potential.

Site-treatment combinations were divided into two groups according to P sufficiency as indicated by grain yield at maturity (Figure 4.4). The *sufficient* group included the highest P treatment at every site, and all treatments with grain yield not differing significantly (by LSD test; $p<0.05$) from the highest P treatment. The *deficient* group included all treatments with grain yield differing significantly from that of the corresponding highest P treatment. The calculated curve indicated for each group was that which resulted in the best fit to the data among several functions used. It is not suggested that the function type has any physiological significance.

A larger proportion of the crop's total P accumulation was generally taken up earlier in the P-sufficient treatments and sites (Figure 4.4); e.g. the shoots contained half of the amount of P that they would contain at maturity approximately five days later for the P-deficient group than for the sufficient group. This delay was somewhat greater than the typical delay in maturity associated with P deficiency in these studies.

Earlier P uptake in higher P treatments is also evident in data presented by Black (1970). In many cases, shoot P accumulation from after about the heading stage exceeded that determined at maturity, particularly for some of the P-sufficient treatments. Loss of shoot P in solution, and as dried leaves not wholly recovered in the harvest sample at maturity, would account for some apparent P losses. Net uptake of P during grain filling (starting during about the eighth week after emergence) clearly played a minor role in providing P to the grain, in comparison with P re-translocation within the shoot. Maximum shoot P accumulation occurred at the heading stage in the study of Boatwright and Haas (1961); however, yields were low and response to P was small. Elliott et al. (1997a) reported maximum P accumulation at or before anthesis for field-grown

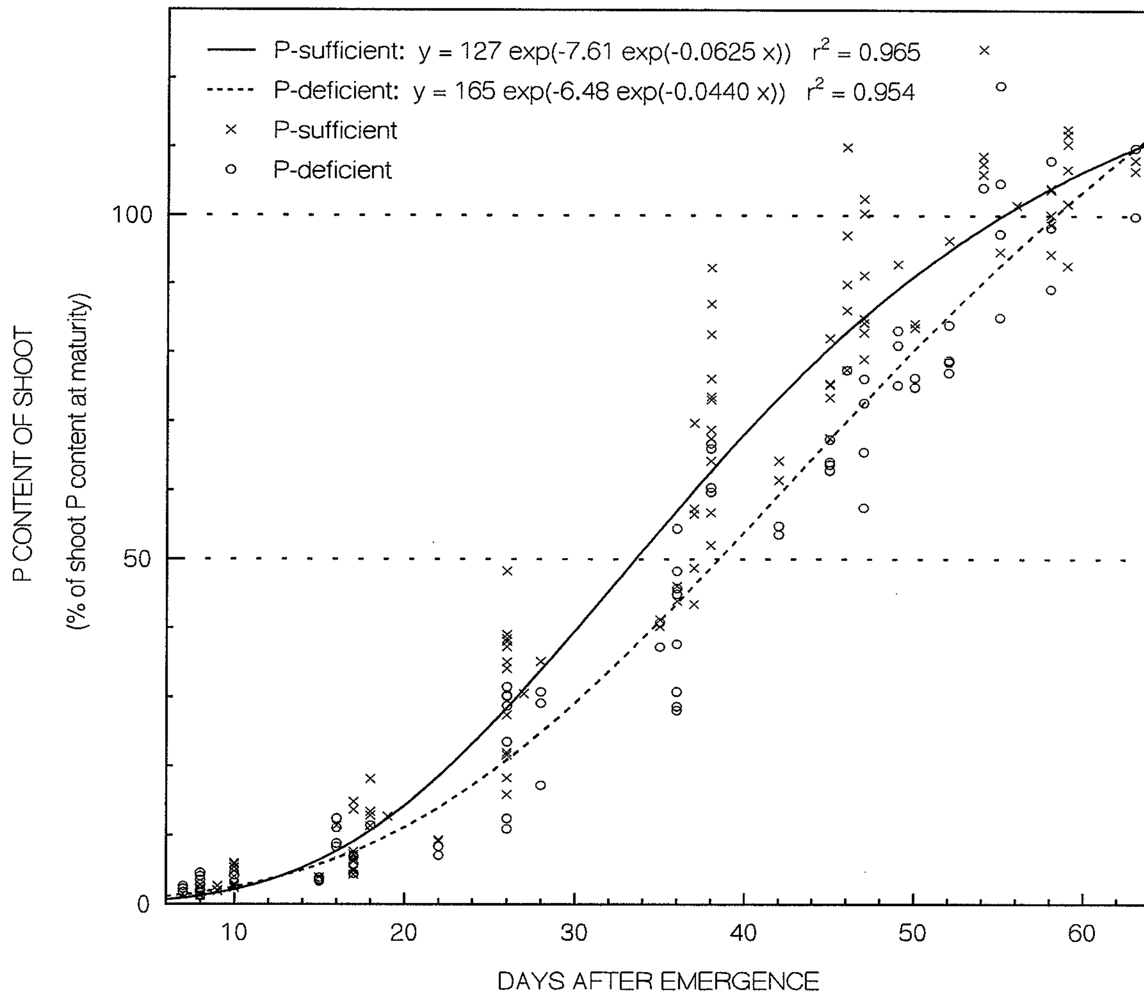


Figure 4.4 Shoot P accumulation through the growing season at all sites and sampling times, as a percentage of shoot P at maturity for the same site and treatment. "P-deficient" treatments are those which produced significantly less grain at maturity than the highest P treatment at the same site.

wheat; no net P uptake occurred beyond the booting stage in P-sufficient treatments at some sites.

Mean grain P concentrations ranged from 2715 to 4150 mg kg^{-1}, only a 1.5-fold variation, while straw P concentrations varied 6.9-fold, from 146 to 1006 mg kg^{-1} (Table 4.3). The corresponding ranges reported by Selles et al. (1995) were 3300 to 4100 mg kg^{-1} P in spring wheat grain, and 300 to 530 mg kg^{-1} in straw, in a study with narrower ranges of soil P levels and P application rates, and generally lower yields. Their grain:straw P concentration ratios ranged from 7 to 12 , compared to 3.7 to 19 in the current study. Racz et al. (1965) reported wheat grain P concentrations as high as 5000 mg kg^{-1}, but yields were less than 1.40 t ha^{-1}. Addition of sufficient P at sowing raised Australian wheat grain P to 0.4%, but the field-grown grain from southern Australia typically contained only 0.25% P (Lipsett 1964). The range of wheat grain P concentration at four sites in another P study in Australia was approximately 0.13 to 0.30% (Elliott et al. 1997d)

Grain and straw P concentrations generally increased with applied P within sites, and with soil P among sites. The Osborne site was a notable exception; P concentrations were affected little by applied P despite the strong growth response. The closeness of the relationship between whole-shoot P concentrations and grain yield P response declined after the boot stage (Section 3.4.2); this trend appears to have continued to maturity. Concentrations of tissue P at maturity were higher in 1991 than in 1990, when similarly P-deficient conditions are compared. This was likely due to the different pattern of soil moisture conditions between the two years. The latter part of the growing season was much moister in 1991 than in 1990, facilitating P uptake during later growth stages in 1991. The Osborne site was again of particular note, with relatively high concentrations of P in both grain and straw in all treatments, despite severe deficiency of P

limiting grain production. Overall, concentrations of P in the mature grain or straw were not good indicators of P deficiency or response.

Fertilization with P had inconsistent effects on harvest index (i.e. the fraction of the shoot dry weight that was grain at maturity; Table 4.3). Harvest index increased with P applied at the severely P-deficient Elm River91 and Osborne sites in 1991. However, harvest index was affected by applied P at only two of the five sites in 1990, and the treatment receiving no P fertilizer had the highest harvest index in both cases. The difference may again be related to the more favorable moisture conditions for grain filling in 1991, when more of the seeds may have filled well to contribute to grain yield; in 1990 more of the seeds may not have reached a harvestable size. Elliott et al. (1997a) also found only small effects of P on harvest index for field-grown wheat.

Although the effects of P stress on harvest index were small and variable in this study, consistent increases in root:shoot ratios due to P deficiency have been observed in other work (e.g., Chapin and Bieleski 1982, Elliott et al. 1997a, Hylton et al. 1965, Sachay et. al. 1991, Sheppard et al. 1986, Smyth and Chevalier 1984). In summary, the fraction of the total plant that is grain is consistently reduced by P deficiency, but the fraction of shoot that is grain is affected variably and to a smaller degree.

The fraction of the shoot P that was contained in the grain at maturity was reduced by P fertilization at all sites except for Osborne (Table 4.3; harvest index for P). This resulted from the much greater effect that applied P had on concentrations of P in the straw than in the grain. Overall, the average fraction of the shoot P contained in the grain was 81.9% for the 21 P-sufficient treatments, and 85.2% for the 14 P-deficient treatments. Individual means, however, ranged from 68 to 91%, similar to the range reported by Elliott et al. (1997b) from a glasshouse study with wide variation in plant P nutrition. The wide range in straw P concentrations and

accumulations should be considered in modeling P in the soil-plant system, since it influences the plant P being returned to the soil and the nutritive composition of the crop residues.

Grain yield is a product of the number of heads, the mean number of kernels per head, and the mean kernel weight - the components of grain yield. At the Reinfeld site, which was slightly deficient in P for maximum grain yield, added P increased grain yield almost entirely through increased numbers of heads; i.e. greater tillering (Table 4.4). Most or all of the spring wheat grain yield response to P fertilizer in other studies has been due to increased numbers of heads (Black 1970, Black 1982, Elliott et al. 1997a). Black (1970) also reported that the number of adventitious roots per plant was very closely correlated with both number of heads (r^2=0.94) and grain yield (r^2=0.93). In our study, mean numbers of kernels per head actually showed a slight decreasing trend as applied P increased at the Reinfeld site. The importance of head numbers in yield response to P fertilizer suggests that P deficiency limits yield at an early growth stage; number of kernels per head and their weight are determined later in the growing season.

At the severely P deficient Elm River91 and Osborne sites, increases in all three components of yield resulting from P fertilization contributed to the large grain yield responses. The largest yield increases were related to head numbers and kernels per head at the Elm River91 site, and to head numbers at the Osborne site. A large number of short, immature late tillers with unproductive heads were observed at these two sites, and were included in the head count. The low-P treatments had the largest proportion of these unproductive heads. Such heads were also counted separately in the harvests of the 0-0 and 20-100 treatments of one block from the Elm River91 site, and accounted for 24% of the total number of heads in the 0-0 treatment, and 15% in the 20-100 treatment. If only the productive heads had been counted, the apparent effect of P fertilization on the number of kernels per head would have been less pronounced, and the effect on number of heads correspondingly greater.

Table 4.4 Components of grain yield at 1991 field sites.

Site and P Treatment	Grain Yield	Number of heads	Mean number of kernels per head	Mean kernel Weight
	kg ha^{-1}	heads m^{-2}		mg
Reinfeld				
0-0	2416 b[z]	467 c	20.0 a	25.8 a
5-0	2664 ab	506 b	19.8 a	26.6 a
20-0	2718 a	510 b	19.7 a	27.0 a
0-20	2692 a	522 ab	19.4 a	26.7 a
20-100	2804 a	557 a	19.1 a	26.4 a
LSD (0.05):	250	38	1.4	1.4
Yield loss fraction[y]:		1.15	-0.30	0.15
Elm River91				
0-0	1024 d	362 c	11.5 c	24.3 d
5-0	1574 c	442 b	13.1 c	27.2 c
20-0	2394 b	476 b	17.5 a	28.8 b
0-20	2511 b	542 a	15.5 b	29.3 b
20-100	3447 a	567 a	19.3 a	31.5 a
LSD (0.05):	388	61	1.8	1.4
Yield loss fraction:		0.37	0.42	0.21
Osborne				
0-0	1171 c	406 c	13.1 c	21.9 d
5-0	1339 c	416 c	14.3 b	22.4 cd
20-0	1948 b	512 b	15.5 b	24.5 b
0-20	1703 b	494 b	14.5 b	23.8 bc
20-100	3328 a	706 a	17.4 a	27.1 a
LSD (0.05):	254	56	1.1	1.5
Yield loss fraction:		0.53	0.27	0.20

[z] Means followed by the same letter within a group are not significantly different by Duncan's multiple range test (P= 0.05).

[y] fraction of the grain yield reduction in the 0-0 treatment (by comparison with the yield in the 20-100 treatment) attributable to this component of yield.

A closer examination of the plants, with documentation of the main stem and tiller growth stages and of the specific leaf axils from which tillers present had emerged, may have provided additional information relevant to the timing of plant stress due to P deficiency. The progress of plant vegetative development for cereal grains has been documented in detail, including the

timing of initiation of each specific tiller under favourable conditions (Klepper et al. 1982). Stress at tiller initiation results in missing tillers or delays in tiller initiation. The very late tillers produced at our two sites that were extremely deficient in P suggest that the rainfall received approximately 5 to 6 wk after crop emergence helped to improve crop P nutrition (by improving soil conditions for uptake of soil P). Improved early tillering (especially the T1 and T2 tillers) resulted from P fertilization in the study of Goos et al.(1994).

Some of the effects of P deficiency on yield components, especially mean kernel weight, may be indirect. For example, at the Osborne site leaf diseases appeared to affect the lower P treatments more severely than those higher in P, and may have limited grain filling to result in lighter kernels.

Plant growth and shoot dry matter accumulation in this section, and tissue P concentrations in Section 3, have been presented as functions of chronological time (days after emergence). A general indication of how time after emergence related to crop phenological development was given in Section 3.4.1. Rates of plant growth and development depend on growing conditions, especially on temperature and radiation, and also on stress factors such as deficiency of P. Presentation of growth, tissue P concentrations, and CNC's as functions of plant developmental stage, using a system of expression such as that of Haun (1973), could have provided results and interpretive criteria which would be applicable over a wider range of growing conditions.

4.5. Conclusions

The effects of P deficiency on several growth parameters were quantitatively characterized for field-grown spring wheat. Shoot dry matter accumulation was restricted within one to two weeks from emergence under P-deficient conditions, even where some P fertilizer was applied with the

seed; visual observations concurred. The relative growth rates of the crop were reduced due to P stress for only the first two to five weeks from emergence. Subsequently, relative growth in P-deficient treatments was similar to or higher than that in high-P treatments, so a partial "recovery" was effected in some cases, though some degree of P stress may still have been present.

The fraction of the leaf tissue that was dry matter was approximately 0.18 from one to four weeks after emergence, then gradually increased over the following four weeks to about 0.32. Corresponding fractions for the whole shoot were similar in magnitude, but remained low for an additional one to two weeks, then rose more rapidly. Dry matter fractions increased with the degree of P deficiency within most sites, in the whole shoot more so than in the leaf.

Total shoot P accumulations were 4 to 19 kg ha^{-1} at maturity, with proportional uptake occurring later under P deficiency. The straw contained 9 to 32% of the shoot P and showed a much wider proportional variation in P concentration than the grain.

Effects of P nutrition on harvest index were relatively small and variable. Analysis of the components of grain yield at three sites revealed that the grain yield response to P at the slightly deficient site was entirely due to a larger number of heads. However, at the two very strongly responsive sites substantial proportions of the yield response were also attributed to more kernels per head and heavier kernels.

5. SMALL-SCALE SPATIAL VARIABILITY OF AVAILABLE PHOSPHORUS IN SOIL

5.1 Abstract

Spatial variations in nutrient availability within normal field soils on a small scale (<0.1 m) have not been determined, but are likely to influence nutrient uptake by plants. Plant availability of fertilizer residual P in particular may be affected by non-uniform distribution due to the slow dispersion of P out from fertilizer granule sites after initial reaction with the soil. This study quantified variability of soil available P in field soils at least a year after fertilizer application.

Soil P extractable with $CaCl_2$ and $NaHCO_3$ solutions was determined for 1-cm^3 samples from the surface 15 cm of undisturbed columns of four Manitoba soils. Substantial spatial variation in P was found in each case, with CV's ranging from 13 to 135% within soil, depth, and extraction method. The relative variability in $CaCl_2$-P was greater than for $NaHCO_3$-P, but the two tests revealed the same spatial patterns. Much of the maximum difference in extractable P occurred over 2-3 cm distances in most cases, both horizontally and vertically. The small scale of the localization of zones of higher P availability were also indicated by geostatistical analysis of the test levels (semi-variograms).

Spatial variability in available soil P at this scale may play a substantial role in the residual availability of P from fertilizers (especially under reduced tillage systems) and in the negative effects of tillage on availability of soil P to plants.

5.2 Introduction

Substantial spatial variability of soil properties has long been established as the rule more than the exception. For many properties, such variation has been studied at a wide range of scales, from the broadest geographic region (e.g. reconnaissance soil survey), to the landscape, to the pedon. Most studies which have examined soil spatial variability of nutrients on the sub-meter scale have been concerned with changes related to depth (e.g. as a function of tillage systems, by Unger 1991), to short-term nutrient fluxes and transformations in and around fertilizer nutrient application zones (for P, by Khasawneh et al. 1974, and Rehm et al. 1995), or to plant root effects on the rhizosphere soil (Jungk 1990). Giesler and Lundström (1993) demonstrated considerable variation in several soil chemical parameters at about the decimeter scale which were not related to the above factors. Raun et al. (1998) determined "microvariability" in plant growth and several soil characteristics among 0.09-m^2 plots; soil samples were taken from the whole 0-15 cm depth zone. They believed that differences in soil test parameters exist at the centimeter scale horizontally, and found that composites of eight soil cores from each 0.09-m^2 area provided less reproducible estimates for extractable P than for other soil characteristics.

Despite limited quantification, small-scale spatial variability of soil nutrients is recognized as a factor influencing nutrient availability and uptake by plants. Jackson et al. (1990) stated that "soil microsites rich in available nutrients are an important source of mineral nutrients for plants in many environments" and showed how three plant species increased the uptake capacity of their roots for P in nutrient-rich soil patches. Sachay et al. (1991) interpreted the longer, less branched rooting pattern of P-starved maize to reflect an effort by the plant to explore a larger soil volume in search of small areas rich in phosphate. Russell (1973) described the phosphate potential of fertilized soil as "a plateau of fairly constant potential with troughs of lower potential around each fertilizer particle . . . This extremely patchy distribution would be expected to last a

fairly long time in an undisturbed soil because of the low solubility of the phosphate in the soil water and the low diffusion coefficient due to the strong phosphate buffering of the soil."

The persistence of the non-uniformity of phosphate potential resulting from fertilization (or plant P uptake, or other phenomenon) and its influence on availability of the P to plants are not clear. Studies of P movement from fertilizers in soils have usually terminated while there were still very high concentrations of P in the retention zones. Very large amounts of P fertilizer have usually been applied, and often to columns allowing flux in only one direction; this has resulted in much further P transport than would occur from normal granules in the field. Lewis and Racz (1969) reported the extent of P movement in ten Manitoba soils in three weeks from pockets of powdered MAP and diammonium phosphate. About 90% of the P was within 2.0 cm of the application site in the calcareous soils, and within 2.5 cm in the noncalcareous soils. Each pocket of fertilizer contained 27 mg of P, which is approximately five times as much P as in a normal fertilizer MAP granule. Eghball et al. (1990) determined the degree of P movement over a period of 94 d out from bands of ammonium polyphosphate supplying 15 to 60 kg P ha^{-1}. The applied P was retained within 1.8 to 3.9 cm of the band centers, which was 1.1 to 5.2 % of the soil to the 30-cm depth. Without band disturbance, the predicted longevity of substantially increased P levels in the bands was 2.6 to 6.5 yr, depending on P rate and soil. Sander et al. (1990) found that the residual value of fertilizer P to the second crop following fertilization was greater for P banded below cultivation depth than for P applied at depths subject to mixing by tillage prior to the second crop.

Soil disturbance by cultivation or mixing can reduce subsequent plant P uptake (Miller et al. 1992, Williams and Simpson 1965). The effect has been examined intensively with corn, and has usually been attributable largely to decreased mycorrhizal colonization of the roots or otherwise reduced effectiveness of the mycorrhizae in P uptake (Evans and Miller 1988,

McGonigle et al. 1990). However, results of Evans and Miller (1988) suggest that other factors may also be involved. Fungicide treatment that reduced mycorrhizal infection intensity to very low levels did not alleviate the disturbance effect on P uptake. Also, soil disturbance reduced P uptake for spinach and rape (non-mycorrhizal) to a similar extent as for wheat (mycorrhizal). In the same study, soil P test levels ($NaHCO_3$-P) were determined after cropping in both the undisturbed and disturbed (crushed to pass a 0.5-cm sieve) treatments. Soil test P was consistently lower (averaging 17% lower) in the disturbed treatment for each of the four crops used, at both the 0-6 and 6-12 cm soil depths, despite smaller amount of P having been taken up by the crops from that treatment. These effects of soil mixing were not discussed by the authors, but may be due to the change in the spatial pattern of the soil available P on mixing. Soil from previously higher-P zones would be brought into closer contact with the bulk of lower-P soil. More rapid reaction of soluble P from the high-P soil with low-P soil could result from reduced transport distances for P in solution.

Studies of P fertilizer placement show that limiting the proportion of the rooting volume in which the fertilizer is placed will increase availability of the P to plants (Yao and Barber 1986). However, in the case of residual fertilizer P, non-uniform spatial distribution of the P in the soil has not often been recognized as a factor that may be enhancing availability and slowing further reaction of the fertilizer reaction products with the soil. McKenzie et al. (1989) observed that crops may not respond to added P fertilizer on soils with a history of P application, even if the soil P test level is still considered deficient. Non-uniform distribution of the residual P may enhance its plant availability to a greater degree than is reflected in a soil test using a bulk mixed sample.

The objective of this study was to characterize the small-scale spatial variability of available P in previously fertilized soils.

5.3 Materials and Methods

Three undisturbed cores of soil were collected from each of four locations in Manitoba in May 1991. Sharpened aluminum cylinders of 20 cm diameter were pressed approximately 18 cm into the soil, all within a minimal area (<1 m^2) at each location, dug up, and brought into the laboratory. All sites had been in annual field crop production (with P fertilization) for many years and had last received P fertilizer in the spring of 1990. Soil type and management practices varied widely among sites (Table 5.1). Except for the zero-tilled site (Osborne), the soils had been cultivated in the fall of 1990, but not in the spring of 1991 prior to collection of the cores. An additional undisturbed core was also taken from another location within a few hundred meters of the Reinfeld site (soil similar to the Reinfeld cores, except lower in sodium bicarbonate extractable P). Cylinders of undisturbed soil were stored frozen until they could be processed.

The cores were thawed and wetted to approximately field capacity. The aluminum cylinders were cut off, and the cores were cut vertically through the center to expose a vertical face of soil. The core orientation in the field had been marked, and the three faces of soil from within each

Table 5.1 Site and soil characteristics for soil P variability study.[z]

Site (soil series)	Legal location	Texture	pH	Carbon		$NaHCO_3$-P
				Organic	Carbonate	
				(%)		mg kg^{-1}
Reinfeld	NW25-5-5W	FL	6.9	1.9	0.00	11
Osborne[y]	NE19-6-1W	C	7.8	3.9	0.32	39
Meharry	SE14-25-21W	CL	7.8	3.4	1.82	9
Meharry+P[x]	SE14-25-21W	CL	7.7	3.7	1.03	9

[z] Analytical data for bulked 0-15 cm depth soil. Texture by hand estimate, pH in 1:1 soil:water suspension, organic C by wet oxidation with dichromate (Yeomans and Bremner 1988), carbonate-C by titrimetry (Bundy and Bremner 1972), and $NaHCO_3$-P by method of Watanabe and Olsen 1965

[y] under zero-till soil management for previous 10+ years; all other sites cultivated.

[x] P fertilizer supplying 175 kg ha^{-1} P_2O_5 was broadcast/incorporated May 1990

site were cut at 60° angles from each other. A specially constructed sampling device was used to remove three horizontal 15 x 1 x 1 cm sections of soil from each soil face, centered within the 0-5, 5-10, and 10-15 cm depths (Figure 5.1). A fourth section was also sampled vertically from the soil surface to the 15-cm depth. Each strip of soil was then cut into 15 1-cm^3 (1x1x1 cm) samples, to yield a total of 60 samples per core. The three cores per site from each of four sites were sampled in this manner. In addition, the single core taken near the Reinfeld site was sampled to recover all 255 1-cm^3 samples from a cut vertical face 15 cm across by 17 cm deep. The small samples were air-dried and crushed until most of the sample would pass a 60 mesh sieve.

Concentrations of phosphate extractable by calcium chloride ($CaCl_2$-P) and by sodium bicarbonate ($NaHCO_3$-P) were determined for each of the samples. For $CaCl_2$-P, 500 mg of soil was shaken for one hour with 2.5 mL of 0.01 M $CaCl_2 \cdot 2H_2O$. The suspensions were then centrifuged for 15 min at 940x*g*, and the supernatant filtered through a small cotton wool plug. $NaHCO_3$-P was determined by the Olsen procedure (Watanabe and Olsen 1965), using 300 mg of soil with approximately 60 mg of washed activated carbon to decolorize and 6 mL of 0.5 M $NaHCO_3$ at pH 8.5. Suspensions were shaken for 30 min and filtered through Whatman #2 paper. For both analyses, extractions were conducted in capped 15-mL centrifuge tubes with end-over-end shaking. During the shaking, suspension temperatures rose from 20±1°C to 22-24°C. Phosphate in the extracts were determined colorimetrically as a phosphomolybdate complex according to the procedure of Murphy and Riley (1962), with additional sulphuric acid added to neutralize the bicarbonate. Preliminary extraction trials were conducted to validate methods used, since the small sample sizes available necessitated modification of some of the normal procedures and equipment. The small-scale methods used were found to yield results similar to normal procedures, with good precision and consistently negligible phosphate levels in extraction blanks.

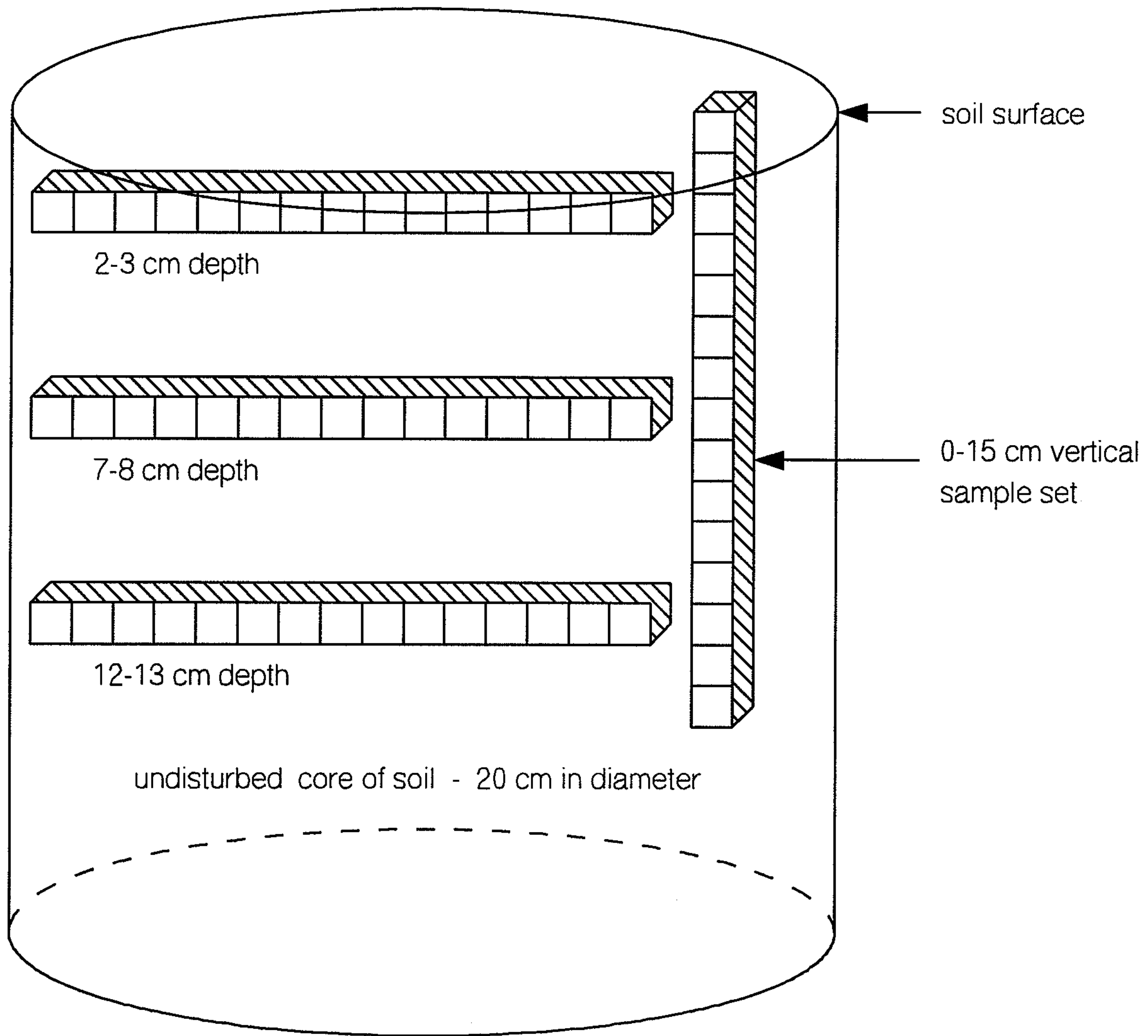


Figure 5.1. Locations of 1-cm^3 samples taken from undisturbed cores of soil.

For the 255 samples from the single core taken near the Reinfeld site, only $NaHCO_3$-P was determined. Analyses conducted up to that point in the study showed that the results of the two extraction methods were highly correlated within soil type, and showed the same spatial patterns.

5.4 Results and Discussion

Results of all extractions for the four soils are illustrated (Figures 5.2 to 5.6). Both $CaCl_2$-P and $NaHCO_3$-P were determined on all samples to provide measures of the P *intensity* and *quantity* factors, respectively (Olsen and Khasawneh 1980). As such, $CaCl_2$-P levels are expressed on the extract basis (cf. soil solution P activity), and $NaHCO_3$-P levels on the soil basis (representing the solid phase reserve of labile P in the soil). These methods chosen for the study were not those normally used for soil P quantity and intensity assessment, but were the most appropriate considering the large number and small size of samples to be analyzed. The $NaHCO_3$-P test is also of particular interest in that it is widely recommended and used by soil testing laboratories world-wide for assessing the P fertility status of neutral to calcareous prairie soils, and it has been more widely field-calibrated and used in Manitoba than other soil P tests.

The obvious close relationship between P concentrations as determined by the two tests (Figures 5.2 to 5.6), within soil types, suggests that the spatial variations reflect real differences in P fertility within the soil. If changes in the quantity *or* intensity parameter alone had occurred, with an opposite or no change in the other, then the effect may have been related to variations in other soil characteristics with the effect on P fertility of the soil questionable. For example, a higher $CaCl_2$-P level may not reflect greater P fertility if it is correlated with higher sand content (Kamprath and Watson 1980).

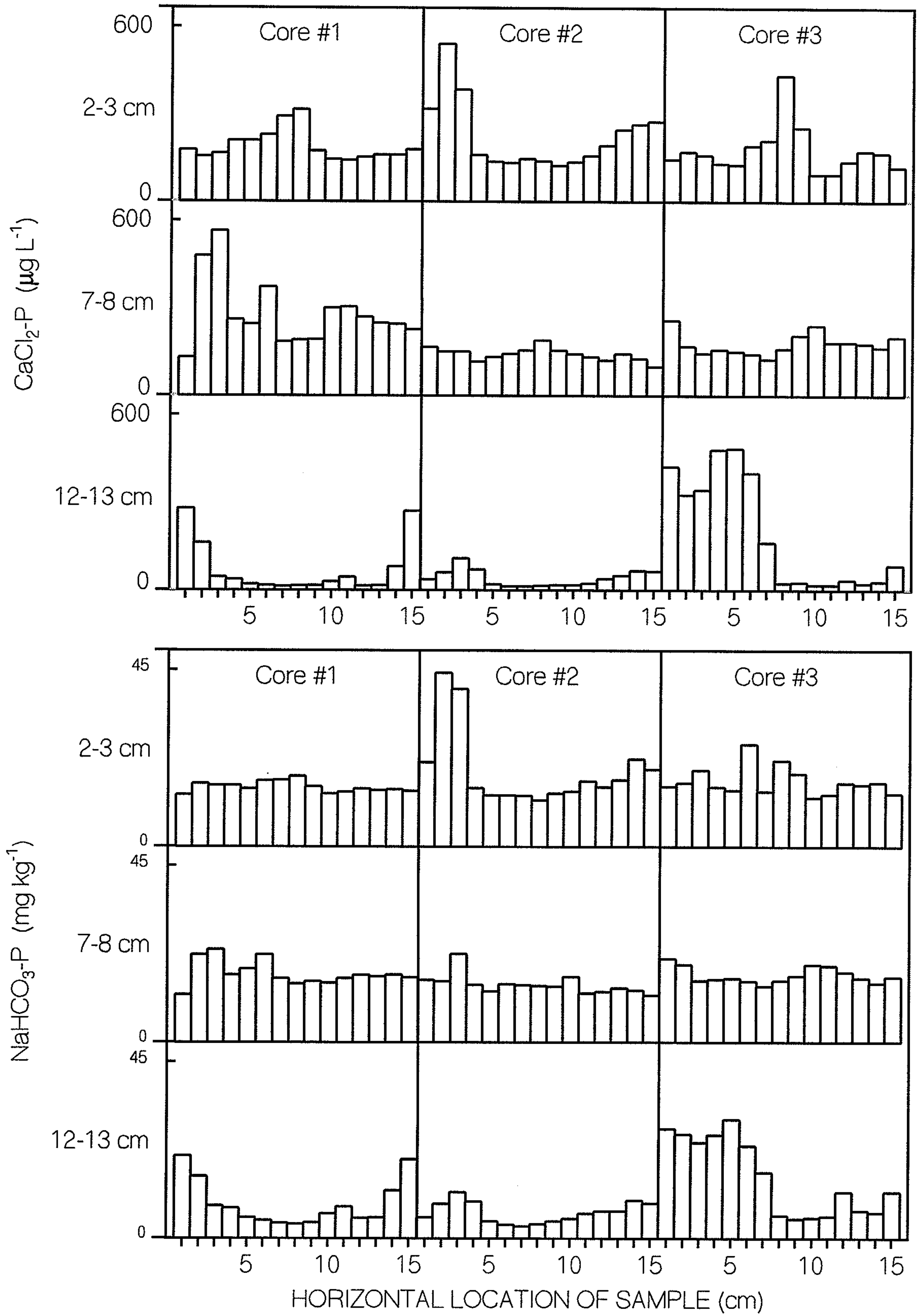


Figure 5.2. Extractable P levels in horizontal sample sets of Reinfeld soil.

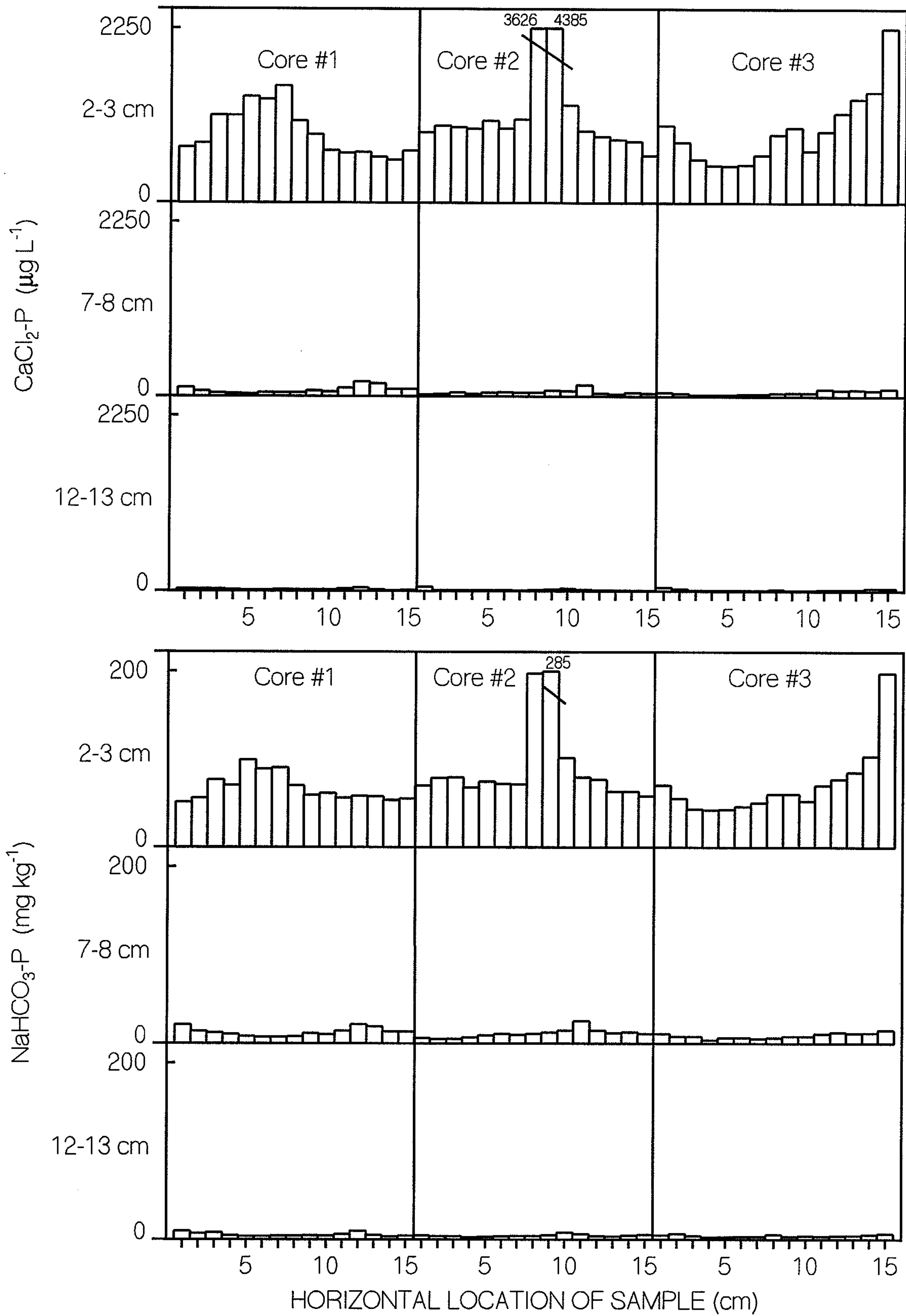


Figure 5.3. Extractable P levels in horizontal sample sets of Osborne soil.

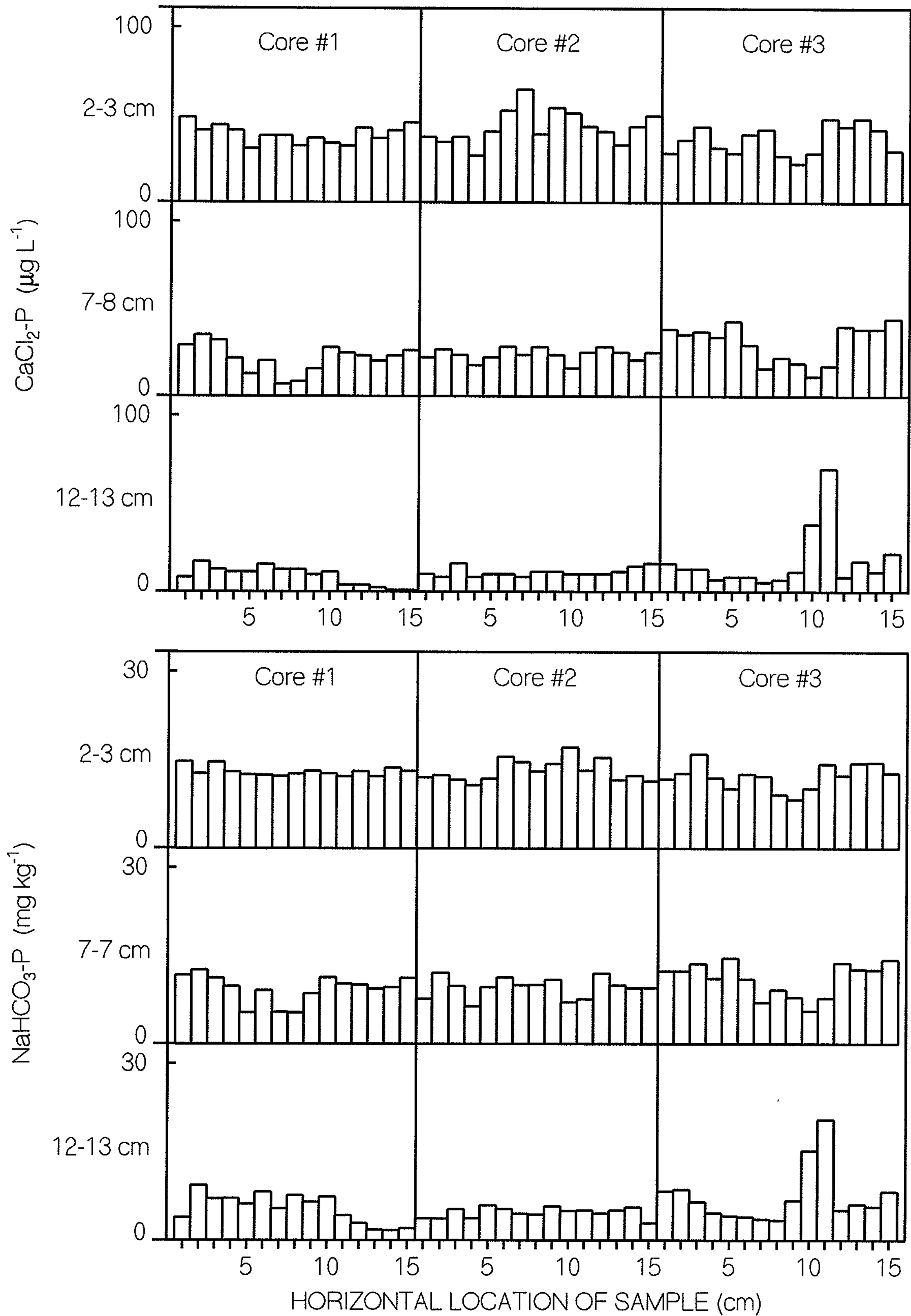


Figure 5.3. Extractable P levels in horizontal sample sets of Meharry soil.

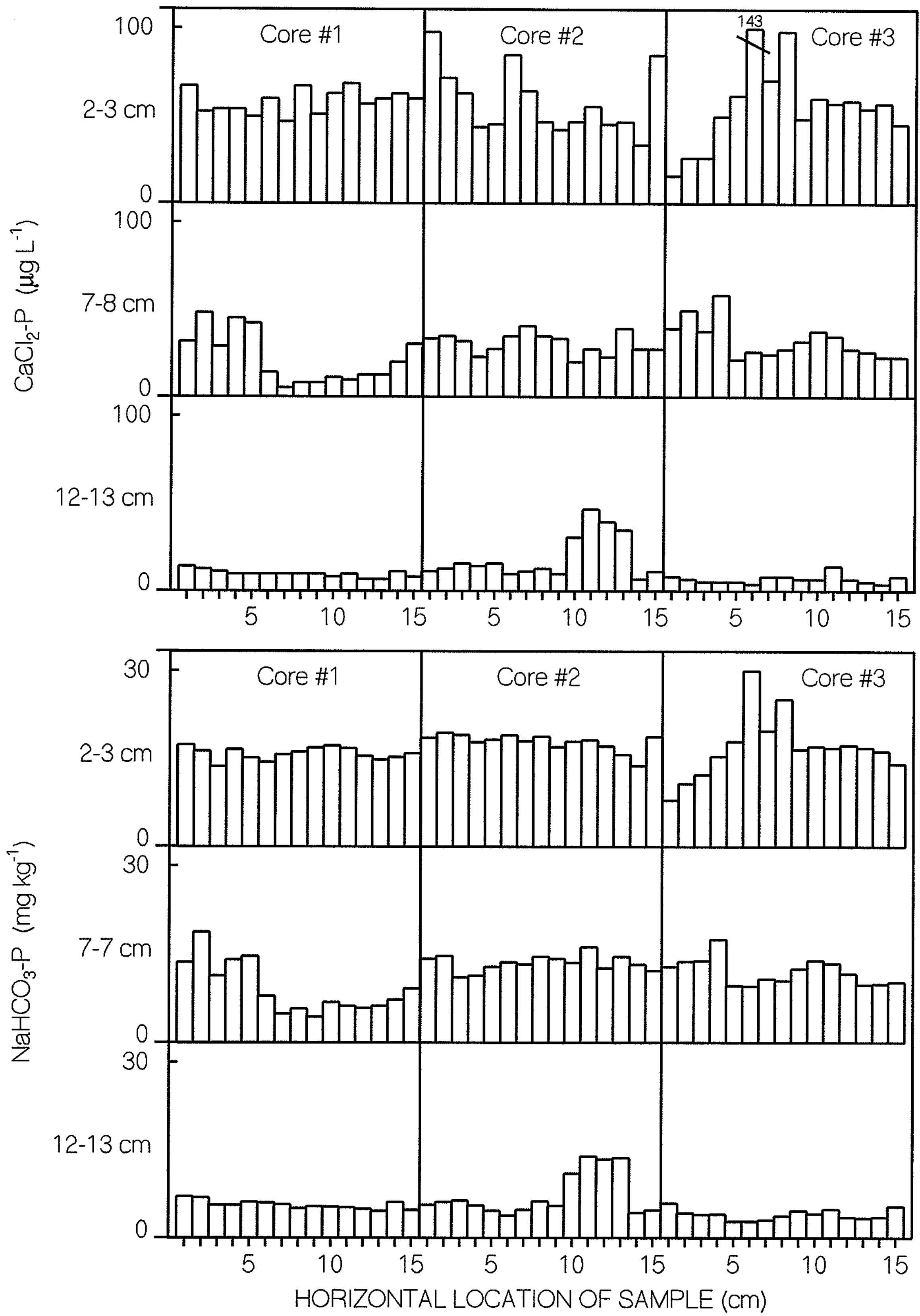


Figure 5.5. Extractable P levels in horizontal sample sets of Meharry+P soil.

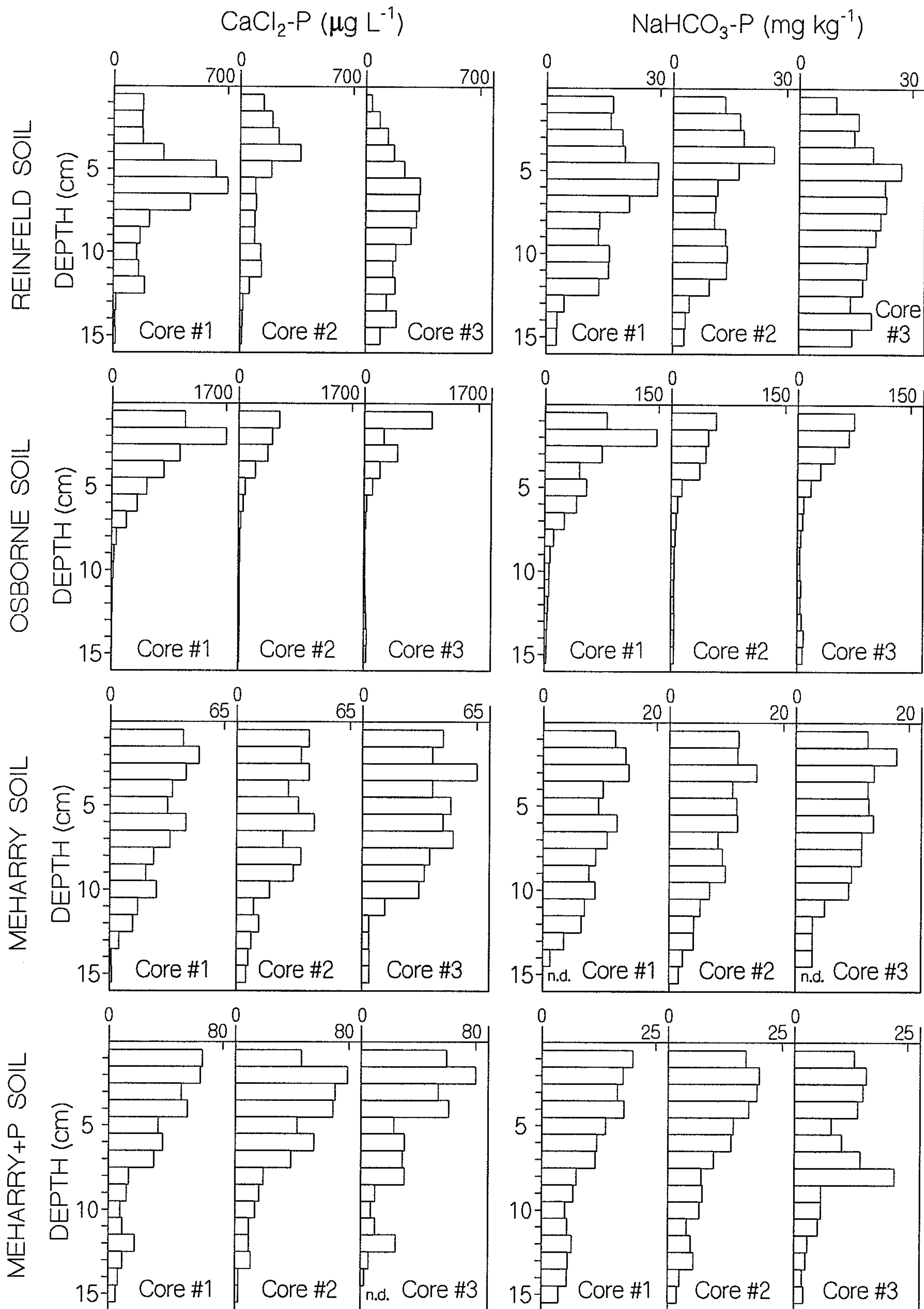


Figure 5.6. Extractable P levels in vertical sample sets of all soils.

The relative variability in $CaCl_2$-P was greater than that for $NaHCO_3$-P. Yao and Barber (1986) observed that addition of P fertilizer increased P intensity relatively more than P quantity, due to declining P buffering power as soil P levels increased.

In the Reinfeld soil at 2-3 and 7-8 cm deep, $CaCl_2$-P varied about 2 to 4-fold, and $NaHCO_3$-P about 1.5 to 3-fold, within the 15-cm soil strips (Figure 5.2). Greater relative variation was present at the 12-13 cm depth, possibly because this was below the normal depth of cultivation (hence low *background* P levels), though occasional deeper tillage might have incorporated surface soil to this depth over a small part of the total area. Two of the three vertical sample sets showed a large drop in P levels at the 12-cm depth. Most of the total range in P levels within sets, horizontally or vertically, often occurs over distances of only 2 to 3 cm, indicating persistence of considerable P gradients even in this sandy loam soil one year after P fertilization. However, none of the P levels were extremely high in this soil.

Most of the available P in the higher-P, zero-tilled, clay soil (Osborne; Figures 5.3 and 5.6) was strongly localized within the surface 4 to 7 cm depth. Phosphorus was retained closer to the surface in this case than in some other vertical P distribution studies which included no-till sites (Robbins and Voss 1991, Weil et al. 1988). This degree of P stratification at the surface was unexpected, since the normal method of P application at this site had been shallow banding, rather than surface broadcasting. The consequences of this distribution on plant P nutrition will depend upon root activity in the surface few cm of soil. Fortunately, root activity and nutrient uptake near the surface is normally relatively high under zero-tillage (Hargrove 1985).

The Meharry soil showed the least spatial variability in P of the four soils examined (Figure 5.4). Comparing the two Meharry soils sampled, the variability at the two shallower sampling depths was slightly higher in the Meharry+P soil, which had been treated with a heavy application of P fertilizer a year before this sampling (Figure 5.5). If the applied 175 kg ha^{-1} of P_2O_5 was

incorporated into the 0-10 cm depth soil, the soil would contain approximately one fertilizer granule per 65 cm^3, so high-P sites should not be expected to be numerous.

Mean extractable P concentrations, and their coefficients of variation, provide objective support to the preceding qualitative observations (Table 5.2). Analysis of 15 replicates of one sample ($NaHCO_3$-P) indicated that the CV that could be attributed to analytical variability alone was 5%.

Results of $NaHCO_3$-P analysis of the 255 samples from the single core taken near the Reinfeld site revealed two small relative *hot spots* in terms of P fertility (Figure 5.7). These zones had $NaHCO_3$-P concentrations two to three times as high as samples from areas only 1 to 2 cm away.

Coefficients of variation (Table 5.2) indicate the relative degree of variation present, but little about the spatial scale over which it occurs. Data from the horizontal sample sets were used to construct semi-variograms (Figure 5.8). The semi-variance statistic [$\gamma(h)$] indicates the variance

Table 5.2 Means and coefficients of variation for extractable P concentrations in horizontal sample sets.[z]

	Depth (cm)				**Depth (cm)**			
Soil	**2-3**	**7-8**	**12-13**	**all**[y]	**2-3**	**7-8**	**12-13**	**all**
	mean $CaCl_2$-P ($\mu g\ l^{-1}$)				**CV for $CaCl_2$-P (%)**			
Reinfeld	195	199	101	165	45	45	135	71
Osborne	1060	59	19	380	68	63	54	168
Meharry	39	25	12	25	21	35	86	56
Meharry+P	58	28	12	33	35	41	75	74
	mean $NaHCO_3$-P (mg kg^{-1})				**CV for $NaHCO_3$-P (%)**			
Reinfeld	17.0	16.4	10.3	14.6	36	17	74	45
Osborne	77.0	10.6	4.5	30.8	57	43	38	135
Meharry	12.7	10.0	5.8	9.5	13	24	55	40
Meharry+P	16.9	11.4	5.6	11.3	19	29	46	49

[z] within soil type, but combining data from all three cylinders.

[y] data from 2-3, 7-8, and 12-13 cm depths combined.

HORIZONTAL LOCATION OF SAMPLE (cm)

0 5 10 15

DEPTH (cm)

0 5 10 15

$NaHCO_3$-P ($mg\ kg^{-1}$): 18 15 12 9 6 3 0

Figure 5.7. Soil $NaHCO_3$-P levels within a 15x17 cm vertical grid.

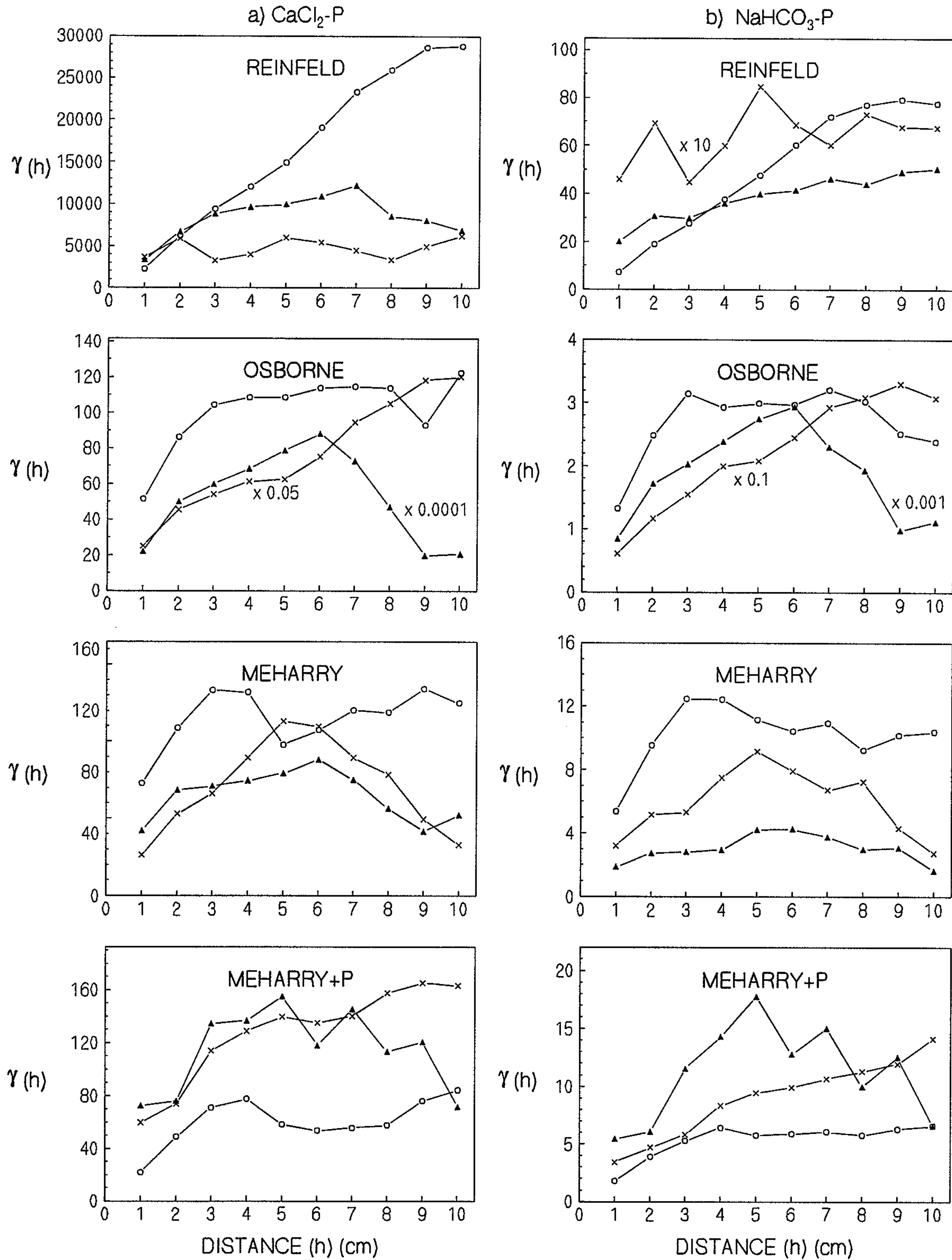


Figure 5.8. Semi-variograms for a) $CaCl_2$-P, and b) $NaHCO_3$-P, for horizontal sample sets for each of the four soils.

in test values for samples which are a specified distance (h) apart (Upchurch et al. 1988), and is calculated for each value of h:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2$$

where N(h) is the number of pairs of points separated by the distance h, $z(x_i)$ is the test value at location i, and $z(x_i{+}h)$ is the test value at distance h from location i. For Figure 5.8, the point location for reference of each 1-cm^3 sampled volume was the center of the volume, so adjacent samples were considered 1 cm apart. As distance increases, N (and hence reliability of γ) declines; values of γ for h = 11 to 14 are not presented due to their small N. The values for all three cores within a site were pooled; i.e. variation was assumed to be the same in all horizontal directions, or *isotropic*.

The database is small for this type of analysis, and zones high in P were few, so some of the semi-variograms are erratic or excessively influenced by the presence or location of one or two high-P zones. For example, a high-P sample in the centre of a horizontal sample set did not affect $\gamma(h)$ for h>7, because no samples were taken more than 7 cm from the centre sample.

In most cases the semi-variogram curves would pass near the origin if smoothed and extrapolated back to h=0. This low *nugget variance* indicates that measurement error was low and that an acceptably small scale for sampling was used (Trangmar et al. 1985). The semi-variograms increase consistently only to about the 3-cm distance. In half the cases, γ at the 2 or 3 cm distance was at least 90 % as great as the mean γ for h=5 to 10 cm. Even for adjacent samples (h=1), γ was at least one-third of the mean γ for h=5 to 10 cm in three-quarters of the cases. This supports earlier observations that most of the variation in extractable P occurs over short distances (2-3 cm). Trangmar et al. (1985) cited values for semi-variogram parameters for several sets of soil and related data, but the sample spacings were twenty to one million times

greater than the 1-cm used in this study; appropriate data for direct comparison were not found (and, according to Raun et al. 1998, do not exist).

Spatial variability in plant-available P in soil at the scale observed in this work has implications for recommended soil sampling protocols for soil fertility assessment. The diameters of soil cores from hand-operated soil probes are only 1.5 to 2.0 cm, which is similar in size to some of the pockets of higher-P soil found. Therefore, the extractable nutrient concentrations for an individual core might be less representative of the surrounding soil for P than for more mobile nutrients, which would more rapidly diffuse into a larger volume of soil. Use of larger-diameter core samples may improve precision of soil testing for P, so long as subsequent sample handling protocol ensures adequate crushing and representative sub-sampling of the whole soil sample taken from the field.

More conclusive interpretation of the results from this study would have been possible if more detailed information had been collected on the tillage and P fertilization history of the sampled sites, especially the tillage practices for the three conventionally tilled sites.

5.5 Conclusions

Soil P extractable with $CaCl_2$ and $NaHCO_3$ solutions was determined for 1-cm^3 samples from undisturbed columns of four soils. Substantial spatial variation in P was found in each case, with CV's ranging from 13 to 135% within soil, depth, and extraction method. The relative variability in $CaCl_2$-P was greater than for $NaHCO_3$-P, but the two tests revealed the same spatial patterns. Much of the maximum difference in extractable P often occurred over 2-3 cm distances, both horizontally and vertically. The small-scale localization of zones of higher P availability in the

soil was also indicated in most of the semi-variograms prepared from the data. Spatial variability in available soil P at this scale may play a substantial role in the residual availability of P from fertilizers (especially under reduced tillage systems), and in the negative effects of tillage on availability of soil P to plants.

6. DISSOLUTION OF OCTACALCIUM PHOSPHATE DURING SOIL PHOSPHATE EXTRACTION BY THE OLSEN SODIUM BICARBONATE PROCEDURE

6.1 Abstract

Phosphate fertilizers interact with soil components to form reaction products that serve as sources of P available to crops for many years. Effective soil tests must correctly reflect the relative availability to plants of the P in the various residual and native P forms present.

Dissolution of Ca and Mg phosphates representative of fertilizer reaction products formed in Manitoba soils was studied under conditions of the Olsen sodium bicarbonate test for plant-available P. Compounds known to be very good P sources to plants dissolved rapidly, whereas very little P in hydroxyapatite (which is relatively unavailable to plants in non-acid soils) came into solution. Octacalcium phosphate (OCP), known to be an important form of residual fertilizer P in Manitoba soils, dissolved at a moderate rate in Olsen extractant without soil present, but very slowly with soil or soil solutes present. Factors affecting OCP dissolution during Olsen extraction were further investigated.

Oxidation of the soil organic matter reduced the retarding effect of soil on OCP dissolution, suggesting that organic species were in part responsible. Of nine inorganic ionic species tested, only Mg^{2+} and Fe^{2+} substantially affected (reduced) rates of OCP dissolution. The retarding effect of Mg^{2+} was strong at levels present during soil extraction, and the effect increased greatly though not regularly with Mg^{2+} concentration. Although Mg^{2+} addition reduced precipitation of Ca^{2+} during extraction, Mg^{2+} also had some more direct mechanism of retarding OCP dissolution.

Dissolution of added OCP was exceedingly slow in the presence of soil, suggesting that the Olsen procedure may under-predict the value this form of residual P to plants. Clearly, OCP dissolution during extraction of soil was very limited by factors other than its solubility.

Determination of cations in solution during Olsen extraction of soils revealed that the solutions were highly supersaturated with respect to calcite.

6.2 Introduction

The phosphate in soluble P-containing fertilizers reacts with other species in the soil to form phosphate reaction products. Though less soluble than the original fertilizer compounds, these reaction products are more available than the bulk of native soil P and persistent enough in most western Canadian soils to provide P to crops for ten or more years (Roberts and Stewart 1987). Since much of the residual fertilizer P is supplied by these reaction products, it is important that soil tests recover them to an extent that properly reflects their availability to plants.

Phosphate fertilizer reaction products directly identified in studies with Manitoba soils include dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP), and additionally in high-Mg soils dimagnesium phosphate trihydrate (DMPT) and trimagnesium phosphate 22-hydrate (TMP) (Racz and Soper 1967, Strong and Racz 1970). These products are in general agreement with those identified by Bell and Black (1970b) from studies involving slightly acid to alkaline (including calcareous) soils. Other P compounds containing NH_4^+, Mg^{2+}, or other ions applied with the phosphate, have also been identified at the reaction site. Indirect evidence in the form of soil phosphate potentials, from Manitoba (Ridley and Tayakepisuthe 1974), Saskatchewan (Sadler and Stewart 1977), and Colorado (Havlin and Westfall 1984, Olsen et al.

1983), point to OCP in particular as an important reaction product governing P availability in many fertilized soils.

Extractants used in fractionation of soil nutrients and in soil testing for fertility assessment are selected based on some particular proposed mechanism(s) for bringing into solution more-or-less specific chemical fractions of the nutrient. In the case of soil fertility testing for P, the objective is to dissolve/desorb the various forms of soil P in proportion to their contribution to plant-available P. In general, measures of *labile* P as provided by isotopic exchange with ^{32}P or by anion exchange resin extraction are the most applicable when a wide range of soils are involved. Such methods are least reliant on specific chemical mechanisms of extraction which may be more effective for some forms of P than for others of similar plant-availability.

Kumar et al. (1991a, b) determined the extractability of P added to four soils in six chemical forms representative of soil-fertilizer reaction products. They used a modified Jackson and Chang soil P fractionation procedure (Williams et al. 1971) and eight soil test P extraction methods. The fractionation was generally ineffective in reflecting the form of P added, and the recovery of added P in each fraction varied among soils. For example, P added as apatite should have been recovered in the final step supposedly specific for Ca-P compounds. However, 41-66% of P added to soils as apatite (and 100% of apatite-P without soil present) was extracted prior to the final step. Proportions of added P recovered by the soil test P extractions also varied widely among the compounds added, the tests used, and the four soils, not always in the predicted manner based on the chemical mechanisms of extraction associated with each test. The same P compounds were added to a soil to test the availability of the various forms of added P to wheat in the greenhouse (Kumar et al. 1992). Though none of the soil P tests used were very effective in reflecting the availability of the P to plants across the range of P compounds used, the Colwell test (which extracts with a $NaHCO_3$ solution) was superior to the others; no

exchange resin based method was included. In an evaluation of results from many P test studies including widely ranging soil types, Sibbesen (1983) concluded that $NaHCO_3$ methods were superior to methods based on acidic extractants, but not as good as anion-exchange resin methods; plant P accumulation was the basis for evaluation.

Soil P extraction with $NaHCO_3$ solution is the most widely used and recommended P test for neutral to alkaline soils, including calcareous soils. It is usually carried out as originally proposed by Olsen et al. (1954) or with the modifications recommended by Colwell (1963). The major mechanisms of P extraction cited by Olsen et al. (1954) are: 1) enhanced dissolution of Ca phosphates due to repression of Ca^{2+} activity in the presence of CO_3^{2-}, and 2) replacement of surface phosphate ions by HCO_3^-, CO_3^{2-}, and OH^-.

If Ca^{2+} activity during the Olsen extraction is maintained at the equilibrium level controlled by the solubility of calcite, and if all Ca phosphates dissolve completely to their solubility limits in that solution, then very high P test levels should be obtained even in soils containing only relatively insoluble Ca phosphates known to be of very low plant-availability (e.g. hydroxyapatite). However, soil test levels are too low to suggest that such dissolution occurs. The studies reported here determined the degree of recovery of P from selected specific soil-fertilizer reaction products, during Olsen extraction. A range of investigations then further elucidated factors influencing OCP dissolution. Although the Olsen test has been the subject of much study (e.g. Barrow and Shaw 1976a,b, and subsequent papers in the series), little is known of the behavior of specific fertilizer residual P forms and compounds during soil extraction by this or other methods.

6.3 Materials and Methods

6.3.1 Preparation of P Compounds

The following five compounds were used, with abbreviation, formula, and source indicated:

i) Dicalcium phosphate dihydrate - **DCPD**. $CaHPO_4 \cdot 2H_2O$. Prepared by the method of Moreno et al. (1960).

ii) Octacalcium phosphate - **OCP**. $Ca_8H_2(PO_4)_6 \cdot 5H_2O$. Prepared by the method of LeGeros (1985; method a).

iii) Hydroxyapatite - **HAP**. $Ca_{10}(PO_4)_6(OH)_2$. The purchased compound was used (Mallinckrodt #4288), after removal of the fraction not passing a 100-mesh sieve. The citrate-soluble fraction was also removed by washing, using the following steps (with centrifuging): water - 1.08 M ammonium citrate (pH 7) - water - 1.08 M ammonium citrate (pH 7, 65°C) - water (4X) - acetone (2X) - acetone (wash in buchner funnel).

iv) Dimagnesium phosphate trihydrate - **DMPT**. $MgHPO_4 \cdot 3H_2O$. The purchased compound was used (Sigma #7782-75-4).

v) Trimagnesium phosphate 22-hydrate - **TMP**. $Mg_3(PO_4)_2 \cdot 22H_2O$. Prepared as described by Racz (1966, p. 13) - slow dropwise addition of solutions of $MgSO_4$ and Na_2HPO_4 into a stirred suspension held at pH 10 by NaOH addition.

Each phosphate compound was dried at 35°C, and ground as required to pass a 100-mesh (150µm) sieve. Compounds were analyzed by x-ray powder diffraction (XRD), and by determination of Ca or Mg (atomic absorption) and P (as phosphate; Murphy and Riley 1962) after dissolution in 1M HCl, with the following results. Elemental concentrations below are expressed as percentages of those in the pure compound (i.e. of theoretical).

i) **DCPD**. 101/98% Ca/P. Well-crystallized, with no evidence of other phases.

ii) **OCP**. 99/97% Ca/P. X-ray diffraction pattern same as reported by LeGeros (1985) for OCP from stirred systems; not strongly crystallized. A distinct reflection corresponding to a *d*-spacing of 7.61 Å (LeGeros [1985] did not report the pattern in this range) suggested some DCPD was be present. No evidence of other phases.

iii) **HAP**. 87/100% Ca/P. X-ray diffraction pattern characteristic of HAP; no evidence of other phases.

iv) **DMPT**. 114/101% Mg/P. Well-crystallized, with no evidence of other phases.

v) **TMP**. 107/99% Mg/P. X-ray diffraction not done.

The above compounds were diluted 100X (w/w) for use, due to the small weights required. Silica (SiO_2, approximately 240 mesh/65μm; Fisher) was used as the diluent, after it was thoroughly washed with the following solutions: 2M NaOH - water - 5M HCl - water - 0.5M $NaHCO_3$ - water (8X). After each wash, solutions were decanted after settling about 10 min; this also removed finer particles so that none remaining would pass a Whatman #5 filter. Diluted phosphate compounds were thoroughly mixed, and subsamples were determined to be of consistent composition (based on phosphate concentration).

6.3.2 General Method for Dissolution in Sodium Bicarbonate Solution

Sodium bicarbonate extractions were carried out as per the Olsen and Sommers (1982) procedure, using 0.5 M $NaHCO_3$, adjusted to pH 8.5 with NaOH. The extractant (100 mL) plus 5 g of soil (and/or indicated amounts of other compounds) was shaken in 250-mL capped bottles for 30 min at room temperature (22±2°C) on a reciprocating shaker at 3 cycles s^{-1}. Suspensions were then filtered by gravity through Whatman #5 filter paper. Orthophosphate in the filtrates was determined colorimetrically as the reduced phosphomolybdate complex (Murphy and Riley 1962); suphuric acid was added to neutralize the alkalinity of the extractant.

6.3.3 Dissolution of Selected Calcium and Magnesium Phosphates

All five phosphate compounds previously described (section 6.3.1) were extracted, in both fresh $NaHCO_3$ extractant and in the $NaHCO_3$ extract of the Riverdale soil (Table 6.1) without the soil present. The soil extract was prepared using the normal extraction procedures as previously described, but on a larger scale, and with centrifuging and vacuum filtration to recover a large volume of extract. Carbon dioxide was bubbled through the soil extract prior to use to bring the pH back down to 8.5. Extract of the Stockton soil referred to in Table 6.1 was used only in the next described experiment (Section 6.3.4).

The silica-diluted P compounds were added to the extractant to supply 100 µmol of P per litre of extractant. Each was extracted in triplicate, in both fresh extractant and soil extract, for each of the following times (dissolution time followed by gravity filtration time):

3 min	(2 min)
30 min	(3 min)
300 min	(3 min)

The 300-min dissolution time was omitted where dissolution was essentially complete at 30 min.

Table 6.1 Characteristics of soils used for soil extract preparation.[z]

Soil series	Texture	pH	Organic C	Carbonate-C	$NaHCO_3$-P
			—— g kg^{-1} ——		mg kg^{-1}
Riverdale	SiCL	7.5	24	8.4	5
Stockton	LS	6.0	12	<0.1	10

[z] 0-15 cm depth soil; texture by hand estimate; pH in 0.01 M $CaCl_2$; organic C by wet oxidation (Yeomans and Bremner 1988), carbonate-C by titrimetry (Bundy and Bremner 1972), and $NaHCO_3$-P by method of Olsen and Sommers (1982).

6.3.4 Effects of Solutes in Extractant on OCP Dissolution

Octacalcium phosphate was extracted for 30 min using the Olsen procedure as described in section 6.3.2, with 16 variations (Table 6.2). The treatments included addition of many of the species that would be present from the soil in solution during $NaHCO_3$ extraction. Concentrations were selected to be similar to, or moderately in excess of, maximum concentrations suspected during soil extraction. Compounds used to supply the ions were chosen to minimize confounding effects of other added ions.

Table 6.2 Extracting solutions for study of effects of solutes in extractant on OCP dissolution.

Trtmnt. No.	Extractant		
1	Control – unamended $NaHCO_3$ extractant		
2	Riverdale soil extract		
3	Stockton soil extract.		
4	Riverdale-OM - Riverdale soil extract, organic matter removed		
5	Stockton-OM - Stockton soil extract, organic matter removed		
6	$KHCO_3$ - 0.5 M $KHCO_3$ (pH 8.5) used in place of $NaHCO_3$		
	Solute added to $NaHCO_3$ extractant		
	Ion	**Ion concentration**	**Source**
		mM	
7	Mg^{2+}	2.0	MgO
8	K^+	1.0	$KHCO_3$
9	Fe^{2+}	0.1	$FeCl_2 \cdot 2H_2O$
10	Mn^{2+}	0.1	$MnCl_2 \cdot 2H_2O$
11	NH_4^+	0.1	NH_4OH solution
12	SO_4^{2-}	1.0	$Na_2SO_4 \cdot 10H_2O$
13	Cl^-	1.0	NaCl
14	NO_3^-	1.0	$NaNO_3$
15	F^-	0.1	HF
16	Citrate	0.1	$Na_3C_6H_5O_2 \cdot 2H_2O$
17	Acetate	0.1	$NaC_2H_3O_2 \cdot 3H_2O$

A fine precipitate formed in the extractant with added manganese chloride. The extractant with added ferrous chloride became slightly turbid after several days. Other treatment compounds appeared to remain in solution. Preparation of the "soil extract" extractants was previously described (section 6.3.3). The "-OM" soil extracts were prepared from the corresponding soil extracts by adding 250 to 300 ml of 30% H_2O_2 solution per litre of extract, in small portions while heating, and boiling down to the original extract volume. After cooling, a yellow-brown precipitate which had formed during the treatment was filtered out (Whatman #42 paper), and CO_2 was bubbled through the solutions to adjust the pH back down from almost 10.0 to 8.5.

Phosphate was determined in each of the 17 extracting solutions, with and without added phosphate in solution, to establish background levels and check for interferences; recovery of added P in solution was 100±3% in all cases.

Octacalcium phosphate was added to each treatment to supply 0.1 mmol of P per litre of extractant. Standard extracting conditions previously described were used, with three replicates of each treatment. Only phosphate was determined in the filtrates.

6.3.5 Effects of Magnesium Concentration on OCP Dissolution

The influence of Mg on OCP dissolution was determined over a range of Mg concentrations and dissolution times.

Magnesium was added to Olsen extracting solution to prepare extractants with 0.00, 1.11, 3.33, and 10.00 mM added Mg. The Mg source was MgO, which was dissolved in a slight excess of HCl, and neutralized with NaOH. Sodium chloride was added to all extracting solutions to bring them to 20.0 mM in Cl. The $NaHCO_3$ source used to prepare the extractant contained some Mg as a contaminant, sufficient to give a Mg concentration of 0.012 mM in the "0.00" mM Mg treatment.

At the start of the dissolution, OCP (not diluted with silica) was added to each solution to supply 0.1 mmol of total P per litre of extractant. Extractions were carried out in one-litre bottles, and were not replicated. Portions of the suspensions were sampled (while agitating to maintain suspension uniformity) and filtered ten times, from 8 min to 98.5 h after the initial mixing.

The extractions with 0.00 and 3.33 mM Mg in the extractant were also carried out with soil added to the suspension. The Riverdale soil (Table 6.1) was used, in the amount that would be present during Olsen extraction (5 g of soil per 100 mL of extractant). The extractions with soil added were carried out both with and without OCP added to supply 0.1 mmol of OCP-P per litre of extractant.

A second set of 24-h OCP dissolutions was carried out for the 0.00 and 10.00 mM Mg treatments, using 0.5 (rather than 0.1) mmol of added OCP-P per litre of extractant (no soil). After 24 h, the solids in suspension were filtered, washed with acetone, and analyzed by XRD to identify the solid phases present. The solids were also analyzed for acid-soluble Ca, Mg, and P, and the filtrates for P.

6.3.6 Effects of Other Selected Variables on OCP Dissolution

This study was carried out to describe and explain factors influencing OCP dissolution during Olsen extraction, by simulating some of the possible conditions existing and species present during soil extraction. Phosphate in solution was determined over dissolution times from about four minutes to two days. Extractions were not routinely replicated. During the time course each of the trials, portions of the suspensions were poured off repeatedly from the single large extraction for each treatment.

The following treatments were used. Octacalcium phosphate was added to provide 10, 100, or 1000 μM of OCP-P in suspension, designated below by *-10*, *-100*, or *-1000*, respectively. Where *soil* is indicated, the Riverdale soil (Table 6.1) was used at 5 g of soil per 100 ml of extractant.

Soil	- Soil only; no added OCP
OCP-10, -100, and -1000	- OCP only
Soil-10, -100, and -1000	- Soil and OCP added
Psol-10, -100, and -1000	- Dissolved P from K_2HPO_4 added to extractant at concentration equal to that of the OCP-P to be added for each treatment
Soil+Psol-10, and -100	- As above, with soil also
Sand-10, and -1000	- silica sand, ground to <150 μm and washed, added at 5 g L^{-1}
Charcoal-10, and -1000	- Charcoal powder, washed, added at 10 g L^{-1}
$CaCO_3$-10, -100, and -1000	- $CaCO_3$ (<150 μm powder) added at 50 mM (equal to amount present during Olsen extraction of as soil containing 10% $CaCO_3$). XRD identified only the calcite form
$CaCl_2$-10, -100, and -1000	- $CaCl_2{\cdot}2H_2O$ added at 12.5 mM, to supply the same amount of Ca^{2+} into solution as a soil containing 25 cmol(+) kg^{-1} of Ca^{2+} exchangeable under Olsen extraction conditions.

All added solids indicated in the above treatments were mixed with the OCP prior to extraction. For the Psol treatments, we added potassium phosphate to the extractant before addition of OCP.

6.3.7 Cations in Solution

Concentrations of selected cations in solution were determined under Olsen extraction conditions, during both OCP dissolution and soil extraction. This trial was conducted as a

follow-up to the study described in section 6.3.4, which had shown OCP dissolution to be retarded in the presence of Mg^{2+} and Fe^{2+}.

For the OCP dissolution study (no soil), OCP was added to all treatments for a final concentration of 0.1mM of OCP-P. Six added cation treatments were used at each of eight added Ca^{2+} levels (factorial), as indicated below. Extractions for each of the 48 resulting treatments were not replicated.

Treatment (Added cation; mM)	Added Ca (mM)
none	0
Fe (0.0075)	0.037
Fe (0.075)	0.111
Mg (0.667)	0.333
Mg (2.0)	1
Mg (6.0)	3
	9
	18

To establish the treatments, all cations were added as their chlorides, and all treatments were brought up to 48 mM in total added Cl using NaCl. Sodium carbonate solution was also added to the extractant for each treatment, in molar concentrations equal to the added $CaCl_2 \cdot 2H_2O$, to prevent a reduction in pH resulting from precipitation of $CaCO_3$ (this was determined to be effective).

Calcium, Mg, Fe, and phosphate were determined in the suspension filtrate after 30 min of dissolution (cations by atomic absorption spectroscopy).

For the soil extraction portion of this study, Olsen extractions of eight Manitoba soils with widely varying characteristics were conducted (Table 6.3). Washed $CaCO_3$ was added to the soils at 0, 1, and 10 % of the soil weight (addition of $CaCO_3$ had been previously shown to

Table 6.3 Characteristics of soils used for determination of cations in Olsen extracts of soils.[z]

Soil series	Texture	pH	Organic-C	Carbonate-C	Ca	Mg
			—— $g\ kg^{-1}$ ——		- $mg\ kg^{-1}$ -	
Riverdale	SiCL	7.6	26	5	6780	370
Willowcrest	LS	7.8	9	1	1670	90
Lakeland	SiCL	7.8	40	7	7340	1680
Stockton	LFS	6.6	12	0	960	110
Osborne	C	7.7	41	8	9740	1390
Plum Ridge	FL	7.9	27	7	6180	680
Reinfeld	FL	6.8	26	0	2230	390
Glenboro	L	5.4	53	0	3220	630

[z] 0-15 cm depth soil; texture by hand estimate; pH in 0.01 M $CaCl_2$; organic C by wet oxidation (Yeomans and Bremner 1988), carbonate-C by titrimetry (Bundy and Bremner 1972), and cations by N NH_4OAc extraction (1:20).

accelerate OCP dissolution without soil present). The suspensions were filtered after 15 and 30 min of extraction, and concentrations of phosphate, Ca, Mg, Fe, and Mn were determined in the filtrate. In addition to the eight soil treatments, a blank (no soil) treatment was used, and a treatment with 5 mM of added $CaCl_2 \cdot 2H_2O$ instead of soil.

6.4 Results and Discussion

6.4.1 Dissolution of Selected Calcium and Magnesium Phosphates

Concentrations of phosphate in the filtrates (less those in the corresponding blanks) are presented as fractions of the P added as the phosphate compounds (Figure 6.1). The percentages are numerically equal to the micromolar concentrations of phosphate-P in the extracts. Blanks averaged 0.1 μM (for fresh extractant) and 7.6 μM (soil extract) in P.

Dicalcium phosphate dihydrate and DMPT dissolved rapidly, with 90-100 % in solution within 30 min. Dissolution in $NaHCO_3$ extract of soil was slightly slower than dissolution in fresh

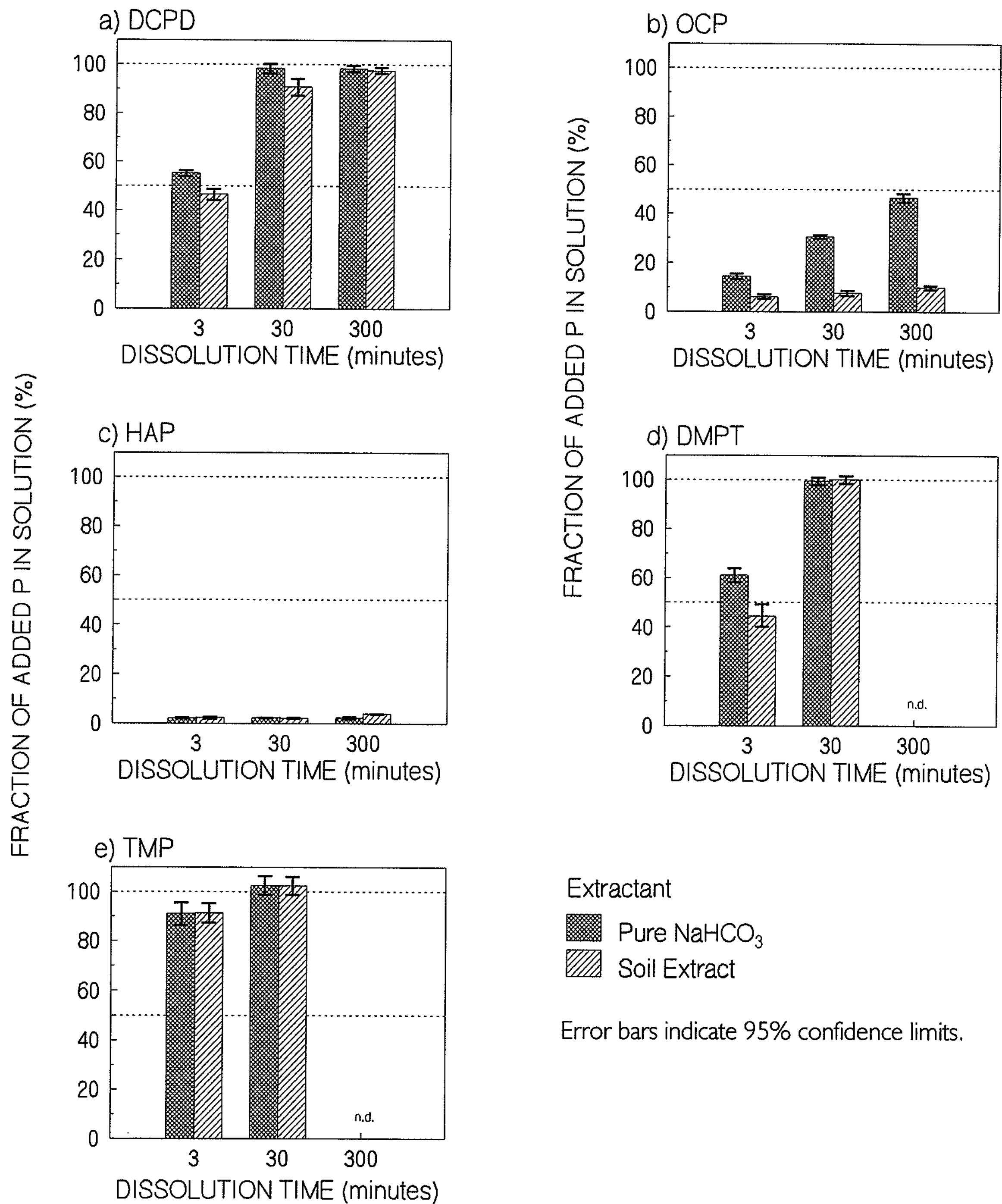


Figure 6.1 Dissolution of all P compounds in $NaHCO_3$ extractant.

extractant. Both DCPD and DMPT are important early reaction products of soluble phosphate fertilizers in Manitoba soils (Racz and Soper 1967, Racz and Soper 1970, Strong and Racz 1970). Despite the relatively high solubility (hence, plant-availability; Lindsay 1979) of these compounds, they can persist for more than a year in non-acidic Manitoba soils (Strong and Racz 1970), so could be important sources of residual P. Consequently, a high degree of dissolution during soil test P extraction, as found, is desirable. In high-Mg Manitoba soils, DMPT can transform to TMP (Strong and Racz 1970), which is also soluble enough to be a good source of plant-available P (Lindsay 1979): TMP was dissolved very rapidly in our study (Figure 6.1e).

Very little if any HAP dissolved (Figure 6.1c). The similar results among extraction times suggest that the small amount of P that was in the filtrate may be surface-displaced P or P in particles small enough to pass the filter. Kumar et al. (1991b) reported 4 % extraction of apatite-P without soil present using the Colwell P test method, which employs the same extractant as the Olsen procedure, but a longer extraction time and wider extractant:soil ratio. Recovery of apatite-P was -1 to 2 % with soil present. In contrast, the five soil test procedures in the same study that employed acidic (pH<6) extractants recovered 47 to 85 % of apatite-P in the absence of soil, and 3 to 84 % of added apatite-P (mean - 44 %) with soil present. Hydroxyapatite is representative of most of the stable native inorganic P in neutral to alkaline prairie soils, but its solubility above pH 6.5 is too low for it to supply significant amounts of P to plants (Roberts and Stewart 1987). Few studies have shown fertilizer residual P to convert to such insoluble forms in the soil.

The extraction time and presence of soil solutes affected dissolution of OCP much more than the dissolution of the other compounds (Figure 6.1). After five hours of dissolution, almost half of the OCP-P was dissolved in fresh extractant, but only 10 % in soil extract. The retarding effect of the soil was probably even greater than suggested by this result, since some of the P dissolving

initially from the added OCP was likely a soluble phosphate contaminant that the OCP contained. In this and all subsequent trials with this OCP source, a minimum of about 4 % of the "OCP-P" came into solution very quickly, regardless of conditions or the presence of soil or other added species, and even when further dissolution of OCP was exceedingly slow. The rapid dissolution of this fraction of the "OCP", along with X-ray diffraction evidence (section 6.3.1), suggests that about 4 % of the phosphate in the OCP used was present as DCPD.

Solubility products for all five compounds studied (even HAP) are many times higher than necessary to permit complete dissolution of amounts added in pure Olsen extractant.

In the above (and following) work, the fraction of the P compound *dissolved* is assumed equal to the fraction of its phosphate present in the filtrate. However, the occurrence of processes that might have removed phosphate from solution prior to filtration cannot be ruled out in some cases; e.g. incongruent dissolution or solid phase transformations to produce new solid phases, and surface exchange or adsorption of ions. Also, some small phosphate-containing particulates likely passed the filter paper in most cases. The gravity filtration method used was compared with millipore filtration (0.2 μm) for OCP dissolving in fresh extractant, after about 22 and 55 % of the OCP had dissolved. For both extraction times, the concentration of P in the millipore filtrate was lower, by almost 3 % of the total P initially present in the suspension. Gravity filtration required at least two minutes, during which time dissolution was still occurring. As a result, the actual extent of dissolution may be over-estimated considerably for very short extraction times.

Interpretations of the observed dissolution rates are qualitative and comparative only. Meaningful quantitative interpretations of results would require information about the compounds and dissolution conditions which is not available, such as the effective specific surface area of each solid throughout its dissolution. Huffman et al. (1957) determined these in

their study of dissolution of several Ca phosphates. Even if such information had been obtained for the compounds in the present study, calculated rates would have limited application to the same compounds formed *in situ* in the soil, for which the corresponding information would not be available.

Due to the likely importance of OCP as a fertilizer residual P source, the subsequent studies were undertaken to determine the factors influencing OCP dissolution during soil P extraction with sodium bicarbonate.

6.4.2 Effects of Solutes in Extractant on OCP Dissolution

As in the study discussed above (Section 6.4.1), approximately 30% of the OCP was dissolved within 30 min in unamended $NaHCO_3$ extractant (Figure 6.2). Only 7 to 8% of the OCP dissolved within 30 min in both untreated soil extracts (probably an even smaller fraction of the actual OCP, since the DCPD contaminant in it likely dissolved completely in all treatments). Kumar et al. (1991a) also reported that the presence of soil greatly reduced phosphate extraction from six added P sources, during a modified Chang and Jackson P fractionation procedure. Possible sorption of dissolved P by the soil could have been responsible for some of the effects. Soils were also found to greatly reduce P extraction from the same P compounds during several standard soil P test procedures (Kumar et al. 1991b), including extraction with 0.5 M $NaHCO_3$. For example, 96% of the phosphate "sorbed onto calcite" (which may have been OCP-like in chemical form, based on its characteristics and method of preparation) was extractable with 0.5 M $NaHCO_3$ without soil, but only 8 to 20% if soil was present; P sorption to the soil could account for little of the difference.

Peroxide treatment to oxidize organic matter in the soil extracts reduced by one-half to two-thirds the retarding effect of soil solutes on OCP dissolution (Figure 6.2). Therefore, organic species

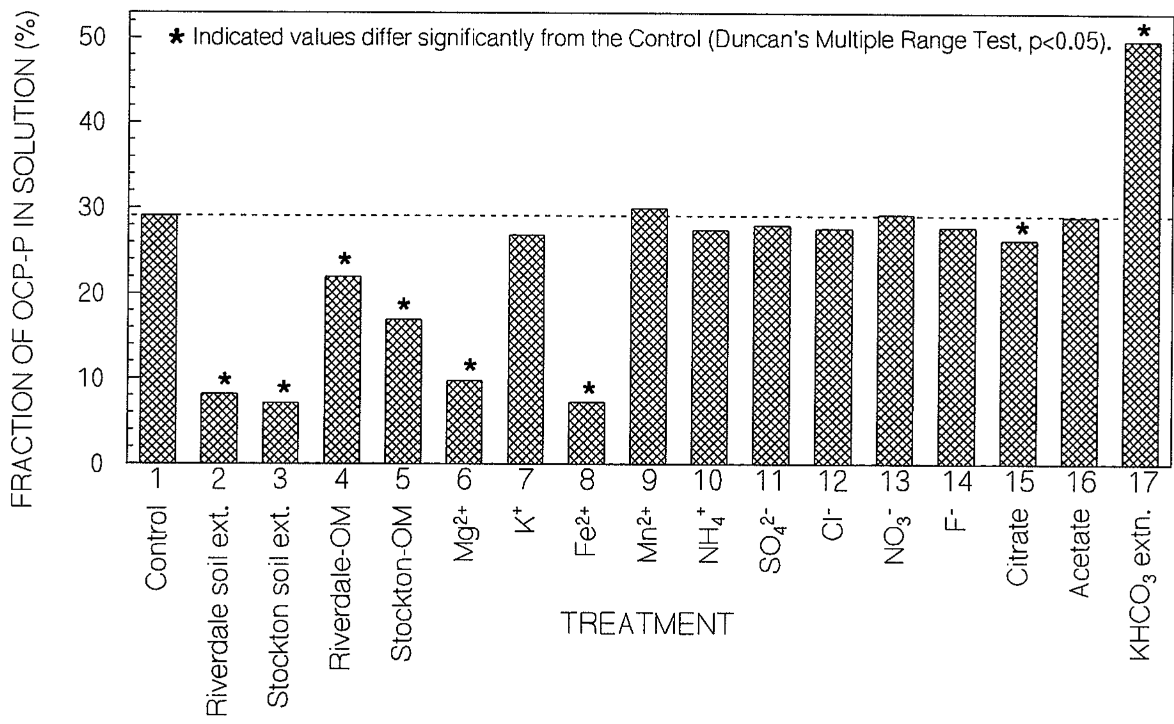


Figure 6.2 Effects of extractant composition on OCP dissolution in 30 minutes.

may have been responsible in part for the inhibition of dissolution. However, the effect of peroxide treatment may also have been through changes in the activities of inorganic species by removal of chelating agents, oxidation, precipitation, etc. Citrate slightly reduced the rate of OCP dissolution, while the other specific organic anion added (acetate) had no effect.

All four inorganic anions added, both monovalent cations, and Mn^{2+} did not affect the rate of OCP dissolution. Both Mg^{2+} and Fe^{2+}, however, strongly retarded dissolution, to degrees similar to those of the soil extracts.

The influence of solutes on OCP dissolution has not been previously studied, so explanations and mechanisms to explain the results are speculative. Some of the better-known effects of various species on precipitation and hydrolysis of Ca phosphates are comparable to the present observations on dissolution; similar mechanisms may be involved.

Inskeep and Silvertooth (1988) found that precipitation of HAP was inhibited strongly by fulvic and humic acids (cf. components of the organic matter in the soil extracts in the current study), and more weakly by lower molecular weight organic acids, including citric acid. The proposed mechanism of inhibition was adsorption of the organic ligands on the HAP seed crystals, blocking crystal growth sites. Octacalcium phosphate is structurally related to HAP (Brown 1962). Organic solutes from soil may similarly retard OCP dissolution if adsorbed. Dissolved organic matter also inhibits calcite precipitation (Suarez et al. 1992). This could indirectly reduce dissolution of Ca phosphates during Olsen extraction of soils, by causing more Ca to remain in solution.

The effects of Mg^{2+} on precipitation and hydrolysis of Ca-phosphates are well documented. Magnesium inhibits hydrolysis of OCP and amorphous Ca phosphates to HAP (Brown et al. 1962, LeGeros and LeGeros 1984, Termine et al. 1970, Posner et al. 1984), as well as direct precipitation of OCP and HAP (Nancollas 1984, Zawacki et al. 1986). Brown et al. (1962)