Plant Phosphorus Requirements and Soil Phosphorus Reactions as Influenced by Temperature

by

S.C. Sheppard

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PLANT PHOSPHORUS REQUIREMENTS AND SOIL PHOSPHORUS REACTIONS AS INFLUENCED BY TEMPERATURE

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STEPHEN C. SHEPPARD

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

Experiments were conducted to characterize the effects of soil and root temperatures on the response of wheat plants to fertilizer P, the reactions of soil and fertilizer P, and the physiological requirement of wheat plants for P. Emphasis was placed on the dynamic nature of the soil-plant P system.

The response to broadcast and band-applied fertilizer P was examined in a soil-plant system with soil temperatures of 10, 15, 20 and 25°C. Growth curves, vegetative yield, tissue P concentrations and root proliferation in the fertilizer band were among the measurements made.

Plant growth rate was markedly reduced at the lower soil temperatures. However, the optimal amount of broadcast P varied only slightly due to temperature at either the three-leaf stage or at final harvest (33 to 36 days after planting). The response to band-applied P was greater at lower soil temperatures and there was evidence that band-applied P was more toxic at higher soil temperatures. Root proliferation in the P-fertilizer band was significant only at 10°C. The relationship of yield to tissue P concentration varied with temperature but it was hypothesized that this phenomenon was due primarily to temperature-induced differences in physiological development.

An incubation/extraction study was conducted using two soils incubated at 10, 15, 20 and 25°C with both carrier-free ^{32}P and ^{32}P-labelled fertilizer P. The soils were extracted using dilute KCl, $NaHCO_3$, anion exchange resin, and short term plant uptake. Increased extraction (or growth) temperature increased the amount of P desorbed. However, increased incubation temperature accelerated the rate of fixation such that less P was desorbed.

The effect of extraction temperature was established within one hour of extraction and the effect of incubation temperature was established within one day of incubation. The rates of reaction beyond these times were relatively independent of temperature.

Desorption curves were measured by use of varied soil:solution ratios. The intercepts increased with higher extraction temperature and in some cases the slopes decreased. The latter indicated a greater P buffer capacity at higher temperatures.

Extension of these incubation/extraction studies to 12 Manitoba soils indicated that the effects of temperature on soil P varied among different soils.

The final study was a solution culture experiment wherein plant P use efficiency was evaluated at 10, 15, 20 and 25°C root temperatures and eight solution P concentrations. Growth curves were measured by weighing the plants at intervals. Plants were harvested at the six-leaf stage and at a 4-g fresh weight stage. Additional plants were switched to a high solution P concentration and their response was assessed to define the plant P status of the plants harvested previously. Rapid changes in tissue P concentration, tissue dry matter content, shoot to whole plant ratio, and relative growth rate were useful for this assessment.

The P use efficiency was highest at 10°C indicating that with a slower growth rate (due to temperature), the physiological requirement for P was less. The relationship between P use efficiency and relative growth rate was linear.

Throughout the study, the dynamic aspects of the soil-plant system were apparent. The desorption of soil and fertilizer P depended upon the effect of temperature on the opposing processes of P fixation and P solubility. Thus, the net effect depended upon the reaction state or "age" of

the P. The P status of wheat depended upon supply of P but also upon the adaptive responses of the plants including modification of root proliferation, shoot:root ratios, tillering, translocation, and ultimately growth rate. The physiological plant P requirements also appeared to change with time and temperature. Thus, the overall response of plants to P fertilization was clearly due to a balance of numerous factors.

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INTRODUCTION

Soil fertility research strives toward improving the recommended techniques and recommended amounts of fertilizer application. A major incumbrance in this research is the variability imposed by yearly weather conditions on the response of crops to fertilization. Knowledge of the effect of weather on crop response to fertilizers greatly improves the ability to interpret experimental results. However, this knowledge may also be useful to extrapolate to "normal" or long-term-average weather conditions. If this extrapolation were possible, the fertilizer recommendations could be based on probabilities derived from many years of meteorological data.

An example of the use of this facility would be the interpretation of the results of a field experiment, not only under the weather conditions experienced, but also with respect to the variation of weather conditions for that experimental site over many years. This concept is viable today because of computerized crop simulation models. Thus, with knowledge of weather-crop response interactions, with long-term weather records (now generally available), and with detailed measurements of a specific site, it might be possible to extrapolate observed crop response to fertilizers in one year to the normal or extremes of ten years on the same site.

The weakest link in this concept is the knowledge of weather-crop nutrient response interactions. This is especially true in the context of the detailed measurements required for state-of-the-art computer simulation.

The purpose of this thesis was to identify some of the important variables and processes and to provide experimental data on how these variables and processes were affected by environmental conditions. Phosphorus was chosen as the nutrient to study because it is recognized to be subject to weather related variability. Temperature was chosen as the environmental variable since it has profound effects on both soil chemistry and plant physiology. A literature review examined the various relevant processes. This was followed by experimentation which included a combined soil-plant experiment to examine the whole system and then experiments dealing with specific aspects of the independent soil and plant components.

Chapter I

LITERATURE REVIEW

Introduction

The high efficiency of modern agriculture is based on the selective use of technology to achieve the greatest financial return for the cost of the technological inputs. In the case of field crops, the selection of the optimal inputs becomes probabilistic since year to year variations in weather account for large differences in productivity and large differences in the response of crops to the applied technology. Thus, before technological research can be applied to crop production, the methods must be evaluated over a number of years. This is an extremely costly procedure.

Soil fertility research (which forms the basis for the application of fertilizer technology to field crops) is particularly susceptible to year to year variations in weather. The final outcome of the research which is knowledge of the most profitable rate of application of a particular nutrient is an integration of soil and plant processes throughout the entire growing season. Daily changes in weather potentially influence all of these processes and thus the range of possible outcomes is magnified phenomenally.

The effects of weather in modifying the response of crops to P fertilization has been well documented (for effects of soil temperature, see reviews by Nielsen and Humphries, 1966; Sutton, 1969; Simpson, 1961). For instance, Ferguson (1964) analysed data for wheat grown on the Canadian Prairies from 1936 to 1962 and observed that up to 59% of the variation in

crop response to P fertilizer was explained by measurements of rainfall. Similar observations of weather influence on response to P have been reported from throughout the Prairie and Great Plains regions (Nutall et al., 1979; Read and Warder, 1974; Fixen and Carson, 1978; Power et al., 1961) and also from more humid regions (Mack, 1965; Voss et al., 1970; Bichelhaupt et al., 1979).

Several researchers have utilized information on weather and the interaction between weather and crop response to develop fertilizer recommendation procedures (Fixen and Carson, 1978; Van der Paauw, 1967). Zentner and Read (1977) demonstrated that measures (or predictions) of stored soil water at time of seeding could be used to economic advantage in adjusting fertilizer recommendations. Bickelhaupt et al. (1979) used regression models to adjust foliar analysis of *Pinus resinosa* to a "normal season" based on precipitation and degree-day summations of the previous season. However, more extensive use of weather-crop response information with a forecast objective is obviously limited by the inability to accurately forecast the weather.

Little or no use has been made of historic weather information in soil fertility investigation. Clearly, if the results of soil fertility research must be interpreted in a probabilistic manner, then analysis of the source of variation (principally weather) should be relevant. The limitation to date has been the inability to systematically associate weather patterns with crop response. Thus,accurate simulation models would be useful.

The simplest models to relate crop response to weather patterns are statistical models such as regression equations. Typically, these models use summations of meteorological data over specific time periods as input variables. The weakness of these models is their inability to deal with

systems that are dynamic on a smaller time scale than the data summations. Furthermore, since the specific processes influenced by weather conditions are not described, unforeseen interactions between processes may not be reflected in the predictions. Thus, a process or mechanistic model would be more suitable.

A process model implies a conceptual accumulation of known processes into a rational network. The advantage over regression models is the flexibility to include any relevant process and the approximation of natural sequences and inter-relationships. Frequently, process models are dynamic in relatively short time intervals, an ideal basis for dealing with environmental effects on annual crops. Nye et al. (1976) proposed and subsequently built this type of model to predict plant growth response to nutrient supply.

The use of a process model implies a detailed knowledge of the processes and the factors which influence the processes. Thus, the following literature review examines the temperature-dependent processes in the soil-plant system. Furthermore, it identifies variables and processes which require further experimentation.

Soil Factors

A Summary of the Soil P System

The supply of P to the plant root represents an integration of soil P reactions and processes. As the plant removes P from the soil solution, the soil system buffers the decrease in solution P concentration by processes of dissolution and desorption of inorganic solid-phase P, mineralization of organic P and diffusion of solution-phase P from adjacent soil. These processes are limited by equilibrium levels and by reaction rates.

The chemistry of inorganic P in soil is very complex and whether precipitation/dissolution reactions or adsorption/desorption reactions control the P concentration in the soil solution has been unresolved (for extensive reviews, see Berkheiser et al., 1980; Nancollas et al., 1979; White, 1980). Recent information suggests that the reactions of P probably intergrade between adsorption and precipitation both in relative strength of bonding and in mechanism (Berkheiser et al., 1980; Nancollas et al., 1979). Fertilizer P applied in pelleted form is a special case because it reacts initially in a highly concentrated micro-region around the pellet site which leads to precipitation of the added P as discrete crystalline products (Sample et al., 1980). When solution P concentrations are much lower, more typical of the bulk soil, the initial reaction of P with the solid phase is probably adsorption. However, it appears that adsorbed P may metamorphose into more crystalline forms. Thus, with the exception of special cases such as the fertilizer pellet reaction site, it appears most appropriate to consider soil solid-phase P as a continuum of forms resulting in a range or profile of equilibrium solution concentrations. This approach is further justified by evidence (Freeman and Rowell, 1981; Nancollas et al., 1980) that surface reactions which may involve a huge number of possible complexes have substantial influence on the soil P chemistry. Thus, the terminology used in this review will describe solution to solid-phase P reactions as "sorption", the reverse as "desorption" and the equilibrium solution concentration as "solubility". "Fixation"will be used to describe reactions leading to decreased solubility, regardless of phase change and "P supply" will be used to describe the potential amount of P which could be released from the soil to a sink, usually the plant root.

The various fixation reactions in the soil occur simultaneously (and

some in chain series), each with specific stoichiometric, thermodynamic and kinetic properties. Many of the reactions are severely limited by reaction rate such that thermodynamically less-favoured products persist, resulting in a metastable equilibrium. For example, hydroxyapatite (HA) is the most stable common Ca-P mineral but dicalcium phosphate dihydrate (DCPD) is formed first and can persist for considerable periods in soil (Nancollas et al., 1979).

This concept of soil P is illustrated diagrammatically in Figure 1 where the height of each bar represents the amount of soil P in each specific form (represented as separate bars) and the width represents the solubility of that form of P. Thus, this figure indicates that most of the soil P is sparingly soluble. The solution P concentration tends to be in equilibrium only with the most soluble form, but the formation of the less soluble forms is limited by reaction kinetics. The term "P solubility profile" is proposed to describe the relative amounts of P in each of the solubility classes.

The desorption of P by a plant root or a chemical extractant presumably is the result of the solubilization of successive forms of P until the most soluble form remaining is that which achieves a quasi-equilibrium with the desorption system (quasi because these desorption processes are short-term relative to the overall rate of fixation). For example, $NaHCO_3$ extractions desorb much more P and extract P forms of much lower solubility than water extractions. Using a series of extraction systems varying in intensity (for example a range of soil:solution ratios), the P solubility profile can be evaluated and is commonly presented as a P desorption curve (equilibrium P concentration versus the amount of P desorbed). Following depletion of the more soluble forms of P, the soil P system reacts over a relatively long time period to replenish these forms (Mack and Barber,

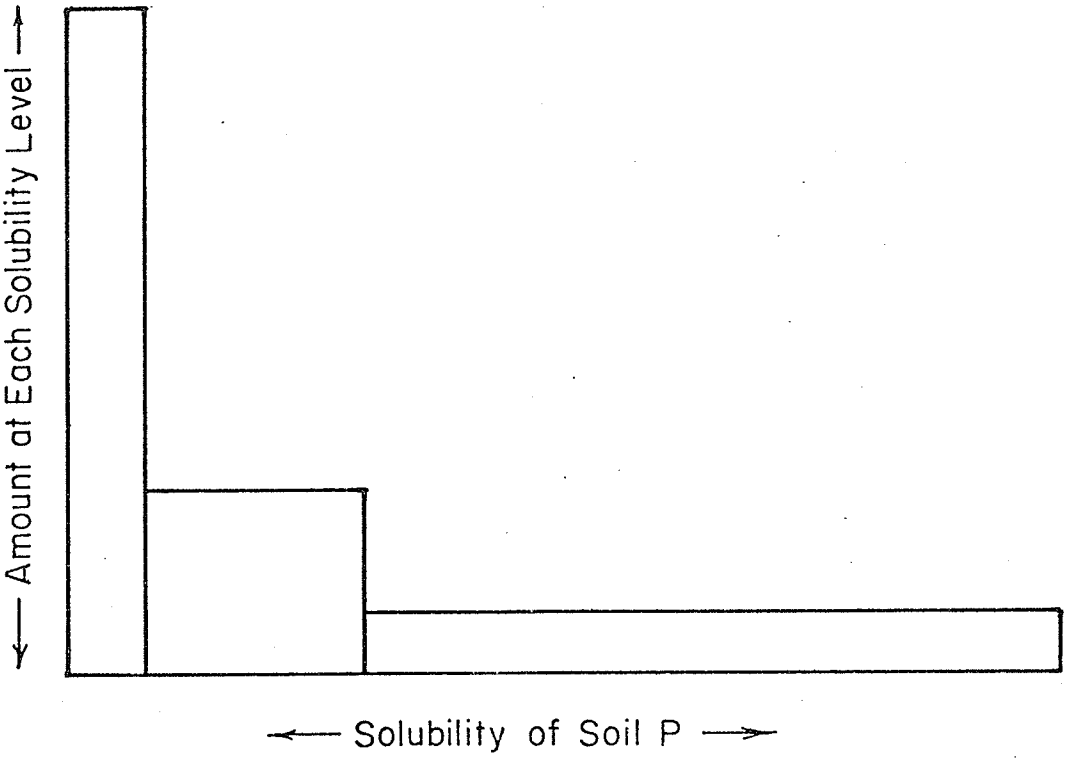

Figure 1: Conceptual diagram of the amount of soil P in three hypothetical solubility groups.

1960a), indicating that fixation reactions are reversible.

Numerous methods have been used to characterize soil inorganic-P solubilities and reactions. Only references which examined the effect of temperature will be cited. The analytical detection limits for the sparingly soluble native soil P represents a major difficulty. One solution to this problem has been to employ concentrated electrolytes (Kuo and Lotse, 1974) or anion exchange resins (Cooke and Hislop, 1963) which extract large portions of the native soil P. A second approach has been to label the soil P with radioactive P (Gunary, 1963) so that sensitive radiological analysis can be used to detect water or weak-electrolyte soluble P. A third approach has been to add large amounts of P to the soil and to incubate these soils long enough that the added P reacts to resemble the forms of native soil P (Barrow, 1979; Kuo and Lotse, 1974). Sufficient P can be extracted from these soils by water and weak-electrolytes for conventional analytical procedures.

These methods have been applied to studies of simple "extractable P" (Cooke and Hislop, 1963), adsorption and desorption rates (Kuo and Lotse, 1974), long-term P fixation rates (Barrow, 1980), adsorption isotherms (Singh and Jones, 1977) and desorption isotherms (Barrow, 1979a). Another important method of soil P analysis, that of solubility diagrams, has not been used in investigations of soil temperature effects primarily due to the lack of knowledge of the effect of temperature on the solubility products and activity coefficients of the dominate soil P species.

Organic P is the description used for P bound into organic complexes but belies the fact that the labile fraction of organic P undergoes continuous mineralization balanced by continuous immobilization (Stewart et al., 1980). These processes are biologically mediated (and thus would have

temperature optima typical of biological systems) but are also closely balanced.

A further complexity in the soil P system was illustrated by Qureshi et al. (1978) who showed that the distribution of P varied throughout the soil micromorphology. They found the amount of P to decrease markedly over a 300 μm transect from a pore surface into a soil aggregate. Plant roots (with the exception of root hairs and mycorrhizal hyphae) probably only contact the surface of the aggregate. Conversely, chemical extractants may cause dispersion of the soil (especially when coupled with rigorous grinding procedures) and thus may extract P from throughout the soil regardless of original position in the soil micromorphology.

Temperature Effects on the Soil P System

Numerous researchers have reported increased solubility of P when temperature increased (see review by Sutton, 1969). This finding indicates that P desorption is an endothermic reaction. Isotopic exchange studies by Gunary (1963) and Arambarri and Talibudeen (1959c) extended these results by showing that the size of the labile pool (the amount of soil ^{31}P in isotopic equilibrium with applied ^{32}P) increased at higher temperatures. Thus, not only the desorption equilibrium was affected by temperature but the reactivity or exchangeability of undesorbed forms was increased. Arambarri and Talibudeen (1959c) also showed the rate of isotopic exchange to increase with temperature.

Anion exchange resins desorb P in a manner similar to that of a plant root since they act as a sink for P and deplete the solution P concentration, rather than relying on mass action displacement of solid-phase P by competing anions (e.g. $NaHCO_3$). Cooke and Hislop (1963) found that the

amount of P extracted by anion exchange resins also increased with temperature.

Singh and Jones (1977) demonstrated the importance of the equilibrium P concentration to plant uptake. They incubated soils with varying amounts of P so that the same equilibrium P concentration was present across a range of temperatures (they found both sorption and desorption to increase with temperature). Much more P was required at lower temperatures to achieve the same equilibrium P concentration. The growth of lettuce plants on these soils was closely related to the P concentration in the soil, regardless of temperature (the plants were harvested at a uniform developmental stage). Their conclusion was that the desorption of soil P was the primary cause of the effect of temperature on plant response to P.

Several effects of temperature on the soil P system were demonstrated by Mack and Barber (1960a). They chose soil from a heavily P-fertilized field and incubated it at -20.5 or 2.7°C for nine months. These samples were subsequently leached (aerobically) at 16 or 32°C for 70 hours. The higher extraction temperature resulted in the desorption of more P, regardless of incubation temperature (Table 1). They also concluded that the rate of desorption increased with temperature although no kinetic analysis was shown. Their data also revealed that the desorption of P was decreased when incubation temperature increased. Similar results were reported by Beaton and Read (1963) and a possible mechanism, that of more rapid conversion of DCPD to octocalcium phosphate (OCP), was demonstrated by Racz (unpublished data). Beaton and Read (1963) attributed the effect of increased incubation temperature to a more rapid fixation of recently applied P to less soluble forms. The soils chosen by Mack and Barber had not been fertilized with P for three consecutive field seasons and thus they concluded

TABLE 1

Amount of Water-Soluble Phosphorus Leached at 16 and 32°C from Soil Preconditioned at -20.5 and 2.7°C*

Preconditioning Temp. (C)	Leaching Temp. (C)	Mg P Leached per 1000 g Soil			
		10 h	30 h	50 h	70 h
-20.5	32	2.8	7.0	10.0	12.2
2.7	32	3.1	7.0	9.3	11.5
-20.5	16	1.8	4.7	6.0	7.5
2.7	16	1.3	3.1	4.2	5.1

*Data from Mack and Barber (1960, Figure 2)

that changes in the form or specific surface of the soil P during incubation was responsible for the effects of incubation temperature. However, their soils were collected in the fall after a cropping season. It is plausible that P released from plant or microbial tissues (which both would have been declining in mass following the growth season) and P released by shifts in the equilibrium of P toward desorption during warm season may have constituted a "recent source" of soluble P. The fixation of this P would have been affected by incubation temperature. Furthermore, freezing temperatures impose a partial sterilization of soil which could have led to release of much of the P normally immobilized in microbial tissues. Thus, the results of Mack and Barber may have been due to fixation rates and/or their use of freezing temperatures.

Barrow (1979b) was the first to clearly partition the processes in the

soil P system and to examine the temperature dependence of each component. He identified solution P, adsorbed P in equilibrium with the solution P and firmly-held P as the three dominate phases of soil P and considered them linked in sequence by reversible reactions. He thoroughly described the soil P system by varying temperature, soil:solution ratio for extraction, incubation time and extraction time. He found that desorption was endothermic, involving a change in heat content that was large enough such that the equilibrium varied significantly with temperature. Furthermore, he found that the rate of fixation and the rate of release between the adsorbed and firmly-held phases of P were both increased by temperature with a Q_{10} of approximately 3. However, these reactions did not involve a change in heat content and thus the equilibrium between these phases was independent of temperature. The reactions between adsorbed and solution P were considered instantaneous.

Kuo and Lotse (1974) observed a much smaller Q_{10} for desorption of P and concluded it was a diffusion-controlled process.

The implication of Barrow's findings are that two opposing effects of temperature are most likely to be observed. At higher temperatures, solubility of the more soluble forms of P will increase but fixation of P to less soluble forms may also increase. The latter reaction is known to be very slow and therefore time and temperature become equivalent variables. This relationship was used by Barrow (1979b) to study the nature of soil P. He incubated soils at 62°C for ten days after adding P to simulate the reactions which would have occurred over much longer reaction periods at lower temperatures. Since the rate of fixation (and hence the potential effect of temperature on fixation) decreases as equilibrium is approached, the age or reaction state of applied P in a soil becomes an important con-

sideration. The fixation of recently applied P is affected more by temperature than is the continued fixation of aged or previously applied P. Thus, to predict the overall effect of temperature on P solubility, the age of the P in the soil must be known.

This difficulty may be illustrated by the results of Chien et al. (1982). They applied P to soils and incubated one subsample at 25°C and other subsamples at a range of temperatures. These soils were subsequently extracted for P at a range of temperatures. When extraction was conducted at the same temperature as the prior incubation, the amount extracted decreased as temperature increased. Conversely, when prior incubation was at 25°C, more P was extracted as extraction temperature increased. The model proposed by Barrow (1979b) suggests that in the former case, fixation was the dominant process whereas in the latter case, solubility was the dominant process. The results of Chien et al. would conform to this model if it could be argued that 25°C was an optimal temperature for P fixation and thus P sorbed at this temperature was the most "aged". The data of Chien et al. emphasize the difficulties which may arise due to the opposing mechanisms of temperature effects on soil P.

Barrow's three phase system may be considered a simplification of the P solubility profile described earlier. His results indicate that more soluble P forms are more dependent on temperature in terms of solubility than are less soluble P forms. Thus, the solubility profile is modified by temperature and therefore, it would be anticipated that parameters such as the desorption curve slope would reflect this modification. Although the importance of this parameter has been recognized (Sutton, 1969), the author has not found references in the literature testing this hypothesis. Barrow (1979a) avoided this consideration by utilizing a constant for the slope

parameter in his mathematical formulations.

The contribution of organic P to the nutrition of fertilized field crops is generally considered negligible (Stewart et al., 1980). Case et al. (1964) concluded that net mineralization of organic soil P was an important factor in the modification of plant response to P due to temperature. However, this response was evident 5 to 11 days after planting. Their description of methods suggests that the soils were dry prior to planting, although it was not explicitly stated. Thus, it seems unlikely that substantial net mineralization could occur this rapidly and furthermore, the usual microbial population-burst following re-watering (Lund and Goksoyr, 1980) could have resulted in net immobilization. Few other reports implicate soil organic P as an important factor in the effect of temperature. Temperature also affects the release of P from manures (Abbott and Lingle, 1965) which may have further consequences in inorganic P reactions (El-Baruni and Olsen, 1979).

Nye (1977) examined the limiting steps in P uptake by plant roots and concluded that the rate of phosphorus uptake per unit root is much greater than the influx of phosphorus due to mass flow with the result that the phosphorus supply very close to the root is depleted. The consequence of this is a steep gradient in phosphorus concentration which drives an effective diffusion process. In general, the rate of diffusion has a Q_{10} of about 1 and thus is less affected by temperature (although still significantly) than desorption processes (Barrow, 1979b).

Diffusion processes may also occur within the soil fabric in response to P gradients across soil peds, as observed by Qureshi et al. (1978). Thus, diffusion processes may control the desorption of soil P from within soil peds. Kuo and Lotse (1974) observed relatively low temperature dependence

of desorption from lake sediments and concluded that the processes were controlled by diffusion.

Temperature Effects on Fertilizer Reactions

The microsite of the reaction between a fertilizer pellet and the soil is unique due to the extremely high concentrations involved. Reactions at these sites are important to plant response to fertilizer and have received attention (Sample et al. 1980).

The solubility of fertilizer salts increases with temperature (Hinman et al., 1962) and both the rate of dissolution of relatively soluble fertilizer materials (Beaton et al., 1965) and the final size of the reaction zone (Beaton and Read, 1963) are greater at higher temperatures. Less soluble sources of P may be less temperature dependent in rate of dissolution (Chein et al., 1980). Since the initial reactions are relatively rapid, the major significance of these temperature effects is on the size of the reaction zone. This may have implications for the interception and utilization of the fertilizer by plant roots (both because of interception probability and because of potential phytotoxicity if the zone is too small).

Longer term reactions at the pellet site approach those already discussed for soil P. Hinman et al. (1962), Beaton et al. (1965), Sanyal and Deb (1976) and Barrow (1974) all observed decreases in fertilizer P availability as temperature increased. Barrow (1974) reported a Q_{10} of 3 for fertilizer P fixation.

Summary of the Soil Factors

Opposing effects of temperature have been clearly identified. The

solubilities of the more soluble forms of P in the soil increase with increasing temperature but this effect may be lessened by an increased rate of fixation to less soluble forms. Thus, the age or degree of fixation of the P in the soil is relevant to the overall effect of temperature on the desorption of soil P. The concept of a P solubility profile (characterized by P desorption curves) was proposed but no direct information on how temperature modifies this profile was found.

Plant Factors

A Summary of Plant Processes

The mineral nutrition of plants, inclusive of the processes of uptake of nutrients by the roots, translocation within the plant and the ultimate physiological role of the nutrients, has been intensively researched (see recent reviews by Clarkson and Hanson, 1980; Mengel and Kirkby, 1980). The physiological role of P (and the other essential elements) requires that a specific range of concentrations be present in the plant tissues for normal function. Thus, as a plant grows, it accumulates more P so that the tissue P concentrations are maintained (André et al., 1978). If the rate of P accumulation is slower (due to inadequate soil fertility) than the rate of dry matter accumulation, then the plant has several mechanisms it may invoke to maintain the tissue concentrations. Phosphorus from older, less physiologically active tissues is translocated to the growing tissues. This process reflects a lower physiological requirement for P by mature tissues relative to growing tissues but may also be associated with senescence of the older plant tissues. The plant may also increase the P uptake capacity at the root surface (Loneragan and Asher, 1967), the overall size of the root system relative to the shoot (Loneragan and Asher, 1967), and

the proportion of roots in a P-enriched zone in the soil (Drew, 1975). Furthermore, P deficiency predisposes the roots to infection by vesicular arbuscular mycorrhiza which in turn provide another route for P uptake by plant roots (Gerdemann, 1974). The final adaptation to a limited P accumulation rate is a decrease in plant growth rate. Although this may not immediately affect economic yield, it is this decrease in growth which in practical agriculture dictates the requirement for fertilization.

The range of mean tissue P concentrations which allow optimal growth is relatively broad. This is due to the ability of plants to tolerate P (and other essential elements) at concentrations above the specific physiological requirement (possibly by means of isolating the excess P in the cell vacuoles (Loughman, 1968)). This luxury consumption of P has obvious adaptive significance when the potential variability in nutrient supply (for example, due to weather conditions) is considered. Luxury consumption may be an essential component of nutrient accumulation in that young plants frequently accumulate large amounts of P (resulting in high tissue P concentrations (Hanway, 1961c)). This internal store of P may buffer the P status of the plant when it is older.

The phenomena of luxury consumption and adaptability to nutrient stress cause difficulties in the study of plant P uptake and nutrition. For example, since plant P uptake rate varies due to plant P status, the latter must be defined. With the exception of complete exclusion of P from the plant growth medium, plant P status is difficult to define. As another example, tissue P concentrations are functionally related to yields only when P supply is limiting. Otherwise, the concentrations reflect luxury consumption which may be governed by completely different environmental factors. Thus, information on plant P uptake cannot be interpreted (as

often is done) as measures of P supply unless proof of P status (deficient versus sufficient P) is provided.

A Summary of Plant Response to Temperature

Temperature has a profound influence on the growth of plants (or any other organisms) with discrete minima and maxima (below and above which growth will not continue) and optima which vary among species. The optimal temperature for root growth may also differ from that for shoot growth. Temperature modifies most reactions and processes in the plant and the observed optimal temperature is actually the temperature at which productive processes have the greatest advantage over destructive processes.

The largest component of living plants, water, undergoes various structural changes including discrete anomalies in the temperature range of biological importance. Trincher (1981) found discrete anomalies at 15, 30, 45 and 60°C and continuous anomalies throughout that temperature range to be due to transformations between three structural classes of water. Thus, even the most basic component of plant chemistry was modified by temperature.

Temperature influences reaction equilibria and reaction rates of both inorganic and organic compounds throughout the plant. The structure of organic compounds and complexes are also altered with the consequence that cellular-scale organelles and structures are possibly altered. Allen (1981) attributed anomalies caused by temperature in leaf membrane responses to changes in protein and lipid structures.

Temperature also mediates the interaction of plants with pathogenic and synergistic organisms. Hallem (1981) observed extensive decomposition of root cortex at temperatures above that optimal for the plant growth.

Cooper and Tinker (1981) noted that the transfer of P from mycorrhizae to the plant was increased by temperature.

The effects of temperature on plant growth are manifest throughout the plant. However, the meristematic regions (root tips and shoot apices) are especially critical because all growth is initiated at these points. Thus, for example, the location of the shoot apices of cereals (which are below or near the soil surface during the vegetative growth stage and during floral initiation) relative to the soil/air temperature gradient is very significant to shoot growth. The effect of air temperature on photosynthetic tissues is also very critical to plant growth.

Response to temperature is expressed by the plant as changes in growth rate and development rate. These rates are not necessarily closely linked. Differences in overall or final yield probably only occur when "chronological" timelimits prevent comparison at equal, temperature dependent, "plant growth times". Thus, for example, the final yield of deterministic plants grown at various temperatures is similar if all the plants are allowed to mature (this example presumes that the rate of development is parallel to the rate of growth). Clearly the definition of the "plant growth time" used to compare growth between temperatures can have a substantial effect on the interpretation of data.

This review will deal primarily with the effects of soil temperature on P supply to the plant and the plant P status. In natural systems, soil and air temperatures are covariate although the absolute differences are dependent on edaphic factors. With the exception of processes unique to the root system (to be discussed), the effects of soil and air temperatures on plant P status are probably similar.

The Effect of Temperature on Plant P Nutrition

The foregoing summaries emphasized that plants have adaptive mechanisms which, within limits, will maintain P accumulation in step with dry matter accumulation. Obviously, certain absolutes, for example the physiological requirement for P, also dictate the plant P status. Temperature has an effect on all processes in the plant and therefore it is moot to catalogue the components of plant P accumulation and utilization that respond to temperature. However, temperature effects on the relationship between P and dry matter accumulation, the adaptive mechanisms of the plant to varied P supply, and the absolutes of plant P nutrition are relevant topics.

P and Dry Matter Accumulation Rates

The relationship between nutrient accumulation rates (and the two components uptake rate and translocation rate) to dry matter accumulation rates have been examined by several authors. These studies necessitate another factor (for example, temperature but more usually species, age, etc.) to provide a range of growth rates in order to examine the relationship. White (1972) compared three legumes all adapted to low P soils and found that the species with the lowest growth rate also had the lowest P uptake rate and vice versa. Chapin (1974) found a similar relationship among sage species adapted to various temperature regimes (latitudes). However, comparison across species may reflect many species-specific phenomena.

A comparable relationship was examined within species by André et al. (1978) who compared plants at different ages. They found the uptake rates of N and K to be especially well related (positive, curvilinear) to root

relative growth rate. Newman and Andrews (1973) compared trimmed and untrimmed root systems and concluded the same relationship held for P. However, growth rates, both relative and absolute, show distinct ontogenic trends (Elias and Causton, 1975) and thus using plant age to provide a range of growth rates relative to uptake rates may implicate features of plant aging.

Pitman (1972) varied the growth rates of barley by varying light intensity and photoperiod and found that the translocation rate of K from the root to the shoot was closely related to growth rate. Raper et al. (1977) imposed temperature differences on tobacco seedlings and measured uptake and dry matter accumulation rates. After an initial acclimation time at each treatment temperature, neither relative uptake nor relative dry matter accumulation rates varied with temperature. However, a range of growth rates was established by the duration of the acclimation period and on this basis, they found a 1 to 1 relationship between P accumulation rate and dry matter accumulation rate. Their experience with temperature acclimation of plants also suggests that uptake and growth rates are more sensitive to a change in temperature than to the specific temperature.

Thus, although few studies have dealt specifically with temperature effects, it appears to be generally true that uptake rates correspond closely to growth rates and therefore, temperature may cause similar modification to both rates.

Specific effects of temperature on P uptake were noted by Bravo-F and Uribe (1981) who noted anomalies (breaks in the Arrhenius plots) of P uptake rates at 13 and 22°C. These may have been related to changes in membrane structure (Allen, 1981) and thus may not have been closely related to plant nutrient demand (growth rate). Holobradá et al. (1981) also found

efflux (loss of P from roots) to vary with temperature and to exhibit the same temperature response as uptake. This may result in an optimal temperature for net P uptake that does not correspond to the optimal temperature for growth. However, these phenomena would be rendered less significant by the balancing of uptake and growth rates which, as initially speculated, appears to occur.

Optimal Tissue P Concentration

The functional relationship of yield to tissue nutrient concentration was reviewed by Bates (1971) and found to be difficult to interpret since plant age, environmental conditions, the plant parts analyzed and species as well as varietal differences all influenced the relationship (Figure 2). The typical relationship consisted of the "Steenbjerg effect" (an apparent non-functional relationship), a linear portion (where growth was a direct function of the tissue concentration), an asymptotic portion (where yield no longer responded to the nutrient) and a toxic portion. The optimal or critical concentration was usually defined at the point where the linear and asymptotic portions joined.

The variation of the optimal concentration with growth conditions has not been carefully examined. Moller Nielsen and Friis-Nielsen (1976, I-III) have re-examined the classical approaches to plant nutritional status and have suggested a novel and useful analysis. In the second paper in the series, they examined the inter-relationship of nutrient elements based on Liebig's law of the minimum. They hypothesized that optimal concentrations of one nutrient, e.g. P, depends upon the growth potential established by another nutrient, e.g. N. Therefore, the only truly fundamental information about response of a crop to P concentration is obtained

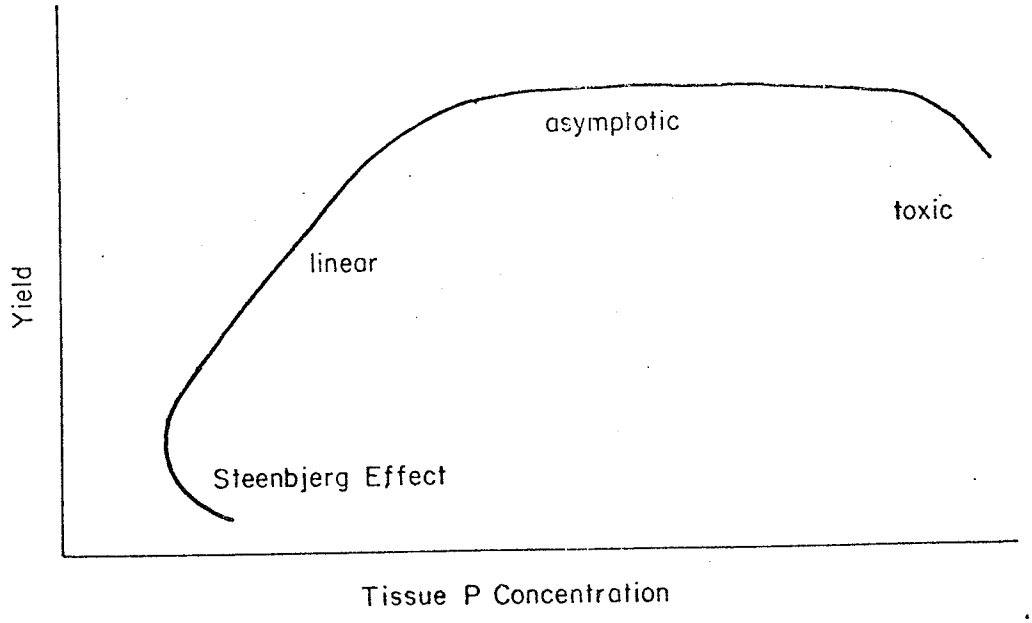

Figure 2: Idealized relationship between yield and tissue P concentration (from Bates, 1971).

when all other factors are optimized. Although this concept is generally recognized, few researchers have proof that their data satisfy these requirements. Moller Nielsen and Friis-Nielsen propose a "borderline" analysis. Theoretically, in a complete population of P-concentration versus yield data points, the borderline of the body of data represents cause-and-effect relationships. The upper borderline involves the situations where highest yield was obtained for the given P concentration, implying all other nutrient concentrations were optimal for that yield level. They use the term "relatively optimal concentration" to describe the concentration of these other nutrients in this situation.

Although a true population of data points cannot be assembled, Moller Nielsen and Friis-Nielsen used a very large number of observations as a reasonable approximation. They found that when growth was suppressed by N deficiency, the "relatively optimal" P concentration was also lower. This concept may also be applied to temperature effects. Thus, plants growing slowly due to unfavourable temperatures may be more (or less) efficient in the use of P. This argument is supported by considering the physiological role of P in plant energetics. Less energy transfer (growth) may implicate a lesser (or greater) optimal tissue P concentration.

This argument leads to the hypothesis that the optimal P concentration may vary with the relative growth rate of the plant (both terms represent intensity parameters). If so, this relationship may hold true over various growth-limiting factors and may bridge the necessity of evaluating changes in the optimal P concentration due to each factor.

Evidence that the optimal P concentration varies with temperature is provided by several researchers. These few studies are based on the optimal P concentration for maximum final yield and the harvests are

usually based on development stage rather than a constant time interval. Such data are difficult to interpret in terms of relative growth rate but appear to be the best available to relate temperature to optimal P concentration.

Balvoll (1970) conducted a trial to compare lettuce nutrient requirements at several temperatures. The first harvest cut clearly demonstrated that the optimal P concentration was 0.30% at 21°C and much above 0.37% at 12°C. Yield, relative to the highest P treatment and determined at widely different times and yield levels, decreased from 21°C to 12°C. However, in the first cut, plants grown in cooler soils were allowed to accumulate much more dry weight than those in warmer soil, thus confounding the effects of plant size and temperature on relative growth rate. Balvoll felt that the P response of the second cut would have been strongly influenced by the P uptake of the first cut. Therefore, these data must be interpreted with care.

Fulton and Findlay (1966) found a different effect of temperature on the optimal P concentration of oats. Optimal P concentration (measured in the grain at harvest) of 0.37, 0.39 and 0.50% were found at 13.0, 18.5 and 24.0°C respectively and a slight decrease in optimal P concentration was noted as moisture supply increased. Their yields were recorded at a uniform morphological stage (presumably at different times) and decreased as temperature increased. The similarity with the results of Balvoll is that optimal P concentration appeared to decrease as conditions became more favourable for growth.

Power et al. (1964) presented data to further support this observation. They grew barley at temperatures ranging from 7.2 to 26.7°C with a range of P supply levels. Although they achieved a yield plateau only at the

optimal temperature for growth (15°C), the shape of their response curves occurred in a consistent pattern. By extrapolation of their data (shown as Figure 3) the optimal P concentration appears to decrease as temperature rises from 7.2 to 15°C and increase as temperatures exceed 15°C. This study has the further advantage that one harvest date was used and all the plants except those at the 7.2 and 11.0°C were at approximately the same stage of phenological maturity. Therefore, the analogy of yield to relative growth rate might appear appropriate.

A linear relationship between optimal concentration and temperature was shown for Mn in soybeans by Ghazali and Cox (1981). The optimal tissue concentration for Mn was lowest at 27.5°C which coincided with the highest relative growth rate.

Other data relating optimal P concentrations to relative growth rate relied on factors other than temperature to provide variation in growth rates. The relationship did not hold across species in the studies of Asher and Loneragan (1967) and Rorison (1968) although Christie and Moorby (1975) found it to hold across three grass species. The converse relationship was shown by White (1972) for three legume species. He found the lowest relative growth rate (across species) was associated with the lowest optimal tissue P concentration.

The data presented by Terman et al. (1977) (with considerable interpolation from the figures presented) suggested that when N supply limited growth, the optimal tissue P concentration was lowest when growth (response to N) was greatest. Thus, the optimal tissue P concentration was inversely related to growth rate.

Johansen et al. (1980) observed that relative growth rate and the optimal tissue P concentration decreased similarly with plant age. They

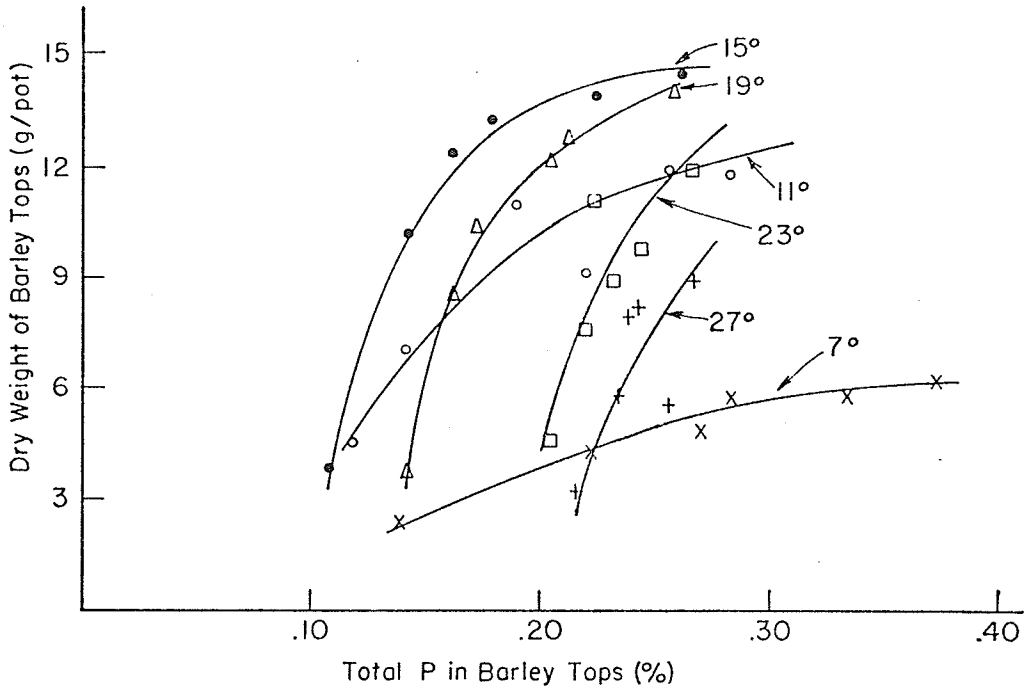

Figure 3: Response of barley shoot yields to tissue P concentration as influenced by soil temperature (data from Power et al. 1964a).

found the relationship to be reliable enough to propose measurement of relative growth rate as a means to interpret tissue P analysis of pasture species (where plant age is unknown).

These various findings suggest that the optimal P concentration may be lower in species that grow more slowly. This could be attributed to a lower physiological activity or to more time for translocation within the plant which thus increases the efficiency of P utilization. This direct relationship also holds for plants of the same species at different ages. However, when plants of the same age and species are controlled by an environmental variable (temperature or nitrogen), the optimal P concentration is lowest under the conditions where growth rate is highest. This implies the P utilization efficiency is maximum under the same conditions that growth rate is maximum. The data of Moller Nielsen and Friis-Nielsen (1976) (where N supply controlled growth) may conflict with this conclusion although these authors did not present growth rate data.

In summary, optimal tissue P concentrations vary with temperature. The possibility of explaining this variation (and variations due to other growth factors) in terms of relative growth rate was explored but apparent conflicts were found. The limited data for temperature effects suggest that optimal tissue P concentration is at a minimum at the optimal temperature for growth. Ontogenic trends for relative growth rate, tissue P concentration and optimal tissue P concentrations lead to difficulties in interpreting these data.

Root System Responses to Temperature

The function of the root system is intimately affected by soil temperature (as compared to the shoot which is indirectly affected by soil temperature). Thus, processes specific to the root system require attention.

The shoot:root ratio decreases in plants under P deficiency, presumably because the roots retain the P they require prior to translocation of P to the shoot and hence are "less deficient" than the shoot (Loneragan and Asher, 1967). This has the apparent adaptive advantage of exploring the soil most effectively to encounter sufficient additional P to supply the plant. Nátr and Purs (1970) observed that at lower temperatures, the shoot:root ratio also decreased but these workers did not assess the P status of the plants. Levesque and Ketcheson (1963) found that temperature and P supply interacted in their effects on the shoot:root ratio. The ratio decreased as temperature decreased (26 to 10°C) at low P supply but reached a minimum at a mid-range temperature (18°C) when P supply was adequate. Thus, the shoot:root ratio changes initiated by temperature treatments were due to temperature-mediated P deficiency. Adequate P supply limited the effect.

Root system morphology has also been observed to change due to temperature. Geotropism (Sheppard and Miller, 1977) and the number of lateral roots (Hunsigi and Ketcheson, 1969) both responded to temperature. These modifications would be significant to plant P status if they affected the total P uptake capacity or the concentration of roots in a localized source of P. The former is related to the shoot:root ratio and, considering the adaptability of the plant, it seems unlikely the plant would maintain P uptake capacity (an energy requiring process) beyond its requirements. The latter could refer to either a P profile gradient (decreasing with depth) or a localized, band type placement of fertilizer P.

Root proliferation in a localized, P-enriched zone has been studied in some detail (Drew, 1975). However, very little research on the effect of temperature on root proliferation has been reported. Related aspects of

soil-fertilizer chemistry have been reported. Blanchar and Caldwell (1966) demonstrated that the size of the fertilizer reaction zone increased with increased fertilizer solubility. Since the solubility of common P fertilizers increase with temperature, the resultant reaction zone will be larger, less concentrated and hence less phytotoxic. However, Baker et al. (1970) showed that the phytotoxicity of fertilizer materials increases with temperature. Clearly, research is required to examine this problem.

Individual root morphologies may also respond to temperature. Nátr and Purs (1970) observed roots to be longer per unit weight at higher temperatures. Richards and Passioura (1981) reported smaller seminal root xylem at higher growth temperatures. Onderdonk and Ketcheson (1973) reported abnormally thick roots at low temperatures. Thus a greater longitudinal extension of the roots at higher temperatures (as opposed to a radial extension of individual roots) may result and this may benefit the plant P status.

Root development through the phases of differentiation (at the tip), elongation, root hair development, cell wall suberization and lateral root development has a marked effect on P uptake. Bowen (1970) showed that the rate of development was delayed at lower temperatures so that a high uptake capacity was maintained along a greater proportion of each root. This may have been an adaptive response to cold-induced P deficiency.

The population of rhizosphere organisms is very important to plant P uptake. Although generalizations across all rhizosphere species may be inappropriate, Rovira (1965) found that the maximum temperature for these organisms corresponded to that for the host plant. Thus, the microbial activity was closely linked to the productivity of the plant and therefore benefits in terms of plant P supply would also correspond to the maximum P requirement.

In summary, root processes and properties, including shoot:root ratios, root system and individual root morphologies, and root-microorganism interactions, are all influenced by temperature. However, the link between temperature effects and plant P status, although often presumed, has not been established in many studies. Because the plant roots have numerous pathways for adaptation to plant P stress, it is difficult to differentiate effects of temperature per se from effects of temperature induced P deficiency.

Conclusion

Weather induced nutrient deficiencies result from a low rate of supply by the soil relative to a high rate of demand by the plant. These processes are buffered by the capacity of the plant to store and relocate excess nutrient (the result of capitalizing on a higher net rate of supply usually early in the plant's growth) and the ability of the plant to modify uptake capacities relative to nutrient requirements. The ultimate adaptation by the plant is a diminished growth rate which implies that the rate of demand has been limited to the rate of supply.

Soil temperature, as the weather-related variable considered, has profound influence on most soil P and plant P processes. The soil P processes which determine the rate of supply of P are generally physical-chemical processes. An increase in temperature increases the solubility of soil P, increases both the rates of sorption (fixation) and desorption, and increases the rate of diffusion. The age or fixation state of the soil P becomes very important in predicting the net effect of temperature. The concept of a P solubility profile was proposed but the effect of temperature on the shape of this profile could only be speculated.

The plant exhibits multiple pathways of adaptation to P deficiency. The effect of temperature on these pathways has been investigated in the literature. The P accumulation rate and the P requirement (optimal P concentration) were related to plant growth rate. Other processes such as shoot:root ratios, root system and individual root morphologies, and root rhizosphere processes were reported to vary due to P status. However, the connection of these processes to temperature effects has not been extensively researched.

Chapter 2

DIRECTION OF RESEARCH

The review of the literature indicated that many studies have been conducted on soil temperature-P nutrition problems. Soil processes, such as long term fixation as well as adsorption and desorption rates have been carefully examined and described by numerous mathematical models. The problems not well researched are 1) the association between soil extraction studies and plant uptake and 2) the effect of temperature on soil depleted of P in the short term (such as adjacent to a plant root). Thus, short term plant uptake studies and desorption curve descriptions relative to temperature were proposed. In support of these soil testing procedures, fixation and isotopic dilution rates and desorption rates were also examined. Plant processes are more complex and involve an adaptive, elastic component. Thus, it seemed appropriate to relate P status to growth rate and to test the hypothesis that the effects of temperature were due to modifications in growth rate.

An initial study was proposed to examine the plant-soil system with emphasis on shoot growth rates, the response to fertilizer P, and the response to fertilizer P placement. Temperatures of 10, 15, 20 and 25°C were chosen for this study and were maintained for both the soil extraction and plant P status studies.

Chapter 3

PLANT RESPONSE TO P AT FOUR SOIL TEMPERATURES

Introduction

Extensive research has examined plant response to fertilizer P under varied soil temperature. The phenomenon usually observed is that more fertilizer P is required when soil temperatures are below that optimal for growth. Some researchers (Singh and Jones, 1977; Nielsen and Cunningham, 1964) have attributed this to soil processes whereas others (Black, 1970; Case et al., 1964) have attributed it to plant processes. Most probably, the phenomenon is the result of the balance between plant growth rate, root extension rate, soil P supply rate and fertilizer fixation rate, all operative in the rapidly changing temperature regimes of spring and early summer (Grant et al., 1972).

Ketcheson (1957) suggested that increased P fertilization may allow the plant to overcome some of the growth limitations imposed by low soil temperatures. This conclusion implies that low soil temperatures may induce P deficiency in soils normally adequate in P supply for plant growth.

The consequence of research concerning soil temperature-fertilizer P requirement interaction has been the recommendation to band-apply P near or with the seed so that in cold soils, the plant roots immediately encounter relatively high concentrations of fertilizer P. However, little or no research has examined the response of plant roots (e.g. proliferation) to band-applied P under varied soil temperatures.

The purpose of this experiment was to examine the yield response of wheat to fertilizer P at soil temperatures of 10, 15, 20 and 25°C. Ferti-

lizer P was mixed throughout the soil (broadcast) or banded near the seed and growth response throughout the vegetative growth period was measured. At the final harvest, root distribution relative to the band-applied P and leaf tissue P concentrations were measured. The results are discussed with emphasis on the effects of temperature and time on the plant response to fertilizer P.

Methods and Materials

Soil Preparation and Planting

Surface soil obtained from the 0 to 15 cm-depth of an Elm River silt loam was selected for study. The soil contained low concentrations of $NaHCO_3$ extractable P (Table 2) and P fertilization on the collection site had increased yields. The soil was air dried, passed through a 10 mm sieve and thoroughly mixed prior to use.

TABLE 2

Properties of the Soil Used in the Experiment

Texture	silt loam
pH (soil-water paste)	7.6
$NaHCO_3$ extractable P (ppm)	6.2
CH_3COONH_4 extractable K (ppm)	290
NO_3-N (ppm)	32.4
CO_3 (%)	6.2
Moisture holding capacity (g water/g dry soil)	0.237

The soil containers were 36-cm lengths of 10-cm inside-diameter sewer pipe, each doubly lined with polyethylene bags. The soil was placed in the containers in layers, the bottom 15 cm unfertilized and the top 15 cm fertilized (diagrammatic, page 195). The layer between 2 and 4 cm from the soil surface was defined as a band zone for the application of N and certain P treatments (to be described subsequently). The layers of soil in the containers were filled using pre-weighed aliquots of soil to achieve the depths required. The potting bulk density was 1.17 g/cm^3.

The soil for the band zone was further prepared by passing it through a 2-mm sieve. This removed straw and other plant debris and thus facilitated recovery of roots from the soil. The band zone was demarcated by horizontal discs of fibreglass screen (2 and 4 cm below the soil surface) and was divided vertically by a rigid plastic plate. Thus the zone was split to form two semi-circular halves that could be fertilized independently.

The top 15 cm of soil received N, K, S, Zn, Cu and Fe at concentrations of 75, 100, 41, 6, 2 and 2 ppm, respectively, mixed throughout. The N was applied as NH_4NO_3 and the other elements were as sulphates. An additional amount of N was applied to the band zone, equal amounts to both halves, to provide a total of 100 ppm N (based on the 0-15 cm depth of soil). This band application of N was applied to all treatments but was intended to elicit a N-P interaction when P was also applied to the band. The coupled application of N and P is well established as an advantageous technique and thus was considered a standard treatment procedure.

Three methods of P application were employed as treatments:

1. Broadcast P was applied as a solution of NaH_2PO_4 which was sprinkled and mixed into the top 0-15 cm of soil. The application and mixing was done in several stages.

2. Banded P was applied in 10 mL of a NaH_2PO_4 solution, labelled with 3.11×10^7 dpm ^{32}P, to one half of the band zone. This solution also contained the amount of N intended for one half of the band zone. A comparable 10 mL of solution containing only N was applied to the opposite half of the band zone. These solutions were pipetted evenly onto the dry soil surface of the band zone and thus did not wet the entire 2-cm depth of the zone. The soil below the band zone had been previously wetted to the moisture holding capacity (0.237 g water/g dry soil) so that leaching of the band zone did not occur.
3. A combination application of both broadcast and banded P involved the same treatment as the banded P but also included the broadcast application of one concentration of P.

The amounts of P for the broadcast treatments were 0, 5, 10, 20, 40 and 80 ppm, based on the 0-15 cm layer of soil (1375 g dry soil). The banded treatments involved the same total amounts of P as the 5, 20 and 80 ppm P broadcast treatments but the P was concentrated into one half of the band zone only. The combination treatments repeated the amounts of P from the banded treatments and included an additional 20 ppm P mixed throughout the 0-15 cm layer. Two replicates were prepared.

Eight seeds of spring wheat (*Triticum aestivum* cv Neepawa) were placed in a row one cm below the soil surface and directly over the vertical plastic divider of the band zone. The soil surface was covered with a 5-cm depth of white Perlite (expanded volcanic lava available commercially as a soil conditioner) to thermally insulate the soil and additional water was added to achieve the moisture holding capacity of the soil throughout the container. Planting was within one day of band-fertilizer application.

The containers were placed in four water baths which regulated the soil temperatures at 10, 15, 20 and 25°C (see Appendix A for description of

water baths). The water baths were located in a growth chamber which provided day and night air temperatures of 18 and 12°C, respectively with light for 18 of each 24 hours.

Plants were thinned to four plants per container at the two-leaf stage. Periodic watering to the moisture holding capacity (field capacity in the containers) was conducted by weighing each container and was done before one half of the water was lost. An additional 100 ppm of N was added in the irrigation water at approximately the 5 to 6 leaf stage.

Data Collection During the Experiment

Growth measurements were made throughout the experiment to enable comparison of treatments at common development stages. A method was developed to estimate plant weights based on length measurements of the leaf, main-shoot and tiller-shoot plant components. This method was used to obtain measurements of growth throughout the experiment without destruction of the plants.

The specific length measurements taken were: a) the leaf length from the auricle to the tip of mature leaves, b) the main-shoot length from the perlite surface to the tip of the most recently emerged leaf and c) the tiller-shoot length, characterized by the length of the last fully-expanded leaf on the tiller (see diagrammatic, page 198). After each mature, fully expanded leaf was measured, it was marked by removing a 0.5 cm portion of the leaf tip. These leaves were not measured again (since they had ceased expanding) but their lengths were included in subsequent measurement records. One plant in each pot was used for the successive length measurements.

Estimates of plant component weights were derived from calibration equations which related weight to length using third-order polynomial equations for each plant component. The calibration equations were developed

on samples collected at harvest and had R^2 values of 90, 44 and 45% for the leaf, main-shoot and tiller-shoot components, respectively (data shown in Figure 35, Appendix E). The poorer fit of the main-shoot and tiller-shoot data reflected the anatomical variability within these components since both leaf and stem tissues were present in varying proportions.

The estimated weights of the various plant components were then summed to derive the whole, single plant weight on each measurement date. The growth curves were constructed from these single plant weight estimates. The smoothness of the curves (e.g., Figure 6) attest to the value of this method.

A more direct test of the method was possible at harvest by weighing the single plant in each pot which had been used for length measurements. The growth curves were extrapolated to the harvest date and the weight estimates were found to be highly correlated to the measured single plant weights ($r = 0.98$). Thus, it was concluded that this method was a good indicator of plant response.

Data Collection at Final Harvest

The shoot fresh and dry weights were recorded at final harvest for each container and the roots were recovered from the band zone of the band- and combination-applied P treatments. To recover the roots, the band zone was cut away from the bulk soil at the bottom screen. The plants were washed free of soil while the roots were still intact with the plant crowns and then the root samples were collected by cutting them off at the top screen. The plastic divider effectively separated the roots in each half of the band zone. Root proliferation in the P-fertilized band was computed as the difference in the root dry weight between the two halves of the band

zone divided by the root dry weight in the nonphosphated, control half.

The first replicate was harvested 33 days after planting and the second replicate three days later. This delay between harvests allowed the root washing procedures for the first replicate to be completed as soon as possible after the shoots were harvested. A randomized complete block statistical design was used to overcome differences between the replicates.

The plant tissues were wet-ashed using 2:1 (by volume) HNO_3:$HClO_4$ (Appendix B). The ^{31}P concentrations in the digests were determined using the ammonium molybdate-antimony-blue-colorimetric method. The ^{32}P content was measured by monitoring Cerenkov radiation (in aqueous media) using a Beckman 7500 liquid scintillation counter. Counting efficiency was determined for each sample by adding an internal standard of ^{32}P (Appendix C).

Results

Growth Response to Soil Temperature

Soil temperature effects were observed very shortly after planting as a delay in germination and emergence of approximately one day per 5°C decrease in temperature from 25 to 10°C. Optimal shoot growth occurred at 20 to 25°C throughout the experiment which differed from the optimal temperature for shoot growth of 12°C reported by Hallem (1981). At harvest, the average dry weights of non-deficient plants were 6.0, 6.5, 3.3 and 1.2 g at 25, 20, 15 and 10°C, respectively. Root growth, observed through the plastic bag liners, was also influenced by temperature but, even at 10°C, the roots had penetrated to the bottom of the containers within 15 days.

Phenological development was delayed by cold soil temperature. At harvest, the plants at 10°C had just begun floral initiation (double ridges just evident on the dissected apical meristem (Williams, 1974)), the

plants at 15°C had well-formed heads but no stem elongation and the plants at 20 and 25°C were approaching the boot stage with substantial stem elongation. Thus, growth responses measured at harvest reflected both direct effects of temperature and effects of differences in developmental stage which were the result of temperature.

Yield Response to Broadcast P

Plant response to P application was established very soon after emergence and was apparent in length measurements at 5 to 17 days after planting for 25 and 10°C, respectively. These differences persisted until harvest (Figure 4). The large differences in yield, compounded by the three-day delay in the harvest of the second replicate, resulted in much larger variances among the data at 25°C than at 10°C. To decrease this inhomogeneity of variance, a $\log_e$ (natural logarithm) transformation was applied to the yield data (Steel and Torrie, 1960). This transformation resulted in more uniform variance (Figure 33, Appendix E) and therefore allowed statistical tests among temperatures. The data presented in Figure 4 are in the linear-scale but the means and statistical tests were computed in the $\log_e$-scale.

Significant yield increases at P concentrations up to 40 ppm occurred at 10, 15 and 20°C and up to 20 ppm at 25°C. The interaction in yield response to P between 20 and 25°C (Figure 4) was significant with significant differences between the yields only at 0 and 10 ppm P. This may indicate a greater release of native soil P at 25°C than at 20°C (Barrow, 1979b) and this may in turn explain the diminished response to fertilizer P at 25°C. These differences in yield at harvest were confounded by attendant differences in developmental stage.

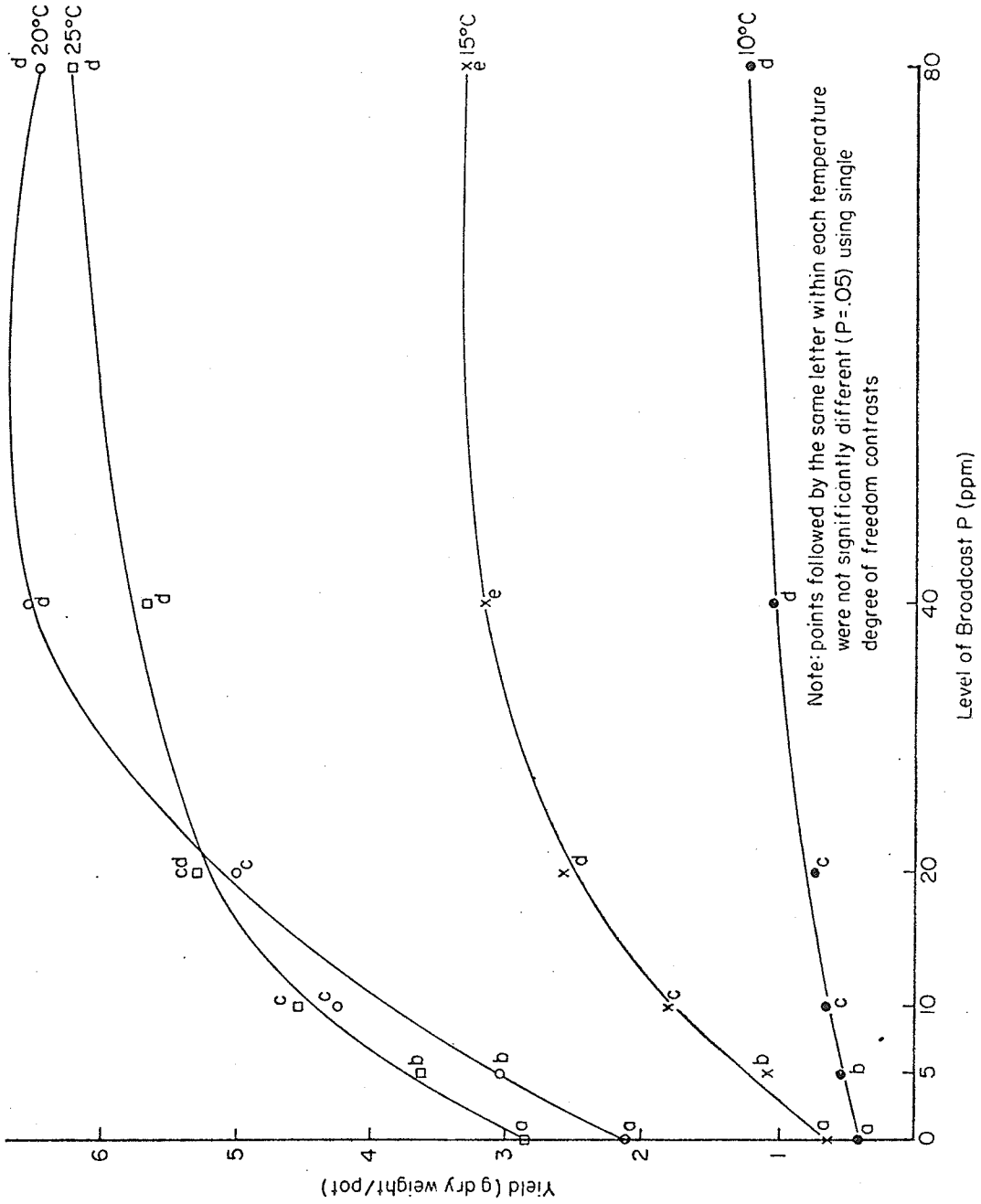

Figure 4: Shoot dry matter yield in response to broadcast P at four soil temperatures.

Growth at a common, three leaf stage of development (Figure 5) was interpolated from the growth curves. The response patterns were similar among the various temperatures, particularly among 10, 15 and 20°C. This coincided with the observations at final harvest that the optimal concentration of P for yield was not influenced by these temperatures. The response pattern at 25°C appeared more gradual than that at the other temperatures and this reflects the decreased responsiveness to fertilizer P at 25°C observed at final harvest.

The similarity in the response patterns at the three leaf stage to those at final harvest attest to the parallel nature of the growth curves (Figure 6). Thus, differences in growth among fertilizer P treatments that were established early were maintained throughout the experiment. Exceptions occurred at 20 and 25°C where there was evidence of accelerated growth at 80 ppm P (increase in slope at 80 ppm P relative to that at 40 ppm P) near the final harvest date. Thus, if the harvest had been delayed, these treatments may have yielded relatively more. The growth curves at 10 and 15°C did not show this pattern, probably because they were at earlier developmental stage. This late response to the highest P rate may have been associated with the development of the true stem and head observed in these plants. Such developments would have been major sinks for P in the plant and thus might have competed with photosynthetic activity for plant P. If so, the plants at the high P treatments would have been better able to maintain photosynthetic activity and hence growth. Thus, the late response to 80 ppm P couldhave been due to a late physiological requirement for P.

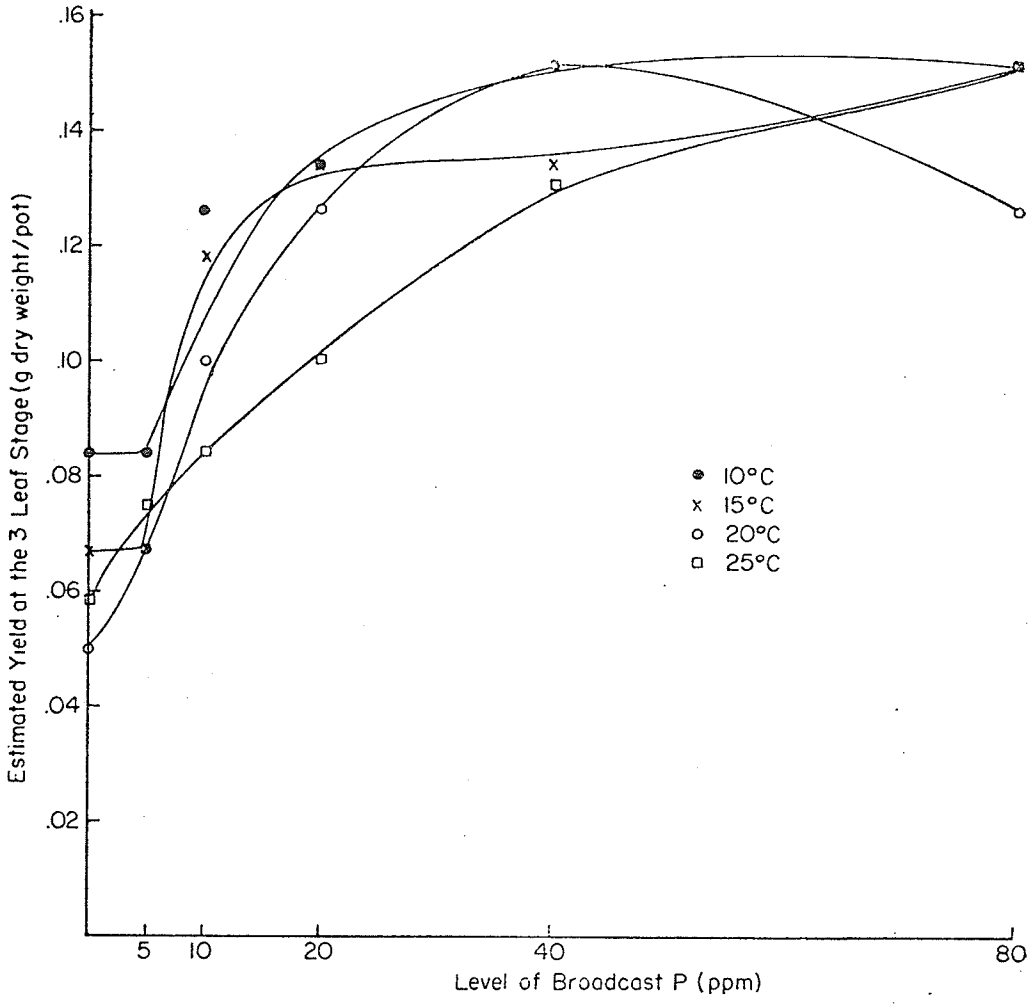

Figure 5: Estimated shoot dry weight at the three leaf stage in response to broadcast P at four soil temperatures.

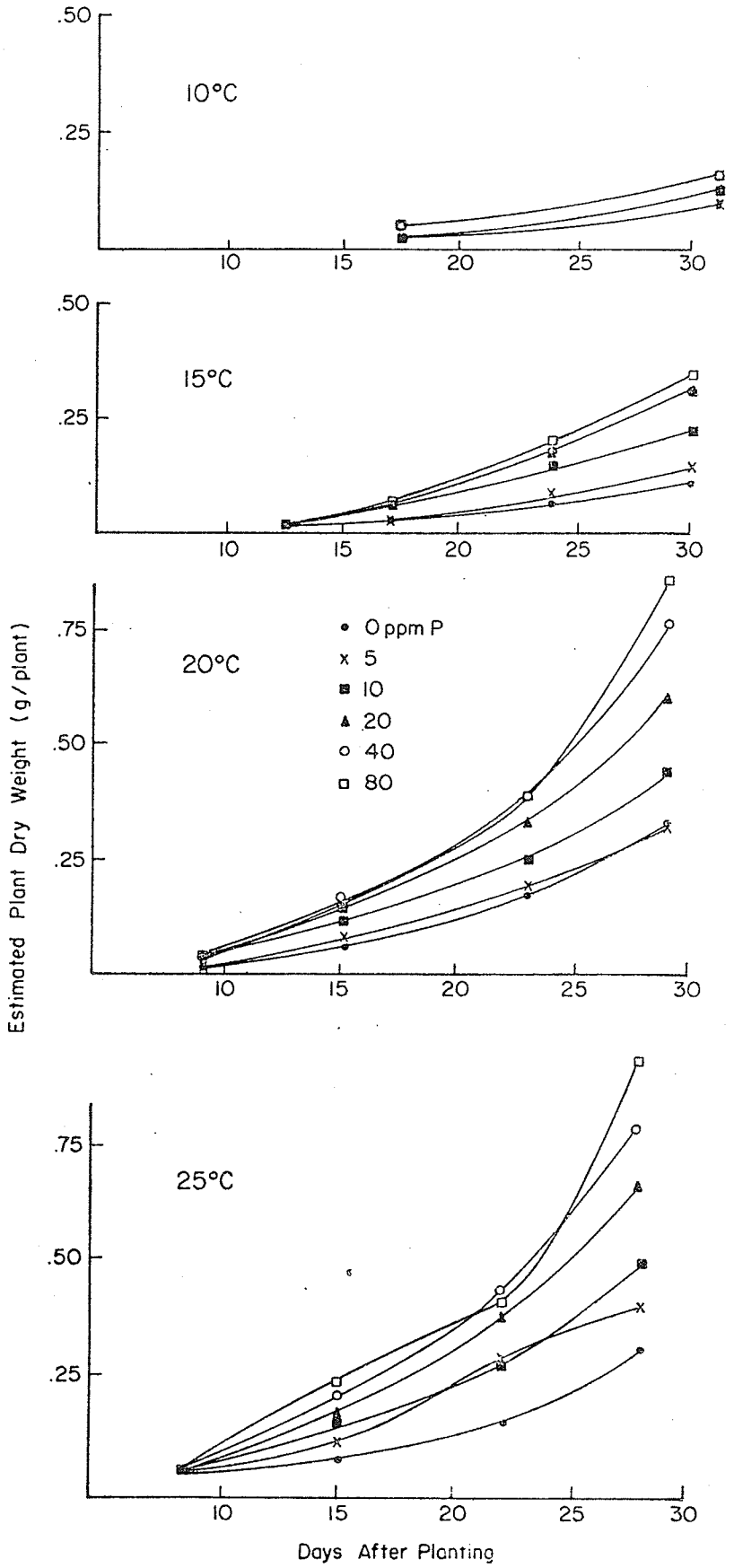

Figure 6: Growth curves with time for the broadcast P treatments at four soil temperatures.

Tissue Concentrations in Response to Broadcast P

Tissue concentrations of P represent another response criteria for comparison of treatments. The typical relationship (Bates, 1971), when P limits growth, is a concomitant increase in yield with tissue P concentrations up to an "optimal" P concentration. Above the optimal level, yield is independent of tissue P concentration.

Very large differences in tissue P concentrations were required to effect small changes in yield at 10°C (Figure 7). The optimal P concentration appeared to be above 0.37% in the first replicate since the curve did not approach a yield plateau. The curve for the second replicate was clearly shifted from that of the first replicate and an approach to a yield plateau seemed to occur near or above 0.30% P. The shift in the curves between replicates was attributed to the three-day delay in harvest of the second replicate. Growth that occurred during this interval shifted the curve upward and probably diluted the plant P concentration. Thus, the growth interval also shifted the curve to the left. This conclusion was substantiated by total plant P uptakes which were almost identical between the replicates.

The optimal tissue P concentrations at 15°C appeared to be 0.28% P in the first replicate and near but above 0.25% P in the second replicate. Thus,as temperature increased, the optimal tissue P concentrations were slightly lower. This is in agreement with Power et al. (1964a) who found the optimal P concentration to be lowest at the optimal temperature for growth. The shift of the curves between replicates was again apparent.

The optimal tissue P concentration at 20°C was clearly defined as 0.19% P in the first replicate and 0.14% P in the second replicate. Thus, as temperature increased, the optimal tissue P concentration was again lower but also, as time increased, the optimal tissue P concentration was

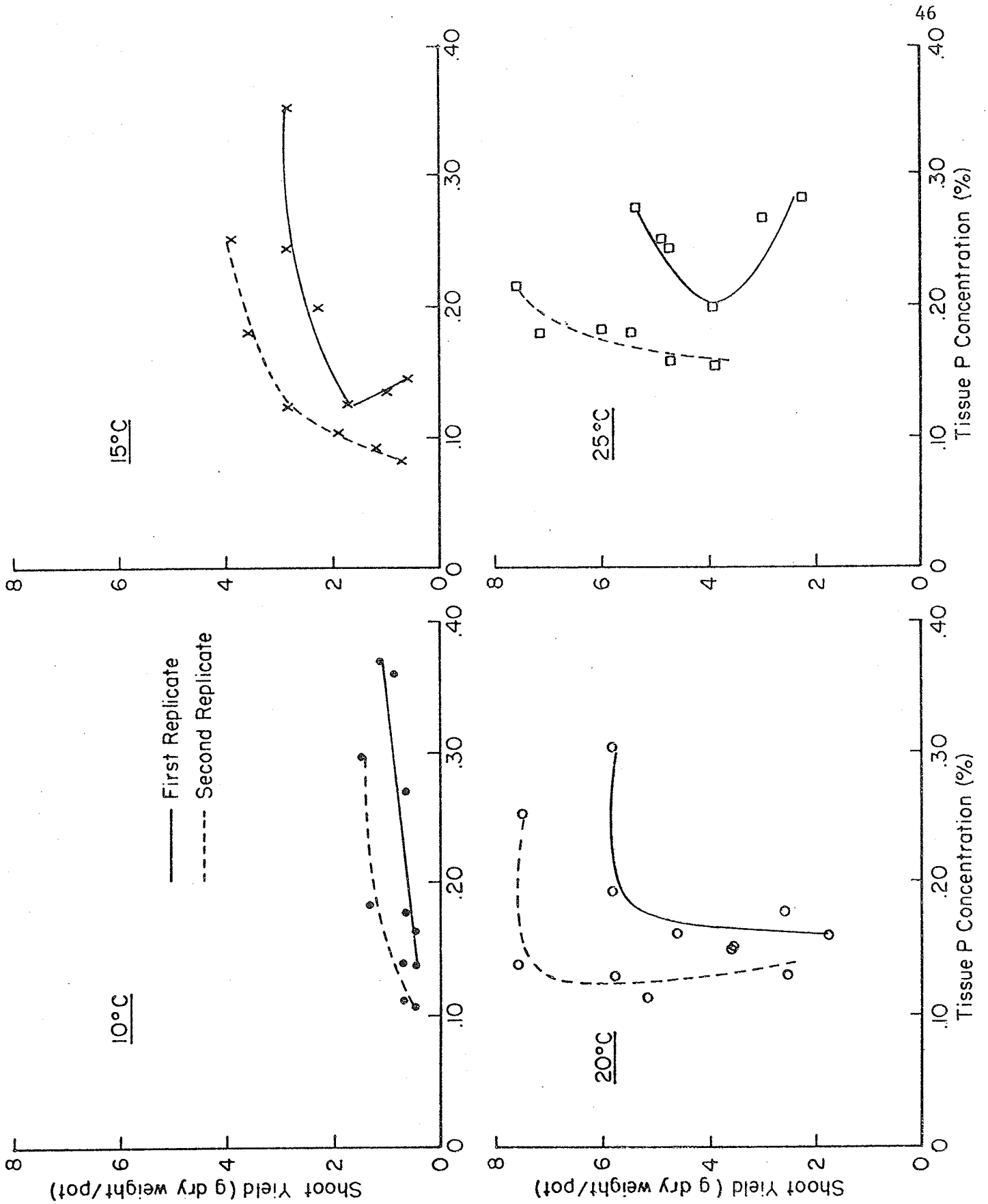

Figure 7: Yield versus tissue P concentrations at four soil temperatures, plotting replicates separately.

lower. This latter finding raises the question of whether temperature per se or whether temperature-induced differences in development (essentially a time-based phenomenon) were responsible for the changes in the optimal tissue P concentration. The developmental stage may be important to whole plant optimal P concentrations since, as the plant grows, mature tissues become an increasingly larger portion of the whole plant. Compared to actively growing and meristematic regions, these mature tissues have lower specific P requirements. Thus, the whole plant optimal P concentration may decrease as the plant develops.

The yield versus tissue P concentration curves from plants at 25°C did not have clearly defined optima or may not have been functional relationships. This could imply either that all the plants were quite deficient in P or that none of the plants were deficient. Previous discussion proposed that the plants at 25°C were subject to a late requirement for P due to development of the head and stem. These data support this hypothesis in that all plants at 25°C may have been deficient and the optimal P concentration may have increased due to the presence of the reproductive tissues.

The observations as discussed for the plants at 20 and 25°C lead to the hypothesis of a change in P requirement linked to developmental stage. The apparent effects of temperature were interpreted as effects due largely to differences in developmental stage. The less mature plants (10 and 15°C root temperatures) were responsive to P throughout the range of treatments and over a wide range in tissue P concentrations. Thus, most of these plants were deficient in P, suggesting relatively high P requirements. The more mature plants (20°C root temperatures) were less responsive to P with apparent luxury concentrations of tissue P at the highest P-application levels. Thus, the P requirements may have decreased at this stage of de-

velopment. This conclusion was supported by the observed effect of time (between replicates) on decreasing the optimal P concentration. The most mature plants (25°C root temperatures) all appeared to be responsive to P and this was associated with the advanced development of the reproductive tissues. Thus, the P requirements may have increased again as the plants approached maturity.

Johansen et al. (1980) found that the optimal tissue P concentrations decreased as the plants matured whereas maximum P uptake by plants has frequently been associated with head development. However, little published data clearly addresses the issue of plant P requirements as they change with time and growth conditions.

Yield Response to Band Applied P

Comparative response to "broadcast" and "band" applied P is presented in Table 3. An interaction at final harvest between the response to P application and soil temperature was statistically significant. At 10°C, banded P at 5 and 20 ppm P increased final yield more than the same amounts of broadcast P. There was no significant difference between the banded and broadcast treatments at 80 ppm P. At 15 and 20°C the advantage to band application was significant only at 5 ppm P, and at 25°C, no significant advantage to band application occurred. This result conformed to a common observation that band application of P is most important in cold soils (Webb, 1977).

A comparison of broadcast to band application at the three leaf stage showed similar trends (Table 3). At this early stage, band application of 5 ppm also provided a slight advantage at 25°C, indicating that cold soil may not be a prerequisite for an advantage to band application. However,

TABLE 3

Yield Response to Band and Broadcast Applied P
at the Three Leaf Stage and at Final Harvest

Temp.	Application Method	At Final Harvest P Level (ppm)				At Three Leaf Stage[1] P Level (ppm)			
(°C)		0	5	20	80	0	5	20	80
		g dry weight/pot				g dry weight/pot			
10	broadcast	0.43	0.539	0.746	1.25	0.08	0.08	0.13	0.15
	band	-	0.691	1.08	1.13	-	0.11	0.15	0.15
15	broadcast	0.64	1.09	2.56	3.33	0.07	0.07	0.13	0.15
	band	-	1.61	2.53	2.68	-	0.07	0.13	0.10
20	broadcast	2.13	3.05	5.03	6.50	0.05	0.07	0.13	0.13
	band	-	3.66	5.30	5.69	-	0.13	0.15	0.13
25	broadcast	2.87	3.64	5.33	6.25	0.06	0.07	0.10	0.15
	band	-	3.64	4.52	4.93	-	0.10	0.10	0.08

1. interpolated from growth curves

2. means joined by a line are not significantly different ($P \leq 0.05$) using single degree of freedom contrasts between band and broadcast treatments within P application level and temperature (for the final harvest data only)

in this case, the early advantage to band application did not persist as growth continued.

P banded at 80 ppm was significantly detrimental to final yield at 15 and 25°C (relative to broadcast P) with a tendency to be detrimental at 10 and 20°C. Growth at the three leaf stage was similarly influenced. This may have resulted from toxicity in the application band although shoot yields were above those of the controls. Baker et al. (1970) demonstrated increased toxicity response at higher soil temperatures but the present results were not conclusive since a continuous or linear effect of temperature did not occur. The results of root weight measurements will be discussed subsequently.

Further support of this hypothesis of toxicity at 25°C was provided by the growth curves of the band treatments. In general, the growth curves of the band treatments, like those of the broadcast treatments (Figure 6), did not intersect. The exceptions were at 25°C where the growth at the 80 ppm P application increased in rate and exceeded the growth at 20 ppm P at about 18 days after planting. This may be an example of the late P requirement as suggested previously, but in this case, it occurred at an earlier date and at a lower mean tissue P concentration. Another explanation of this effect is that the toxicity of the band decreased with time. Barrow (1979b) found that higher temperatures increased the rate of P fixation to less available, and hence less toxic forms. Therefore, this observation at 25°C may be indicative of a temperature effect of fertilizer P reaction rate.

Response to banded P when 20 ppm P was broadcast is shown in Table 4. The response to banded P decreased as temperature increased such that at 25°C there was no significant response to banded P. Two mechanisms were

TABLE 4

Yield Response to a Combination of 20 ppm P Broadcast with Various Amounts of Band Applied P

Temperature	Band Applied P (ppm)			
	0	5	20	80
10°C	0.75[1] a	0.97 b	1.17 c	1.13[2] bc
15°C	2.56 a	3.03 ab	2.72 ab	3.11 b
20°C	5.03 a	6.12 b	6.00 ab	6.42 b
25°C	5.33 a	5.19 a	5.16 a	5.84 a

1. g dry weight/pot, the mean of two replicates averaged in the ℓn transformed mode

2. values followed by the same letter were not significantly different ($P \leq 0.05$) using single degree of freedom contrasts (within each temperature)

Root Weights in the Band Zone and Root Proliferation

The root weights harvested from both the P fertilized and control halves of the band zone varied significantly with temperature (Table 5). In each case, the root yields increased from 10 to 20°C but were distinctly lower at 25°C. Both halves of the band zone received one quarter of the total, original N application which would have given a localized concentration of up to 810 µg N/g band zone soil (see subsequent description of computation of localized P concentration in the band zone). Thus, the decreased root growth in the band may have resulted from greater N toxicity at higher temperatures. However, it seemed unlikely with regular surface irrigation that the toxic effect would have persisted until harvest. Visual observation of root growth in a comparable system (Appendix D) confirmed the lower root growth at this depth at 25°C and led to the speculation that the whole root system morphology may have been modified. Perhaps at 25°C, geotropic reaction (Sheppard and Miller, 1977) or lateral root initiation (Bowen, 1970) was modified such that root growth was more extensive deeper in the soil column.

Proliferation of roots in the P-enriched band was defined relative to the control half of the band zone to compensate for overall effects of the band zone as shown at 25°C. Proliferation was observed in most of the containers but substantial variability (CV = 74%) was characteristic of this data and few significant differences were obtained (Table 6). The significant proliferation at 10°C supported the hypothesis that plants in cold soil benefit most from band application of P. It could be concluded that P deficiency was greatest at 10°C, thus inducing proliferation in the P-enriched zone. However, Drew and Saker (1978) showed that proliferation (relative to a control rooting zone) decreased with the age of the plant.

TABLE 5

Root Weights in the Band Zone as a Function of Temperature

Parameter	Temperature			
	10°C	15°C	20°C	25°C
Root weight in phosphated half (g)	0.37 b	0.42 b	0.54 a	0.34 b
Root weight in control half (g)	0.13 b	0.19 a	0.21 a	0.20 a
Proliferation[1]	2.5 a	1.4 b	1.5 b	0.8 b
Shoot/Root ratio[2]	9.8 b	14.1 b	24.4 a	25.5 a

1. Calculated as (the difference in root weight between the halves)/(root weight in the control half), computed on individual-pot data

2. Calculated as (shoot dry weight)/(root dry weight in the control half)

- means followed by the same letter within each row were not significantly different ($P \leq 0.05$)

TABLE 6

Root Proliferation in the Fertilized Band Zone Relative to the Unfertilized Zone Opposite the Band[1]

Temperature (°C)	Treatment	Level of Band-Applied P (ppm) 5	20	80
10	Band[2]	1.8	3.6*	3.6*
	Combination[3]	2.2	3.1*	0.8
15	Band	1.0	1.7	2.3
	Combination	0.6	1.6	1.0
20	Band	1.6	1.6	0.8
	Combination	0.5	2.0	2.3
25	Band	1.2	0.8	0.5
	Combination	1.4	0.6	0.3

1. see Methods and page 195 for explanation, calculated as (the difference in root weight between the halves of the band zone)/(the root weight in the control half).

2. all of the P was applied in the band zone

3. 20 ppm P was broadcast as well as the band-applied P

* significantly different from 0 at $P \leq 0.05$

Thus, the greater proliferation observed at 10°C may have resulted from the earlier stage of plant development at this temperature.

Drew (1975), in a review article, concluded that root proliferation was induced by P deficiency. In this experiment, as previously noted, it appeared that P deficiency was possibly alleviated in the more mature plants at higher temperatures. Thus, with the inducement gone, root proliferation at these temperatures would have been diminished.

The absolute amounts of proliferation (calculated as the difference in root weights between the halves of the band zone) were 0.24, 0.23, 0.33 and 0.14 g at 10, 15, 20 and 25°C, respectively. Although there was the same differential in root growth at 10 and 15°C, relative proliferation as defined in Table 6 decreased with increasing temperature (2.5 and 1.4 respectively) due to the larger amount of roots in the band zone at 15°C. In this way, a differential in root growth established early in plant development (and recognized then as proliferation) would have been masked by further, non-differential root growth. Such a phenomenon may be particularly relevant for wheat where the growth of nodal roots, radiating in all directions from the plant crown, constitute a major portion of the root system near the crown on a weight basis.

In contrast to these arguments that proliferation may have been a function of time rather than temperature, visual observations of a comparable system (Appendix D) showed very little or no proliferation at 25°C at any time whereas proliferation was apparent at lower temperatures. The present data were not sufficient to reconcile whether time or temperature was the important factor.

Proliferation of roots in the P band also occurred when 20 ppm P was broadcast. Since 20 ppm P broadcast gave yields close to the maximum, it

would be anticipated that the P deficiency was substantially less than in the treatments without broadcast P. Thus, less inducement for proliferation would be expected. However, similar degrees of proliferation were observed, suggesting that proliferation occurred regardless of plant P status.

Another explanation of this result lies in the relative concentrations of P in the soil that the seedling roots encountered. The seed placement was approximately 1.0 cm from the band zone and therefore the roots would have encountered the contrasting halves of the band zone very shortly after germination. The band applications resulted in localized concentrations in the P-fertilized half of 160, 650 and 2600 µg P/g dry soil for the 5, 20 and 80 ppm treatments respectively.[1] The control half of the band zone had either the native soil P concentration (in the band treatments) or 20 ppm P broadcast-applied P (in the combination treatments). In comparison to the P-fertilized half, the difference between the native soil concentration and the 20 ppm P was minor. Thus, root proliferation between the halves of the band zone would not have been influenced by the addition of 20 ppm P broadcast as in the combination treatment.

The similarity in proliferation between the banded and combined treatments was confirmed by a similarity in fertilizer P uptake from the band zone. Virtually identical amounts of band-applied P (differentiated by the presence of ^{32}P labelling) were absorbed from the band regardless of the broadcast P applied. However, more non-band-applied P was absorbed when 20 ppm P was broadcast. Thus, band-applied P comprised less of the total plant P in the combination treatments.

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[1]Calculated by assuming that the 10 mL of solution containing the band P treatment wetted 42.2 g of the band zone soil to the moisture holding capacity.

The proportion of band-applied P relative to total plant P is shown in Figure 8. Increases in the amount of band-applied P increased the portion of this nutrient in the plant. As temperature increased, generally less of the plant P was derived from the band zone, suggesting that the more extensive root systems of these larger, more mature plants availed them to more of the non-band or bulk soil P. This implies that the importance of the band to plant P nutrition diminished with plant age.

Shoot/Root Ratio

The shoot/root ratio is known to decrease during nutrient deficiencies resulting in a greater portion of the photosynthate produced by the plant available for root proliferation. Approximations of the shoot/ root ratio in this experiment were possible by using the dry weight of the roots in the zone opposite the P band as the denominator. In general, the shoot/ root ratio increased with temperature (Table 5) and was significantly correlated to yield ($r = 0.78$). Thus, at lower temperature, relatively more photosynthate was partitioned toward the roots and this may have afforded the root proliferation observed.

The increase in the shoot/root ratio with temperature may have been due to: a) a direct effect of temperature, b) the result of ontogenic development which was more advanced at higher temperatures, and/or c) the result of a change in the plant P status from deficient to sufficient as the plants grew.

Discussion

Soil temperature had several influences on the soil/plant system. The decreased yield response to fertilizer P at 25°C provided evidence that more native soil P was available to plants at higher soil temperatures

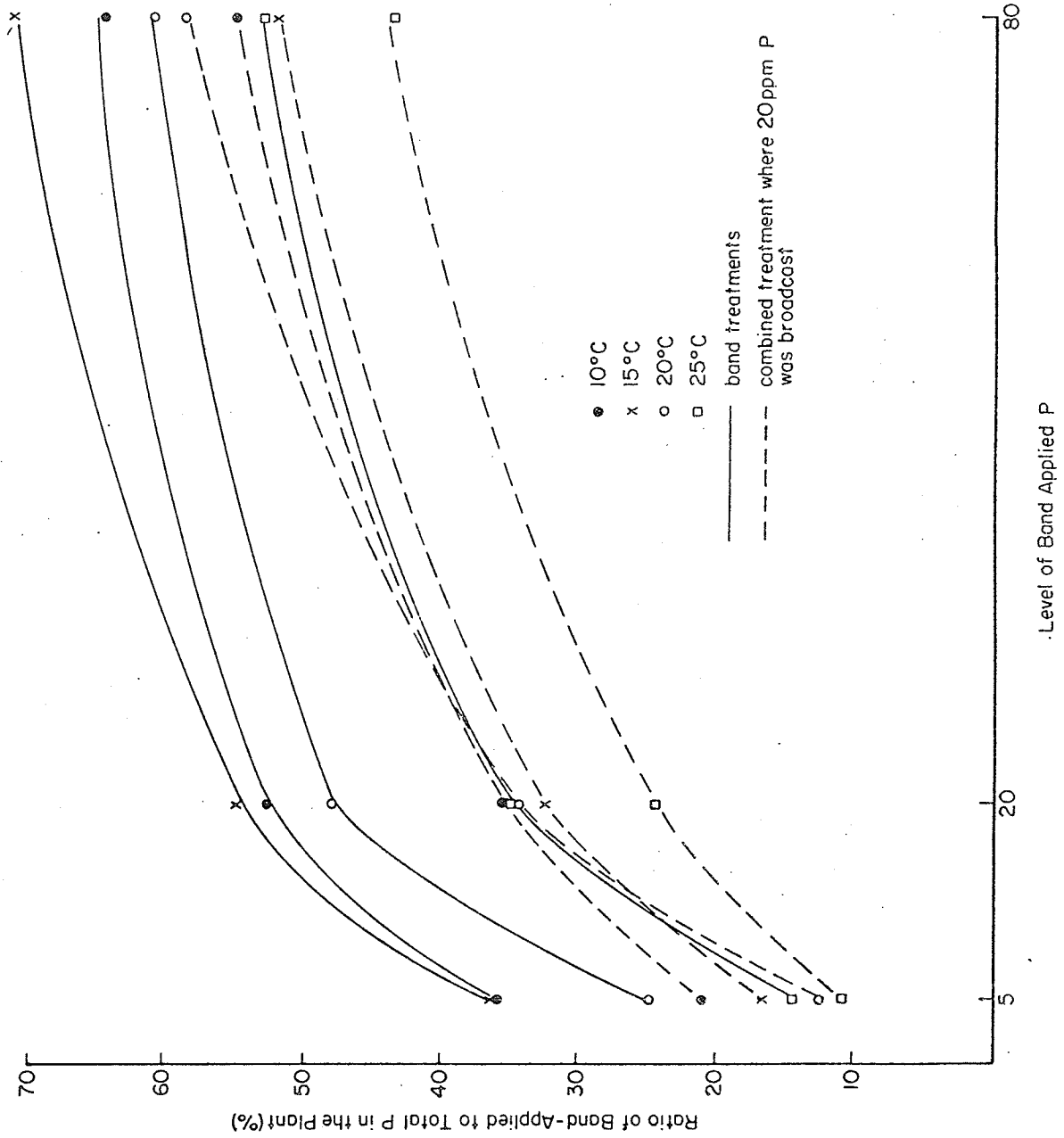

Figure 8: Ratio of band-applied P to total P in the plant.

(Figure 4). Increased temperature also appeared to decrease fertilizer P solubility based on apparent recovery from toxicity in the fertilizer P band at 25°C. However, this effect was probably masked in most treatments of this experiment by the plant response to temperature which, as a biological system, would have been more affected in the opposite direction than the P reaction system.

The most notable influence of temperature on plant growth was on physiological age. Thus, temperature either directly modified plant processes or was manifest through differences in physiological age.

This experiment provided evidence that the plant P status changed with age. Thus, when comparisons were made on a chronological rather than physiological time scale, age dependent effects were also associated with temperature. The change in plant P status with physiological age was hypothesized based on the late response in growth rates to 80 ppm P and the leaf tissue analysis of samples collected three days apart at each of the four temperatures (Figure 7). The conclusion from these observations was that P deficiency was general very early in the experiment, was alleviated in plants receiving high P applications during vegetative growth, and may have occurred again at the onset of reproductive growth. This conclusion was supported by the observation that root proliferation in the P fertilizer band was most apparent in the younger plants which implied that P deficiency was most acute in the younger plants.

The practical implication of the change in plant P requirement with time is that tissue critical-P concentrations cannot be determined based on yield at an arbitrary stage. If the plant P status changes with time, there is no guarantee that tissue P concentrations represent the growth limiting factor at the time the samples are collected unless response to P

at that time can be demonstrated. The general practice is to sample tissues at a stage where P deficiency has been commonly detected. However, it must be recognized that this is a statistical criterion and that exceptions are to be expected under varied growth conditions. Very little research information is available to judge plant P status under a variety of conditions and physiological ages.

Further response of the plant system to temperature was manifest in the response to band-applied P. The yield advantage to band-applied as opposed to broadcast-applied P was evident at harvest particularly at 10°C. Root proliferation in the band at harvest was also most pronounced at 10°C. However, both these phenomena could have been the effect of physiological age and decreased P deficiency with time. There was evidence that at the three-leaf stage, band-applied P was an advantage to growth regardless of soil temperature, but the advantage did not persist until harvest. This emphasizes the need to not only find efficient application methods such as banding, but also to define if and under what conditions the effect of these efficient methods will persist until final yield.

The effects of temperature in this study generally progressed in a parallel manner from 10 to 20°C (see, e.g. Figure 4). However, the responses at 25°C were often unique. This may indicate that 25°C was above the optimal temperature for growth. Hallem (1981) found that at temperatures above optimal, extensive bacterization of the root cortical cells occurred. Thus, the unique results found at 25°C may indicate that different processes mediated the response to temperature.

Conclusions

Yield response, tissue P concentrations, fertilizer use efficiency and

root proliferation were reported as a function of amounts and methods of P application as well as soil temperature. Cursory examination of the data suggested that in a cold soil, plants responded to larger applications of P, they utilized band applied P more efficiently and they proliferated roots to a greater degree when the P was applied in a band. However, the plants were harvested at the same chronological age and there was considerable evidence that many of these phenomena were due to differences in physiological age. Growth curves were used to examine response at a similar physiological age and very little difference in response could be found. Thus, temperature differences were confounded by developmental differences and a dynamic interpretation of the data was required.

In summary:

1. Soil temperatures modified plant growth and development rates with the most rapid growth and development at 25°C.
2. The yield response to fertilizer P was independent of temperature at 10, 15 and 20°C but slightly lower at 25°C.
3. Tissue analysis data suggested a change in plant P status with time.
4. Band-applied P in non-toxic amounts was efficiently utilized by plants at all temperatures.
5. Roots proliferated in a N-P fertilizer band and this was especially significant at 10°C.
6. It was hypothesized that many of the observed effects of temperature, including that of root proliferation at 10°C, could have been the result of the change in P status with time. This change was associated with temperature because of the effect of temperature on physiological age.
7. Further research on the soil chemistry and plant nutrition aspects of this problem are required to fully understand the effects of temperature on plant response to P.

Chapter 4

EXTRACTABLE P AT FOUR SOIL TEMPERATURES

Introduction

There is little question that temperature influences both the supply of P from the soil and the uptake and utilization of P by the plant. It is more difficult to attribute the effects observed in the combined soil-plant system to responses by either the soil or the plant. The effects of temperature on soil P supply can be examined with extraction procedures but it must be recognized that these procedures are only an approximate indication of the chemical environment which surrounds a plant root. Conversely, utilizing plants to infer effects of temperature on soil P supply is made difficult by the overriding effect of temperature on plant growth.

The purpose of these experiments was to examine the effect of temperature on soil P supply. Several methods were examined including extraction with $NaHCO_3$, extraction with an anion exchange resin, construction of desorption curves, measurement of desorption rates and measurement of short term plant uptake. The major objective was to choose the best method to characterize the response to temperature and to determine if this response varied from soil to soil.

Choice of Methods

Soil phosphorus is held in the soil by a range of mechanisms which intergrade in terms of chemistry and binding strength among adsorption, precipitation and perhaps occlusion. Plant roots may have access to a relatively broad spectrum of these forms of P, particularly due to the rhizo-

sphere reactions (Soon and Miller, 1977), root hairs (Barley and Rovira, 1970) and mycorrhizal associations (Gerdemann, 1974) known to enhance P solubilization. Because of these specific enhancement mechanisms, it is very difficult to chemically simulate the phosphate solubilizing properties of roots. For this reason, it was felt essential to use plants to characterize the response of soil P to temperature. However, a unique system was required so that soil response could be differentiated from plant response.

The requirements of the desired system were best satisfied by a short term transplant system (Beaton and Read, 1963). Plants were grown under uniform conditions and high fertility (excluding P) prior to introduction to the treatment soil. Thus, they were growing rapidly and had a high P requirement which should have maximized the P uptake capacities per unit of root. These plants were transplanted onto the treatment soil and grown for a very short period which further decreased the effects of treatment on plant growth. The goal of this procedure was to maximize plant P uptake per unit of root so that differences between treatments could be attributed to effects in the soil system. Adjustments for differences in root mass were required.

Chemical extraction methods were used to support the short term plant uptake studies. Sodium bicarbonate was used because of its wide use as a P soil test. In one experiment, an anion exchange resin was used since it was chemically less severe than $NaHCO_3$ but still extracted a large amount of P.

Another technique was extraction to prepare P desorption curves. This involved measuring the equilibrium solution P concentration when varied amounts of P had been desorbed per unit of soil. The advantage of this characterization was that it described the solubility of the most soluble

forms of P in the soil and also described the P buffer capacity or "solubility profile" of P in the soil.

The term "solubility profile" was chosen to describe the concept that soil P exists in a continuous gradation of forms resulting in a continuous range of solubilities. A soil with a low P buffer capacity (the buffer against change in the solution P concentration) would contain relatively small amounts of P of each form or solubility. Thus, if a set amount of P was desorbed from this soil (analogous to the uptake by a plant root of P required for growth) the most soluble forms of P would be exhausted and the resultant solution P concentration would be low, in equilibrium with a relatively insoluble form of P. Conversely, a soil with a high buffer capacity would release the same total amount of P but would exhaust fewer of the more soluble forms of P. As a result, the final solution P concentration would be relatively high, in equilibrium with the more soluble forms of P. Thus, the solubility profile describes the amount of P at each solubility, progressing from the most to the least soluble forms.

The desorption curves were derived by extracting soils with a range of solution to soil ratios (Figure 9a, for hypothetical case). These data for hypothetical soils A, B and C showed the amount of P desorbed when the sink for P (the volume of solution) was varied. These data were replotted (Figure 9b) by computing the equilibrium solution P concentration (computed as the amount of P desorbed divided by the solution to soil ratio) and using this as the ordinate. Thus, the desorption relationships were curvilinear, shown in Figure 9b intersected by lines representing the solution:soil ratios.

The data could be more easily described and compared in a linear form. In this case, transforming the ordinate to the $\log_e$ scale resulted in

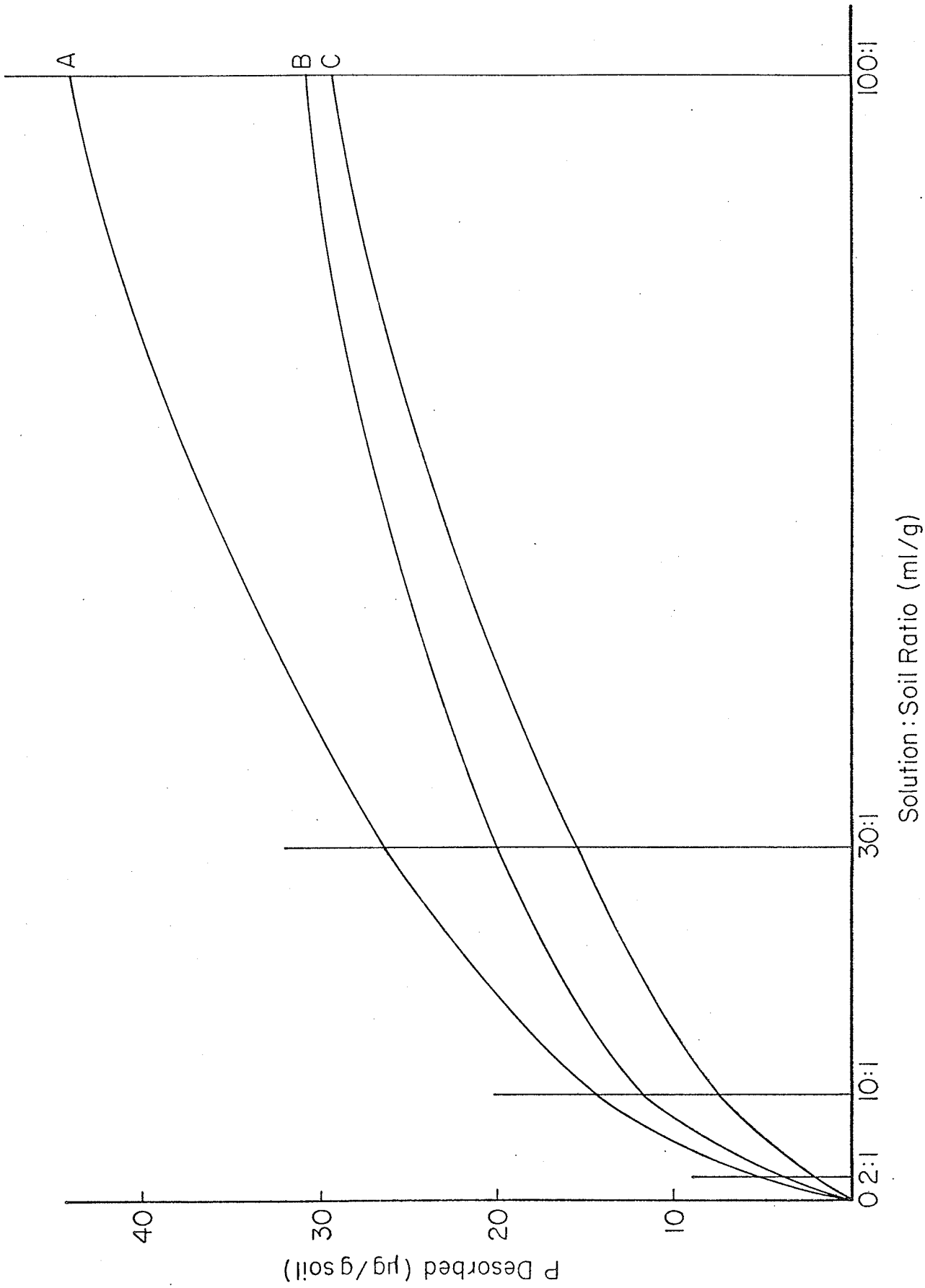

Figure 9a: Desorption of soil P from three hypothetical soils as influenced by solution:soil ratios.

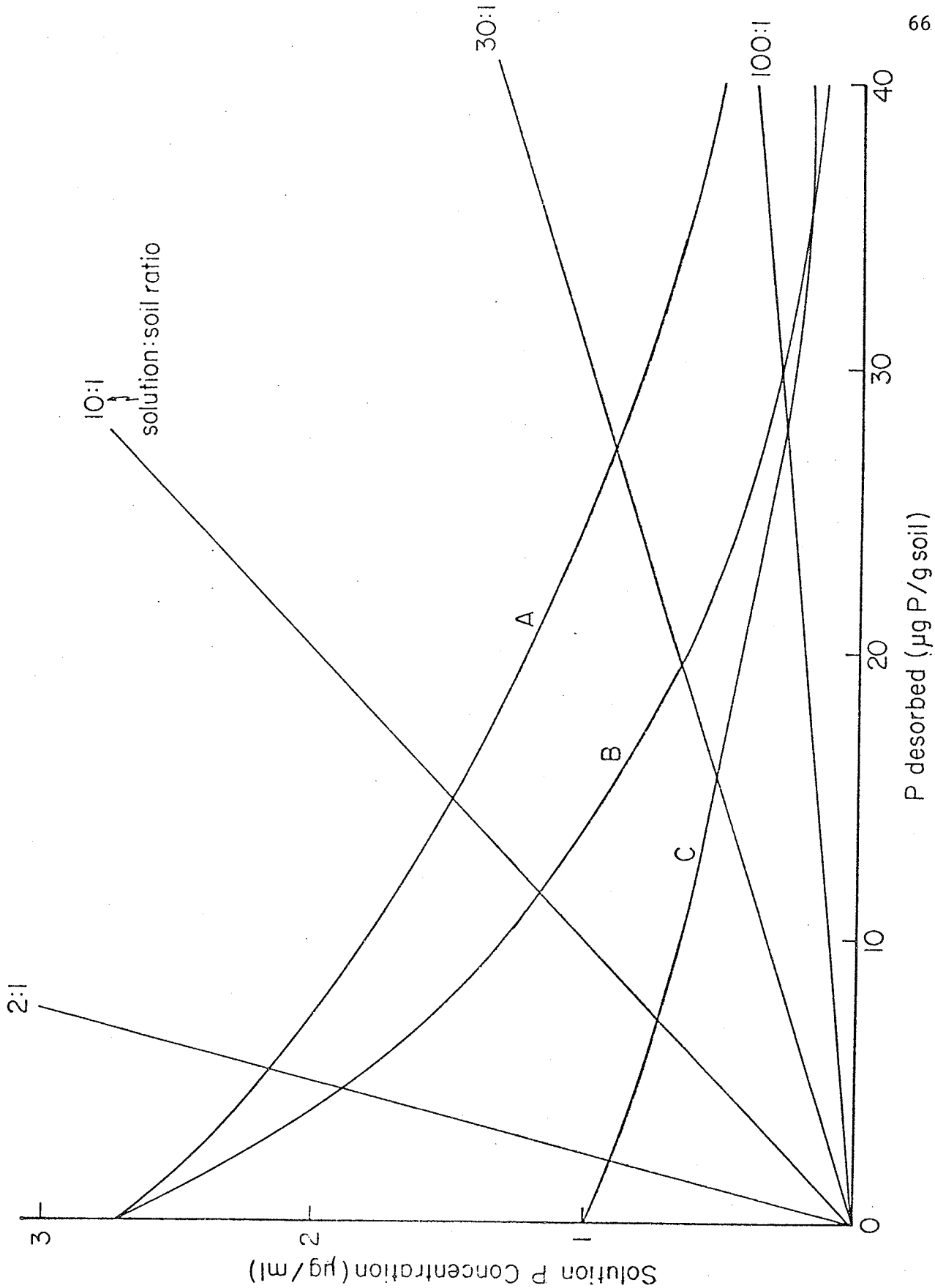

Figure 9b: Desorption curves computed for the hypothetical soils in Figure 9a.

linear functions (Figure 9c) which were described by intercepts and coefficients or slope terms. The lines representing the solution: soil ratios became curvilinear. The desorption curve intercept was interpreted as the solubility of the most soluble form of P in the soil since, as this point was approached, the smallest amounts of P were desorbed from the soil implying that only the most soluble forms of P were involved. The slope was interpreted as an indicator of the soil P buffer capacity or solubility profile because it described, for a unit of P desorbed from the soil, how large a change in solubility had occurred. A steeper slope (larger absolute regression coefficient) indicated a lesser buffer capacity. Thus, it described how many of the more soluble forms of P were exhausted. The model equation involving the intercept (a) and the slope (b) was:

$$\log_e (\text{equilibrium solution P concentration}) = a + b\ (\text{amount of P desorbed/g soil}) \qquad [1]$$

Methods, Experiment A

Two major experiments were conducted. In experiment A, P supply was examined as a function of temperature in P-fertilized and unfertilized soils, whereas in experiment B, P supply was examined as a function of temperature in a range of soils. Each major experiment entailed several sub-experiments.

Two soils were chosen for this study, one being the Elm River silt loam soil used in the previous response experiment (Chapter 3) and the other an Almasippi loamy fine sand also known to be deficient in P. Pertinent soil properties are included in Table 7. The soils were air-dried and sieved to pass a 2 mm mesh.

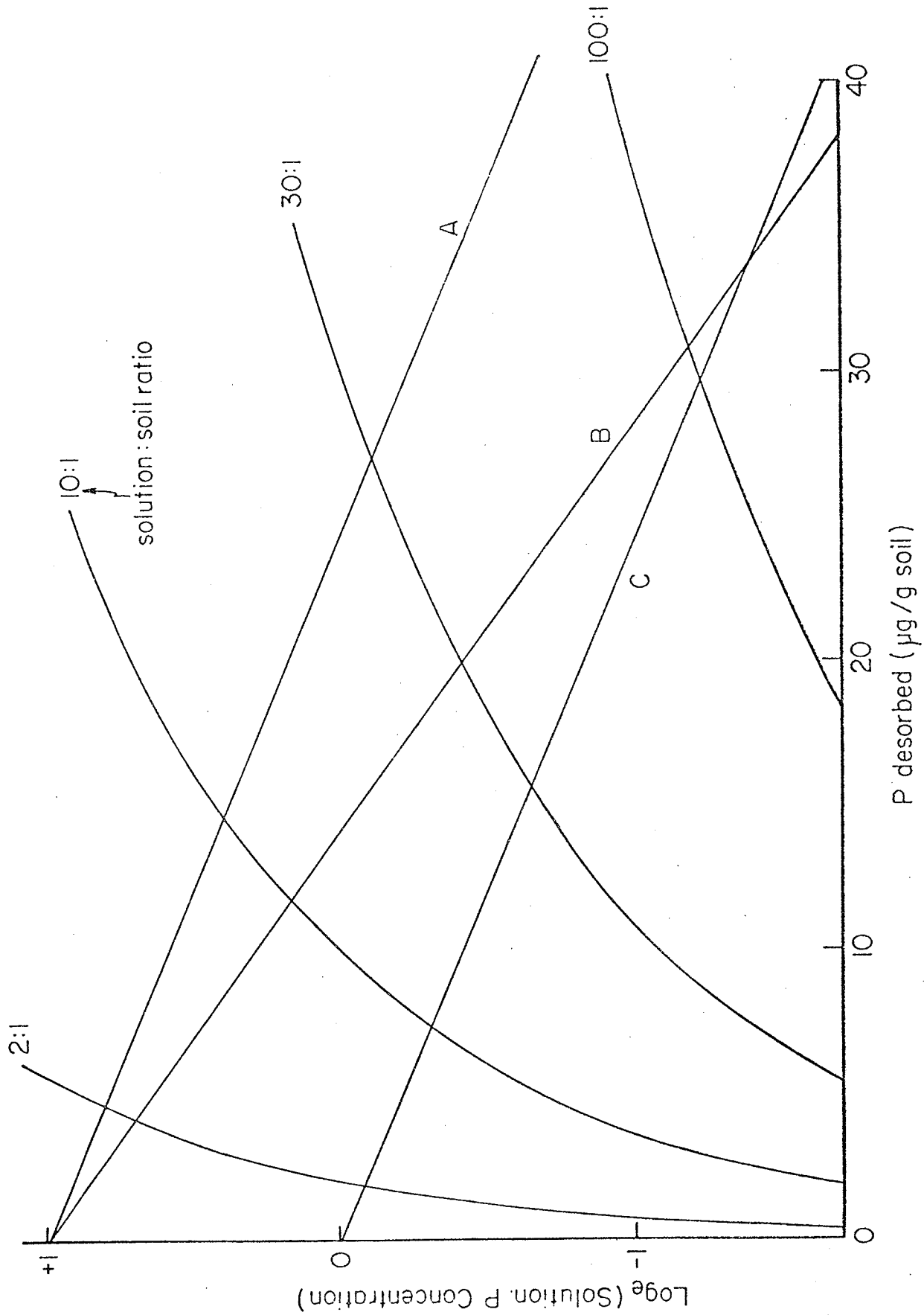

Figure 9c: Desorption curves presented in $\log_e$ - linear plot.

TABLE 7

Soil Properties of the Soils Selected for Study

Soil Name[1]	Great Group	Texture	P $NaHCO_3$ Extractable (µg/g)	pH in 1:1 Soil:Water	CO_3 (% CO_3)	NO_3-N $CaCl_2$ Extractable (µg N/g)	K NH_4OAc Extractable (µg K/g)	SO_4-S $CaCl_2$ Extractable (µg S/g)	Potting Bulk Density (g/cm^3)
Balmoral	Rego Humic Gleysol (Carbonated phase)	SiC	18.5	8.1	10.6	17.8	410	20+	1.00
Almasippi	Gleyed Rego Black	LFS	6.9	6.9	0.05	6.4	189	8.2	1.25
Inwood	Gleyed Rego Dark Gray (Carbonated phase)	CL	20.0	7.9	12.0	26.8	204	10+	1.16
Manitou	Orthic Black	CL	25.1	6.8	0.20	7.6	475	9.6	0.93
Lakeland	Gleyed Rego Black (Carbonated phase)	SiCL	18.8	7.9	18.2	13.4	262	5.8	1.09
Lundar	Gleyed Rego Black (Carbonated phase)	L	15.5	7.7	3.4	37.4	277	10+	0.97
Newdale	Orthic Black	CL	75.0	7.5	1.8	65.0	282	10+	1.10
Plum Ridge	Gleyed Rego Black (Carbonated phase)	LFS	9.0	8.1	8.0	19.4	160	20+	0.88
Elm River	Cumulic Regosol (Carbonated phase)	SiL	16.3	7.8	6.2	32.4	290	9.0	1.27
Snowflake	Orthic Black	CL	5.8	7.1	0.30	4.6	508	5.0	0.83
Stockton	Orthic Black	LFS	7.5	6.8	0.0	1.4	115	2.0	1.33
Wellwood	Orthic Black	L	11.4	7.1	0.37	8.6	288	6.8	1.04

1) soil series or association name

The soils were incubated moist prior to the experiment to avoid artifacts of dry soils (Barrow and Shaw, 1980; Lund and Goksoyr, 1980) by wetting to the moisture holding capacity (field capacity in the incubation containers) and then slowly allowing them to dry (with mixing) to a workable moisture content. This was judged to occur at approximately 12% water by weight for both soils. Each soil was weighed into 2250 g (2000 g dry weight) aliquots for each of two replicates, two P application rates and four temperatures (a total of 32 aliquots). The soils were sealed in doubled polyethylene bags and placed to incubate in the respective temperature control baths set at 10, 15, 20 and 25°C (for description of baths, see Appendix A). The period of moist incubation prior to P treatment was 13-14 days for the first replicate and 20-21 days for the second replicate.

After the period of moist incubation at the respective temperatures, the soils were treated with 5.33×10^4 dpm ^{32}P/g dry soil either carrier-free or with 40 µg ^{31}P/g dry soil as NaH_2PO_4. These treatments were applied in 120 mL of water as a fine mist to each of the moist soil (2000 g dry weight) aliquots. The soil was spread very thinly and the spray was added in four applications with thorough mixing of the soil between applications. An additional spraying and mixing procedure using distilled water was employed to moisten the soils to their moisture holding capacity. The soils were sampled for measurement, resealed in polyethylene bags and returned to the temperature control baths.

The soils were subsequently incubated, with periodic sampling, at their respective temperatures for 53 (rep 1) to 61 (rep 2) days.

Moisture Contents

The various measurements to be described were based on weighed aliquots

of moist soil calculated to provide approximately the required amounts of dry soil. The actual dry weights were determined by measuring the soil moisture contents at each sampling time. Very little moisture loss occurred during the incubation period.

Specific Activities

The specific activities were measured by extracting 50 g dry soil with 450 mL 0.02 M KCl in 500 mL Nalgene bottles. The bottles were agitated end-over-end in their respective water baths (Appendix A) for 22 hours. The suspensions were allowed to settle for an additonal two hours in the baths and then the supernatants were filtered under suction through #42 Whatman filter paper. The filtration was done at room temperature but the relatively clear supernatants filtered rapidly enough (approximately 15 minutes) that the temperature changed only slightly. The filtrate was acidified with five drops of concentrated H_2SO_4 and a 250 mL sample was retained. These samples were evaporated to dryness in a warm oven (75°C) and then redissolved in 20 mL of 0.5 N H_2SO_4. The concentrated samples were analysed for ^{32}P and ^{31}P (analytical methods described subsequently).

Specific activities were determined on all 32 lots of soil immediately after P treatment, three further times throughout the P treatment incubation period, and in a factorial of four incubation temperatures and two extraction temperatures (to be described subsequently) at the end of the incubation period.

Desorption Curves

Desorption curves were obtained by extracting the soil with 0.02 M KCl in varied soil-solution ratios as described by Vaidyanathan and Nye (1970).

Ratios of 0.5, 0.15, 0.05, 0.01 and 0.003 (soil dry weight/solution volume) were approximated with the exact moist soil weights recorded for each sample. The solution volumes were 20 mL for the 0.5 and 0.15 ratios and 40 mL for the other ratios. Screw-cap, 50 mL test tubes were used and they were agitated end-over-end in their respective temperature control baths for 22 hours.

The extraction suspensions were allowed to settle for an additonal two hours in the baths prior to filtration through #42 Whatman filter paper. The filtrate was collected in polyethylene scintillation vials and was analysed for ^{32}P and ^{31}P.

The five extraction ratios were used on the 32 lots of soil immediately after P treatment and again at the end of the incubation period. At the later time, the five ratios were each duplicated. The intercepts and slopes of the desorption curves were estimated by linear regression. The effect of temperature on these parameters was tested by including temperature as a linear effect in a multiple regression of the desorption curves (see footnote, Table 12).

Sodium Bicarbonate Extractable P

Extraction with 40 mL of 0.5 N $NaHCO_3$ at pH 8.5 was similar to the desorption curve extractions. A soil-solution ratio of 0.05 was used and 1.0 g of $NaHCO_3$-washed charcoal was included to decrease organic matter colouration of the extracts. The analytical procedures and the measurement dates were the same as for the desorption curve extractions.

Statistical treatment of the data involved analysis of variance utilizing a randomized complete block model with temperature as the main plot. The P treatments were analysed separately but the data from the start and

end of the incubation were combined since the respective error variances were comparable.

Desorption Time Measurements

Desorption of P as influenced by extraction time and temperature was examined using the extraction system described for specific activity determinations. Sub-samples of the extraction suspensions were filtered at 1, 2, 4, 6, 24, 30 and 48 hours after the extraction was begun. The 20-30 mL subsamples were removed from the extraction bottles 15 minutes before the intended filtration time and were allowed to settle. At the filtration time, the supernatant was filtered with #42 Whatman filter paper and the filtrate was collected in polyethylene scintillation vials for ^{32}P analysis.

The seven desorption times were used on the 32 soil lots at the end of the incubation period and were usedfor a factorial of four incubation temperatures and two extraction temperatures (to be described subsequently).

The desorption versus time curves demonstrated a rapid initial reaction followed by a slower reaction up to 48 hours. Three statistical models were used to describe the data. The first was a segmented, polynomial regression model of solution P concentration (PC) on time (t) where the first segment was a quadratic function (equation 2) to fit the initial rapid reaction and the second was a linear function (equation 3) to fit the longer-term reaction:

$$PC = a + b\ (t) + c\ (t)^2 \qquad [2]$$

$$PC = d + e\ (t) \qquad [3]$$

The join-point (t^o) of the segments was defined as the point where the slopes (equation 4) and the estimates at t^o (equation 5) of the two func-

tions were equal:

$$b + 2c\ (t^{o}) = e \qquad [4]$$

$$(t^{o}) = (e-b)/2c$$

$$a + b\ (t^{o}) + c\ (t^{o})^{2} = d + e\ (t^{o}) \qquad [5]$$

$$d = a + (b - e + c(t^{o}))\ (t^{o})$$

Equation 5 reduced the number of unknown coefficients to four. This segmented model was fitted to the data for each temperature, soil and P treatment using a Gauss-Newton iterative scheme (SAS, 1979) until the residual sums of squares changed by less than 1×10^{-8}.

The second statistical model was a linear function relating the natural logarithm of the amount of P desorbed per unit of soil to the natural logarithm of the extraction time (Sharpley et al., 1981). This model was fitted to the data of each temperature, soil and P treatment by linear regression.

The third statistical model described simultaneous diffusion-limited desorption and a first order desorption reaction (Wiles, D.R. personal communication). This model was appropriate since the soils were not rigorously dispersed and therefore diffusion within aggregates was a plausible rate-limiting process. The model related solution P concentration to $a(t)^{\frac{1}{2}}$ as the diffusion-controlled P (hence the square root function) plus $b(1-e^{c(t)})$ as the first order desorption reaction where the concentration tended toward b, modified by the rate coefficient c. This model was also fitted using the iterative scheme provided by SAS (1979).

Short Term Plant Uptake

Short term plant uptake was measured by transplanting week-old wheat

plants (*Triticum aestivum* cv Neepawa) onto a small sample of soil and growing the plants for one week. The wheat plants were started in screen-bottomed pots (7.6 cm inside diameter x 8 cm high made from ABS sewer pipe and fibreglass screen), three-quarters filled with acid-washed granitic gravel (2-5 mm diameter, washed with HCl and rinsed with distilled water). Twenty-four seeds were pregerminated in weigh-boats and the best 20 seeds were placed 2 cm deep in the gravel. The plants were grown in the growth chamber (see Chapter 3) and were watered daily with full strength Hoagland solution, minus P (see Appendix I). An excess number of transplant pots was prepared to allow selection for uniformity.

The seedlings were at the one to two leaf stage with some roots penetrating the screen-bottom of the transplant pots at the end of one week. The pots were watered and then placed on the surface of preweighed aliquots of moist treatment soil (approximately 250 g dry weight). The soil filled the bottom 4-cm depth of a 15 cm deep container. The entire unit (transplant pot plus soil container) was weighed and then floated in the temperature-controlled water baths.

The plants were grown the second week at the treatment soil temperatures with bidaily watering to the original weight with distilled water. The plants were harvested at the end of the second week by washing the soil away from the roots with distilled water and collecting the roots below the screen. There was no visible loss of roots during washing. The root samples were manually cleaned of remaining detritus, dried at 85°C and weighed. The plant shoots were counted and all of the plant material above the screen (including some roots, the residue of the seed and the leaves) was washed free of gravel, dried at 85°C and weighed. The plant samples were analysed for ^{31}P and ^{32}P (method described subsequently) and the P content

(defined as the total amount of P) in the plant material above the screen, expressed as the total P content per 20 plants, was used as the measure of plant-available soil P.

Several notes about the method are required because this method of short term plant uptake measurement was unique to this study. Firstly, the actual number of plants per experimental unit varied slightly despite steps taken to ensure uniformity. However, there was no correlation between the actual number of plants and either single-plant weight or single-plant P uptake. Thus, inter plant competition was not a confounding factor in this study.

Secondly, root growth below the screen was highly dependent on soil temperature, ranging from averages of 0.11 g/20 plants at 10°C to 0.56 g/20 plants at 25°C. The roots at 20 and 25°C had thoroughly explored the soil with noticeable concentration of the roots at the bottom of the soil containers. In contrast, the roots at 10°C were much less prolific with no concentration at the bottom of the soil containers. The roots at 10°C were also slightly thicker than those grown at higher temperatures, similar to the response of corn roots reported by Onderdonk and Ketcheson (1973).

The morphological differences among the roots due to temperature precluded a direct, arithmetic computation of P uptake per unit of root. Clearly a linear relationship between uptake and root length would not have existed if it included the roots at 25°C which had congregated at the bottom of the pot. Therefore, a curvilinear relationship was assumed and was implemented by using analysis of covariance. Consequently, P uptake was adjusted by the covariates root-weight and the square of root-weight.

The transplant method of short term plant uptake was used on the 32 soil lots immediately after P treatment and at the end of the experiment.

At the later time, the temperature treatments included a factorial of four incubation and two growth temperatures (to be described subsequently). The statistical design was a randomized complete block split-plot with temperature and time as the main plot.

Temperature Changes

At the end of the incubation period in experiment A, measurements of specific activity, desorption rates and short term plant uptake were conducted at the incubation temperatures and at a new temperature imposed for the extraction or growth period. Soils from the 25°C incubation temperature were extracted (or plants grown) at 25 and 15°C. Similarly, from 20°C at 20 and 10°C, from 15°C at 15 and 25°C and from 10°C at 10 and 20°C. Thus, a factorial arrangement of four incubation temperatures (10, 15, 20 and 25°C) and two extraction or growth temperatures (the incubation temperature and a new temperature above or below the incubation temperature) was imposed.

Methods, Experiment B

Six carbonated soils and six non-carbonated soils were chosen to represent the range of agronomic soils in Manitoba. The properties of each soil are shown in Table 7. The soils were air-dried and sieved to pass a 2 mm mesh.

Experiment B was conducted in two separate sub-experiments, a soil extraction experiment and a short term plant uptake experiment. However, the soils were prepared in the same manner for each sub-experiment. Because of the range in soil textures and structures, a constant volume of each soil was deemed a better criterion than constant weight for the comparison of

plant response. Thus for both sub-experiments, the addition of carrier-free ^{32}P was based on a constant volume of soil. Conversion to a weight basis was possible using the bulk densities shown in Table 7.

The soils were moist-incubated at approximately half their moisture-holding capacity for one week in the treatment temperature baths. After the moist-incubation, carrier-free ^{32}P was added to the soil (1.78×10^7 dpm/200 cm^3 soil) in four applications using a pipette and thoroughly mixing the soil between applications. Additional water was added to achieve the moisture holding capacity and the soil was sealed in plastic containers and floated in the respective temperature control baths.

Soil Extraction

The soils were subsampled for extraction two weeks after the ^{32}P was added. Desorption curve extractions and $NaHCO_3$ extractions were conducted as described for Experiment A. An additional extraction using an anion exchange resin was included.

The hydroxyl form of AG 1-X8 resin[1] was prepared by rinsing with 1:1 concentrated HCl:H_2O to form a chloride resin. The resin was rinsed with distilled water and dried by eluting with acetone. The dried resin was weighed into 2.0 g aliquots and each was sewn into a nylon mesh bag (10 cm long x 1.5 cm diameter (Sibbesen, 1977)). Each mesh bag was held rigid by a 10 x 1.5 cm rectangle of fibreglass screen which was sewn into the bag. The resin bags were moistened with 0.2 M KCl and refrigerated until required.

The resin bags were placed in 50 mL screw-cap test tubes with approximately 2 g dry weight (exact weight recorded) of moist treatment soil and 40 mL of distilled water and were agitated similarly to the $NaHCO_3$ extrac-

1. BIORAD refined DOWEX styrene resin, 3.2 meq/g dry resin, 20-50 mesh range (U.S. Standard).

tions. At the end of the extraction, the resin bags were removed from the tubes and rinsed free of soil with distilled water. The P was liberated from the resin by shaking each bag for 24 hours (at room temperature) with 40 mL of 0.5 N HCl. A 20 mL sample of this solution was retained for ^{32}P and ^{31}P analyses.

This sub-experiment entailed seven extraction systems, twelve soils and four temperatures. The lack-of-fit to a linear model for temperature effects was used as the error term.

Short Term Plant Uptake

The short term plant uptake measurements in Experiment B were slightly different than those reported in Experiment A. The gravel was replaced with Perlite (expanded-lava soil conditioner) because the white Perlite absorbed less radiant heat, had a higher moisture holding capacity and was less dense than the gravel (thus making the transplant plus soil-container units more buoyant). However, the roots above the screen penetrated the Perlite which then could not be removed. Therefore, only the leaves above the Perlite surface were harvested for P uptake measurements. Other procedures were the same as reported in Experiment A.

The initial proposal was to use the pretransplant leaf P contents as a "control" and to ascribe any increase in P content after transplanting to soilsupplied P. However, the P content of the leaves occasionally decreased during the growth period. A subsequent experiment to examine this phenomenon (Appendix G) showed that when external sources of P were low, the seedling P concentration was rapidly diluted by growth. The P derived from the seed was initially distributed to both the shoot and roots, but, as growth continued and the tissue P concentrations decreased, there was a

repartitioning of P toward the root system. Thus, leaf P contents of the controls decreased during the growth period. A regression model was developed to predict this decline in ^{31}P content of the controls so that the contribution of soil P could be estimated for Experiment B. The variation of the seed P in the leaf was small relative to the measured soil plus seed P (about 5%) for most of the soils.

A second approach to this problem involved a conceptual model of plant P uptake. The leaf ^{31}P content was comprised of seed-derived and soil-derived ^{31}P. The seed-derived ^{31}P was proportional to the number of plants per pot (which ranged from 10 to 20 plants/pot in Experiment B). The soil-derived ^{31}P could be computed as the product of the leaf ^{32}P content, all of which was derived from the soil, and the inverse of the soil ^{32}P specific activity. Thus, the model was written as:

$$\text{leaf } ^{31}P \text{ content} = b_1 \text{ (number of plants per pot)} + b_2 \text{ (leaf } ^{32}P \text{ content)} \quad [6]$$

The coefficient b_1 represented the amount of seed-derived ^{31}P in the leaf per plant and the coefficient b_2 represented the inverse of the soil ^{32}P specific activity. The amount of seed-derived ^{31}P in the leaf per plant was shown by an independent experiment (Appendix G) to range from 0.034 to 0.040 mg ^{31}P/plant as temperature increased from 10 to 25°C. The specific activity of soil P was expected to change due to both soil and temperature. Therefore, the coefficients b_1 and b_2 were derived by linear regression and were nested within temperature and within soil and temperature respectively.

The model described the data well (R^2 = 98%) and the coefficients were close to expected values. The coefficient b_1 ranged from 0.041 to 0.067 mg ^{31}P/plant as temperature increased from 10 to 25°C. The mean specific

activity was 806 dpm $^{32}P/\mu g$ ^{31}P but this value was probably an overestimate since b_1 was overestimated compared to preliminary experimental results.

The transplant growth on the soil was initiated one week after the ^{32}P was added so that the harvest date coincided with the extraction date of the previous sub-experiment of Experiment B. The short term plant uptake measurements were done in triplicate (including triplicated soil preparation) on the 12 soils at the four temperatures. A randomized complete block split plot design (with temperature as the main plot) was used.

Methods, General

Analytical Methods

The whole unground plant tissue samples were wet-ashed, using 2:1 (by volume) HNO_3 and $HClO_4$ (Appendix B). The root samples were filtered after ashing to remove suspended particles attributed to residual soil. The ^{31}P content for all samples was determined colorimetrically using the ammonium-molybdate-antimony blue method (Appendix B). Radioactive ^{32}P was measured by monitoring Cerenkov radiation (in aqueous media). Counting efficiencies were determined by internal standard for the soil extracts and by monitoring the spectral shift for the plant digestions (Appendix C). Both a Searle Mark III and a Beckman 7500 liquid scintillation counter were used.

Statistical Methods

Statistical analysis involved the GLM (general linear models), STEPWISE (stepwise regression) and NLIN (non-linear regression) procedures of SAS (1979).

Results and Discussion, Experiment A

As outlined in Methods, Experiment A consisted of several sub-experiments. The experimental variables common to all of the sub-experiments included two soils, two P treatments (carrier-free ^{32}P or ^{32}P plus 40 ppm ^{31}P), four incubation temperatures (10, 15, 20 and 25°C) and in some cases two extraction or growth temperatures. All of the treatments were replicated. The first sub-experiment was to trace the reactions of the applied ^{31}P and ^{32}P with time. For this purpose, chemical extractions (using the specific activity extraction system) were conducted at intervals during the incubation of the P treatments. Once these extractions showed that the soils had reached a quasi-equilibrium, a series of further sub-experiments was conducted. These included $NaHCO_3$ extractions, desorption curve extractions, desorption rate measurements and short term plant uptake measurements. For comparison to the situation prior to incubation, $NaHCO_3$ extractions, desorption curve extractions and short term plant uptake measurements were conducted at zero time (immediately after P treatment). The various sub-experiments will be discussed in the aforementioned order.

The purpose of ^{32}P in Experiment A was twofold. For the unfertilized soils to which carrier-free ^{32}P was applied, the ^{32}P was intended to label the labile pool of soil P and hence increase the detection sensitivity for other measurements. For the fertilized soils where 40 ppm P was added as well as the ^{32}P, the ^{32}P traced the reaction of the fertilizer P. Thus, ^{32}P analysis had a different meaning for each of the two P treatments.

Extractions during the Incubation Period

The reactions of the ^{32}P applied to the soil, especially for the unfertilized soils, involved isotopic exchange of the ^{32}P with soil ^{31}P.

Characteristically, this process proceeds rapidly at first as isotopic equilibrium is attained with the relatively soluble forms of soil ^{31}P. The process slows when the solubility of forms not yet in equilibrium becomes very low and final equilibrium may never be achieved in a soil system. However, during extraction of the soil, the isotopic exchange may be accelerated because the extractant may dissolve some of the less soluble forms of soil ^{31}P which were not yet in equilibrium with the ^{32}P. For this reason, a mild extractant (0.02 M KCl described for the specific activity measurements) was used in this study.

The consequence of using a mild extractant was a low extraction efficiency which, despite a 12.5 fold concentrating step (see Appendix F), did not yield measurable quantities of ^{31}P from the unfertilized soils. The activity of ^{32}P at the end of the incubation of the unfertilized soils was also below detection limits.

The decrease in extractable ^{32}P over 26 days for the unfertilized soils (Figure 10) followed the expected trend. The largest change and greatest variability for both soils occurred between zero and five days, after which the decrease was more gradual. The Elm River soil retained consistently more ^{32}P than the Almasippi soil even at time zero. Since the same amount of ^{32}P was applied in each case, this indicated that the differential between the soils was established during the first 24 hours. These data suggest that although the ^{32}P continued to react with the soil, an incubation time of five days was adequate for carrier-free labelling of soil P.

Isotopic exchange in a simple system (for example with only one form of ^{31}P) would follow mass-action or first-order reaction kinetics that could be described by:

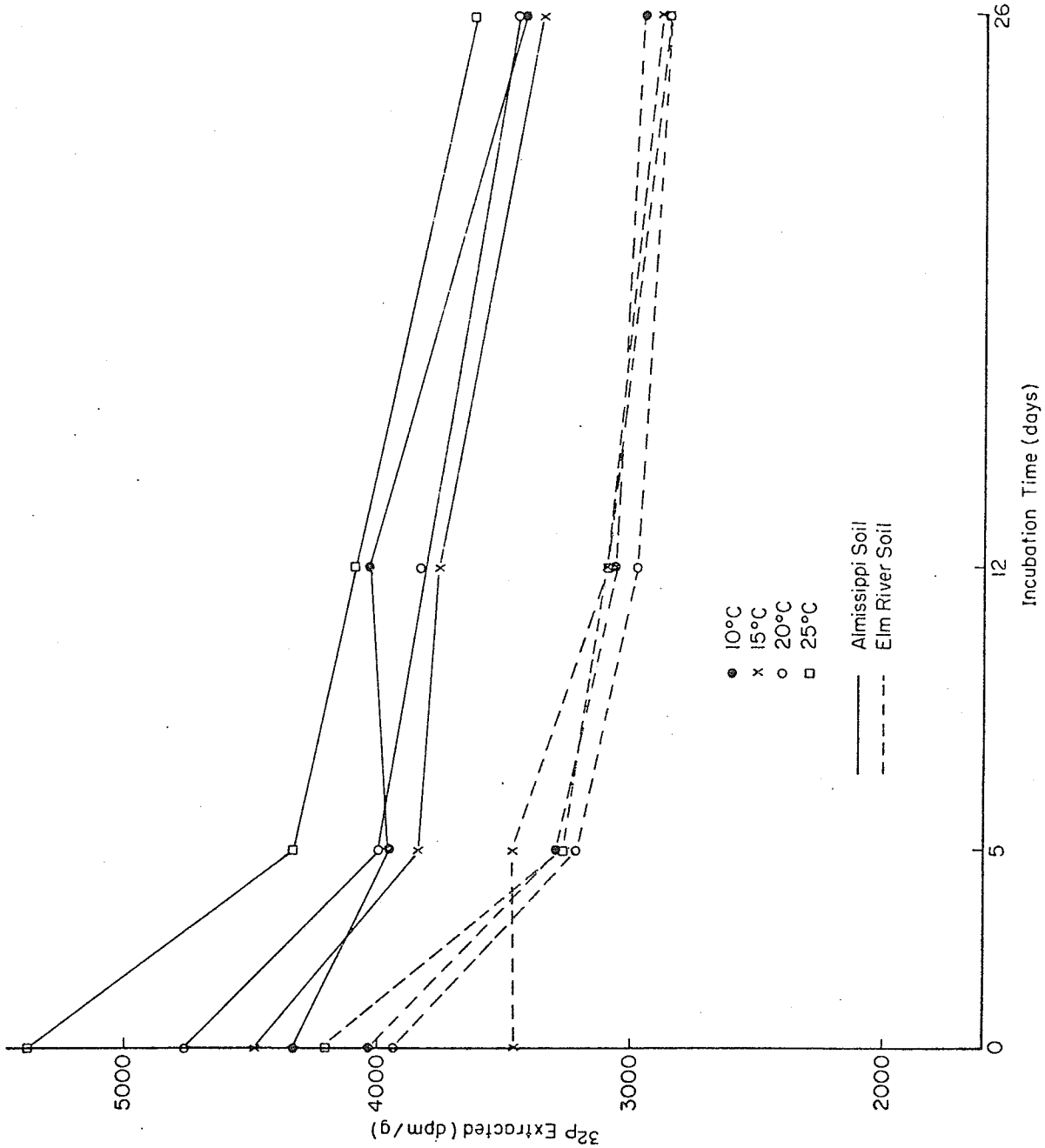

Figure 10: Extractable ^{32}P with incubation time in the unfertilized soils.

$$\log_e (^{32}P \text{ extracted}) = a + b \text{ (reaction time)} \quad [7]$$

Although a soil system is complex and probably involves a series of first order reactions (Weir and Miller, 1962), one for each form of soil ^{31}P, equation (7) was used as an approximation to describe the present data (Table 8).

TABLE 8

Parameters of the Reaction Rate Curves for ^{32}P in the Unfertilized Soils, Described by the Function Log_e (^{32}P Extracted) = a + b (Reaction Time)

Soil	Parameter	Temperature			
		10	15	20	25°C
Almasippi	a(dpm/g)	4360 a	4320 a	4540 a	5010 b
	$b(day^{-1})$	-0.0085 a	-0.0098 a	-0.0109 a	-0.0132 a
	r^2(%)	90	64	96	62
Elm River	a(dpm/g)	3750 a	3500 a	3640 a	3830 a
	$b(day^{-1})$	-0.0105 a	-0.0076 a	-0.0108 a	-0.0125 a
	r^2(%)	68	43	65	67

- Parameters Followed by the Same Letter Within a Row were not Significantly Different ($P < 0.05$) by t test.

The fit of the first-order model to the data was variable with r^2 values varying from 43 to 96%. The residuals showed a trend which suggested that a multiple first-order model may have described the data more closely. However, there were too few sampling times in this experiment to pursue a more complex model.

The equation intercepts for the Almasippi soil increased with temperature, indicating that at time zero, a higher extraction temperature solubilized more ^{32}P. This was counterbalanced by steeper regression slopes such that at 26 days, there was little effect of temperature. The steeper regression slopes at higher temperatures, although not significant, suggest that there was a more rapid isotopic exchange rate at higher temperatures, as shown by Arambarri and Talibudeen (1959). Thus, temperature had opposing effects on ^{32}P solubility, firstly to increase P solubility but secondly to increase the rate of reaction to less soluble forms. Comparable results were found by Barrow (1979b). There were no significant effects of temperature in the Elm River soil although, very generally, the trends noted for the Almasippi soil were present.

The ^{32}P in the fertilized soils was intended to trace the fixation of the fertilizer ^{31}P. Decreases in both extractable ^{32}P and ^{31}P were quite parallel between soils and among temperatures (Figures 11 and 12, soils averaged). The Elm River soil again retained more P than the Almasippi soil. These data were also described using the $\log_e$-linear relationship of equation (7) (Table 9). The fit of the equations was very good (r^2 values of 92 to 97%) but the trend in the residuals again indicated that a more complex model may have been more appropriate.

The regression intercepts decreased as temperature increased although significantly only for the ^{31}P data (analysis of variance of extractable ^{32}P at time = 0 also showed a significant decrease). This suggested that effects on the rate of fertilizer P fixation predominated over effects on P solubility. However, the rates of fixation reactions from the first extraction to the end of the incubation, indicated by the regression coefficients, were affected only slightly by temperature. Thus, the temperature dependent

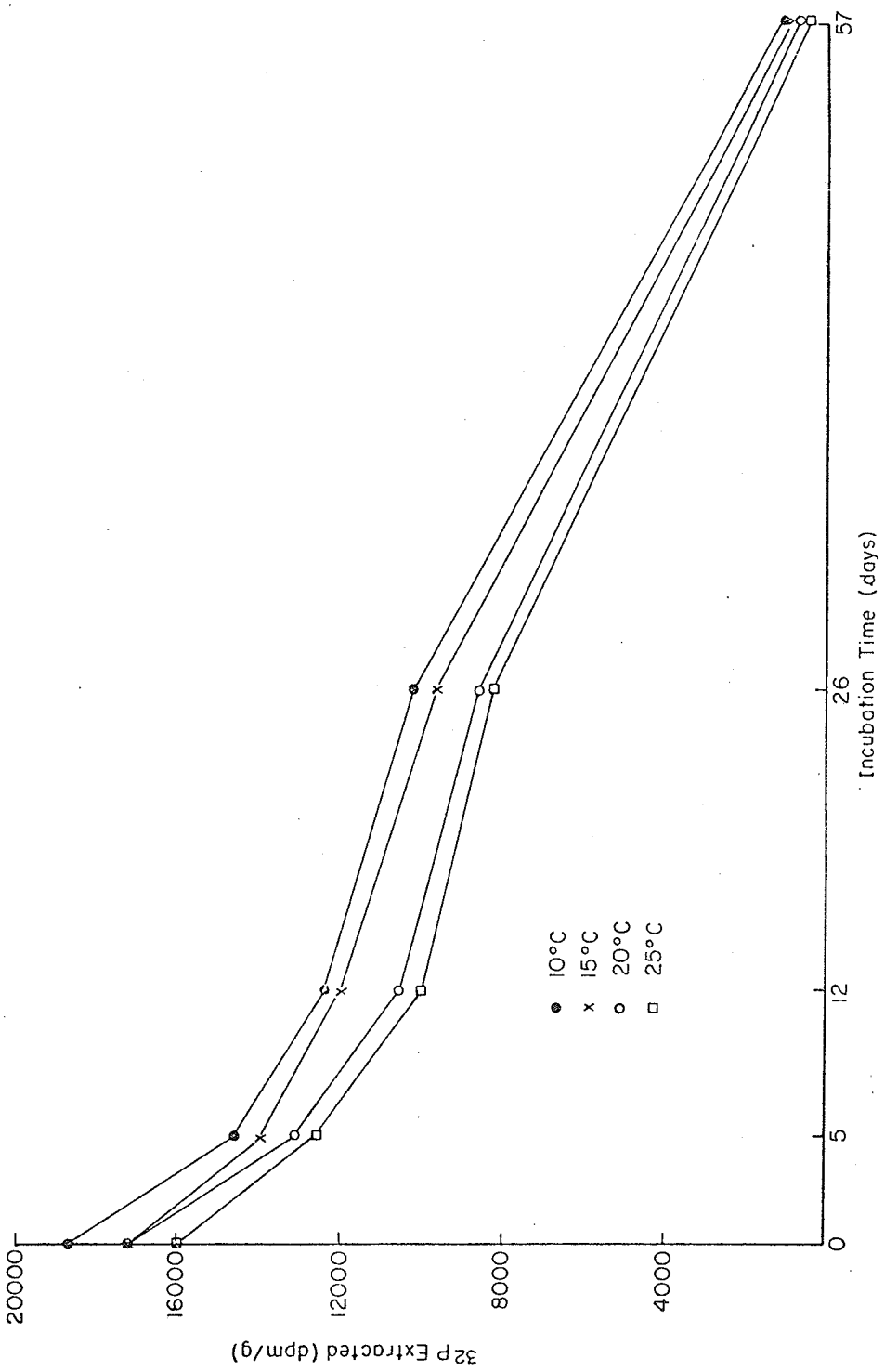

Figure 11: Extractable ^{32}P with incubation time in the fertilized soils, mean of two soils.

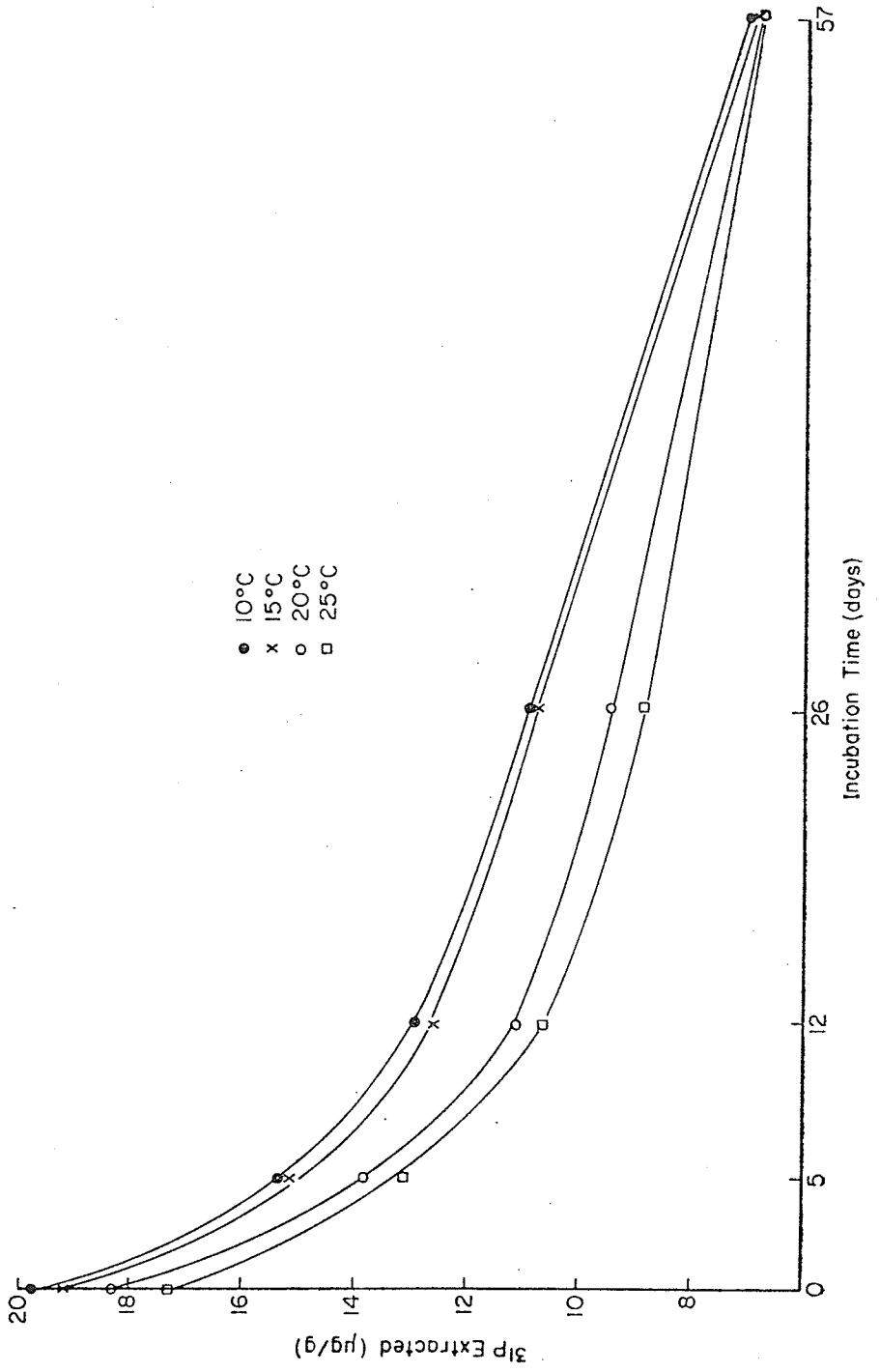

Figure 12: Extractable ^{31}P with incubation time in the fertilized soils, mean of two soils.

TABLE 9

Parameters of the Reaction Rate Curves for ^{32}P and ^{31}P in the Fertilized Soils, Described by the Function Log_e (P Extracted) = a + b (Reaction Time)

Soil and Isotope	Parameter	Temperature 10		15		20		25°C	
Almasippi	a(dpm/g)	22200	a	22000	a	21800	a	19300	a
^{32}P	b(day^{-1})	-0.0463	a	-0.0473	a	-0.0484	a	-0.0474	a
	r^2(%)	93		92		94		93	
Elm River	a(dpm/g)	20300	a	19300	a	17200	a	17300	a
^{32}P	b(day^{-1})	-0.0477	a	-0.0485	a	-0.0505	a	-0.0519	a
	r^2(%)	93		93		94		95	
Almasippi	a(µg/g)	20.7	b	20.9	b	19.7	b	17.8	a
^{31}P	b(day^{-1})	-0.0279	a	-0.0283	a	-0.0275	a	-0.0255	a
	r^2(%)	97		96		97		97	
Elm River	a(µg/g)	17.1	b	16.3	b	14.0	a	13.9	a
^{31}P	b(day^{-1})	-0.0259	a	-0.0253	a	-0.0248	a	-0.0257	a
	r^2(%)	97		97		96		97	

- Parameters Followed by the Same Letter Within a Row were not Significantly Different ($P \leq .05$) by t-test.

phase of the fertilizer P fixation must have occurred during the first 24 hours of extraction. Over this 24-hour period 50 to 60% of the applied ^{31}P was retained by the soils in forms that were not extracted. Similar results were reported by Gardner and Preston Jones (1973).

The regression coefficients from this model had the units day^{-1} and described the rate of reaction regardless of the variable measured. Thus, the coefficients for the ^{32}P and ^{31}P data were comparable. The reaction rate of ^{32}P exceeded that of ^{31}P and thus ^{32}P was not an accurate tracer of the reactions of fertilizer ^{31}P. The higher reaction rate of ^{32}P was probably due to isotopic exchange of the ^{32}P with soil ^{31}P, thus implicating a second, non-reversible reaction to accelerate the loss of extractable ^{32}P. Cho et al. (1970) also found ^{32}P to be an unsuitable tracer for P diffusion studies because of the concurrent isotopic exchange with soil ^{31}P.

Isotopic exchange with soil ^{31}P was confirmed by the observation that specific activities decreased with time from 898 and 963 dpm $^{32}P/\mu g$ ^{31}P at the start of the incubation for the Almasippi and Elm River soils respectively to 249 dpm $^{32}P/\mu g$ ^{31}P at the end of the incubation for both soils. The variability in the specific activities was also greatest at time = 0 but stabilized after 12 days.

In summary, temperature increased the solubility of P but also increased the rate of reaction of recently applied P toward less soluble forms. Thus, opposing effects of temperature were operative and the net effect depended on the reaction system. For example, in the unfertilized soils, the effect of temperature on solubility was predominant whereas in the fertilized soils, the effect of temperature on the rate of P fixation was predominant.

The reactions of both ^{32}P and ^{31}P continued throughout the incubation

period. However, it was concluded that five days was a useful minimum incubation period to achieve some stability in the process of labelling soil ^{31}P with carrier-free ^{32}P. Indeed, many of the reactions of both ^{32}P and fertilizer ^{31}P occurred during the first 24 hours.

Finally, the reaction rate of ^{32}P exceeded that of ^{31}P in the fertilized soils and hence isotopic dilution with soil ^{31}P interfered with the use of ^{32}P as a tracer of fertilizer ^{31}P reactions.

Sodium Bicarbonate Extractable P

The $NaHCO_3$ extraction system clearly differentiated the two soils throughout the incubation period whether fertilized or unfertilized. The Elm River soil had 11.0 µg extractable ^{31}P/g when unfertilized and 41.5 µg ^{31}P/g when fertilized compared to 14.7 and 48.3 µg ^{31}P/g, respectively, for the Almasippi soil. However, the two soils generally responded similarly to the experimental treatments.

There was no significant effect of temperature in the fertilized soils on the amount of extractable ^{31}P (Table 10) whereas with the KCl extraction of the same soils (Table 9) there were decreases in extractable ^{31}P with increasing temperature. As suggested previously, a higher temperature would have increased the solubility of P but would have also increased the rate of fertilizer P fixation. Thus, in the $NaHCO_3$ extraction system these processes appeared to have been counterbalanced whereas in the KCl extraction system, the effect of temperature on the fixation processes predominated. The explanation of this discrepancy may lie in the widely differing efficacies of the extractants. For example, at the end of the incubation period, the KCl system extracted only 4.3 µg ^{31}P/g soil. Therefore, it was concluded that the $NaHCO_3$ system, which recovered a relatively large pro-

TABLE 10

$NaHCO_3$ Extractable ^{31}P at the Start and End of the Incubation Period, Showing the Effect of Temperature and P Fertilization[1]

Temperature	Fertilized Soil		Unfertilized Soil	
	Start of Incubation	End of Incubation	Start of Incubation	End of Incubation
°C		µgP/g Soil		
10	48.4 a	42.3 a	11.7 a	11.1 a
15	47.9 a	42.1 a	11.9 a	12.3 a
20	46.9 a	41.3 a	13.1 a	12.9 ab
25	49.9 a	43.1 a	14.4 a	15.4 b

1 Values are Means of 2 Soils and 2 Replicates

- Means Within a Column Followed by the Same Letter were not Significantly Different ($P \leq .05$), Tested by Single Degree of Freedom Contrasts

portion of the applied ^{31}P even at the end of the incubation period (73%), was not as sensitive to subtle changes in P solubility which were probably manifest in the most soluble forms of soil P.

There was also no effect of temperature on the extraction of ^{32}P from the fertilized soils and this was taken as further evidence of the high extraction efficiency and hence lack of sensitivity of the $NaHCO_3$ extraction system.

There was a significant decrease in the amount of extractable ^{31}P and ^{32}P from the fertilized soils with incubation time (Table 11). This was attributed to the fixation of the fertilizer P to forms not extracted by $NaHCO_3$. However, this decrease (1.1 fold) was relatively small compared to that in the KCl extraction system (4.3 fold, from 18.6 to 4.3 μg ^{31}P/g) and this was again attributed to the insensitivity of the $NaHCO_3$ system to changes in the most soluble forms of soil P.

The recovery of ^{32}P was calculated as a percentage of the 5.33×10^4 dpm ^{32}P/g dry soil that was applied and decreased markedly with time, particularly in the Elm River soil (Table 11). The recovery of fertilizer ^{31}P was calculated as the difference in extractable ^{31}P between the fertilized and unfertilized soils taken as a percentage of the 40 μg ^{31}P/g of fertilizer ^{31}P applied (Table 11). These recovery percentages were greater than those based on ^{32}P, especially at the end of the incubation, and this discrepancy was attributed to isotopic dilution of the ^{32}P with soil ^{31}P of forms not extracted by $NaHCO_3$. This process would have been more pronounced with time. Thus, the previous suggestion that ^{32}P was not an accurate tracer of fertilizer ^{31}P reactions was confirmed.

The amount of soil ^{31}P which was in isotopic equilibrium with the applied ^{32}P (i.e. the labile pool of soil ^{31}P) was calculated using:

TABLE 11

The Effect of Incubation, Soil and Fertilization on the Extractability of ^{31}P and ^{32}P Using the $NaHCO_3$ Extraction System (averaging the effects of temperature)

Soil	Fertilized Soil		Unfertilized Soil	
	Start	End	Start	End
	Extractable ^{31}P (µg P/g soil)			
Almasippi	53.6	44.3	14.5	14.9
Elm River	42.9	40.2	11.1	11.0
	Extractable ^{32}P (dpm/g soil)[1]			
Almasippi	4.77×10^4	3.18×10^4	4.36×10^4	2.62×10^4
Elm River	3.83×10^4	3.29×10^4	3.85×10^4	2.12×10^4
	Recovery of ^{32}P applied (%)[2]			
Almasippi	89.6	59.7	81.9	49.1
Elm River	71.8	61.7	72.3	39.8
	Recovery of ^{31}P applied (%)[2]			
Almasippi	97.8	73.5	-	-
Elm River	79.5	73.0	-	-
	Labile pool (µg ^{31}P/g soil)[2]			
Almasippi	20.3	34.7	17.8	30.4
Elm River	20.3	26.9	15.3	27.5

1. Corrected for 1/2 life to the day the P treatments were applied.
2. Computation described in text
- More complete data presented in Appendix H.

$$\frac{\text{extracted } {}^{32}P}{\text{extracted (fertilizer + soil) } {}^{31}P} = \frac{\text{applied } {}^{32}P}{\text{applied fertilizer } {}^{31}P + \text{soil labile } {}^{31}P} \quad [8]$$

The labile pool increased from 20.3 to 30.6 μg ^{31}P/g soil throughout the incubation period which indicated that a substantial amount of exchange with soil ^{31}P had occurred.

There was a consistent increase in the amount of ^{31}P extracted as temperature increased in the unfertilized soils although this trend was statistically significant only for the data at the end of the incubation period. This finding conformed to the finding of the KCl extraction system in that P solubility increased with increasing temperature. There was no significant effect of temperature on the amount of extractable ^{32}P in the unfertilized soils.

The specific activities of the unfertilized soil extractions at the end of the incubation decreased with increasing temperature as a result of the different responses of ^{31}P and ^{32}P to temperature. This implied that after some equilibration time and at higher temperatures, isotopic exchange was more advanced and thus there was a larger labile pool. At 10, 15, 20 and 25°C, the labile pools at the end of the incubation period were 25.6, 26.8, 31.7 and 32.9 μg P/g soil. This finding conformed to expectation since isotopic dilution in soil is usually a long term reaction and was probably rate-limited during this incubation. Thus, increased temperature would have accelerated the process. A similar trend was not clearly established in the KCl system although the specific activities of the 10°C soils were consistently higher at the end of the incubation period.

The incubation period had no effect on the amount of extractable ^{31}P in the unfertilized soils but, since the soils had been incubated at their respective temperatures 22 to 30 days before the ^{32}P incubation period

began, any changes in the ^{31}P extractability due to moist incubation probably occurred prior to the first extraction. However, the amount of extractable ^{32}P decreased markedly during the incubation (Table 11). The recovery of the applied ^{32}P decreased from 77% to 44% during the incubation period and this was attributed to isotopic dilution with forms of soil ^{31}P which were not extracted. The labile pool in the unfertilized soils (calculated with equation (8) substituting zero fertilizer ^{31}P) changed from 16.5 to 28.8 μg P/g soil during the incubation period. These values are very similar to those calculated for the fertilized soils and thus suggest that the labile pool was not affected by fertilizer ^{31}P application.

In summary, the extractions of ^{31}P and ^{32}P using the $NaHCO_3$ system were useful for demonstrating: (1) fertilizer P fixation, (2) isotopic dilution of ^{32}P with soil ^{31}P with and without the addition of fertilizer ^{31}P and (3) that the two soils differed in P extractability. This system showed effects of temperature in the unfertilized soils but appeared to be less sensitive than the KCl extraction system to some changes in solubility due to temperature and time. It was concluded that the high extraction efficiency of the $NaHCO_3$ system resulted in total removal or masking of the most soluble forms of P but that these were the forms which were modified by temperature and reaction time.

Desorption Curves

The desorption curves offered the advantage of not only describing the solubility of P but also the solubility profile or the amount of P at decreasing solubilities (see Choice of Methods section for further description). The statistical model $\log_e$ (equilibrium P concentration) = a + b (P desorbed per g soil) described the ^{32}P desorption from fertilized soils

well with r^2 values from 69 to 98% (multiple regressions presented in Table 34, Appendix H). The ^{31}P desorbed from the fertilized soils was close to the analytical detection limits and thus not all of the data yielded statistically significant regressions (the r^2 values ranged from 36 to 91%). Similarly, the ^{32}P desorbed from the unfertilized soils was low with few significant regressions. The r^2 values ranged from 0 to 91%.

The desorption curve intercepts (Table 12) which were interpreted as the solubility of the most soluble form of P in the soil generally confirmed the effects on P solubilities shown by the $NaHCO_3$ and KCl (specific activity) extraction systems. More ^{32}P was soluble in the Almasippi soil than in the Elm River soil and the ^{32}P solubility decreased with time in both soils, whether fertilized or unfertilized. Higher temperature decreased the ^{32}P solubility in the fertilized soils, a trend which was most clearly established at the end of the incubation period. The effect of temperature in the unfertilized soils was less clear although in the Almasippi soil at the start of the incubation, ^{32}P solubility increased with increasing temperature, as shown previously by the $NaHCO_3$ extractions. The ^{31}P solubilities were inconsistent with the ^{32}P results probably because most of the regressions were not statistically significant (indicated in Table 13).

The desorption curve slopes (Table 13) for the fertilized soils were markedly steeper at the end of the incubation period than at the start. Thus, the solution P concentrations were more buffered at the start of the incubation such that a large quantity of P had to be desorbed to deplete the more soluble forms of P in the soil. This finding was expected since the fertilizer ^{31}P plus ^{32}P applied at the start would have undergone only superficial fixation reactions and therefore was present in relatively soluble forms. Recoveries of the fertilizer P of up to 75% at high solu-

TABLE 12

Antilog$_e$ of the Regression Intercepts[1] of Desorption Curves

Soil and Isotope	Start of Incubation 10°C	15°C	20°C	25°C	End of Incubation 10°C	15°C	20°C	25°C
Fertilized Soils								
Almasippi ^{32}P	5340	3750	3060	4730 NS[2]	1820	1410	1340	1240 *
Elm River ^{32}P	4060	3410	3080	2200 *	1130	830	610	520 *
Almasippi ^{31}P	1.51	3.18	1.00	3.42 NS	2.56	0.14	0.21	0.22 NS
Elm River ^{31}P	1.89	2.29	2.10	2.21 NS	0.04	0.05	0.12	0.43 *
Unfertilized Soils								
Almasippi ^{32}P	355	406	394	507 *	179	226	204	191 NS
Elm River ^{32}P	309	276	255	252 NS	88	80	-	- NS

1. ^{32}P results as dpm/mL, corrected for decay to the day the P treatments were added; ^{31}P results as µg/mL.

2. Test of the coefficient b_2 in the multiple linear regression $\log_e(\text{P concentration}) = a + b_1(\text{P desorbed}) + b_2(\text{temperature}) + b_3(\text{P desorbed})(\text{temperature})$.

NS - not significant, * - significant at $P \leq 0.05$.

TABLE 13

Regression Coefficients[1] of Desorption Curves

Soil and Isotope	Start of Incubation					End of Incubation				
	10°C	15°C	20°C	25°C		10°C	15°C	20°C	25°C	
Fertilized Soils										
Almasippi ^{32}P ($x10^{-5}$)	-6.17	-5.27	-4.33	-7.50	NS[2]	-10.3	-8.87	-9.55	-10.6	NS
Elm River ^{32}P ($x10^{-5}$)	-5.29	-5.62	-5.47	-4.45	NS	-8.78	-6.79	-5.87	-5.61	*
Almasippi ^{31}P	<u>-0.038</u>	-0.067	<u>-0.017</u>	-0.72	†	-0.220	<u>+0.120</u>	<u>+0.029</u>	<u>+0.044</u>	†
Elm River ^{31}P	<u>-0.061</u>	-0.070	-0.067	-0.054	†	<u>+0.343</u>	<u>+0.229</u>	<u>-0.013</u>	<u>-0.139</u>	†
Unfertilized Soils										
Almasippi ^{32}P ($x10^{-5}$)	-3.24	-5.35	-3.80	-4.60	NS	-3.62	-22.3	-10.6	<u>-0.45</u>	†
Elm River ^{32}P ($x10^{-5}$)	-2.50	<u>-0.73</u>	-1.60	<u>-0.51</u>	†	<u>-0.40</u>	<u>+1.82</u>	-	-	†

1. ^{32}P results as g/dpm, corrected for decay to the day the P treatments were added, ^{31}P results as g/µg.

2. Test of the coefficient b_3 (see footnote, Table 12).

NS - not significant, * - significant at $P \leq 0.05$, † - test not completed because some individual coefficients (underlined) were not significantly different from zero ($P \leq 0.05$).

tion to soil ratios confirmed that the applied P was highly soluble at the start of the incubation. By the end of the incubation period, the fertilizer P had reacted to form less soluble materials. However, the fertilizer P would not have reacted to form one single product but would have formed a series of reaction products. These various products would have different solubilities and would tend toward a profile of P solubilities parallel to those indigenous to the soil. Some of the fertilizer P would remain relatively soluble whereas some would become quite insoluble. Thus the desorption curve slope, which reflects this solubility profile, would become more steep with time.

The desorption curve slopes were not different between the soils at the start of the incubation, probably because they reflected the initial fertilizer reaction products. By the end of the incubation, the fertilized Almasippi soil had significantly steeper ^{32}P desorption curve slopes than the Elm River soil. Thus, although the desorption curve intercepts were higher in the Almasippi soil, the curves of the two soils converged such that when approximately 2×10^4 dpm ^{32}P/g soil was desorbed, the solubility of the remaining ^{32}P was the same in both soils.

The desorption curve slopes of ^{32}P for the fertilized soils at the start of the incubation were steeper than those from the unfertilized soils. Similar results were obtained from the results of Sharpley et al. (1981) (when their data of Figure 2 was plotted into the desorption curve format). This probably reflects the concept that fertilizer P enhances the amount of more-soluble P in the soil with less immediate effect on the less soluble forms.

Temperature had little consistent effect on the desorption curve slopes. The only significant effect of increasing temperature was the less

steep desorption curve slope observed in the fertilized Elm River soil at the end of the incubation period. This trend was the inverse of the effect on the intercept and thus the curves converged (the 10 and 25°C curves intersected when 2.45×10^4 dpm ^{32}P/g soil was desorbed). The less steep slope at the higher temperatures implied that the P buffer capacity was higher at higher temperatures. It was concluded that at higher temperatures, the tendency toward increased P solubility resulted in relatively more ^{32}P remaining in the more soluble forms. Thus, although higher temperature increased the rate of fertilizer P fixation (and hence lowered the desorption curve intercept) the final reaction products were generally more soluble. This conformed to the balance of reaction rates and P solubilities proposed by Barrow (1979b). Because more of the applied ^{32}P was present in more soluble forms, the equilibrium solution P concentration remained higher when a set amount of P was desorbed and therefore the desorption curve slope was less steep. These results would suggest that the constant desorption curve slope term used by Barrow (1979 a and b) was probably appropriate when the incubation of fertilizer P was conducted under constant conditions but may not be appropriate when incubation conditions vary.

In summary the desorption curve intercepts confirmed previous conclusions about the effects of time, soil and temperature on P solubility. At higher temperatures, fertilizer P was less soluble due to the increased rate of fixation and soil P was more soluble due to effects on solubility. The desorption curve slopes added a new dimension to the data by describing the solubility profile of P in the soil. Incubation time markedly decreased the P buffer capacity because, with time, the fertilizer P reacted to form a series of materials with a solubility profile comparable to the

indigenous soil P. Temperature did not consistently modify the desorption curve slope but, in at least one case, the slopes were less steep at higher temperatures. This was interpreted to indicate a shift in the solubility profile such that more P remained in the more soluble forms at higher temperatures.

Desorption With Extraction Time

Desorption of P from soil is seldom at equilibrium and more typically involves a progressive desorption with time. A preliminary experiment (Appendix F) showed continued desorption up to 96 hours using the $NaHCO_3$ extraction system. This process was unaltered by the addition of toluene or by periodic aeration during the extraction process. Thus, microbial and anaerobic processes were discounted and it was concluded that a very slow P desorption process was operative. Since temperature may affect reaction rates as much or more than overall solubility, the desorption process was examined as a function of both time and temperature. This was done using a partial factorial of incubation and extraction temperatures.

The amount of ^{32}P desorbed from the fertilized soils increased rapidly for the first three to ten hours followed by slower desorption up to 48 hours. Similar results were reviewed by Chien and Clayton (1980). The curves were statistically described using the following models:

The desorption curve data was also described by the model $\log_e$ (P desorbed) = a + b $\log_e$ (solution:soil ratio) (Table 35, Appendix H) after Sharpley et al. (1981). The r^2 values ranged from 84 to 99% for the ^{32}P data although there was a consistent trend in the residuals. This model was not discussed in detail because it is less applicable to P nutrition studies.

(A) a segmented model which related solution P concentration (PC) to time (t)

$$PC = a+b(t) + c(t)^2 \quad \text{up to } t = time^o$$

when $PC = d + e(t)$

(B) a log-log model (Sharpley et al., 1981) which related the amount of P desorbed per unit of soil (PD) to time

$$\log_e (PD) = f + g \log_e (t)$$

and

(C) a diffusion-desorption model (Wiles, D.R. personal communication) which related solution P concentration to time.

$$PC = h(t)^{\frac{1}{2}} + i\ (1-e^{j(t)})$$

The R^2 values were the highest for the segmented model (ranging from 99.6 to 100.0%) and therefore the predictions of this model are presented (Figures 13 and 14) and the coefficients discussed in detail. The R^2 values ranged from 51.0 to 96.0% for the log-log model and from 97.1 to 99.9% for the diffusion-desorption model. The latter model was less stable for the iterative convergence scheme, particularly for the coefficient j. This was probably due to the direct competition between the coefficients in that both h and i plus j described the same portions of the desorption rate curves. The coefficients are presented in Tables 37 to 39, Appendix H.

The segmented model was most useful for a description of the data. The intercept (a) represented the ^{32}P concentration at time zero and varied significantly due to temperature. It indicated that more ^{32}P was soluble as extraction temperature increased and less was soluble as incubation temperature increased. When extraction was conducted at the incubation temperature, less ^{32}P was soluble suggesting that the effect of incubation temperature was predominant The significant effect of extraction temper-

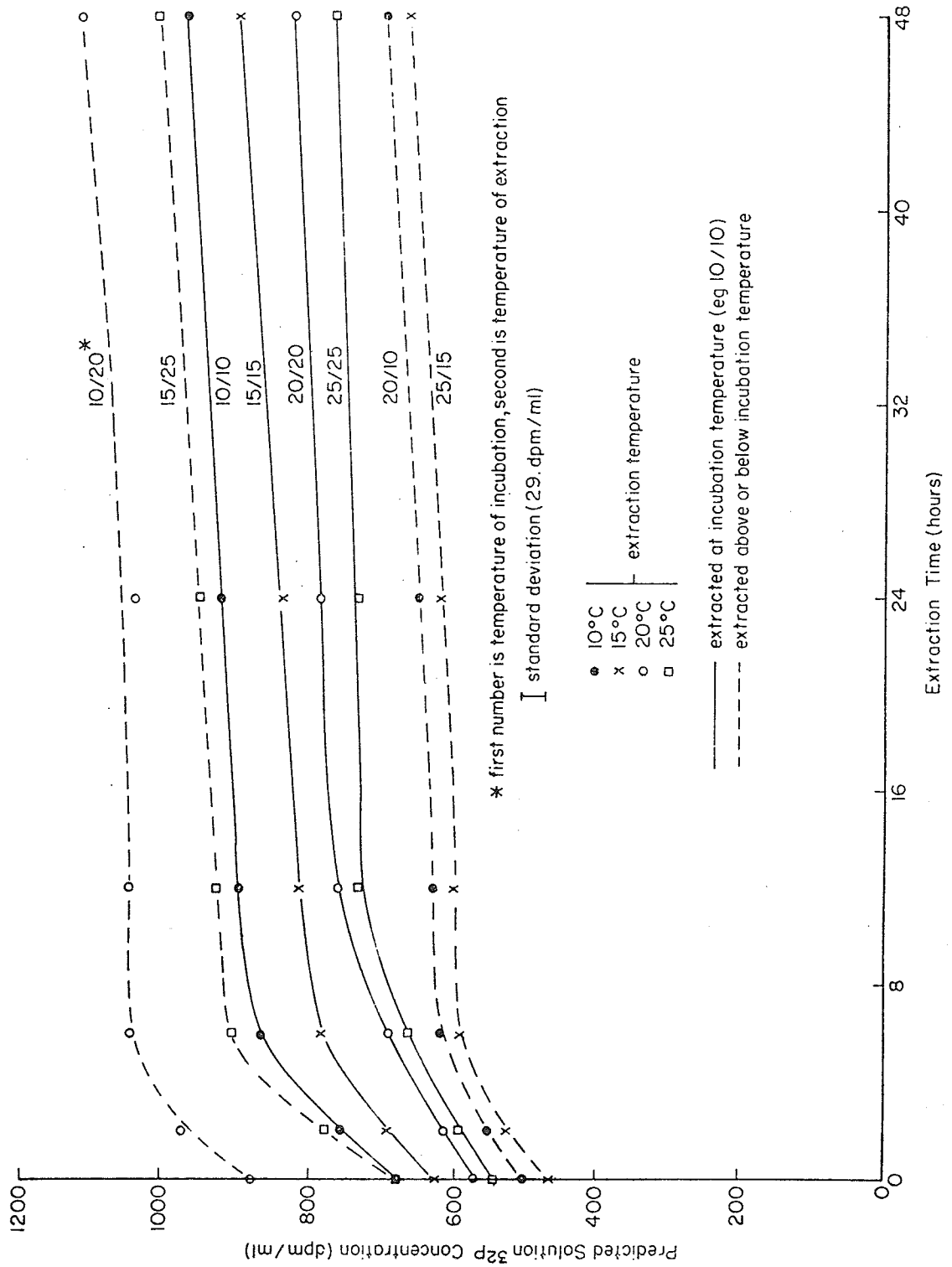

Figure 13: Fitted curves of ^{32}P concentration with extraction time in the fertilized Almasippi soil.

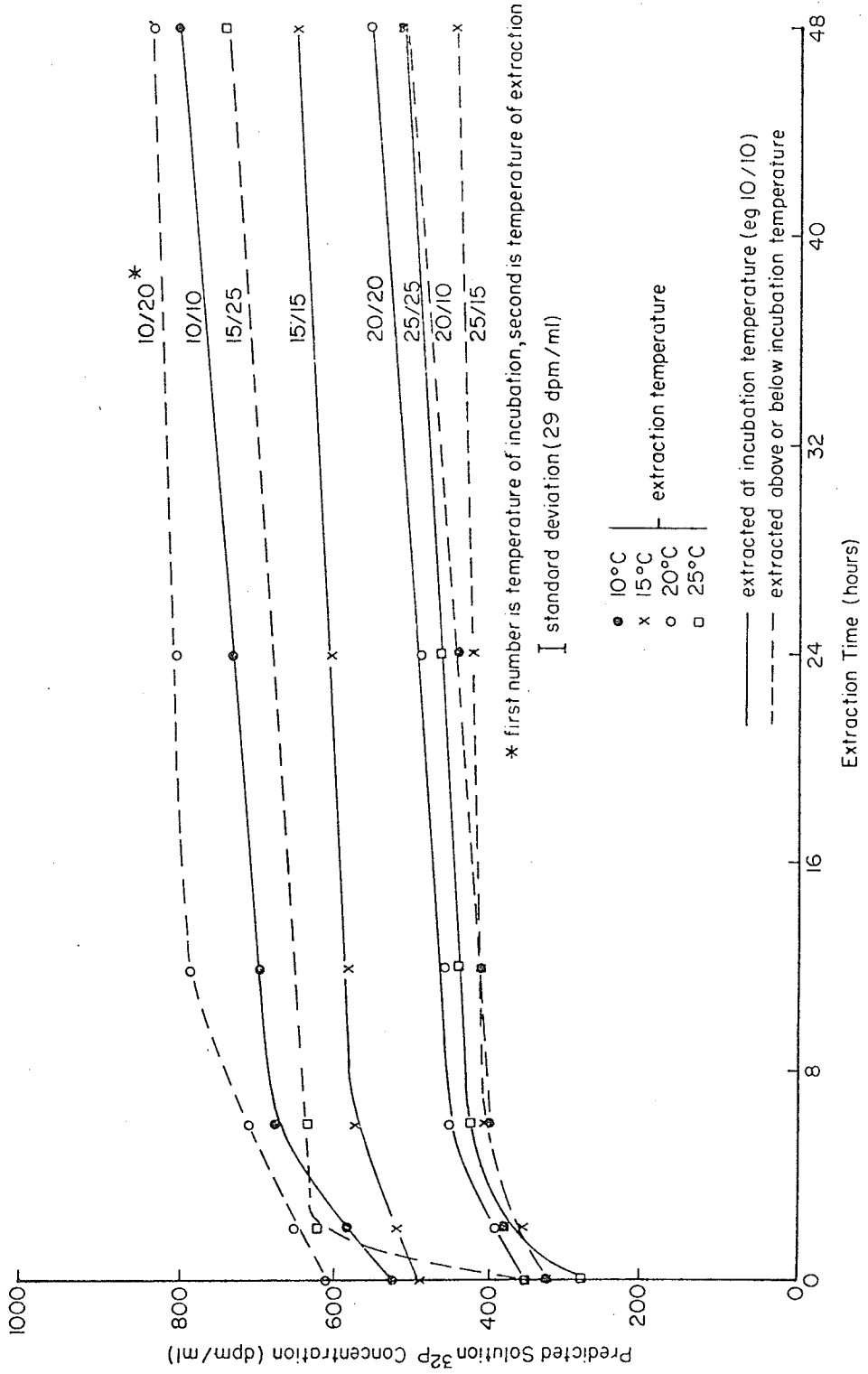

Figure 14: Fitted curves of ^{32}P concentration with extraction time in the fertilized Elm River soil.

ature at time zero emphasized that the effect of temperature was established within one hour of extraction. Thus, desorption rates during this first hour may have been temperature dependent.

The coefficients b and c defined the slope of the rapid desorption phase and thus indicated the rate of desorption. Although both coefficients were generally significantly different from zero (indicating that desorption increased with time), neither coefficient varied consistently with temperature. Thus, desorption rates in the rapid desorption phase must have been limited by a temperature-independent process. Physical dispersion of the soil would be an example of such a process.

The coefficient e defined the slope of the slower, long-term desorption phase. This slope was substantially less than the rapid desorption phase but, in general, the coefficient e was significantly different from zero. Thus, desorption continued during this phase. The coefficient e was not consistently modified by temperature.

The join-point of the two portions of the segmented model ($time^{o}$, see Methods) can be interpreted as an indicator of the transition between the rapid and slower desorption reactions. The join-points were not consistently different among temperatures for either soil. The average join-point for the Almasippi soil was 22.3 hours and for the Elm River soil was 15.2 hours. Since the desorption rates were not different, it was concluded that the difference in join-point between the soils was due to the lesser total amounts of ^{32}P desorbed from the Elm River soil.

The log-log model described the data with the fewest coefficients. The coefficient f represented the amount of ^{32}P desorbed after one hour and followed the trends discussed for coefficient a. The coefficient g represented the overall slope but, as with the coefficients b, c and e,

did not vary consistently with temperature.

The diffusion-desorption model implied the simultaneous operation of two desorption mechanisms. The coefficient j can be interpreted as the rate constant of a first-order desorption reaction. As such, the half life of the reaction can be computed (half life = -0.693/j). The mean half life was 0.25 hours, confirming that the initial reaction rate was very fast. The coefficient h could be interpreted as the amount of soil P subject to diffusion-limited desorption and the coefficient i could be interpreted as the amount of soil P subject to rapid first-order desorption reactions. Both coefficients (but especially i) increased significantly with increases in incubation temperature.

The ^{31}P data for the fertilized soils was more variable (CV of 22.4% compared to 6.2% for the ^{32}P data) and thus the curve fitting technique for the segmented model did not converge in all cases. The log-log model yielded low r^2 values from 1 to 59%. The intercepts (coefficient f) generally followed the trends of those describing ^{32}P desorption but there were no trends among the slopes (coefficient g). The coefficient j of the diffusion-desorption model could not be accurately determined for the ^{31}P data (due to instability in the iterative convergence) but tended toward values less than -27. Thus, the first-order desorption reaction was assumed to be instantaneous and the model was reduced to:

(D) P concentration = $h\ (t)^{\frac{1}{2}} + i$

This model was also proposed by Cooke and Larson (1966). The r^2 values for this abbreviated model ranged from 81.7 to 99.3%. The coefficient h was unaffected by temperature and was not consistently different from zero (mean 0.03 µg/mL). However, the coefficient i was significantly affected by temperature following the same trends as for the ^{32}P data (mean 0.23 µg/mL).

Thus, the desorption versus time curves for the ^{31}P data demonstrated a curvilinear response (means of replicates, soils and temperatures shown in Figure 15) similar to that of the ^{32}P data but the data were more variable.

Since desorption rates were relatively constant, the effects of temperature in the fertilized soils were examined at a single extraction time of 24 hours (Table 14 and Table 40a, Appendix H). In all cases, the ^{32}P and ^{31}P concentration decreased when the extraction plus incubation temperatures increased (the first column in Table 14). Increases in extraction temperature increased the P concentration (contrasting the first and second columns) and increases in incubation temperature decreased the P concentration (contrasting the first and third columns). These trends conformed to previous observations and implied that effects of temperature on P fixation predominated over effects on P solubility when incubation and extraction temperatures were the same.

The amounts of ^{32}P extracted from the unfertilized soils were very small and thus the data were more variable. The r^2 values ranged from 96.3 to 99.6% for the segmented model and 0 to 58% for the log-log model. The coefficient j of the diffusion-desorption model could not be accurately determined and thus the abbreviated model D was used, giving r^2 values of 95.2 to 99.6%. In many cases the coefficients were not significantly different from zero due to the variability of the data. In order to overcome this variability, the data was re-analyzed in an analysis of covariance design using desorption time as the covariate (Table 40b, Appendix H). This effectively averaged the rate curves so that overall temperature effects could be examined (Table 15). The first column in Table 15 shows an inconsistent effect of temperature when extraction was done at the incubation temperature. However, both extraction temperature (contrasting

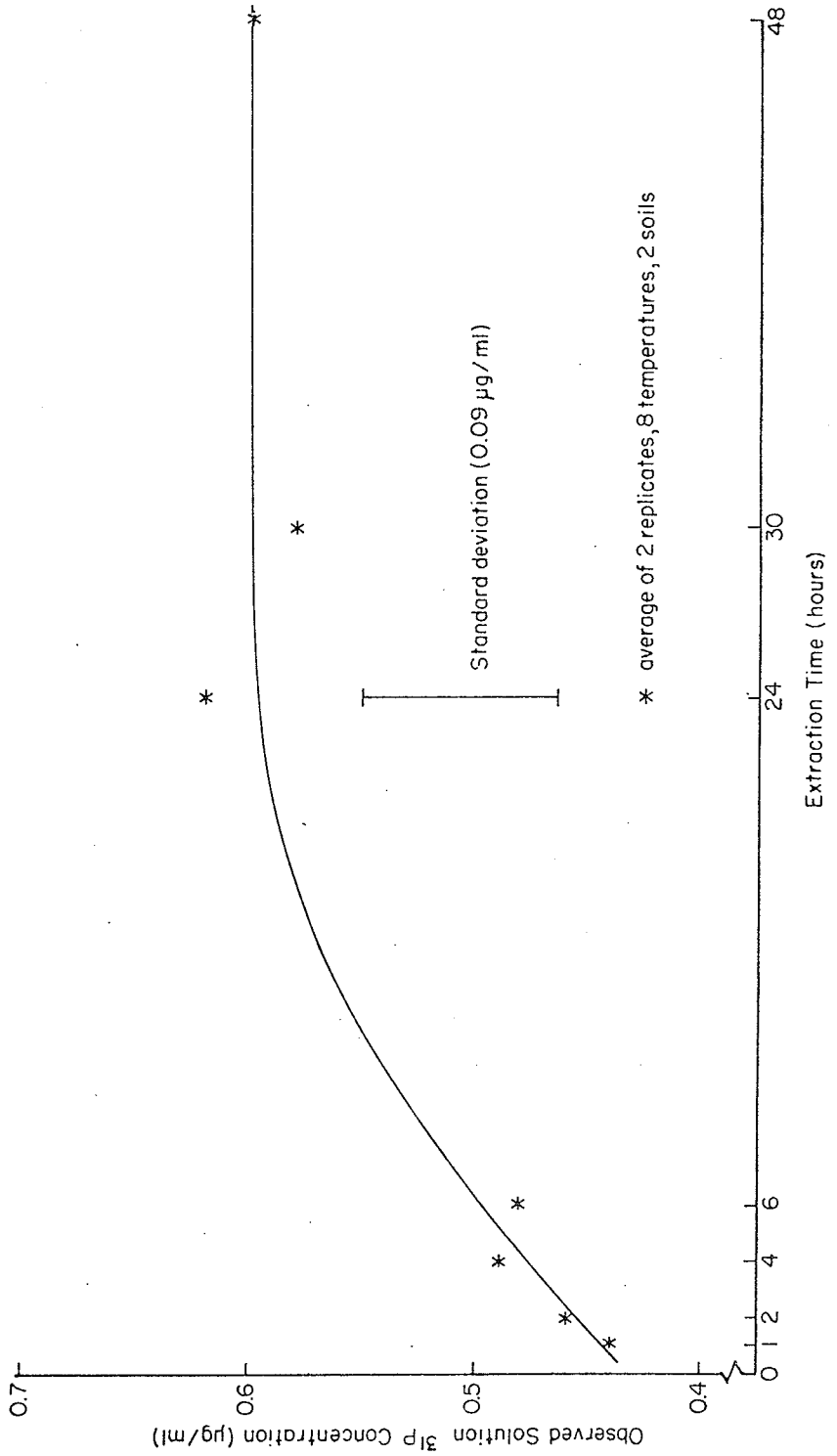

Figure 15: Concentration of ^{31}P with extraction time in the fertilized soils.

TABLE 14

Extractable ^{32}P and ^{31}P from the Fertilized Soils after 24 Hours of Extraction

Soil and Isotope	Extraction at Incubation Temperature		Testing Extraction Temperature		Testing Incubation Temperature	
	Inc/Ext[1]	Extractable P	Inc/Ext	Extractable P	Inc/Ext	Extractable P
Almasippi ^{32}P (dpm/g)[2]	10/10	8120 a[3]	10/20	9310 *[4]	20/10	5610 *[4]
	15/15	7260 ab	15/25	8150	25/15	5290 *
	20/20	6990 b	20/10	5610 *	10/20	9310 *
	25/25	6420 b	25/15	5290 *	15/25	8150 *
Elm River ^{32}P (dpm/g)	10/10	6370 a	10/20	5990	20/10	3590 *
	15/15	5400 ab	15/25	5780	25/15	3640 *
	20/20	4400 bc	20/10	3590	10/20	5990 *
	25/25	4160 c	25/15	3640	15/25	5780 *
2 soils averaged ^{31}P (µg/g)	10/10	5.95 a	10/20	6.41	20/10	4.32 *
	15/15	5.29 ab	15/25	6.62 *	25/15	4.86
	20/20	4.60 b	20/10	4.32	10/20	6.41 *
	25/25	4.47 b	25/15	4.86	15/25	6.62 *

1. Incubation temperature/extraction temperature.

2. Disintegrations per minute adjusted for decay to the day the P treatments were applied.

3. Means followed by the same letter (within the column of 4 values) were not significantly different ($P \leq 0.05$).

4. Means not followed by an asterisk were not significantly different ($P \leq 0.05$) than the mean in the first column on the same row.

TABLE 15

Extractable ^{32}P from the Unfertilized Soils after Adjustment to a Mean Extraction Time Using Independent Desorption Rate Curves[5]

Soil	Extraction at Incubation Temperature		Testing Extraction Temperature		Testing Incubation Temperature	
	Inc/Ext[1]	Extractable P	Inc/Ext	Extractable P	Inc/Ext	Extractable P
		(dpm/g)[2]				
Almasippi	10/10	1340 a[3]	10/20	1560 *[4]	20/10	1070 *[4]
	15/15	1250 a	15/25	1380 *	25/15	1310
	20/20	1280 a	20/10	1070 *	10/20	1560 *
	25/25	1440 b	25/15	1310 *	15/25	1380
Elm River	10/10	740 b	10/20	793	20/10	530 *
	15/15	690 b	15/25	780	25/15	550 *
	20/20	590 a	20/10	530	10/20	793 *
	25/25	660 ab	25/15	550 *	15/25	780 *

5. The values were adjusted by analysis of covariance where the covariate (extraction time) was nested within temperature treatment and soil.

- Other footnotes as in Table 14.

the first and second columns) and incubation temperatures (contrasting the first and third columns) significantly affected P desorption. Thus, in the unfertilized soils, the effects of temperature on solubility and isotopic exchange rate were counterbalanced.

In summary, desorption progressed from 1 to 48 hours but the rates during this time were independent of temperature. The effect of extraction temperature was established within one hour and thus the rates of processes during this time may have been temperature dependent.

The previously speculated effects of incubation temperature (on P fixation rate or ^{32}P isotopic exchange rate) and extraction temperature (on P solubility) were separated by a partial factorial design and shown to be independently significant. In the fertilized soils, the effect of temperature on the rate of P fixation was greater than the converse effect on P solubility. In the unfertilized soils, the effect of temperature on the rate of ^{32}P isotopic exchange was more closely balanced by the effect on P solubility.

Because the desorption rates were quite uniform across all treatments, extraction times standardized at any interval between 1 and 48 hours could be used to characterize P solubilities.

The three models used to describe the desorption versus time data each had useful characteristics. The segmented model was merely descriptive (with no mechanistic basis) but was useful, particularly to test the possibility of continued desorption beyond 24 hours. The log-log model was most easily fitted to the data and involved few coefficients. However, this model did not describe the data as well and the coefficients were difficult to interpret in either a mechanistic or descriptive sense. The diffusion-desorption model had the advantages of a zero intercept (which is

probably theoretically correct) and a mechanistic interpretation. However, the coefficients were strongly interrelated (competitive) such that the iterative convergence scheme was less stable. Interpretation of the coefficients as mechanistic constants must be done with caution because of the interrelationship of the coefficients (i.e. small changes in coefficient h would directly modify coefficients i and j).

Short Term Plant Uptake

The short term plant uptake study was used to directly measure plant available P. The technique involved adjustment of the uptake measurements for root growth which varied with growth temperature (means of 0.11, 0.24, 0.49 and 0.56 g root/20 plants at 10, 15, 20 and 25°C, respectively). Root growth was not affected by other experimental factors and leaf growth was independent of temperature, soil and P treatment. Thus, once the adjustment for root weight was made (Table 41, Appendix H), the uptake measurements were not biased by shoot growth responses to temperature.

The ^{32}P uptake from the fertilized soil (Table 16) was significantly higher, especially at the end of the incubation period, from the Almasippi than from the Elm River soil. The reactions of ^{32}P with the Elm River soil resulted in only 31% of the ^{32}P available at the start of the incubation still being available at the end of the incubation period. The corresponding proportion for the Almasippi soil was 49%. This effect of incubation time was statistically significant.

The effect of temperature was comparable to that observed using the chemical extractions. Uptake increased markedly with temperature when growth occurred at the incubation temperature. Thus, growth temperature effects predominated over incubation temperature effects. This was con-

TABLE 16

Short Term Plant Uptake of ^{32}P from the Fertilized Soils

Start of the Incubation		End of the Incubation					
		Growth at Incubation T		Testing Growth T		Testing Incubation T	
Inc/Grth	^{32}P	Inc/Grth	^{32}P	Inc/Grth	^{32}P	Inc/Grth	^{32}P
			Almasippi Soil (x 10^4)				
10/10[1]	26.7 [2]a[3]	10/10	13.4 a	10/20	50.7 *[4]	20/10	10.2
15/15	56.8 b	15/15	28.4 b	15/25	46.8	25/15	24.4
20/20	115.0 c	20/20	49.7 b	20/10	10.2 *	10/20	50.7
25/25	98.8 bc	25/25	51.2 b	25/15	24.4 *	15/25	46.8
			Elm River Soil (x 10^4)				
10/10	28.6 a	10/10	9.32 a	10/20	43.2 *	20/10	5.53
15/15	53.7 b	15/15	20.6 b	15/25	54.7 *	25/15	17.9
20/20	92.2 bc	20/20	21.2 b	20/10	5.52 *	10/20	43.2 *
25/25	96.8 c	25/25	27.3 b	25/15	17.9	15/25	54.7

1. Incubation temperature/growth temperature.

2. ^{32}P uptake as 10^4 dpm/20 plants adjusted by covariance to a common root weight and corrected for decay to the day the P was added to the soils, analysis of variance was conducted on Log_e transformed data.

3. Means within a column of 4 followed by the same letter were not significantly different ($P \leq 0.05$).

4. Means in the third or fourth columns followed by an asterisk were significantly different ($P \leq 0.05$) from the mean in the second column of the same row (e.g. 13.4 versus 50.7 *).

firmed when the effects were examined independently. An increase in growth temperature consistently and significantly increased ^{32}P uptake whereas an increase in incubation temperature significantly decreased ^{32}P uptake in only one case, although the trend was apparent in most other cases. The observed importance of growth temperature reflected the large biological, as opposed to physical, response to temperature expected in this study.

The ^{32}P uptake from the unfertilized soils (Table 17) demonstrated the same trends as ^{32}P uptake from the fertilized soils but differed in that much less ^{32}P was found in the plants. The effect of incubation temperature in this case was interpreted as an effect on the rate of isotopic dilution.

The ^{31}P uptake from both fertilized and unfertilized soils was more variable (a CV of 16.1% compared to 3.2% for the ^{32}P uptake data) and thus the only significant difference found was a decrease with incubation time in the fertilized soils (Table 18). There was a trend of increased ^{31}P uptake as temperature increased from the fertilized soils at the start of the incubation but no other consistent trends were noted. This lack of precision was attributed to variability in the seed-derived P content of the plants (estimated at 1.69 ± 0.20 µg ^{31}P/20 plants, Appendix G) coupled with the relatively large portion of the measured ^{31}P which was thus not derived from the soil.

The specific activity of the soil-derived P was estimated by subtracting the average seed P content from the total plant ^{31}P. The labile pool of soil ^{31}P was then calculated using equation (8). There were no significant trends in the labile pool due to temperature, P treatment or soil because of the variability in plant ^{31}P content. However, the pool size

TABLE 17

Short Term Plant Uptake of ^{32}P from the Unfertilized Soils

Start of the Incubation		End of the Incubation					
		Growth at Incubation T		Testing Growth T		Testing Incubation T	
Inc/Grth	^{32}P	Inc/Grth	^{32}P	Inc/Grth	^{32}P	Inc/Grth	^{32}P
			Almasippi Soil (x 10^4)				
10/10	13.8 a	10/10	5.73 a	10/20	19.6 *	20/10	5.83
15/15	31.0 b	15/15	15.4 b	15/25	21.9	25.15	12.1
20/20	36.8 b	20/20	15.3 b	20/10	5.83 *	10/20	19.6
25/25	39.1 b	25/25	20.2 b	25/15	12.1	15/25	21.9
			Elm River Soil (x 10^4)				
10/10	4.29 a	10/10	2.47 a	10/20	6.13 *	20/10	1.67
15/15	10.1 b	15/15	5.32 b	15/25	10.1 *	25/15	2.84 *
20/20	12.5 b	20/20	6.03 b	20/10	1.67 *	10/20	6.13
25/25	12.3 b	25/25	7.14 b	25/15	2.84 *	25/15	10.1

- Footnotes as in Table 16.

TABLE 18

Plant Uptake of ^{31}P from the Fertilized and Unfertilized Soils

Start of the Incubation		End of the Incubation					
		Growth at Incubation T		Testing Growth T		Testing Incubation T	
Inc/Grth	^{31}P	Inc/Grth	^{31}P	Inc/Grth	^{31}P	Inc/Grth	^{31}P
			Fertilized Soils				
10/10	3.06 a	10/10	2.97 a	10/20	2.29	20/10	2.83
15/15	3.10 a	15/15	2.72 a	15/25	2.72	25/15	2.41
20/20	3.39 a	20/20	2.32 a	20/10	2.83	10/20	2.29
25/25	3.71 a	25/25	2.34 a	25/15	2.41	15/25	2.72
			Unfertilized Soils				
10/10	1.84 a	10/10	2.08 a	10/20	2.05	20/10	-
15/15	1.70 a	15/15	1.77 a	15/25	2.12	25/15	1.72
20/20	1.60 a	20/20	1.84 a	20/10	-	10/20	2.05
25/25	1.88 a	25/25	1.93 a	25/15	1.72	15/25	2.12

- Footnotes as in Table 16 except that ^{31}P uptake as µg/20 plants, means are average of 2 soils, control plants grown on P-free gravel contained 1.69 µg P/20 plants.

averaged 43 and 95 μg ^{31}P/g soil at the start and end of the incubation period respectively, substantially higher than those measured using $NaHCO_3$ (18.4 and 29.9 μg ^{31}P/g soil respectively). This occurred despite the fact that ten-fold more ^{32}P was removed per unit of soil by the $NaHCO_3$ extraction than by the plants. A difference in specific activity, and hence pool size, measured from the same sample by two different methods can only be attributed to differences in the extraction of soil ^{31}P not in isotopic equilibrium with the ^{32}P. Thus, the plants had access to soil ^{31}P not in isotopic equilibrium and also not extracted by the $NaHCO_3$. Owusu-Bennoah and Wild (1980) demonstrated comparable phenomena that resulted from the highly efficient soil P dissolution by mycorrhizal fungi. It was concluded that the intimate root-soil contact, through specific rhizosphere processes, availed the plants to quite insoluble forms of P in the soil.

In summary, the short term uptake measurements confirmed the trends observed from chemical extractions. The effect of growth temperature was especially pronounced, indicating that temperature effects on the biological system were profound and probably were not fully compensated by the adjustment to a common root weight. The specific activity of the soil-derived P differed from that of $NaHCO_3$ extraction, indicating that the plant roots had access to less readily exchangeable or less labile forms of soil ^{31}P.

Results and Discussion, Experiment B

Experiment B was conducted to examine temperature effects on unfertilized soils more fully and to extend the number of soils investigated. Chemical extraction and short term plant uptake studies were conducted separately and are presented in that order.

$NaHCO_3$ and Resin Extractable P

The soils fell into three categories based on $NaHCO_3$ extractable ^{31}P contents (Table 19). The Newdale soil was quite high, the Almasippi, Elm River, Snowflake, Stockton and Wellwood soils were low and the remaining soils were intermediate. Increased temperature significantly increased the extraction of ^{31}P only for six soils and these, with the exception of the Balmoral soil, were the soils lowest in extractable ^{31}P. It was not clear if there was a mechanistic relationship between the level of extractable P and the response to temperature.

Less ^{31}P was extracted from the Almasippi and Elm River soils in this study than in Experiment A. However, even less (7 and 6 μg P/g soil respectively) were extracted from air-dried samples. Thus, the longer moist incubation period of experiment A may have resulted in more extractable ^{31}P.

The amount of ^{32}P extracted (not shown) did not vary among the soils and was not correlated to the amounts of extractable ^{31}P. The average recovery of the applied ^{32}P was 87%. Thus, it was concluded that the recovery of ^{32}P was maximized and therefore was not related to ^{31}P solubility in each soil. This implied that all of the forms of soil ^{31}P which had achieved isotopic equilibrium were extracted and hence the measured specific activities did not reflect the labile pool of soil ^{31}P.

The resin extractable ^{31}P was more variable (a CV of 33% compared to 6.7% for the $NaHCO_3$ extractions) and fewer trends were apparent than with the $NaHCO_3$ extractable ^{31}P. The resin extractable ^{31}P was generally correlated to the $NaHCO_3$ extractable ^{31}P but the increased variability resulted in fewer significant differences between soils and fewer significant responses to temperature (Table 44, Appendix H). The greater variability

TABLE 19

$NaHCO_3$ -Extractable ^{31}P From 12 Soils at 4 Temperatures

Soil Name	Temperature			
	10	15	20	25
			$\mu g\ ^{31}P/g$	
Balmoral	21	19	22	23 *
Almasippi	9	12	12	13 *
Inwood	23	23	23	24
Manitou	25	23	24	24
Lakeland	21	22	22	23
Lundar	23	23	23	24
Newdale	38	39	39	37
Plum Ridge	19	22	21	21
Elm River	6	8	8	10 *
Snowflake	11	13	12	15 *
Stockton	12	13	15	16 *
Wellwood	17	19	20	22 *

- Row of Values Followed by ' * ' Increased Significantly with Temperature by t-test of Regression Coefficient (Appendix H).

was attributed to the added analytical procedures required to release the P from the resin for measurement.

The average amount of ^{31}P extracted by the resin (28.1 μg ^{31}P/g soil) was larger than that extracted by $NaHCO_3$ (19.8 μg ^{31}P/g soil). However, the amounts of ^{32}P extracted were much less with the result that mean specific activities were 88 dpm $^{32}P/\mu g$ ^{31}P for the resin compared to 281 dpm $^{32}P/\mu g$ ^{31}P for $NaHCO_3$. This may have been caused by isotopic exchange of ^{32}P with ^{31}P in the resin or resin bags or may indicate that the resin and $NaHCO_3$ sampled different fractions of the soil ^{31}P. The $NaHCO_3$ more effectively dispersed the soil and may have extracted ^{31}P from within former aggregates whereas the resin may have desorbed much more ^{31}P and ^{32}P from aggregate surfaces.

In summary, the $NaHCO_3$ extractions demonstrated increased ^{31}P solubility at higher temperatures in some soils. The most responsive soils to temperature were the soils lowest in extractable P. The same differences occurred in the resin extractions but were less useful due to variability inherent with the methodology.

The ^{32}P was very efficiently recovered by the $NaHCO_3$ extractions and thus yielded no information about the soil labile pool. The specific activities differed between the $NaHCO_3$ and resin extractions which may have been the result of extraction of different fractions of the soil ^{31}P.

Desorption Curves

The desorption curves were described with multiple regression such that linear effects of temperature on the desorption curve intercepts and slopes could be examined. The intercepts at the various temperatures can be derived from Table 20 by the formula $a + b_2$ (temperature, °C). Similarly

TABLE 20

Desorption Curve Coefficients[1] for Desorption of ^{32}P

Soil Name	Coefficient				
	a	b_1	b_2	b_3	r^2
	$(Log_e (dpm/mL))^2$	$(x10^{-5} g/dpm)^2$	$(°C^{-1})$	$(x10^{-6} g/dpm.°C)^2$	(%)
Balmoral	5.82	-2.67 *[3]	0.0140 NS	0.42 NS	94
Almasippi	5.17	-0.62 NS	0.0559 *	-0.30 NS	79
Inwood	5.98	-3.95 *	0.0254 NS	1.15 *	80
Manitou	6.45	-7.57 *	0.0043 NS	2.54 *	60
Lakeland	5.93	-0.24 NS	0.0248 *	-1.15 *	90
Lundar	7.36	-7.90 *	-0.0122 NS	2.41 *	92
Newdale	6.77	-3.42 *	0.0394 NS	0.47 NS	85
Plum Ridge	7.74	-3.14 *	0.0019 NS	0.37 NS	94
Elm River	5.38	-1.76 *	0.0218 *	-0.13 NS	90
Snowflake	5.88	-0.95 NS	0.0221 NS	0.01 NS	47
Stockton	5.49	-2.21 *	0.0143 NS	0.39 NS	76
Wellwood	5.35	0.14 NS	0.0091 NS	-0.80 NS	41

1. Equation of the form Log_e (P concentration) = a + b_1 (P desorbed)+b_2(temp) + b_3 (P desorbed)(temperature).

2. ^{32}P expressed as dpm corrected for decay to the day the ^{32}P was applied.

3. Coefficients tested for significant difference from zero (*) at $P \leq 0.05$ (otherwise NS) by t-test within each soil (Appendix H).

the slopes can be derived by the formula $b_1 + b_3$ (temperature, °C). The tests for an effect of temperature involved a t-test of the coefficients b_2 (for effects of temperature on the intercept) and b_3 (for effects of temperature on the slope).

The intercepts generally reflected the same differences among soils shown by the $NaHCO_3$ extractions. Temperature significantly modified the intercepts only in the Almasippi, Lakeland and Elm River soils where the intercepts (and thus solubility of the most soluble forms of P) increased with temperature.

The desorption curve slopes (and thus solution P buffer capacities) were significantly less steep in the Inwood, Manitou and Lundar soils and were significantly steeper in the Lakeland soil as temperature increased. Less steep desorption curve slopes with increasing temperature were observed in one case in Experiment A and were interpreted to indicate a shift in the soil P profile toward more soluble forms. Thus, after a set amount of P was desorbed, the remaining equilibrium solution P concentration was higher at higher temperatures. The steeper slopes observed in the Lakeland soil suggested a shift toward less soluble forms, comparable to the effect of incubation time observed in the fertilized soils of Experiment A. This soil was unique for the highest carbonate content (18.2% CO_3) of the 12 soils studied. It may be that at higher temperatures, reactions in the carbonate system (for example, the loss of CO_2 from the soil water with resultant shifts in pH and carbonate solubility) may have indirectly decreased P solubility. However, the Inwood soil was also high in carbonate (12.0% CO_3) and the opposite effect on desorption curve slope was observed.

The Almasipppi, Lakeland and Elm River soils, in which temperature modified the desorption curve intercepts were not distinguished by other

measured soil properties (Table 7). However, the Inwood, Manitou, Lakeland and Lundar soils in which temperature modified the desorption curve slopes were among the six soils highest in $NaHCO_3$ extractable P and, with the exception of the Lakeland soil, had the highest desorption curve intercepts. The Lakeland soil was also an exception due to less steep desorption curve slopes at colder temperatures. Thus, the effect of higher temperatures on decreasing the desorption curve slopes was related to overall P solubility. It may be that detectable effects of temperature were possible only in soils high in P content.

The desorption curve intercepts and slopes (coefficients b_2 and b_3 respectively, Table 20) were negatively correlated ($r = -0.51$) across the four temperatures with a general trend toward higher intercepts and less steep slopes as temperature increased. Thus, the curves from the four temperatures tended to diverge as more P was desorbed. This implied that in a cooler soil, both the solubility of the most soluble forms of P and the solution P buffer capacity diminished. Thus, temperature effects were not surficial but modified portions of the P solubility profile.

In summary, P solubility was positively correlated to temperature in some soils but the effect was not consistent from soil to soil. In the $NaHCO_3$ extractions, the soils lowest in extractable ^{31}P appeared to be the most responsive to temperature. The desorption curves varied with temperature in some soils. The generalized effect of an increase in temperature was to increase the desorption curve intercept and decrease the desorption curve slope. Thus, the P solubility profile shifted toward higher solubility and hence better buffering of the solution P concentration. However, the effects of temperature were minor relative to the inherent differences among the soils in P solubility.

Short Term Plant Uptake

As in Experiment A, the P uptake measurements in this study were adjusted arithmetically to 20 plants/pot and then adjusted to a common root weight using curvilinear analysis of covariance. In this study the root weight (per 20 plants) varied significantly from soil to soil as well as among temperatures (Figure 16). There was also a significant interaction since in some soils (e.g. the Plum Ridge soil) the root weight increased as little as 2.2-fold from 10 to 25°C whereas in others (e.g. the Lundar soil) it increased up to 4.3-fold.

The effect of each soil on root growth and root growth-response to temperature was not clearly related to specific soil properties. The poorest root growth occurred in soils with low moisture holding capacity, low clay content and low fertility (see Table 7) but these properties occurred in the same soils and thus the specific controlling factor could not be isolated.

The plants in this study were at the same stage for which P deficiency was observed previously (see Chapter 3) and the leaf P concentrations from some soils were close to those observed in a similar preliminary experiment in which the substrate was devoid of P (Appendix G). Furthermore, there was a well defined relationship between root growth and total root P content (Figure 41, Appendix H). Other nutrients were less likely to control root growth since prior to transplanting, the plants were supplied with the other nutrients, probably in excess of requirements. Root growth was not correlated to soil NO_3-N content. Therefore P nutrition was probably the primary factor controlling root growth differences among soils.

The desorption curve data was also analyzed in an analysis of covariance model where the $\log_e$ (P concentration) was adjusted by the covariate $\log_e$ (solutionsoil ratio) (Table 46, Appendix H). This model (after Sharpley et al,, 1981) described the data well but yielded no information beyond that discussed from the desorption curve analysis.

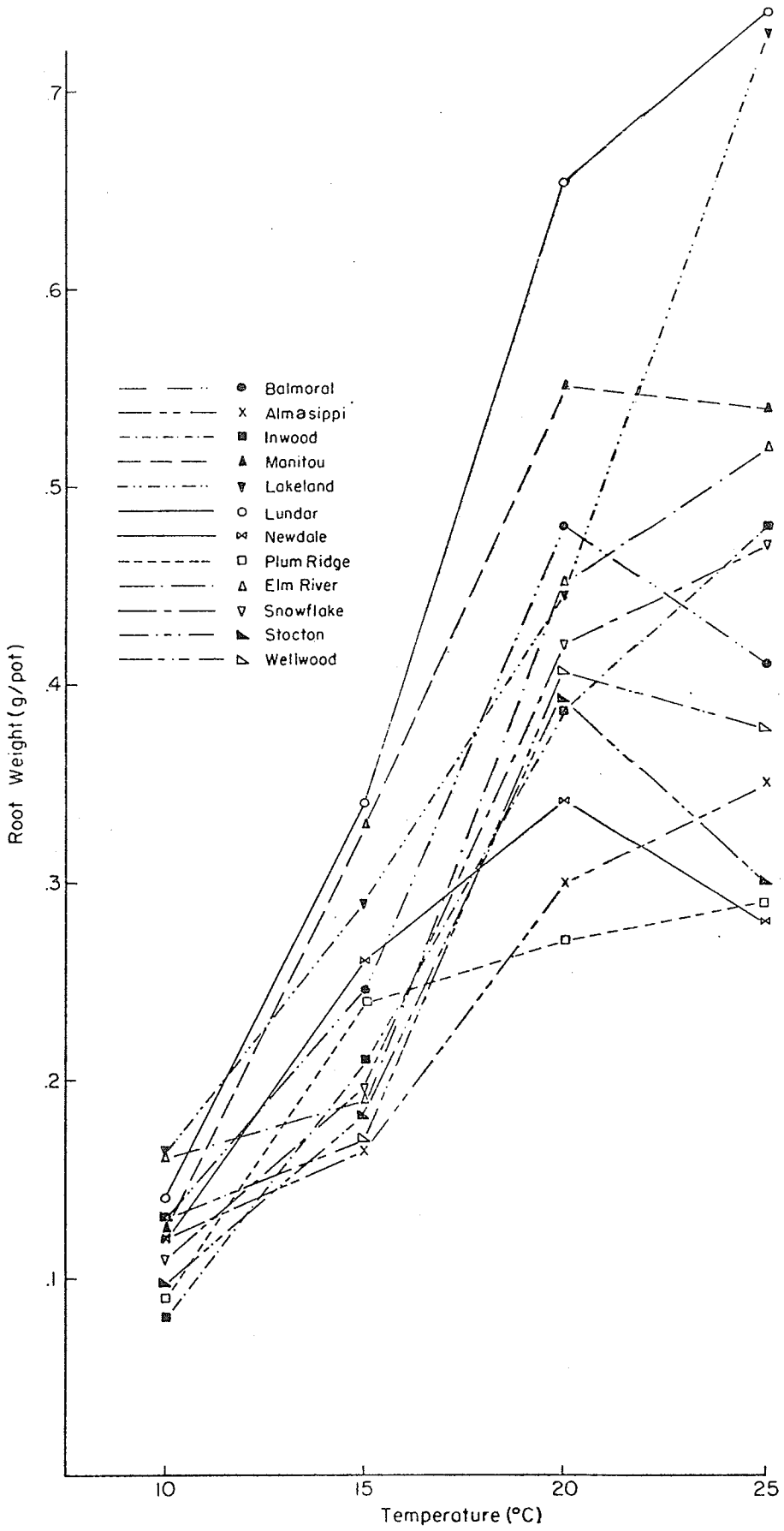

Figure 16: Root weight in response to temperature in twelve soils.

The uptake of total ^{32}P and soil-derived ^{31}P (calculated by subtracting the seed-derived P predicted from a preliminary experiment, Appendix G) (Figures 17 and 18) were similar in that uptake increased with increasing temperature in some soils and not others. The total ^{31}P uptake (both seed- and soil-derived ^{31}P) was very similar to the soil-derived ^{31}P data. Plants grown on the Inwood, Manitou, Lundar and Newdale soils had consistently increased P contents at higher temperatures. The root growth in the Lundar soil in particular but also in the Manitou soil was very responsive to temperature. In contrast, the root growth in the Newdale soil was one of the least responsive to temperature. Thus, although the analysis of covariance may not have been a perfect correction for root activity due to temperature, these results showed that P uptake (and hence P supply of the soil) was a function of temperature in some soils despite the amount of root growth.

The four responsive soils were distinguished by chemical extractions of their P supplies. They had the highest amounts of $NaHCO_3$-extractable P, the highest (with the exception of the Plum Ridge soil) desorption curve intercepts, and the steepest desorption curve slopes. However, the first two of these properties were not modified by temperature. The desorption curve slopes of the Inwood, Manitou and Lundar soils were significantly less steep as temperature increased. Thus, soils which provided the largest response to temperature of plant P uptake were high in extractable P content and had increased buffer capacity as temperature increased. However, of the measurements derived from chemical extractions, only the buffer capacity showed a concomittant response to temperature. This was true for only three of the four soils.

The relationship between plant uptake response to temperature and the

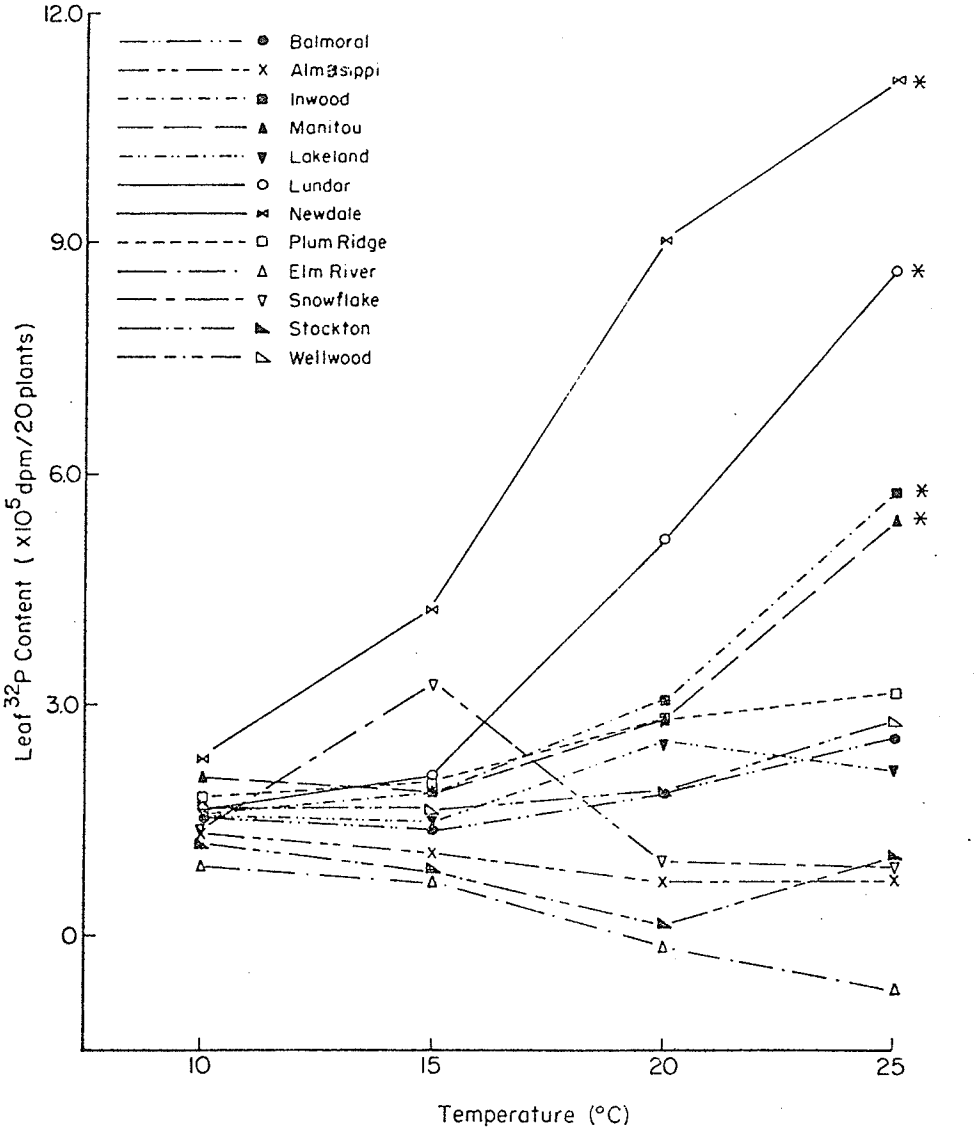

Figure 17: Leaf total ^{32}P content from plants grown in twelve soils at four temperatures, adjusted for root growth.

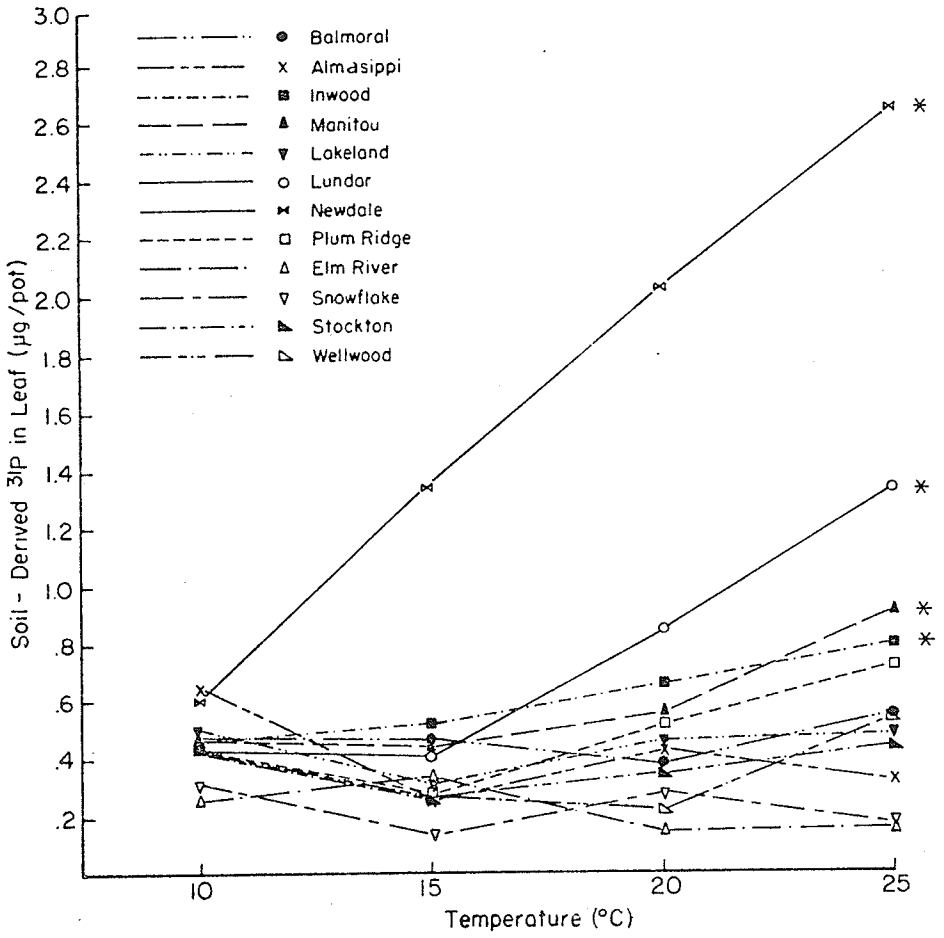

Figure 18: Soil-derived ^{31}P in the leaves of plants grown in twelve soils at four temperatures, adjusted for root growth.

extractable P content of the soils may indicate that only in the soils where the P content was relatively high was the effect of temperature detectable. However, it can be concluded that the effect of temperature on P supply did vary among soils.

There was significant positive correlation between soil NO_3-N content and plant P uptake. However, this was largely due to the Lundar and Newdale soils which were high in both soil NO_3-N and plant-available P. Thus, a causal relationship between soil NO_3-N and plant P uptake cannot be asserted.

The range of 12 soils provided the opportunity to examine isotopic dilution in widely different soil-P systems. Two approaches were used to predict the seed-derived P content of the plants so that the specific activity of the soil derived P could be examined.

The specific activities calculated by subtracting an independent estimate of seed-derived P content (Appendix G) did not change consistently with temperature in any of the soils. The mean specific activity was 469 dpm $^{32}P/\mu g\ ^{31}P$. The specific activities calculated as the inverse of the regression coefficient describing the relationship between ^{31}P and ^{32}P uptake in a multiple regression model (see Methods section) were more consistent. These specific activities were inversely correlated to temperature for every soil except the Snowflake soil. The mean specific activity by this method was 806 dpm $^{32}P/\mu g\ ^{31}P$.

The difference in the mean specific activities between the methods of computation was attributed to an underestimation by the multiple regression model of the seed-derived ^{31}P content in the leaves. Thus, although this method was more precise (a CV of 17% as compared to 266%), the previous method (using an independent estimate of seed-derived ^{31}P content) was

considered more accurate.

The decrease in specific activity as temperature increased indicated that isotopic exchange was more advanced at higher temperatures. This trend was expected since isotopic exchange was a rate-limited process which would have been accelerated at higher temperatures.

The specific activity varied considerably among soils but was not significantly correlated to either $NaHCO_3$ extractable ^{31}P or the desorption curve intercepts. Ranked in order of decreasing size of labile pool, the soils were Snowflake, Plum Ridge, Stockton, Lakeland, Manitou, Lundar, Balmoral, Elm River, Almasippi, Inwood, Newdale and Wellwood.

In summary, plant uptake of P varied with temperature in some soils but was not significantly affected by temperature in other soils. Root growth response to temperature explained much of the variance in plant P uptake among soils but it was clear that root growth was not responsible for all of the effects observed. The four soils which provided a plant P uptake response to temperature were distinguished by high levels of soil P. Three of the four soils were also distinguished by a response of the desorption curve slopes to temperature. Other soil properties were not consistently related to the observed response of plant P uptake to temperature.

Estimates of soil specific activities were inversely related to temperature. However, differences in specific activities among soils were not clearly related to other measured soil properties.

Conclusions

The principal objective of this study was to examine the effect of temperature on the availability of soil and fertilizer P to plants. In the previous experiment (Chapter 3), effects of temperature on the soil and the

plant systems were confounded. A secondary objective was to compare methods of evaluating the effects of temperature on the soil P system.

The Effect of Temperature on Soil and Fertilizer P

The most important finding of this study was the dual role of temperature in modifying P availability in soil. Both chemical extraction and short term plant uptake showed P to be more soluble in soil at higher temperatures. Thus, P desorption was an endothermic reaction, confirming findings of Mack and Barber (1960) and Barrow (1979b). However, fertilizer P was less soluble at higher temperatures due to an accelerated rate of fixation. Thus, the net effect of temperature was variable and was dependent on the system examined. The present results showed that even 57 days after fertilizer P was applied (in the amount required for optimal plant growth), the effect of temperature on rate of fixation predominated over the effect of temperature on solubility (as measured by KCl extraction). Therefore, during the early part of a growing season when P supply is thought to be most critical, higher temperatures may decrease the amount of soluble fertilizer P in the soil. Conversely, P applied in the fall would be preserved in a relatively available form by low winter temperatures.

The desorption curve data showed that increasing temperature not only increased the solubility of the most soluble form of P in the soil but shifted the solubility profile such that the solution P concentration was better buffered. Thus, when a particular amount of P was desorbed at higher temperatures, the remaining forms of soil P were also relatively more soluble. These changes in P buffer capacity (or desorption curve slope) with temperature varied from soil to soil and appeared to be a useful means to predict which soils would change most in P supply due to temperature.

Furthermore, the desorption curve slope cannot be considered constant (Barrow, 1979b) when used to compare different soils or different degrees of fertilizer P reaction (for example, due to incubation at varied temperatures). There was soil to soil variability in the effect of temperature on P supply in both chemical extractions and plant uptake. This response seemed to be related to the overall solubility of P in the soil. Thus, the effect of temperature was probably manifest in all the soils studied but only detectable in the soils with high P solubility.

The relevance of this study to the previous response experiment (Chapter 3) was the information it provided on the soil P system alone. It was suggested in Chapter 3, based on control pot yields, that native soil P may have been more soluble at 25°C than at lower temperatures. This was confirmed. The reactions of the P fertilizer in the band treatments of Chapter 3 would also have been influenced by temperature. The slower root development at 10°C would have been partially offset by a slower fertilizer fixation rate so that when the roots did exploit the band, the fertilizer P was still relatively soluble. However, the short term plant uptake studies showed that the biological system was far more responsive to temperature than the physical, soil reaction system. Therefore root growth response to temperature was more determinate of P uptake than was fertilizer fixation response.

The effect of temperature on fertilizer P fixation rate was established within 24 hours after the P was applied (shown by decreased solubility at higher temperatures). The reaction rates for the next 5-7 days were not greatly affected by temperature. Similarly, the effect of temperature on the desorption reaction was established very rapidly (within one hour) with little further effect of temperature up to 48 hours. Thus the reactions

which were modified by temperature were rapid and, in terms of plant growth, could be considered instantaneous.

Comparison of Methodology

Chemical extractions are crude estimates of the P available to plants. In this study there was evidence, based on the specific activity of recovered soil P, that plants had access to a broader spectrum of soil P than did $NaHCO_3$. Thus, although $NaHCO_3$ extracted large quantities of P, the plant may have desorbed less soluble forms from the zones of soil-root contact. For this reason, plant uptake was an indispensable technique for measurement of soil P supply. However, the root growth response to temperature was probably not completely accounted for by analysis of covariance and therefore the technique was of most use comparing previous temperature (i.e. incubation) treatments.

The chemical extraction procedures each had advantages. The $NaHCO_3$ extraction removed enough soil P that measurements were possible even on the soils lowest in P. However, there was evidence that this extraction removed relatively large quantities of soil P and differences in the more soluble forms of P which were affected by temperature and incubation time were not detected. The resin extractions probably had advantages in extracting forms of soil P similar to those available to the plant root. Refinement of the resin regeneration procedure would be required to decrease variability in the measurement of resin extractable P. The KCl extractions detected subtle differences in soil P due to temperature but extracted very small amounts of soil P and hence concentrations of P in many samples were close to or below detection limits. The KCl extractions to measure desorption curves provided another dimension to the soil P studies by reflecting

the soil P buffer capacity.

In conclusion, each method yielded additional information but, when the amounts of P extracted could be detected, the desorption curves were the single most useful method. They have the further advantage of use in simulations of plant P uptake (Brewster et al., 1976) and other studies of plant P nutrition (Hendricks et al., 1981).

The desorption time study indicated that extraction times of one to 48 hours yielded the same information for comparison of treatments although the amount of P desorbed continued to increase with time. Exchange of ^{32}P with soil ^{31}P also progressed with time but a minimum of five days appeared necessary for the most rapid reactions to be complete.

The tracing of fertilizer ^{31}P reactions using ^{32}P was shown not to be accurate as the ^{32}P exchanged with soil ^{31}P and thus was subject to more reactions than the fertilizer ^{31}P. In terms of P uptake studies, the ^{32}P would be recovered less efficiently than the fertilizer ^{31}P and thus the use of ^{32}P to identify fertilizer P in the plant would result in an underestimate of the actual fertilizer ^{31}P in the plant.

In summary, fixation of fertilizer P was accelerated at higher temperatures and solubility of P was increased at higher temperatures. Thus, opposing effects of temperature may result in apparently conflicting data. However, the effect of higher temperature on plant growth and root extension was much greater than the effect of higher temperature on the fixation of fertilizer P to unavailable forms. Thus, studies on plant response to temperature will be most useful in understanding the overall effect of soil temperature on the soil P supply-plant P requirement balance.

Chapter 5

PLANT P REQUIREMENTS AT FOUR ROOT TEMPERATURES

Introduction

Numerous researchers have studied the effect of temperature on plant P nutrition in soil (Nielsen and Humphries, 1966) and generally concluded that more fertilizer P was required under cold soil conditions. This has been variously attributed to soil and plant processes. However, few researchers (Balvoll, 1970; Power et al., 1964) have considered the possibility that the optimal tissue P concentration for growth (or the plant P use efficiency) may vary with temperature. Temperature directly affects plant growth rate and it is therefore conceivable that under sub-optimal growth conditions, tissue P may be used more efficiently (due to the possibility of sufficient time for movement between active sites within the plant) or less efficiently (influenced in the same manner as overall growth).

Two distinct problems have impeded research on this topic. Firstly, temperature variation imposes changes in the rate of development as well as rate of growth such that comparison of plants between temperatures must be based on some plant stage criteria. In this study, two plant stage criteria were used for comparisons. These were 1) a stage defined by the number of leaves and 2) a stage defined by plant weight. Secondly, tissue P concentrations can change very rapidly with time. In a preliminary experiment (Appendix J), tissue P concentrations decreased 50% in ten days. The plant P status (i.e. whether the plant is deficient or sufficient in P) may also change quickly and therefore, it is necessary to confirm the plant P status

at the time of harvest. This was accomplished in the present study by continuing to grow plants after the defined harvest stage 1) at the initial P supply and 2) at a modified, relatively high P supply. Solution culture was used to facilitate rapid modification of the P supply.

The objective of this study was to determine if the optimal tissue P concentration or P-use efficiency varied due to root temperature. If this relationship was found, a secondary objective was to relate this response to an index of plant growth status, for example relative growth rate. If the exact tissue P requirements were related to growth rates as modified by root temperature, then similar relationships may hold for other growth limiting factors (e.g. water supply, light, other nutrients).

Methods and Materials

Experimental Design

The basic components of the experimental design involved growing wheat plants in solution culture at root temperatures of 10, 15, 20 and 25°C and P nutrient solution concentrations of 5, 10, 20, 30, 40, 50, 100 and 200 ppm P. Two other experimental factors were involved.

Two "harvest-stage criteria" were used to allow comparison among temperatures. The first was based on number of leaves and was defined to occur when the plants had six mature, main stem leaves (termed the sixth leaf stage). The second was based on total plant fresh weight and was defined to occur when the plants achieved 4 g total fresh weight (termed the 4 g fresh weight stage). The use of two harvest-stage criteria was necessary since leaf development and weight gain responded differently to temperature (Appendix J). The harvest-stage criteria were assessed within each temperature treatment but across P supply treatments such that a

single harvest at each stage in each temperature included all of the P supply treatments. The stage criteria were assessed on the plants rated visually to be growing in the optimal solution P concentration.

Three "harvest treatment groups" were used to confirm plant P status at harvest. These consisted of three sets of plants, termed A, B and C. The A plants were harvested when the stage criteria, either 6th leaf or 4 g fresh weight, was met. The B plants grew an additional six to nine days (longer at lower temperatures) at the initial solution P concentrations (i.e. these differed from the A plants only in age at harvest). The C plants were placed in 200 ppm solution P concentrations (regardless of the initial solution P concentrations) at the stage criterion when the corresponding A plants were harvested and were harvested with the corresponding B plants. Thus, differences in response between the B and C plants could be attributed to the change to adequate P supply of the C plants.

Two separate experiments were conducted using the same experimental design but with a small difference in culture methodology (to be described subsequently).

The Solution Culture System

The plants were grown in individual polyethylene bags (40 cm long by 15 cm flat-width providing a solution volume of 1.5 to 2 L) suspended in the temperature controlled water baths. A glass tube (3 mm ID x 14 cm long) was placed in each bag to provide aeration (at approximately 400 cm^3 air/min). The Hoagland nutrient solutions were modified from that of Hoagland and Arnon (1950) by reducing the macronutrient concentrations to one fifth and the micronutrient concentrations to one half (Appendix I). The Fe was supplied as Fe-EDTA. The eight solution P concentrations were

established using KH_2PO_4 and the corresponding K concentrations were adjusted to a uniform concentration using KCl. The solution pH was adjusted to 5.5.

Each solution culture bag held a variable amount of solution (1.5 to 2 L, due to the mechanism used to support the bags). In the first experiment, the bags were filled with premixed solutions. Thus, although concentrations were initially controlled, the total amount in each bag varied proportionally to the bag volume. In the second experiment, the bags were filled with distilled water and then 50 mL of a stock solution was added to provide the same total amount of nutrient as in 2 L of the desired concentration. The plants decreased the solution P concentrations in both experiments relatively rapidly and thus the later method provided more consistent amounts of P to the plants. However, the overall objective was to examine growth relative to tissue P concentrations and therefore decreases in nutrient solution P concentration and differences in total P supply were not important factors. The various solution P concentrations were intended to provide a range of plant P nutritional status.

The nutrient solutions in each plant container were changed every five to ten days depending on the plant development rate as modified by temperature. The solutions were siphoned out of each bag and replaced by freshly prepared solutions.

Plant Culture

The seeds were germinated and grown for one week (to the one leaf, three to five seminal root stage) in a tray of Perlite, watered with tap water. The plants were removed from the Perlite and 576 uniform plants were transplanted into the solution culture system. Three plants were

placed into each solution culture bag.

The plants were held in place by a strip of foam rubber (3 cm wide x 1 cm thick x 45 cm long) which had one end wrapped in plastic film. This film prevented roots and tiller-shoots from penetrating the foam. The three plants were laid on the plastic film with the seed midway across the width of the foam. The foam was rolled lengthwise around the seedling crowns to form a plug (8 cm diameter) which was fitted snugly into the open end of the solution culture bags.

The seedlings were sprayed twice during the first week after transplanting using Captan-Benomyl fungicides to reduce seedling loss. The plants were thinned to one plant per bag one week after transplanting.

Growth Measurements

Plant growth was measured several times before each plant was harvested by removing the plants from the nutrient solutions, removing the foam rubber plugs, allowing the roots to drain of excess water and weighing each plant. These total plant weights were used to compute relative growth rates (RGR as g/g.day) and absolute growth rates (GR as g/day) using equations (9) and (10) respectively.

$$RGR = \frac{\ln (\text{Total FW}_2) - \ln (\text{Total FW}_1)}{(t_2 - t_1)} \qquad [9]$$

$$GR = \left(\frac{\text{Total FW}_2 - \text{Total FW}_1}{t_2 - t_1} \right) \times \left(\frac{\text{Shoot FW}_H}{\text{Total FW}_H} \times \text{Shoot DM}_H + \frac{\text{Root FW}_H}{\text{Total FW}_H} \times \text{Root DM}_H \right) \qquad [10]$$

Total FW, Shoot FW and Root FW represented the respective tissue fresh weight (g) at time (subscript) 1, 2 and H (final harvest), t_1 and t_2 represented the measurement time (days), and Shoot DM_H and Root DM_H represented

the respective tissue dry matter contents at final harvest (g dry/g fresh). Equation (10) estimated the rate of dry matter increase by using the shoot and root to total plant weight ratio and dry matter contents measured at harvest. The results (to be discussed) showed that these latter ratios did not change appreciably with time.

At final harvest (as dictated by the respective stage criterion and harvest treatment group) the shoots and roots were separated, weighed fresh and again after drying for 24 hours at 85°C. In the first experiment, the number of tillers was recorded and in the second, the number of leaves on the main shoot was recorded. The shoots were wet-ashed and analyzed for ^{31}P (Appendix B).

The GLM (general linear models) and STEPWISE (stepwise regression) procedures of the SAS (1979) were used to analyze the data.

Results and Discussion

Stage Criteria and Response to Temperature

The "sixth leaf stage" occurred 20 to 28 days after transplanting and the "4 g total fresh weight stage" occurred 15 to 25 days after transplanting (Table 21). The plants achieved their respective stage criteria in approximately the same number of days (generally within one day) between the two experiments. The "weight" stage occurred five days prior to the "leaf" stage at 25°C but only three days prior at 10°C. A similar trend was noted in a preliminary experiment (Appendix J). Thus, plants at the higher temperatures weighed more at a given developmental stage.

This was attributed to larger individual leaves and more tillers at 25°C in the preliminary experiment (where development relative to plant dry weight was considered). In this study, the lower dry matter content at

TABLE 21

Comparison of Number of Leaves and Total Plant Fresh Weights at the "6th Leaf" and "4 g Fresh Weight" Harvest Stages[1]

	Temperature (°C)			
	10	15	20	25
	"6th leaf"			
Days after transplanting	28	24	21	20
Number of leaves				
"P sufficient"[2]	5.5	5.5	5.3	5.7
overall[3]	4.9	5.1	5.4	5.6
Total fresh weight				
"P sufficient"	4.31	8.21	9.76	8.79
overall	4.09	6.79	8.70	6.95
	"4 g fresh weight"			
Days after transplanting	25	20	16	15
Number of leaves				
"P sufficient"	4.5	4.5	4.3	3.5
overall	4.3	4.3	4.3	3.2
Total fresh weight				
"P sufficient"	3.88	4.87	3.62	3.24
overall	3.59	3.86	3.60	2.84

1. Values are means of two experiments (A plants), except number of leaves which was recorded only for the second experiment.

2. "P sufficient" means are of plants grown at 30, 40, 50 and 100 ppm P.

3. Means of all eight P concentrations.

25°C than at 10°C (Table 22) would indicate more fresh weight for a given accumulation of dry matter. Therefore, because the "weight" stage was defined on a fresh weight basis, the plants at 25°C achieved the fresh weight criteria with less actual (dry matter) growth than plants grown at 10°C.

The "P sufficient" means shown in Table 21 have a mean of 5.5 leaves for the "6th leaf stage" and 3.9 g for the "4 g fresh weight stage". These recorded means are lower than the target values defined by the stage criteria. This occurred because the actual harvests were based on observed optimal growth and thus not all plants at 30, 40, 50 and 100 ppm P (as averaged for Table 21) exhibited optimal growth. The actual number of leaves (for the 6th leaf stage) were fairly uniform across temperatures, thus allowing comparisons between temperatures on a uniform leaf number basis. The actual total fresh weights (for the 4 g fresh weight stage) were less uniform but still showed no linear trend with temperature. Thus, comparisons among temperatures were also possible on a uniform fresh weight basis. The plants harvested by the "leaf number" criteria were variable in weight and conversely, the plants harvested by the "weight" criteria were variable in number of leaves. Thus, the defined stage criteria were generally achieved and responded differently with respect to temperature.

The result of using the harvest-stage criteria was that yield parameters were uniform across temperatures. Thus, shoot and root weights and numbers of tillers and leaves were not correlated significantly with temperature (Table 22).

Several other parameters were significantly correlated to temperature. Most notable were the inverse correlations of dry matter contents and the direct correlations of the growth rate parameters and tissue P concentrations with temperature (Table 22). Shoot dry matter content was also

TABLE 22

Response of Several Plant Parameters to Temperature

Parameters	Temperature (°C)				Correlation (r) with Temperature
	10	15	20	25	
Shoot dry weight (g/plant)	0.26	0.31	0.32	0.27	0.01 NS
Root dry weight (g/plant)	0.14	0.18	0.20	0.16	0.05 NS
Shoot/total weight ratio					
fresh	0.39	0.36	0.33	0.36	-0.20*
dry	0.65	0.63	0.62	0.64	-0.10 NS
Dry matter content (dry/fresh)	x 100				
shoot	17	17	16	15	-0.33***
root	6.0	5.6	4.8	5.0	-0.35***
Relative growth rate (g/g.day)[1]	0.126	0.135	0.170	0.188	0.27**
Absolute growth rate (g/day)[1]	0.043	0.069	0.085	0.086	0.25**
Tissue P concentration (%)	0.49	0.48	0.55	0.64	0.21*
Number of tillers (Exp. 1)	4.5	3.8	3.9	3.5	-0.14 NS
Number of leaves (Exp. 2)	4.6	4.7	4.9	4.6	-0.07 NS

- means of 32 A plants (two experiments, two-stage criteria, eight solution P concentrations).

- NS (not significant), * ($0.05 \geq P \geq 0.01$), ** ($P < 0.01$), *** ($P < 0.001$).

1. Growth rate as increments in total plant weight, measured as fresh weight (and in the case Absolute growth rate, converted to dry weight using dry matter contents measured at harvest).

negatively correlated with solution P concentration (r = -0.35***) and relative growth rate (r = -0.61***). Thus, more rapidly growing plants were more succulent and therefore dry matter content was a useful parameter to identify growth status. P ositive correlationsof growth rates and tissue P concentrations to temperature reflect increased biological activity (dry matter accumulation and P uptake rates) with temperature.

Solution P concentrations were correlated with shoot/total plant weight ratios (r = 0.49*** for fresh weight ratios, r = 0.45*** for dry weight ratios) and number of tillers (r = 0.41***). Clearly, as P supply became more severely limiting, the plant partitioned more dry matter to the root system. This is a well documented response to P deficiency (Loneragan and Asher, 1967). The number of tillers was probably a component of yield response to P supply.

The second harvest of plants grown at the eight initial solution P concentrations provided data to examine the effect of time on the plant parameters (Table 23). During the six to nine day period, the shoot yield increased over three-fold. This was also reflected in increased absolute growth rate, number of tillers and number of leaves. The tissue P concentrations decreased, probably due to biological dilution following the rapid gain in yield. The shoot/total plant and dry matter content ratios were generally unaffected by time. The relative growth rate decreased with time as reported by Elias and Causton (1975) and was interpreted as a "dilution" of the weight of actively growing tissue by the weight of mature tissue which accumulated with time.

Response to the Solution P Concentrations

The overall response to solution P concentrations, averaged across

TABLE 23

The Change in Several Plant Parameters over a 6 to 9 Day Interval[1] Following the Defined Stage

Parameters	Harvest at the defined stage (A plants)	Harvest 6 to 9 days later (B plants)
Shoot dry weight (g/plant)	0.29	1.09
Root dry weight (g/plant)	0.17	0.63
Shoot/total weight ratio		
fresh	0.36	0.35
dry	0.63	0.63
Dry matter content (dry/fresh) x 100		
shoot	16	17
root	5.3	5.3
Relative growth rate (g/g.day)	0.154	0.132
Absolute growth rate (g/day)	0.762	1.90
Tissue P concentration (%)	0.54	0.41
Number of tillers (Exp. 1)	3.9	11.7
Number of leaves (Exp. 2)	4.7	6.2

- means of 128 plants (2 experiments, 2 stage criteria, 8 solution P concentrations, 4 temperatures).

- parameters as in Table 21.

1. The harvest 6 to 9 days later (the second column) was intended to evaluate growth rates in response to P after the defined stage (see Methods for details).

stage criteria and temperature, is shown in Figures 19 to 25. The shoot dry weights at the stage criteria (Figure 19, A plants) were optimal at a solution P concentration of about 30 ppm. For the later harvests (B and C plants), shoot dry weights were optimal at about 50 to 100 ppm P, even though 200 ppm P had been supplied to the C plants (as per harvest treatment, see Methods). Since the increased supply of P to the C plants would have alleviated P deficiency, it appeared that six to nine days was too brief to realize a measurable yield response.

The response of the shoot/total plant fresh weight ratio to P supply (Figure 20) was similar between the A and B plants (those grown continuously at the initial solution P concentrations) with increases in the ratio up to the 200 ppm P solution concentration. However, the C plants (which received 200 ppm P at the defined stage criterion) had uniform ratios typical of the P-sufficient A and B plants. Thus the C plants very rapidly adjusted the shoot/root ratio when P supply became adequate and hence assimilates formerly partitioned to the root were probably available for shoot growth. Presumably, this would have appeared as increased shoot growth if the second harvest had been delayed.

The dry matter contents of both shoots (Figure 21) and roots (Figure 22) were minimized at 50 to 100 ppm P for the A and B plants. The 200 ppm P supplied to the C plants caused rapid changes in the dry matter content to values typical of the P-sufficient A and B plants. This response was comparable to that noted for shoot/total plant weight ratio.

The relative growth rates (Figure 23) had optima comparable to those observed in shoot dry weight (30 ppm P for A plants, 50 to 100 ppm for B and C plants). Of particular note was the higher relative growth rate of the C plants corresponding to the P-deficient B plants (those grown at an

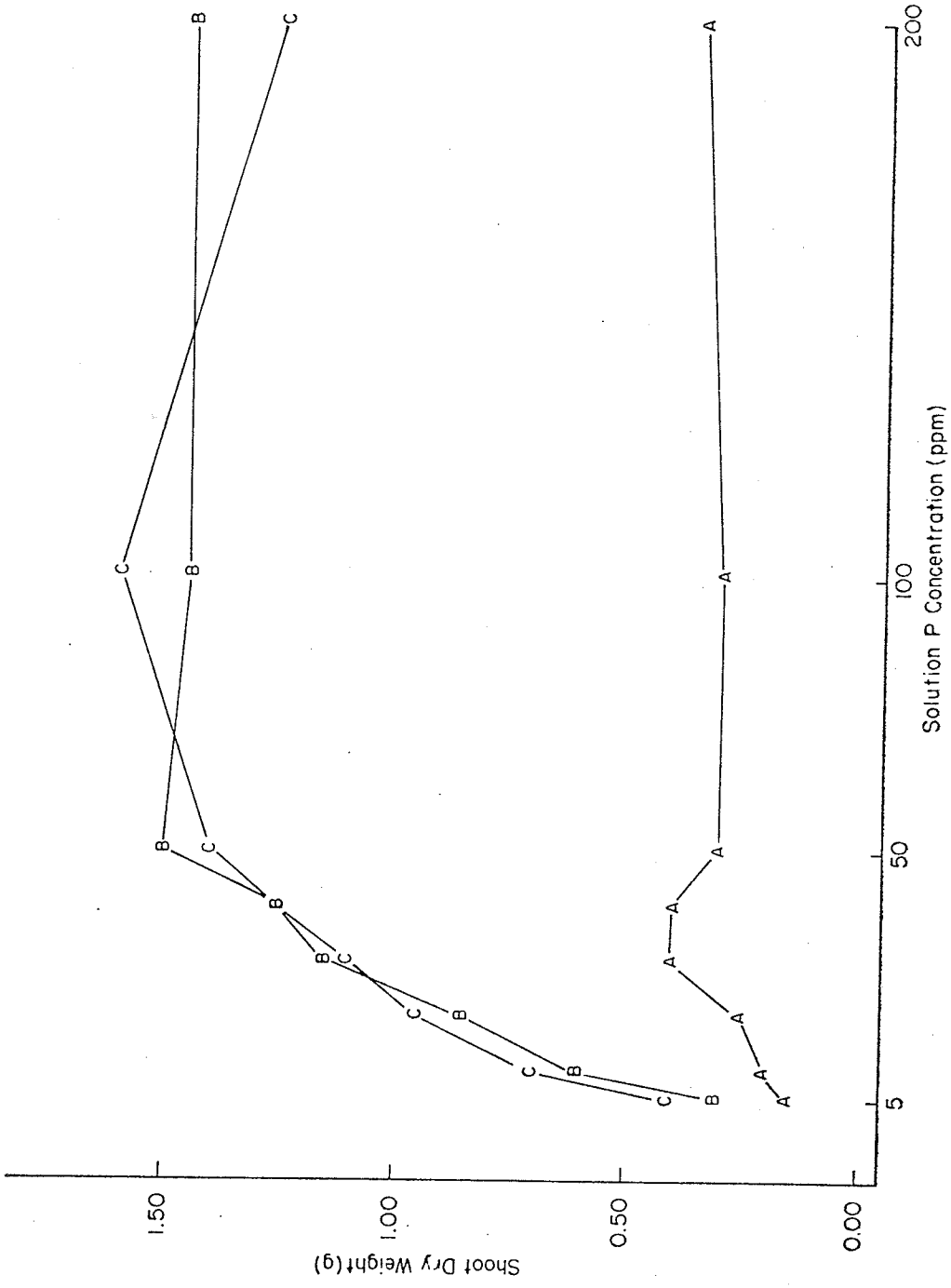

Figure 19: Mean shoot dry weight in response to nutrient solution P concentrations.

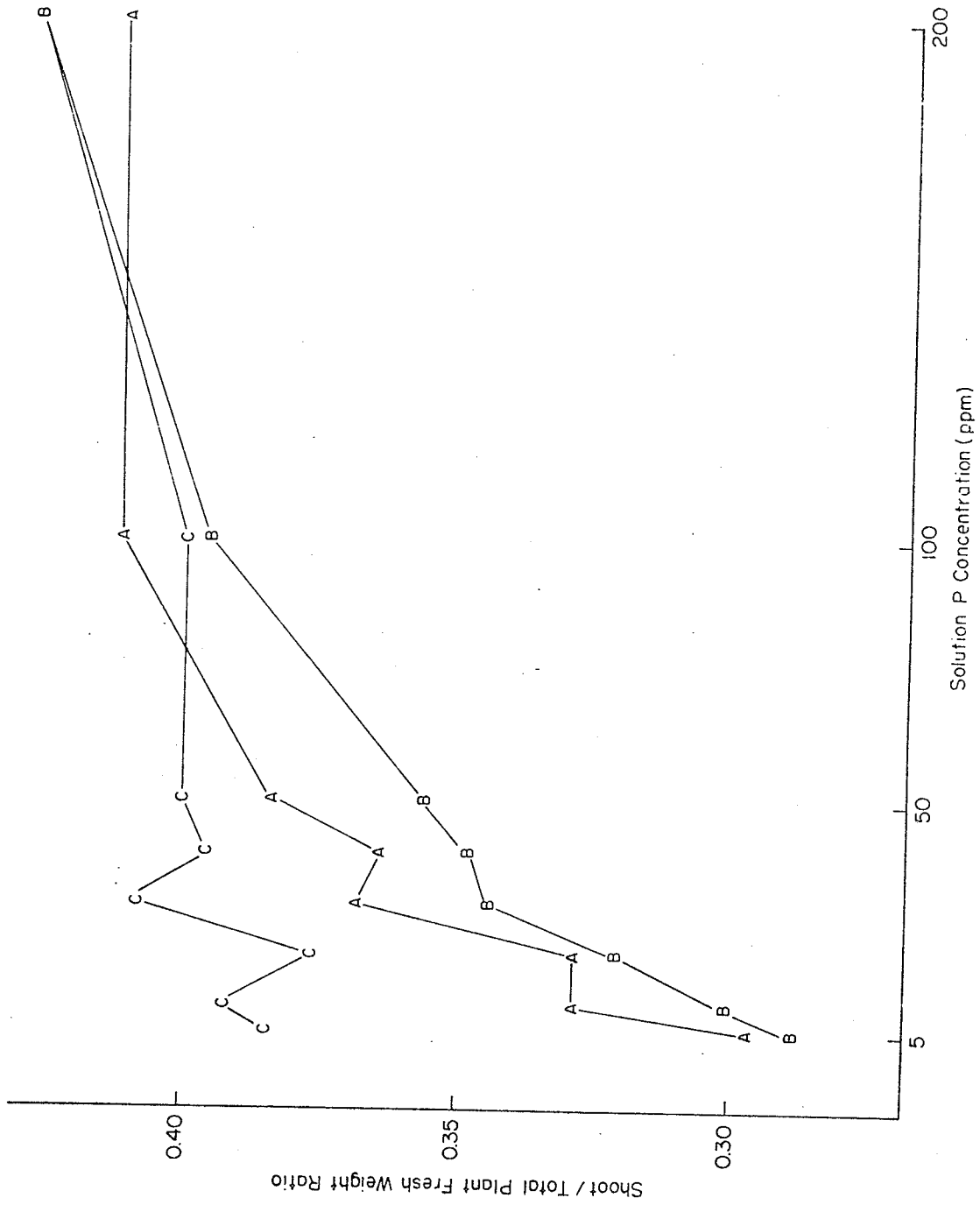

Figure 20: Mean shoot to total plant fresh weight ratio in response to nutrient solution P concentrations.

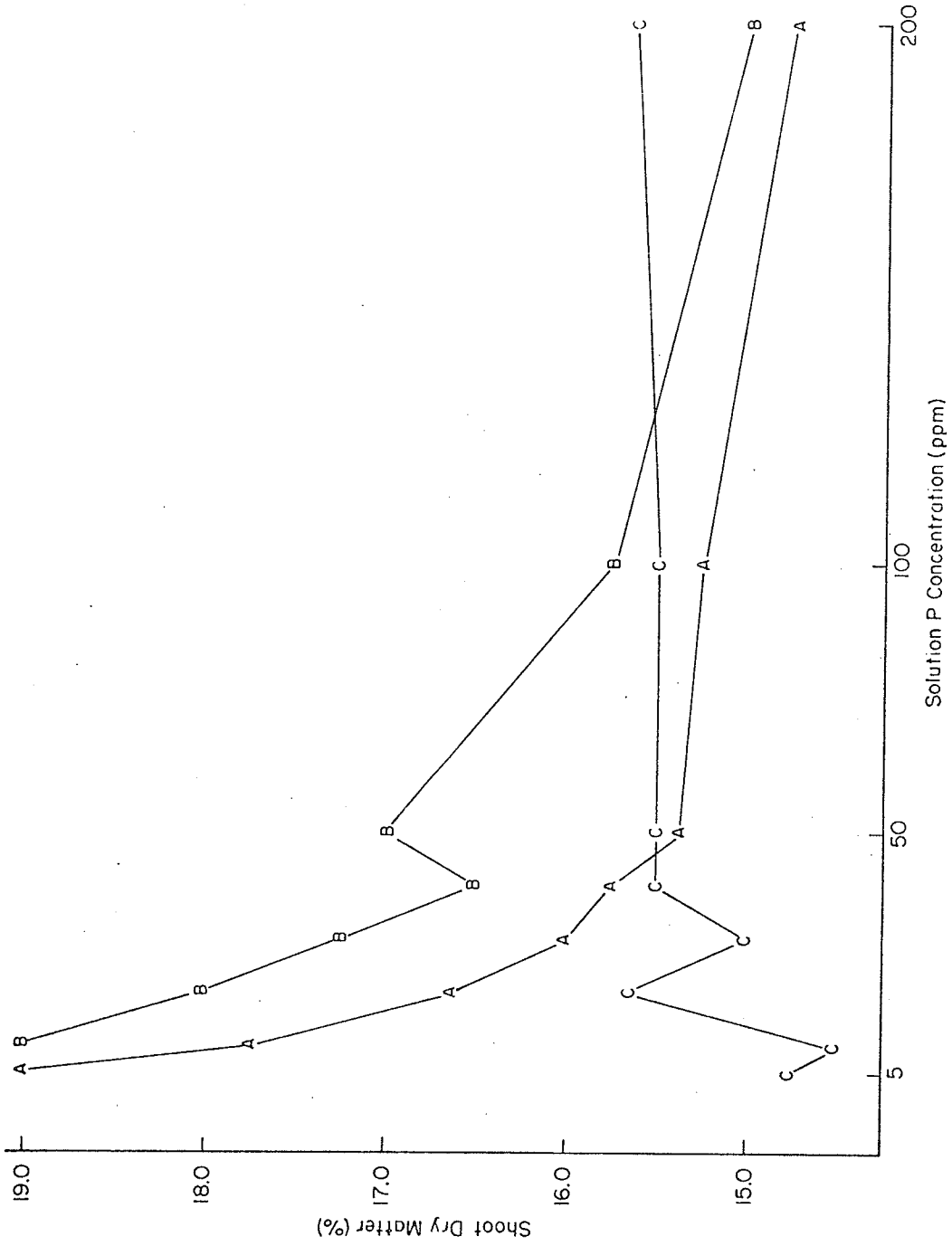

Figure 21: Mean dry matter content of shoots in response to nutrient solution P concentrations.

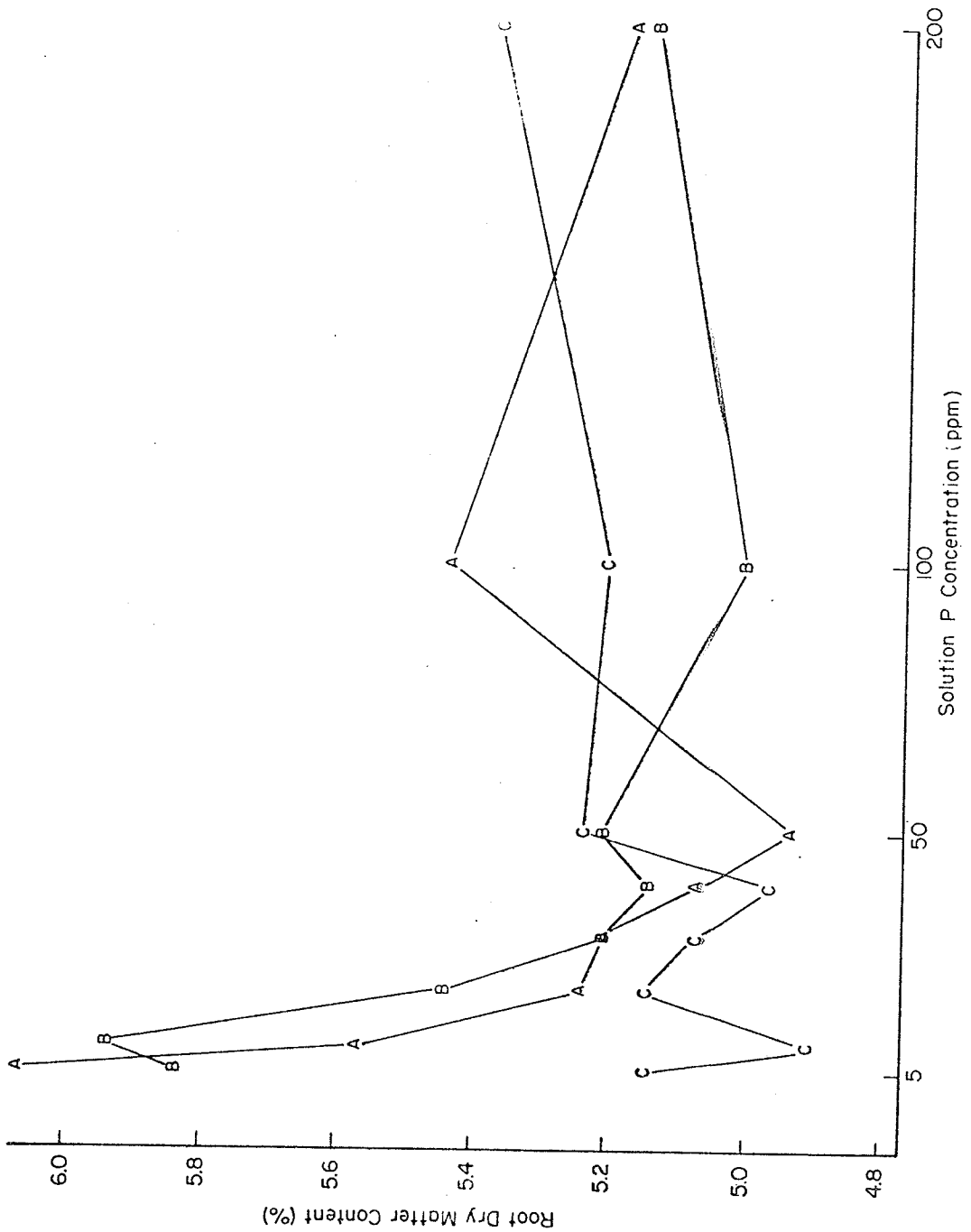

Figure 22: Mean dry matter content of roots in response to nutrient solution concentrations.

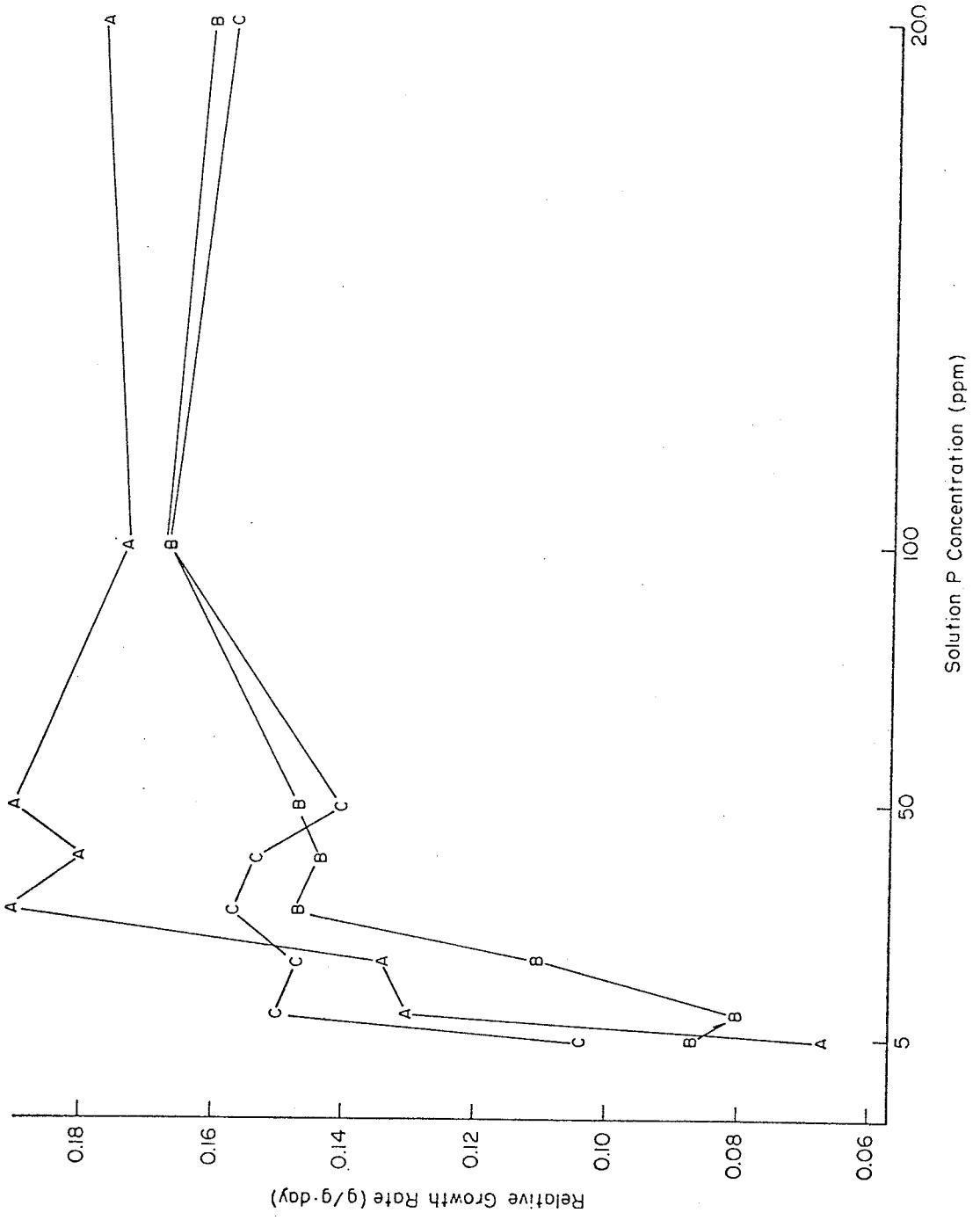

Figure 23: Mean relative growth rate of whole plants in response to nutrient solution P concentrations.

initial solution P concentration less than 100 ppm). It appeared that the 200 ppm P applied to the C plants caused accelerated growth in the plants grown previously in sub-optimal solution P concentrations. This is evidence that the growth response expected from shifts in the shoot/total plant ratio was beginning to materialize.

The absolute growth rates (Figure 24) very closely resembled the shoot dry weight curves. This was expected since absolute growth increase was a direct function of the amount of plant tissue.

The tissue P concentrations (Figure 25) generally increased throughout the range of solution P concentrations for A and B plants, with slight inflections near the solution P concentrations optimal for growth. Thus, P uptake beyond the amount required, that is luxury consumption, probably occurred. Very distinct luxury consumption appeared in the deficient plants which received supplemental P. Clearly, the P uptake mechanisms of these C plants had been optimized as an adaptation to inadequate P supply and were not deactivated rapidly enough following the sudden change to a high P supply. The resulting high tissue P concentrations (as high as 4.5% P in individual plants) were probably toxic. This was noted by Green et al. (1973) under similar conditions of increased P supply.

It was clear from the results presented that yield increases from the B to the C plants were too small to be used to confirm the P status of the B plants. However, several other parameters did respond rapidly enough to show a difference between the C plants and the P-deficient B plants. The response curves (as shown in Figures 19 to 25) of these parameters showed a divergence of the B and C curves at low P supplies (due to P deficiency in the B plants) and a convergence as the P status of the B plants improved. The point of convergence was interpreted as an index of the P status of the

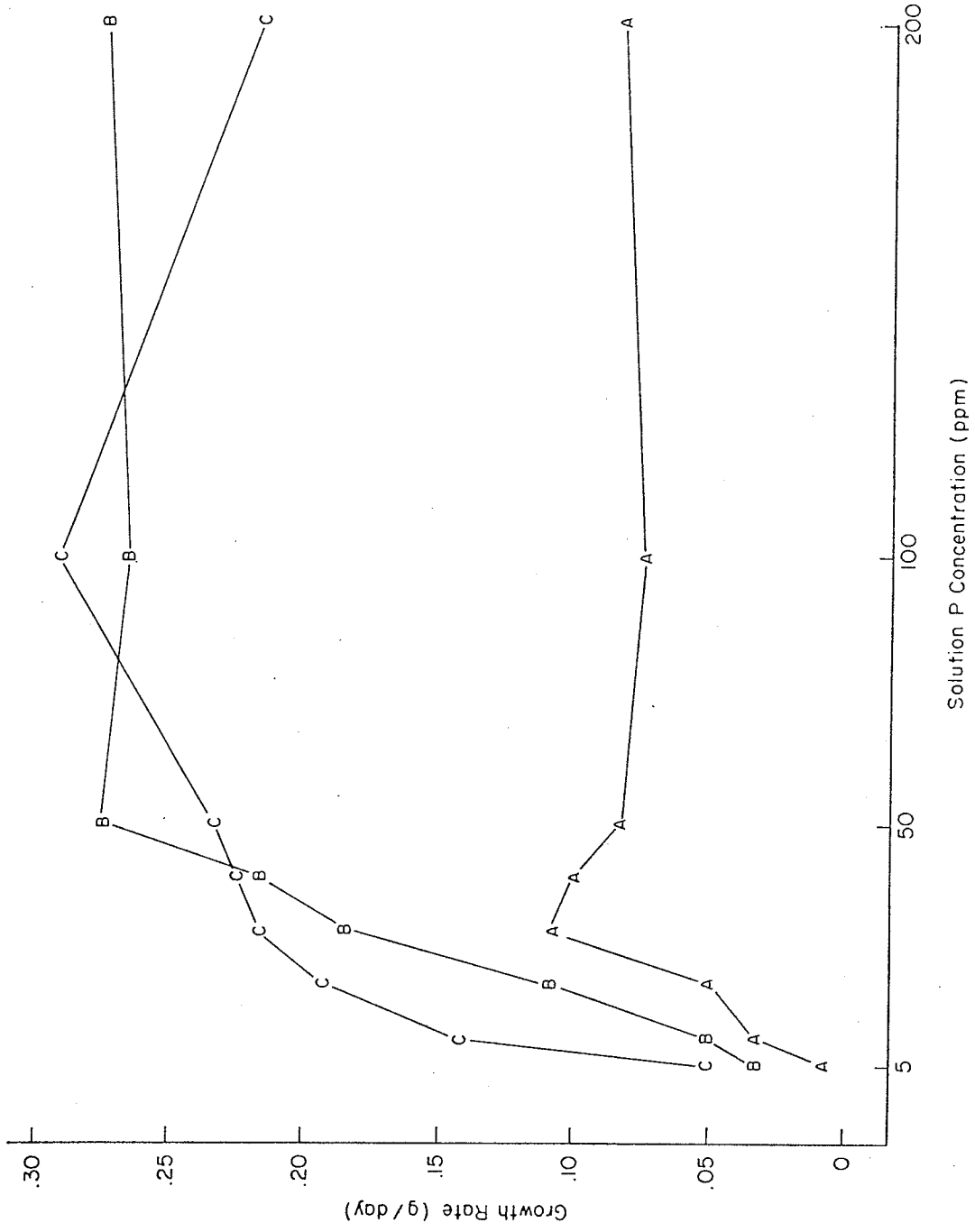

Figure 24: Mean absolute growth rate of whole plants in response to nutrient solution P concentrations.

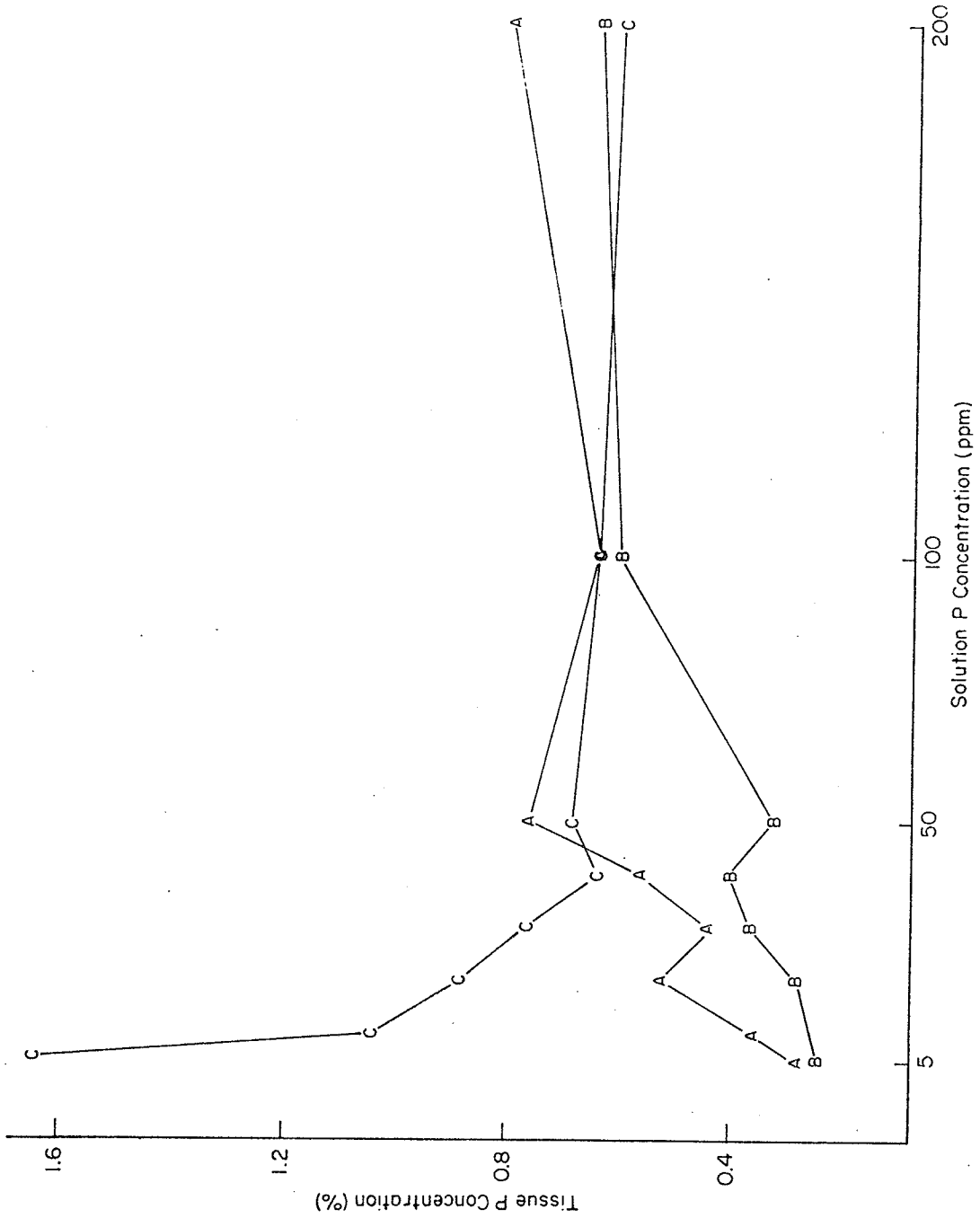

Figure 25: Mean shoot tissue P concentrations in response to nutrient solution P concentrations.

B plants. For example in Figure 20, the curves converge at about 100 ppm P, suggesting that B plants at lower P supplies were deficient.

The convergence points were determined by regressing each parameter for B and C plants on P supply, temperature, harvest stage criterion, and experiment number in a backward stepwise manner. Variables, including all two-way interactions and the square-root of P supply, remained in the equation if their coefficients were significantly different from zero ($P \leq 0.05$). The equation predictions were then examined and the P supply at the points of convergence of corresponding B- and C-plant curves were noted. Convergence was defined to occur when the curves converged to within 5% of the parameter mean. These points were converted to tissue P concentrations (using the equation of tissue P concentration) for presentation in Table 24.

The convergence points consistently decreased as temperature increased for both harvest criteria. The yield parameters of the B and C plants did not differ significantly and thus convergence points of these parameters are not presented. The implication of these findings was that the optimal tissue P concentration may have also decreased as temperature increased. However, a more rapid acclimatization to the new P supply concentration by the C plants at 25°C relative to 10°C would also explain these results, independent of the optimal tissue P concentrations.

In summary, the response of A and B plants to P supply was assessed using shoot dry weight and did, on average, show optima. Confirmation of this response (to assure that the deficiency had not been alleviated with time) was shown by the C plants which responded rapidly in terms of relative growth rate, tissue P concentration, shoot/total plant ratio and shoot dry matter content. Thus, the C plants grown initially at inadequate P

TABLE 24

Tissue P Concentration in B Plants (%) Where the Response of Various Parameters of C Plants Converged With Those of B Plants

Temperature (°C)	Parameter			
	Relative Growth Rate	Tissue P Concentration	Shoot/Total Plant Fresh Weight Ratio	Shoot Dry Matter Content
		"6th Leaf stage"		
10	0.42	0.41	parallel[1]	0.42
15	0.37	0.37	0.42	0.41
20	0.31	0.34	0.40	0.40
25	0.30	0.31	0.39	0.38
		"4 g fresh weight stage"		
10	0.60	0.58	parallel	0.59
15	0.53	0.56	> 0.70	0.56
20	0.49	0.55	0.65	0.54
25	0.47	0.54	0.63	0.53

- The observed parameters were regressed on the supply of P (B and C plants in separate regressions) and the convergence was defined as the lowest P supply where the predictions of the B and C equations were within 5% of the parameter mean. The P supply was converted to tissue P concentration using the regression of tissue P concentrations for B plants.

1. Curves did not converge.

supply concentrations responded to the supplemental P at the time it was applied (the harvest stage criteria), confirming that these plants were deficient at that time.

The results considered thus far have been related to the solution P concentration. However, to be applicable to other systems and because the solution P concentrations were not constant (some depletion was observed), the results and the effects of temperature and plant stage will be considered with respect to tissue P concentrations.

Response to Tissue P Concentrations

The usual criteria for response to tissue P concentrations is the optimal tissue P concentration. In this study, although optima occurred in many cases, some optima appeared to be beyond the range of the data (i.e. yield response continued up to the highest tissue P concentration observed). Assuming, for the sake of computation, that the optimal tissue P concentration in these cases was the highest concentration observed, the mean optima for 10, 15, 20 and 25°C occurred at 0.75, 0.82, 0.93 and 1.01% P for the A plants and 0.58, 0.48, 0.74 and 0.61% P for the B plants. Thus, the optimal tissue P concentration appeared to generally increase as temperature increased. This finding is in apparent contrast to the convergence-point data presented in this study. However, due to the occurrence of few true optima another criterion was used to define plant P status.

A lower optimal tissue concentration implies a more efficient use of P since the maximum yield is achieved with a lower amount of tissue P. The P use efficiency can also be defined as the slope of a total yield versus total P uptake relationship. When P deficiency regulates growth, then this term reflects the yield achieved per unit of P, that is, the efficiency of

P utilization.

The multiple regression approach described previously (for finding the convergence points) was applied to the shoot dry weight and total shoot P content data for the A and B plants. The predictions of the equations for solution P concentrations less than 50 ppm (to include primarily the P-deficient portions of the response curves) were compared by regressing the predictions of shoot dry weight on the predictions of total shoot P content. The linear equations were estimated through the origin. The resulting coefficients (Table 25) were interpreted as the "P use efficiency".

The P use efficiencies increased as temperature decreased. Thus, P utilization was most efficient at lower temperatures and the observed lower optimal tissue P concentration at 10°C than at 25°C was confirmed.

The P use efficiencies were higher for B plants than A plants and were higher at the "6th leaf stage" than at the "4 g fresh weight stage". Thus, as the plants grew larger, the P use efficiency increased. This trend was expected since older plants had more tissues which were no longer actively growing, for example, mature leaves. These tissues would have a P requirement for maintenance only which would be less than that for young, actively growing tissues. Thus the overall P use efficiency, essentially averaging all plant tissues, increased as the plants grew.

The observed response of P use efficiency agrees with inferences drawn from the data presented by Moller Nielsen and Friis-Nielsen (1976) (see Literature Review) but conflicts with the data of Power et al., (1964). These latter authors harvested at a common chronological stage (50 days after planting) for all temperatures and did not confirm the P status of the plants (whether deficient or sufficient) at the time the tissues were analyzed for P. Thus, if the yield response had been determined by an

TABLE 25

Phosphate Use Efficiency of P Deficient Plants
(g dry weight/g plant P)

Temperature (°C)	'A' Plants	'B' Plants
	"6th Leaf stage"	
10	2.45	4.69
15	2.09	4.02
20	1.67	3.61
25	1.39	3.33
	"4 g Fresh weight stage"	
10	1.82	3.57
15	1.73	2.95
20	1.60	2.60
25	1.44	2.38

early, subsequently alleviated P deficiency (as hypothesized to occur for some plants in Chapter 3), the tissue P concentrations at harvest may not have been critical. If so, then the highest yields, which were obtained at the optimal temperature for growth (15°C), would also correspond to the most biological dilution of tissue P. Thus, the tissue concentrations of P would be lowest at the temperature where yield was highest, leading to the conclusion that the lowest optimal tissue P concentration occurred at the optimal temperature for growth. It is suggested, based on the results of the present study, that without confirmation of the plant P status, this conclusion must be reconsidered.

The concept of more efficient P utilization at lower temperatures is consistent with the corresponding biological activity. At lower temperatures, the slower growth would allow more time for P translocation and therefore greater potential for efficient usage by the plant.

The results of the previous soil-plant experiment (Chapter 3) suggested that the plant P requirements were highest at 10°C. It was hypothesized that this phenomenon was due to the earlier stage development of the plants when harvested at 10°C. The present study supports this hypothesis since plant P requirements were less at 10°C when differences in stage of development were eliminated. Thus, the higher plant P requirements noted in Chapter 3 for plants at 10°C was most likely due to the corresponding early developmental stage.

In the present study, the treatment temperatures were applied only to the roots, with some temperature gradient effects on the shoot apex, but no direct effect on the photosynthetic tissues. Thus, apart from P metabolism in the root, the observed effects of temperature on P status must have been indirect. Clearly plant growth rate and development rate were retarded at

the lower temperatures. Thus, the observed changes in P use efficiency with temperature were attributed primarily to intermediate effects on plant growth rate.

The effect of temperature on plant growth rate was characterized by estimating the total plant relative growth rate at each temperature when P supply was optimal. This was done by finding the maxima among predictions from multiple regressions developed as for the convergence-point analysis. The P use efficiencies were significantly, negatively correlated to the optimal relative growth rates (Figure 26) with coefficients of -0.89 for A plants and -0.92 for B plants (correlations across temperatures and harvest-stage criterion). Thus, for plants at approximately the same developmental stage, the effect of temperature on P use efficiency was well explained by relative growth rate.

This finding has important consequences in that such a relationship may hold when other environmental factors limit growth. If so, then the response of plants to P as modified by environmental conditions may be characterized by the effect of those conditions on growth rate alone. This concept has the potential to simplify computer simulations of plant P nutrition.

The relationship changed markedly from the A to the B plants, indicating that developmental stage or plant size does modify the relationship. Clearly, plant development must be considered when evaluating plant P status.

Conclusions

Phosphate use efficiency by the plant was found to be greater at the lower root temperatures and to be negatively correlated to relative growth

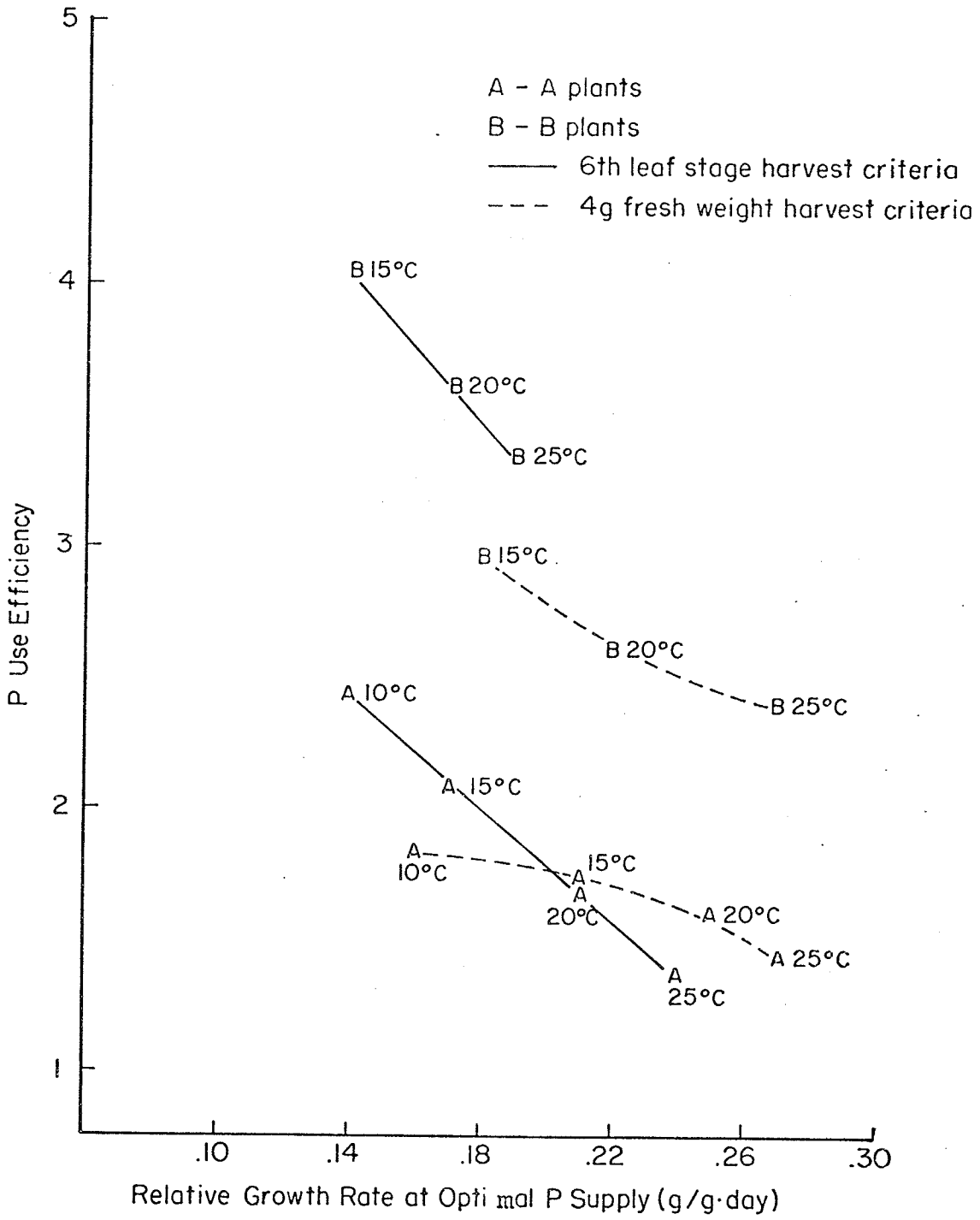

Figure 26: P-use-efficiency as a function of optimal relative growth rate at four root temperatures.

Note that the optimal relative growth rate at 10 °C for the B plants was beyond the present data.

rate. The optimal tissue P concentrations also appeared to be lower at lower root temperatures but too few well defined optima were observed to clearly show this relationship. However, the relationship between the optimal tissue P concentration and relative growth rate, as proposed, may be useful.

This study emphasized the dynamic nature of plant P nutrition. Clearly this experiment required the careful confirmation of plant P status since changes in P supply, growth conditions and biological dilution rapidly changed the tissue P concentrations. Parameters such as shoot/total plant ratios and dry matter contents were particularly useful in this regard since they changed much more rapidly than yield or growth rate in response to changes in P supply.

The implication of these findings in the field are that, although cool soils may impede P supply, the actual requirement for P by the plant is also likely to be lower. Thus, the actual P status of plants growing in cool soils is the result of several opposing, dynamic factors.

Chapter 6

SUMMARY OF FINDINGS

This study was initiated to examine certain aspects of weather-crop response interactions. It was felt that conceptual and simulation models needed more detailed information to interpret the complex systems involved. In this study, root temperature and plant P-nutrition were the conditions examined.

The literature review cited the results of much research in this area. The soil P system has been well examined but few results were found which described the effects of temperature on the P solubility profile. The plant P system has also been well researched but few papers dealt with the specific effect of temperature on plant P requirements. Finally, the linkage of the soil and plant systems, particularly with emphasis on dynamic interactions, has not been well researched.

The present studies approached the problem in three ways. The first involved a soil-plant culture system with varied root temperatures which emphasized the dynamic nature of soil-plant interactions. The second involved extraction of soil P, both chemically and with plants, to thoroughly characterize soil temperature effects. The third involved solution culture of plants to demonstrate requirements for P in a system that was not buffered for P supply.

The results of this study have been presented in three chapters representing the three different experimental approaches. The first, the soil-plant experiment, examined the response of wheat to various concentrations

of broadcast and band-applied P at four soil temperatures. This experiment suggested that the system of plant P nutrition was dynamic. The P status of the plants changed from sufficient to deficient during the experiment, the tissue concentrations of P were diluted rapidly by growth and the plants exhibited adaptability to P supply by proliferating roots in the zone of band-applied P. This later response seemed to be triggered by adverse growing conditions which probably induced P deficiency. However, despite the profound effects of temperature on growth, the optimal concentration of fertilizer P for growth did not change markedly.

The second experimental approach, involving soil incubation and extraction studies, included extraction by short term plant growth. Higher temperatures increased the solubility of soil P as well as the rate of fixation of applied P. This later effect resulted in decreased solubility of fertilizer P during the reaction period. Thus the "age" of recently applied P dictated the apparent effect of temperature on P solubility. The soil P system was described by a solubility profile reflecting a continuum of P forms from relatively soluble to rigidly fixed. Desorption curves characterized this profile and slopes varied with temperature in at least some cases.

The third experimental approach, the solution culture experiment, allowed plant growth to be monitored closely and P supply to be changed accurately and quickly. Thus, it was possible to confirm plant P status at harvest. This study showed that plant P use efficiency was highest when root temperatures were lowest. Thus, under poor growing conditions, the plant has the opportunity to use P more efficiently.

The later two experimental approaches were very useful in outlining several opposing effects of temperature. When the soil was at a relatively

cool temperature, the solubility of soil P was depressed and root growth was severely decreased. However, fertilizer P remained more soluble and plant P requirements in absolute amounts and in terms of rate of supply were less. Thus, it is very conceivable that these processes may be balanced such that fertilizer P requirements are not affected. This balance was observed in the first experiment. The apparent contradiction of the findings of the first experiment to numerous reports in the literature of higher P requirments in cold soils merely reflects a different relationship among the processes involved.

The overall conclusion of this study was that P nutrition was not static, was the result of the balance of various opposing processes, and was modified by the adaptive response of the plant. Thus, the only way to assess the effect of temperature on P fertilizer requirements would be to conduct very realistic and extensive response trials, or to assemble the knowledge of the various processes into a computer simulation. It is hoped the data presented herein will be useful to either approach but will be particularly well adapted to the formation of a computer simulation model.

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Appendix A

DESCRIPTION OF WATER BATHS

Temperature in the water baths (Figure 27) was controlled by continuously cooling the water and then maintaining the desired temperature using heating coils which were thermostatically controlled. The heating coils and thermostats were positioned adjacent to a circulating pump to ensure efficiency. Cooling at 25 and 20°C was due to the temperature gradient between the bath and air temperatures whereas cooling at 15°C was accomplished with a refrigeration "dip cooling" probe. The probe was placed in a partially closed chamber to inhibit water circulation and thus decrease its cooling efficiency to a level in balance with the heater-circulator. A submersible pump was used to eliminate vertical temperature gradients near the cooling probe chamber. Cooling at 10°C employed the same type of dip cooler probe but it was encased in a flow-through chamber and water was forced over the probe to increase its cooling efficiency. Polyethylene glycol at 0.1% by weight was added to prevent icing of the dip cooler probe.

The water in the 25, 20 and 15°C baths was circulated using the heater-circulator pump connected to a hose which forced water to the opposite end of the bath. Water circulation in the 10°C bath and through the flow-through chamber on the dip cooling probe utilized two hoses connected to a submersible pump. The end of the 10°C bath, containing the control apparatuses, was separated by a divider and the heater-circulator pump ensured mixing in this end to improve control efficiency.

Water flow around the heating coil and thermostat of each bath was

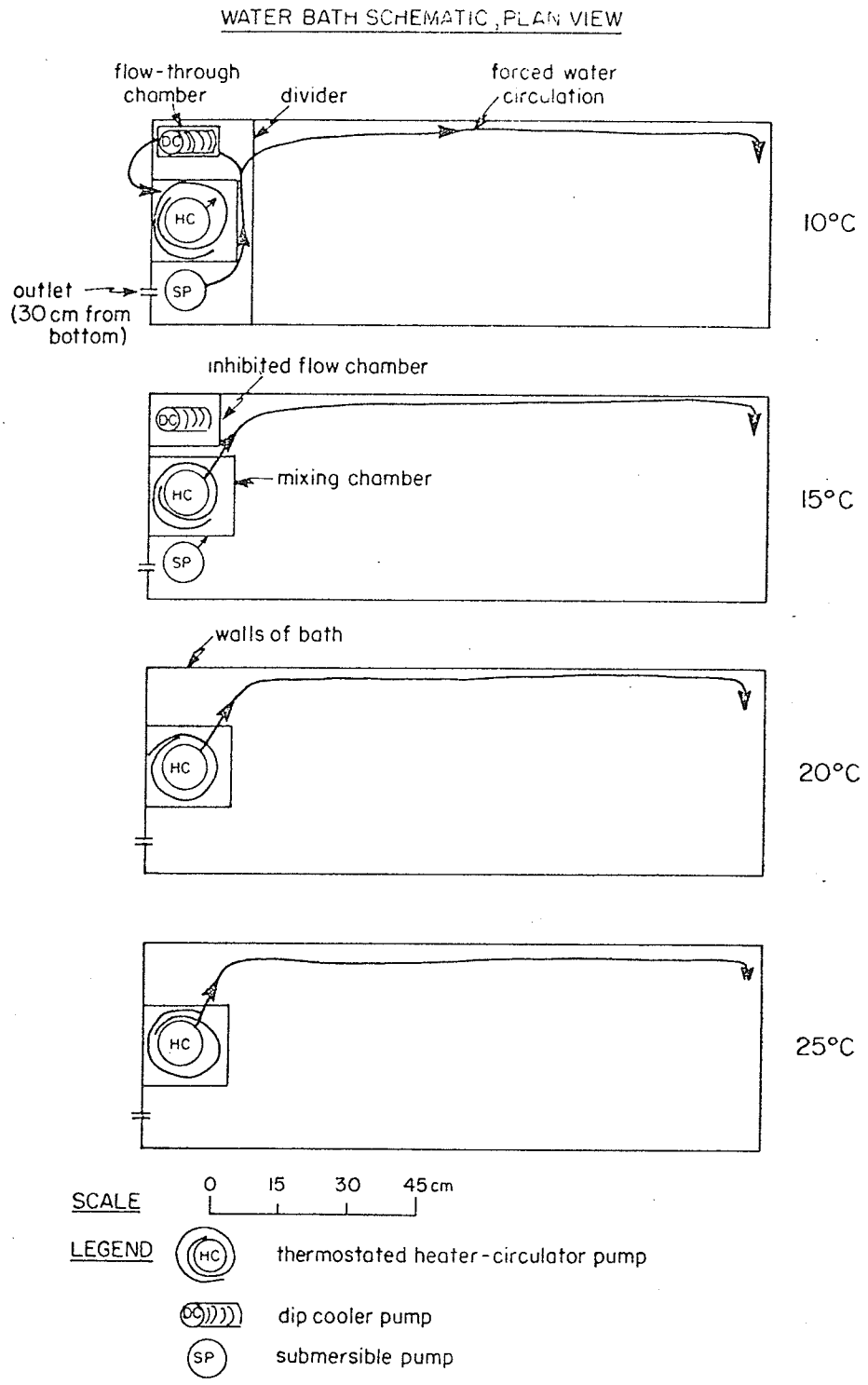


Figure 27: Schematic of the temperature-controlled water baths, plan view.

ensured by encasing each unit in a small mixing chamber which forced the water to flow over the heating coils prior to entering the heater-circulator pump.

Water was added as required and an outlet ensured a maximum water depth of 30 cm. Algal growth (when abundant) was controlled with the addition of 60 ppm $CuSO_4$.

The baths were constructed of 1.9 cm thick plywood, lined with 1 mm rigid plastic, and then sealed with silicon caulking material. They were supported 20 cm above the growth chamber floor to ensure the circulation of the air in the growth chamber.

The growth chamber provided 18/12°C diurnal temperature change. The inlet for air temperature and humidity sampling by the growth chamber control apparata was extended above the baths to the canopy level of the growing plants.

Temperature regulation throughout the baths was $\pm$ 1°C from the intended temperature. Water flow around the control apparata was the most critical controlling factor.

End-Over-End Agitator for the Water Baths

Two end-over-end agitators were designed to operate in the water baths.

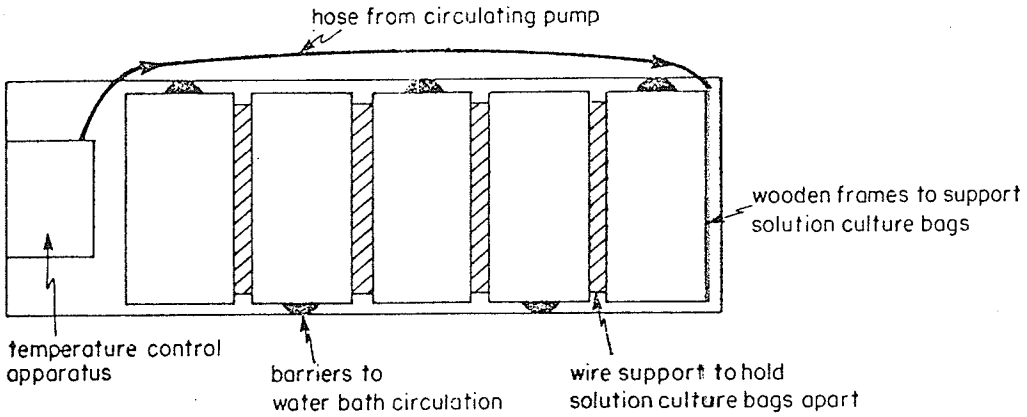
A 1-cm diameter iron rod was rotated 10 to 15 cm below the water surface by means of a 12.7 cm pulley and a V-belt. The V-belt was driven by a 5.1 cm pulley which was connected to a 28 rpm electric motor. Test tubes and bottles were attached perpendicularly to the submerged rod using elastic bands and were thus agitated end-over-end at 11.2 rpm. The agitators were lifted from the baths to achieve access to the test tubes and bottles.

The agitator used for Experiment B of Chapter 4 consisted of one 50-cm long submerged rod which operated in one bath at a time. The second agitator operated four submerged rods each 30 cm long, one in each bath, simultaneously. This was used for Experiment A of Chapter 4 (see Plate 6, Appendix H).

The submerged parts of the agitator were corrosion-susceptible and frequent drying and oiling was required to keep them operable.

Water Bath Adaptation and Containers for the Solution Culture Systems

Wooden frames were placed in the water baths to support the solution culture bags used in the experiment reported in Chapter 5 and Appendix J (see Figure 28). The frames were rectangular boxes (18 x 43 cm, 36 cm deep) which rested on the floor of the water baths (Figure 29). The ends of the boxes were constructed of wood, the sides were of flexible vinyl and the bottoms were open. These boxes were positioned 3 cm apart in the water baths by supports made of wire mesh and plastic pipe so that water could flow between the flexible vinyl sides of the frames. The circulation of the water in the water baths was channeled to flow along the sides of each nutrient culture container.



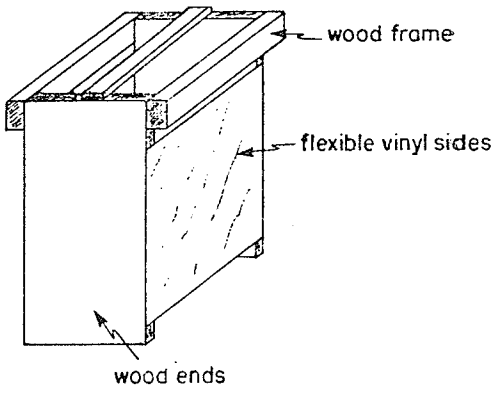


Figure 28: Schematic of a water bath with solution culture frames in place.

Figure 29: Schematic of one solution culture frame.

Water Bath Apparatuses

Cooling Probes

- Model RU-5 Dip Cooler units were obtained from Techne Incorporated. Each used 400 watts at 20°C

Heater-Circulators for 10 and 25°C

- Model 180 Fisher Circu Stat temperature controllers were obtained from Fisher Scientific Company. Each used 1000 watts

Heater-Circulators for 15 and 20°C

- Model Paratherm IM temperature controllers were obtained from Julabo Labortechnik. Each used 1000 watts

Submersible Pumps

- Submersible pumps were obtained from Little Giant Pump Company.

Appendix B

WET ASHING AND ^{31}P ANALYSIS PROCEDURE

Wet Ashing

- place 0.05 to 3.0 g dry weight of plant material, whole or ground, into micro-kjeldahl flask
- add 5 mL concentrated HNO_3 and pre-digest at room temperature for 1-4 hours
- add 2.5 mL concentrated $HClO_4$ and digest at boiling point 2-4 hours, until clear
- filter with #1 Whatman if cloudy
- dilute to 25 or 50 mL with distilled water
- store in plastic scintillation vial

^{31}P Analysis

Reagent A - 500 mL distilled water
- 7.5 g ammonium paramolybdate $(NH_4)_6Mo_7O_{24}.4H_2O$
- 0.14 g antimony potassium tartrate
- 88 mL concentrated H_2SO_4
- dilute to 1000 mL

Reagent B - 2.5 g L-ascorbic acid in 100 mL water
- prepare daily

- dilute sample to 0.05 - 0.60 mm P range
- adjust pH to 2 using 2-4 dinitrophenol as indicator
- combine reagents (4 parts A, 1 part B), prepare daily
- add 1 part reagent to 5 parts diluted sample, allow 5 minutes for colour development
- record absorbance at 885 nm within 3 hours

Appendix C

ANALYSIS OF ^{32}P

Cerenkov radiation, emitted as the beta particles from the decay of ^{32}P passed through aqueous media, was detected using a liquid scintillation counter. This analytical system was chosen because the sample was not contaminated by a scintillation cocktail and therefore could be used for further analyses. Counting efficiencies were improved by using opaque plastic scintillation vials.

Two liquid scintillation counters were used, a Beckman model 7500 and a Searle model Mark III. Unless otherwise indicated, the results presented were obtained using the Beckman counter.

Counting efficiencies for the Beckman counter were estimated using an internal standard or a sample channel ratio that traced spectral shift. Spectral analysis of ^{32}P-Cerenkov radiation (Figure 30) showed the entire activity to be between the window settings of 0 and 650. Colour quenching (controlled experimentally with yellow food colouring) shifted the spectra downward and the 0 to 200 window was used to monitor this shift. Therefore, the sample channels ratio (SCR) was used to estimate quenching (Figure 31) and was defined as (window 0-200)/(window 0-650). This technique also corrected for variations in the colour of the scintillation vials (Smith, 1981).

No effect of chemical quenching with acetone up to 20% acetone by volume was found. Counting efficiencies were uniform through the sample volume range of 5 to 17 mL.

Counting conditions established in the counting program included back-

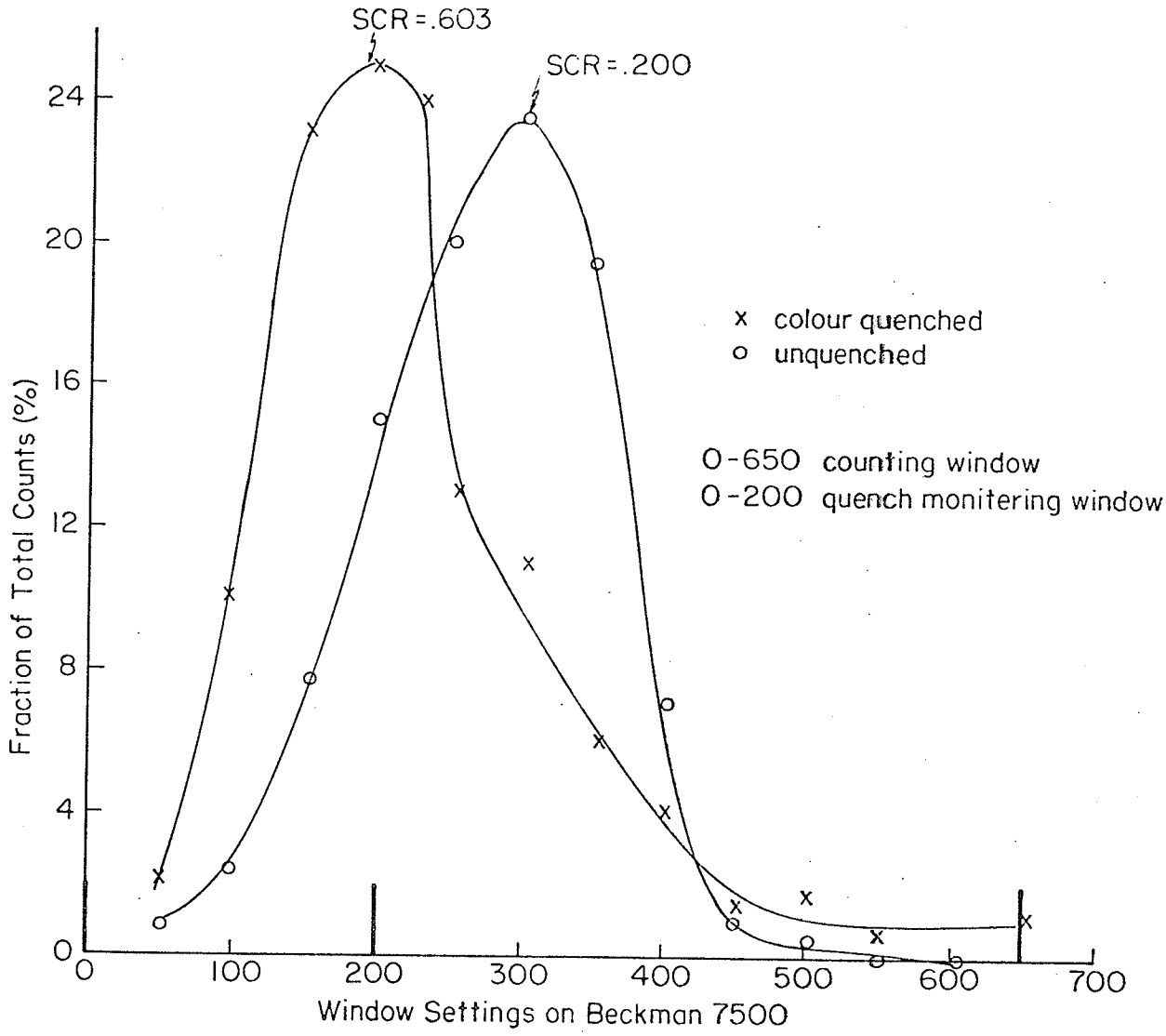
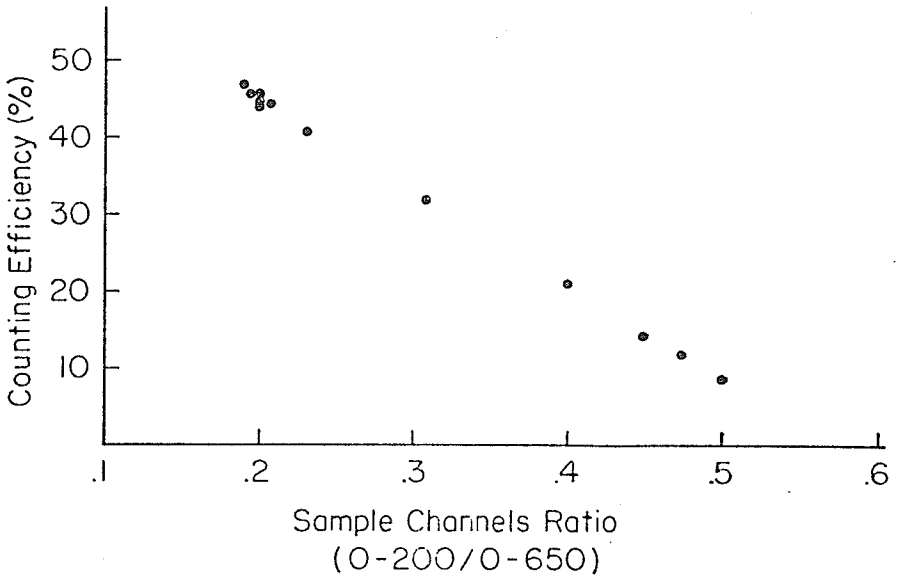


Figure 30: Spectral shift of ^{32}P-Cerenkov radiation due to colour quench using the Beckman 7500 Liquid Scintillation Counter.



$$\text{Efficiency} = 93.73 - 358.68(SCR) + 745.65(SCR)^2 - 743.69(SCR)^3$$

Figure 31: Quench curve for Cerenkov counting of ^{32}P in plastic vials for the Beckman 7500.

ground subtraction, half-life correction during the counting session, counting to 20 min or 2% error (whichever occurred first) and calculation of the sample channel ratio. Counting efficiencies were also calculated from a second count after the addition of a known activity of ^{32}P as an internal standard. Subsequent regression of the internal standard efficiencies versus the sample channels ratio provided a check of the counting procedures. Uniformity of addition of 0.1 mL of internal standard was indicated by a coefficient of variation of 1.11%.

The Searle counter was used for the ^{32}P analysis in Chapter 4, Experiment B. The external standard pulse height (ESP) of the ^{32}P program in this counter was found to be insensitive to quenching in the Cerenkov system. Thus, counting efficiencies for Cerenkov counting in this machine were based solely on internal standard counts.

Liquid scintillation counting using PCS counting fluor was employed with the Searle counter (see Chapter 4, Experiment B). For this system, the ESP of the ^{32}P Program was found to effectively predict quenching (controlled experimentally by addition of acetone) and therefore counting efficiencies for this system were based on the ESP.

Appendix D

VISUAL OBSERVATION OF ROOT PROLIFERATION

Introduction

Studies reported in Chapter 2 showed that root proliferation in a P-enriched fertilizer band was greatest at low temperatures. However, these plants were also physiologically younger than those at higher temperatures and thus it was not clear whether temperature or physiological age was the primary controlling factor on root proliferation. This experiment was designed so that root growth and proliferation could be examined with time. The objective was to determine if root proliferation occurred in young plants at higher temperatures and, if so, was it masked by non-differential root growth as the plants became older.

Methods and Materials

Soil containers were prepared as described in Chapter 3 to correspond to the 20 ppm P banded treatment except that no ^{32}P was used. The soil containers were semi-circular halves of those used in Chapter 3. A clear perspex plate was attached to the flat side so that root development in the soil could be observed and photographed. Planting and watering procedures were as described in Chapter 3.

Duplicate containers were placed in each water bath (at 10, 15, 20 and 25°C) and the plants were grown for 38 days with photographs of the root development taken at 11, 17, 21, 26 and 38 days after planting.

Proliferation was evaluated visually as the proportion of roots in

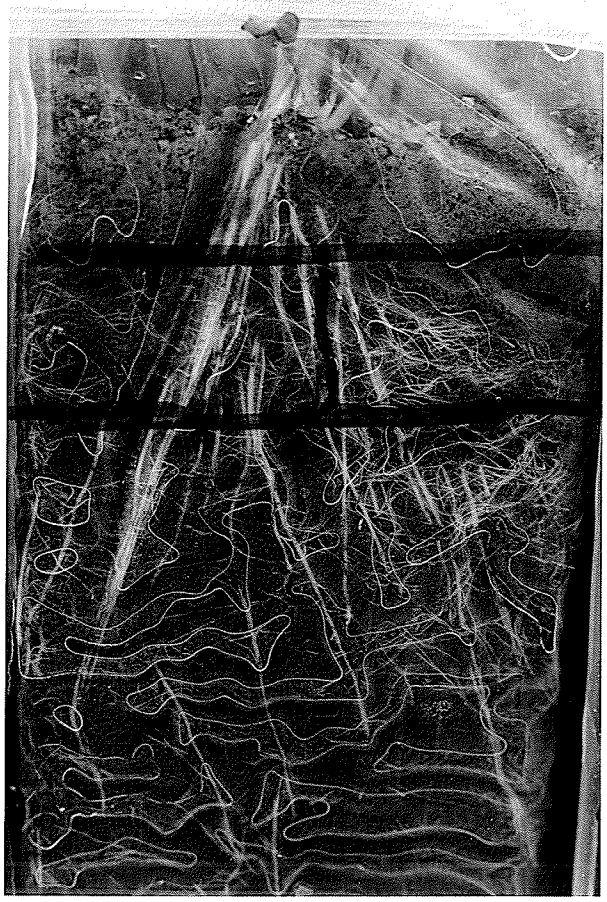
P-fertilized half of the band zone relative to the opposite, control half of the band zone.

Results and Discussion

Eleven days after planting, the roots at 25, 20 and 15°C had penetrated to the bottom of the pots and were visible throughout the soil. At 10°C, very few roots were evident. The roots at the higher soil temperatures were more advanced in terms of lateral root development. Proliferation of roots in the fertilizer band was first observed 11 days after planting, expecially at 15°C but also at 20 and 25°C.

Proliferation of lateral roots during the 11 to 38 days after planting became quite distinguishable in both duplicates at 15°C (Plate 1). At 20 and 25°C, the roots in one container at each temperature showed slight proliferation whereas the other was evenly distributed. At 10°C, the roots were slow to develop and were abundant only after 26 days from planting. A slight proliferation was noted at day 21 for roots at 10°C but was still not very pronounced even 38 days after planting.

Changes in root proliferation with time and hence physiological age were observed, especially at 15°C where proliferation increased with time. However, when the plants at each temperature were compared at day 38, there was evidence of a mechanism by which proliferation, measured on a weight basis, would be diminished at higher temperatures (and hence, in older plants). At 20°C and especially at 25°C, a large number of nodal axial roots were developing at day 38, growing downward in a cone-shaped distribution pattern independent of the P fertilizer band (Plate 2). These roots were much larger in diameter than the lateral roots found to proliferate in the band and hence, on a weight basis, would dominate the root samples.



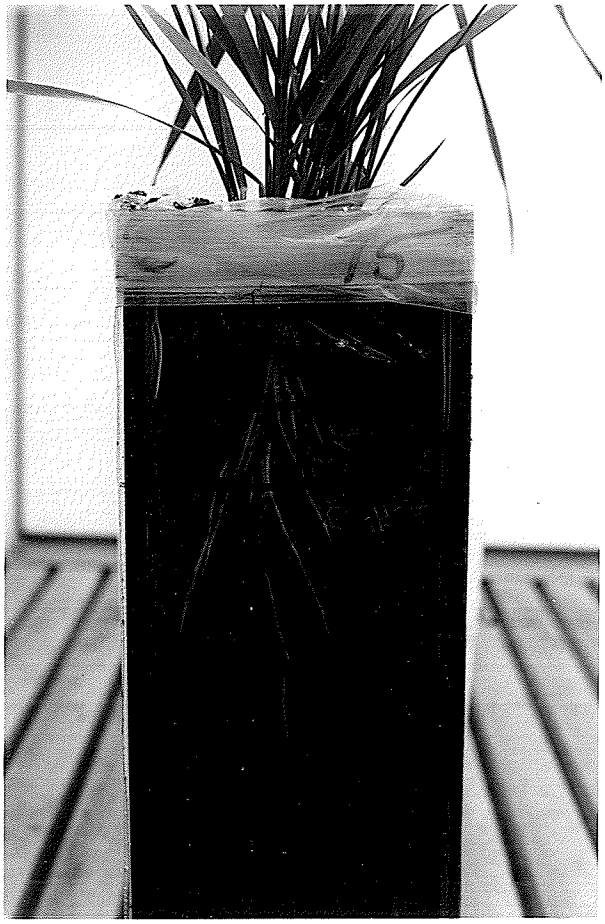


Plate 1: Proliferation of lateral roots in the P-fertilized half of the band zone (right half in photograph)

Plate 2: Extension of nodal roots from the plant crown

Thus, as these roots which developed late in the experiment penetrated the band (in a random manner) their weights would mask differences in lateral root weights and proliferation would appear to diminish.

It was apparent in this study that the variability observed in the root proliferation data (Chapter 3) was not only due to difficulties in root recovery but also due to the random (with respect to fertilizer placement) directions of axial root growth. Both seminal and nodal axial roots originated from the seed or stem and extended into the soil, directed by properties such as genetic parameters, geotropism and soil physical restraints. The location of a fertilizer band several centimeters away from the seed had little or no effect on the original direction of lateral growth. Thus proliferation was manifest in lateral root development from the axial roots which penetrated the band in a random manner. The variability in proliferation was therefore increased by the variable number of axial roots which penetrated the band. This problem was more acute in the clear-front containers since visible proliferation could only occur when an axial root penetrated the band very close to the clear front.

Conclusions

Visual observation of root development around a fertilizer band was instructive of the processes involved. Proliferation was due to lateral root development from axial roots and the axial roots penetrated the fertilizer band on a random basis. Proliferation increased with time as the lateral roots developed in and exploited the fertilizer band. However, nodal root axes which developed late in the experiment and randomly penetrated both the P-fertilized and control bands would have masked proliferation measured on a weight basis.

The effect of temperature on physiological age was distinct and thus lateral roots (which accounted for proliferation) developed earlier at higher temperatures. Furthermore, the nodal root development which would have masked root proliferation measurements was also earlier at higher temperatures. Thus, although visual observation was not adequate to confirm the hypothesis, there was evidence of a mechanism by which root proliferation at higher temperatures would be diminished due to the concomitant advanced physiological age.

Appendix E

DATA WITH STATISTICS FOR CHAPTER 3

The data presented in this appendix are the means of two replicates. Averaging of the two replicates for shoot dry weight was performed on the $\log_e$ transformed data with the antilog_e of these means presented. The data are classified by treatment under the columns "broadcast P", "band-applied P" and "combination-application of P". The levels of P applied to the combination treatments refer to band-applied P, excluding the 20 ppm that was broadcast-applied to these treatments.

A split plot analysis of variance (ANOVA) was conducted using temperature as the main plot and P as the subplot treatments. The coefficient of variation (CV), the degrees of freedom for error (df_{errorb}) and the error mean square ($MS_{e(b)}$) were based on the subplot error term (error b).

The variable descriptions are given in the Methods section.

Plate 3: Respnse to broadcast P at 10 C (in the background) and 25 C (in the foreground), P supply increasing left to right (0,5,10,20,40, and 80 ug/g)

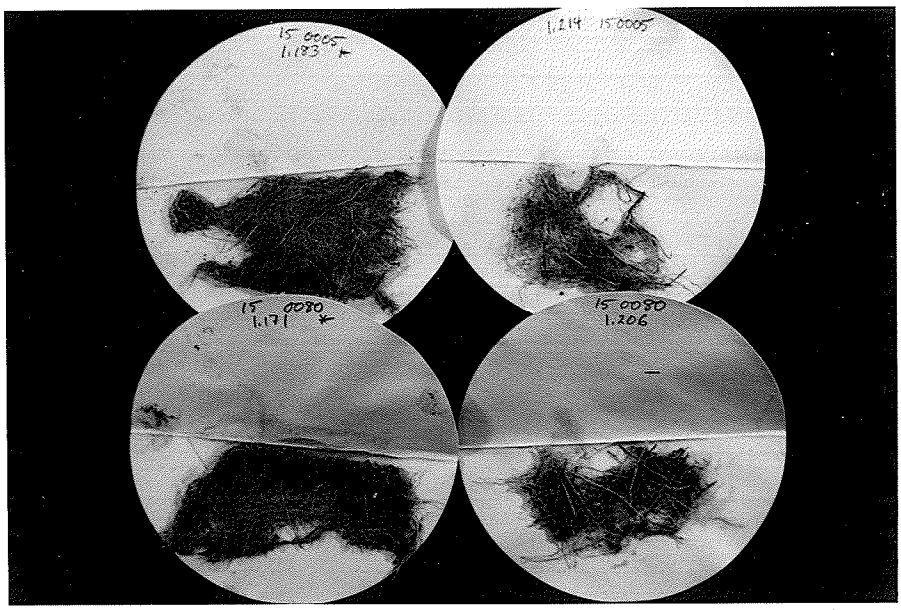


Plate 4: Root growth in the band zone at 15 C, P-fertilized zone on the left (top left at 5 ppm and bottom left at 80 ppm) and control zone on the right

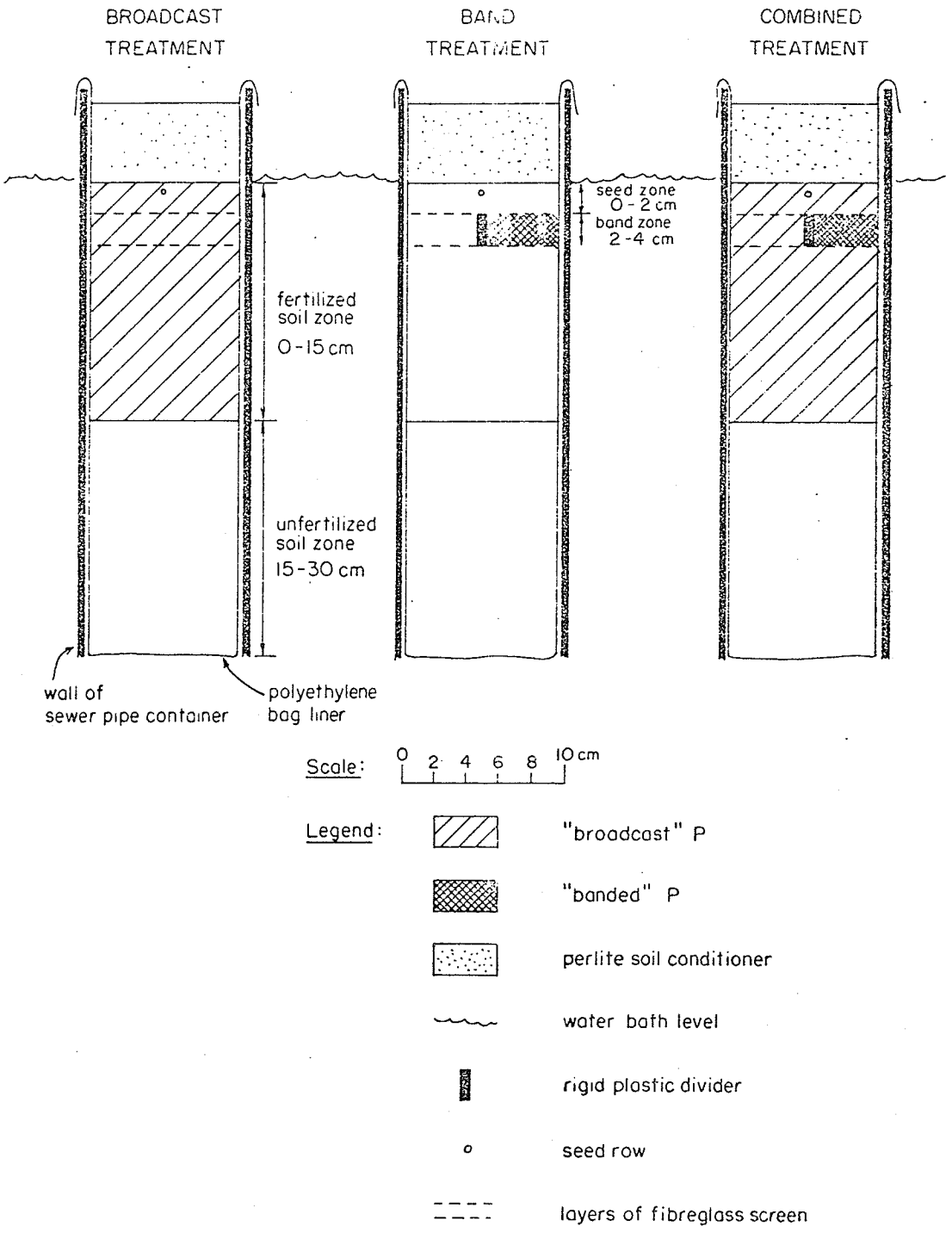


Figure 32: Schematic of treatment placement and planting procedure used in Chapter 3.

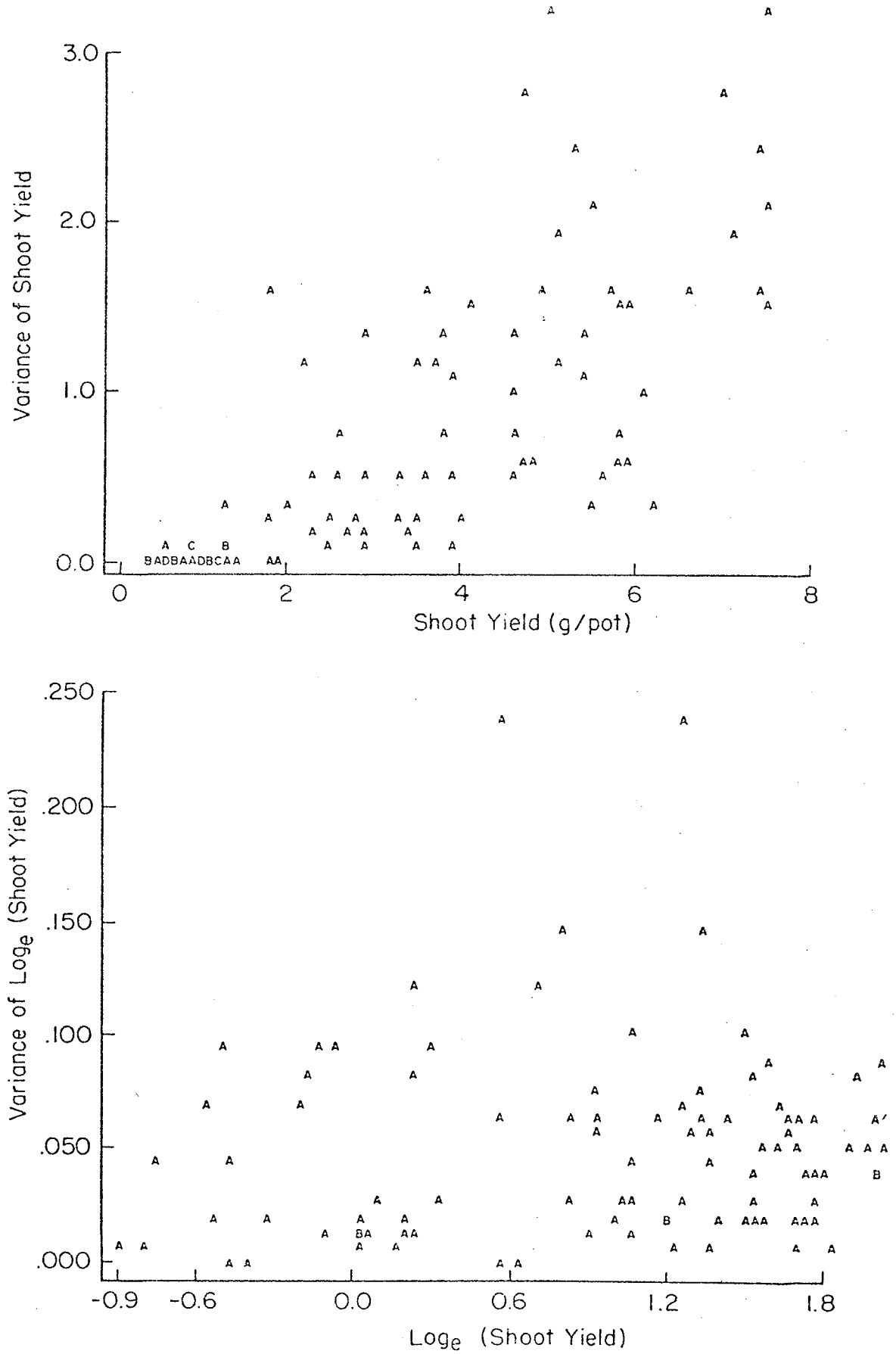


Figure 33: Plots of variance versus means for shoot dry weight data in linear and $\log_e$ - transformed scales.

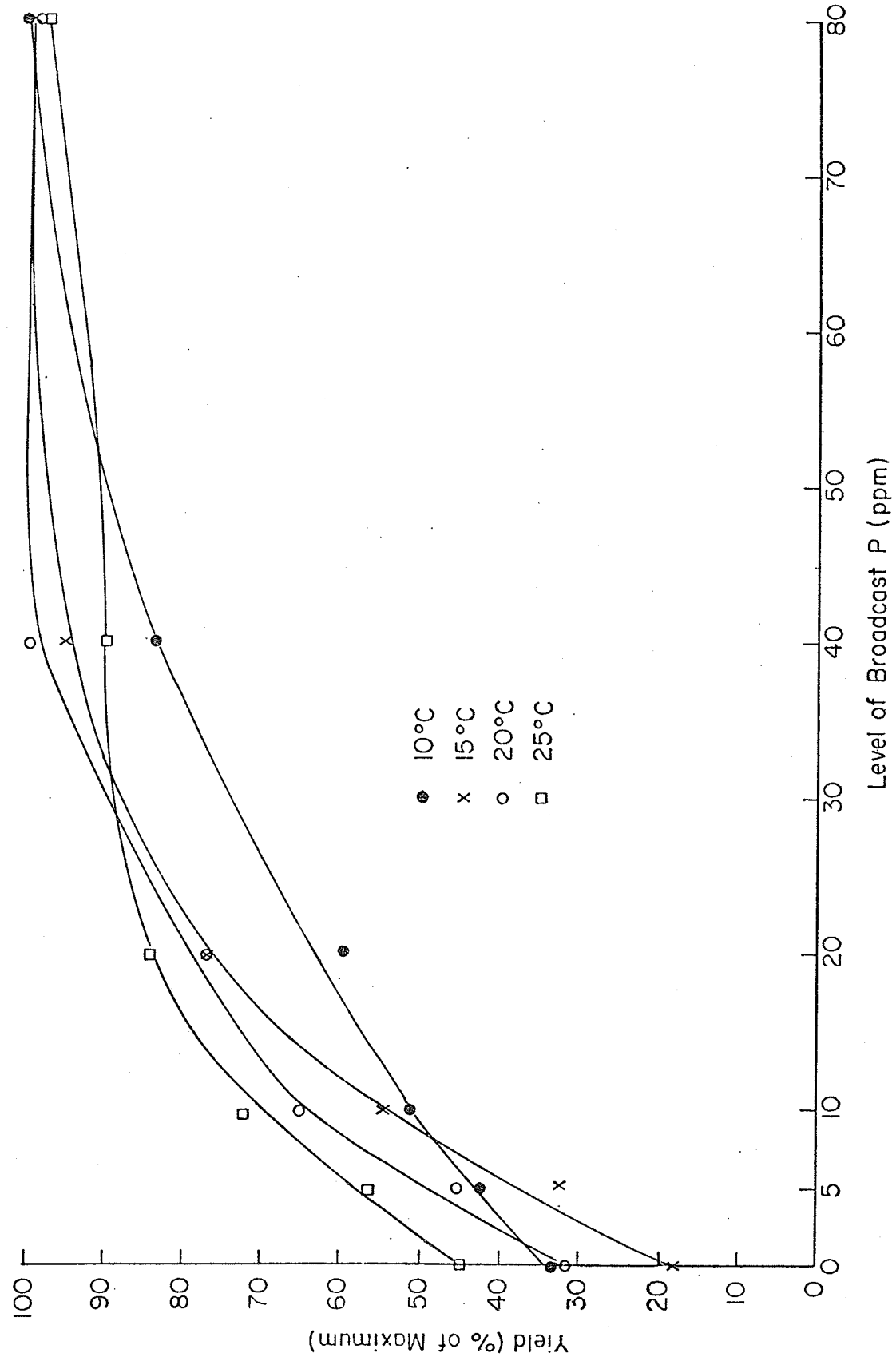


Figure 34: Shoot yield response to broadcast P plotted as a percentage of the maximum yield within temperature and replicate.

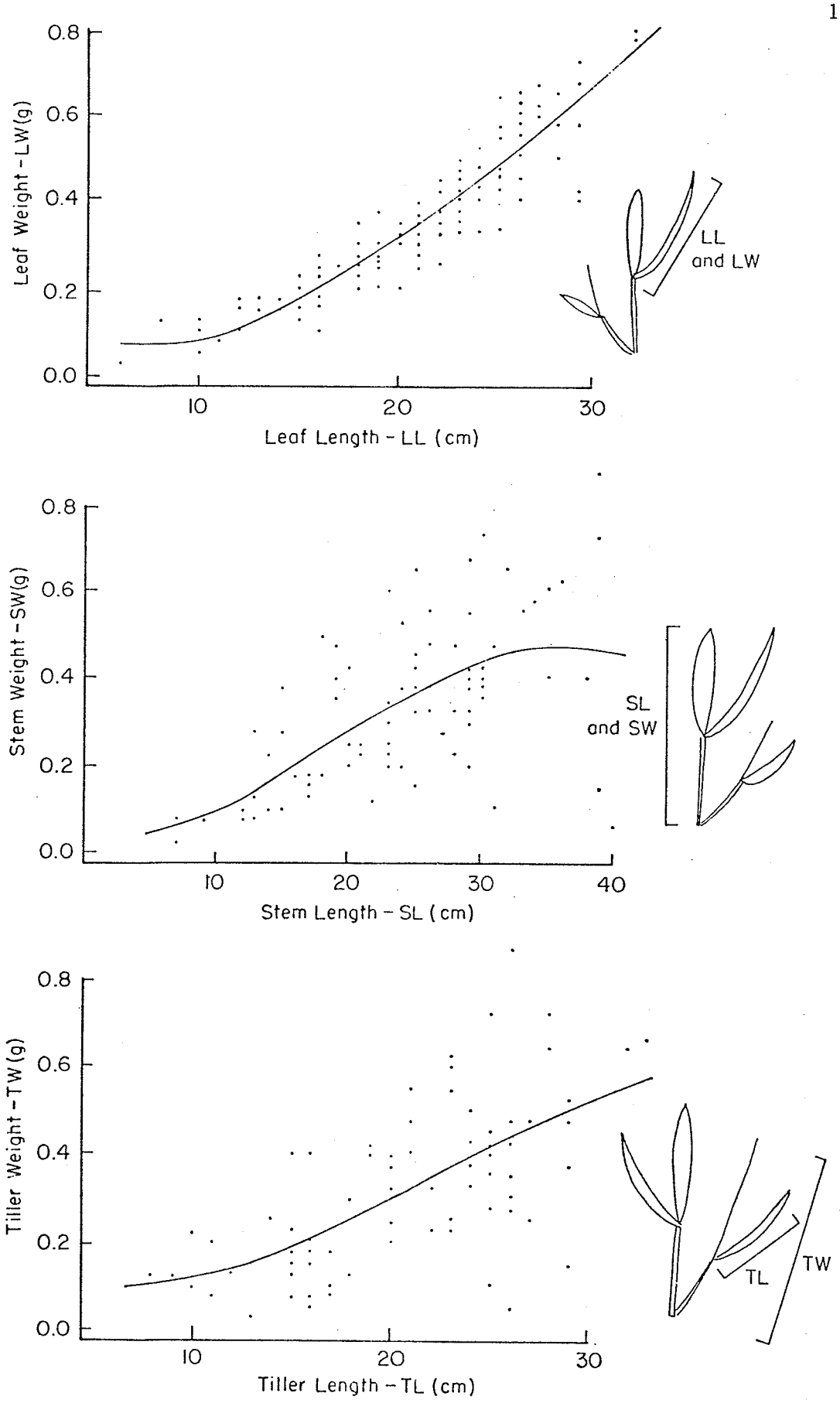


Figure 35: Calibration of dry weights to length measurements of leaf, "stem" and "tiller" tissues.

TABLE 26

Dry Weights of Shoot and Root (g/pot)

Temp.	P level	Shoot[1]			Fertilized Band Zone Root		Opposite Band Zone Root	
(°C)	(ppm)	broad.	band	comb.	band	comb.	band	comb.
10	0	0.428	0.428	0.746			0.12	
	5	0.539	0.691	0.969	0.35	0.25	0.17	0.11
	10	0.649						
	20	0.746	1.08	1.17	0.42	0.39	0.10	0.10
	40	1.05						
	80	1.25	1.13	1.13	0.45	0.34	0.10	0.20
15	0	0.642	0.642	2.56			0.14	
	5	1.09	1.61	3.04	0.48	0.40	0.25	0.26
	10	1.81						
	20	2.56	2.53	2.73	0.51	0.36	0.19	0.16
	40	3.17						
	80	3.33	2.68	3.11	0.46	0.30	0.17	0.15
20	0	2.13	2.13	5.03			0.14	
	5	3.05	3.66	6.12	0.58	0.38	0.22	0.25
	10	4.24						
	20	5.03	5.30	6.00	0.62	0.63	0.22	0.22
	40	6.56						
	80	6.50	5.69	6.42	0.49	0.53	0.28	0.17
25	0	2.87	2.87	5.33			0.12	
	5	3.64	3.64	5.19	0.28	0.34	0.13	0.17
	10	4.57						
	20	5.33	4.52	5.16	0.40	0.40	0.24	0.26
	40	5.71						
	80	6.25	4.93	5.84	0.29	0.33	0.19	0.26
	CV		9.9%		33.1%		30.0%	
	df_{errorb}		44		20		24	
	$MS_{e(b)}$		0.008558		0.018969		0.002966	

1. means of two replicates averaged in the $\log_e$ transformed scale, presented here as linear scale (antilog$_e$ of means), MS_e is of $\log_e$ transformed ANOVA

TABLE 27

Relative Growth Rate (g/g.d)[1]

Temp. °C	Broadcast P level (ppm)	Growth Interval (days from planting)		
		17 - 31		
10	0	0.070		
	5	0.070		
	10	0.104		
	20	0.105		
	40	0.100		
	80	0.104		
		12 - 17	17 - 24	24 - 30
15	0	0.170	0.080	0.073
	5	0.132	0.093	0.088
	10	0.175	0.123	0.083
	20	0.175	0.144	0.094
	40	0.170	0.143	0.087
	80	0.188	0.155	0.084
		9 - 15	15 - 23	23 - 29
20	0	0.145	0.102	0.117
	5	0.191	0.109	0.095
	10	0.212	0.088	0.098
	20	0.209	0.108	0.102
	40	0.233	0.113	0.112
	80	0.214	0.115	0.129
		8 - 15	15 - 22	22 - 28
25	0	0.118	0.085	0.110
	5	0.158	0.135	0.046
	10	0.196	0.101	0.102
	20	0.206	0.113	0.100
	40	0.224	0.109	0.101
	80	0.232	0.082	0.135

1. Calculated as $\frac{(\log_e w + 2 - \log_e w + 1)}{\text{day } 2 - \text{day } 1}$

TABLE 28

^{31}P Concentrations of Shoot and Root (%)

Temp. (°C)	P level (ppm)	Shoot broad.	Shoot band	Shoot comb.	Fertilized Band Zone Root band	Fertilized Band Zone Root comb.	Opposite Band Zone Root band	Opposite Band Zone Root comb.
10	0	1.3	1.3	2.2			1.1	
	5	1.4	1.8	2.4	1.7	1.2	1.1	1.6
	10	1.6						
	20	2.2	2.3	3.0	2.7	1.5	1.7	1.7
	40	2.7						
	80	3.3	3.7	4.3	3.9	3.7	2.8	1.6
15	0	1.2	1.2	1.6			0.9	
	5	1.2	1.6	1.6	1.3	1.9	1.3	1.4
	10	1.2						
	20	1.6	2.3	2.4	1.8	2.2	1.8	1.5
	40	2.1						
	80	3.0	3.1	2.8	3.1	3.1	1.9	2.1
20	0	1.5	1.5	1.4			1.2	
	5	1.6	1.2	1.3	0.8	1.0	1.2	1.1
	10	1.3						
	20	1.4	1.4	1.8	1.6	1.5	1.2	1.4
	40	1.6						
	80	2.8	2.4	2.2	2.6	2.8	1.2	1.8
25	0	2.2	2.2	2.1			1.5	
	5	2.1	2.0	2.1	1.4	1.1	1.3	0.9
	10	1.9						
	20	2.1	1.9	2.3	1.5	1.0	1.0	1.3
	40	2.0						
	80	2.4	2.1	2.3	3.0	1.3	1.7	1.2
	CV		13.4		29.2		24.5	
	df_{errorb}		44		20		24	
	$MS_{e(b)}$		78300.		340679.		124746	

TABLE 29

^{31}P Uptake (in the shoot) (mg P/pot)

Temp. (°C)	P level (ppm)	broad.	band	comb.
10	0	0.5	0.5	1.6
	5	0.7	1.2	2.3
	10	1.1		
	20	1.6	2.4	3.5
	40	2.7		
	80	4.1	4.1	4.9
15	0	0.7	0.7	4.0
	5	1.2	2.3	4.8
	10	2.1		
	20	4.0	5.4	6.6
	40	6.6		
	80	9.9	8.2	8.7
20	0	3.0	3.0	7.2
	5	4.9	4.4	7.5
	10	5.5		
	20	7.2	7.4	10.7
	40	10.5		
	80	18.0	13.2	13.8
25	0	6.1	6.1	11.2
	5	7.4	7.3	10.8
	10	8.5		
	20	11.2	8.5	11.9
	40	11.2		
	80	15.1	10.2	12.9
CV			1.3%	
$df_{error(b)}$			44	
$MS_{e(b)}$			0.012004[1]	

1. means and statistics were computed on $\log_e$ transformed data, means presented in linear scale.

TABLE 30

Uptake of Band-Applied P (mg P/pot)[1] and Shoot/Root Ratio[2]

Temp. (°C)	P level (ppm)	Uptake of Band-Applied P		Shoot/Root Ratio	
		band	comb.	band	comb.
10	0			3.9	
	5	0.43	0.48	5.6	13.7
	20	1.29	1.21	12.6	14.4
	80	2.65	2.67	11.7	6.4
15	0			4.5	
	5	0.84	0.79	7.4	13.1
	20	2.94	2.14	13.8	20.1
	80	5.79	4.51	17.7	21.9
20				15.0	
	5	1.10	0.92	16.7	25.2
	20	3.67	3.65	24.0	29.6
	80	7.85	7.98	21.2	38.9
25	0			24.5	
	5	1.13	1.19	29.6	33.0
	20	2.93	2.90	20.7	21.8
	80	5.47	5.67	26.5	22.1
CV		21.4		34.0	
$df_{(errorb)}$		20		24	
$MS_{e(b)}$		175.9228		39.153558	

1. calculated as (total ^{32}P uptake)/(fertilizer P specific activity)

2. calculated as (dry weight of shoot)/(dry weight of roots in zone opposite band)

TABLE 31

Simple Correlations (r)

Variable		Variable Number										
		1	2	3	4	5	6	7	8	9	10	11
Shoot Dry Weight	1		-	**	***	***	*	-	-	-	***	-
Band Root D_W	2	0.21		-	-	-	*	-	-	-	-	-
Opp. Root D_W	3	0.47	0.25		-	*	***	-	-	-	-	-
Shoot/Root Ratio	4	0.78	0.04	-0.11		***	-	-	-	-	**	-
Single Plant Fresh	5	0.87	0.23	0.29	0.71		-	-	-	-	*	-
Proliferation	6	-0.34	0.34	-0.74	0.13	-0.17		-	-	*	-	-
Shoot % P	7	-0.18	-0.13	-0.10	-0.18	-0.10	0.16		***	***	-	**
Band Root % P	8	-0.27	-0.03	-0.23	-0.20	-0.15	0.13	0.61		***	**	***
Opp. Root % P	9	-0.21	-0.04	-0.37	0.02	-0.12	0.44	0.52	0.64		-	***
Banded Uptake	10	0.52	0.25	0.19	0.40	0.42	-0.06	0.19	0.42	0.19		***
Banded P/Total P	11	-0.10	0.26	-0.13	-0.10	-0.07	0.28	0.43	0.70	0.54	0.68	

\- not significant (i.e. $P > 0.05$)

* significant $0.05 \geq P > 0.001$

** significant $0.001 \geq P > 0.0001$

*** significant $0.0001 \geq P$

Appendix F

EFFECT OF TEMPERATURE AND TIME ON EXTRACTABLE P

Introduction

Experiments were conducted to determine the most appropriate extraction times for use with both $NaHCO_3$ and KCl extractants. The influence of temperature on extraction equilibration was examined in the first experiment. A second experiment was conducted to investigate several processes which may have regulated the extraction process.

Methods and Materials

In the first experiment, the Elm River soil (Table 7, Chapter 4) was incubated moist for two weeks at 10, 15, 20 and 25°C and then extracted using the apparatus described in Chapter 3. The $NaHCO_3$ extraction involved duplicate extractions of 2.25 g moist soil (2.00 g dry weight) in a 50 mL test tube using 40 mL of 0.5 M $NaHCO_3$ (pH 8.5) and 1 g of $NaHCO_3$-washed charcoal. The KCl extraction involved 50.0 g moist soil (44.4 g dry weight) in a 500 mL polyethylene bottle using 450 mL of 0.02 M KCl. Separate samples were extracted using an end-over-end agitator (Appendix A) at their respective temperatures for 1, 24, 48 and 96 hours. After extraction, the samples were allowed to settle briefly and the supernatants were filtered through #42 Whatman filter paper in a temperature-controlled chamber adjusted to the respective extraction temperatures.

The filtrate from the $NaHCO_3$ extraction was analysed directly. Phosphorous concentrations in the filtrates from the KCl extraction system were extremely low and therefore were concentrated prior to analysis. The

samples were acidified with two drops of concentrated H_2SO_4, 200 mL subsamples were evaporated to dryness at 50°C, and were redissolved in 20 mL of 0.5 N H_2SO_4 for analysis.

In the second experiment, variations in the $NaHCO_3$ extraction system were used for the extraction of air-dried aliquots of the Almasippi soil (Table 7, Chapter 4). The variations included (1) the original $NaHCO_3$ system, (2) the addition of four drops of toluene to control microbial activity, (3) the aeration of the extraction suspension by opening the tube to admit fresh air every 6 to 12 hours, and (4) the adjustment of the pH of the suspension if it varied with extraction time.

Separate samples were extracted in triplicate using a reciprocal shaker at room temperature for 1, 6, 18, 24, 48 and 96 hours. The pH of the suspensions was recorded prior to filtration through #42 Whatman filter paper.

The P content of the filtrates was determined as previously described (Appendix B).

Results and Discussion

The effects of time and temperature in the first experiment for the $NaHCO_3$ system (Figure 36) and the KCl system (Figure 37) were very similar. In both systems, more P was extracted at higher temperatures. There was a continuous increase in extractable P as extraction continued up to 96 hours. Measurements of P extracted at 96 hours for 15, 20, and 25°C in the $NaHCO_3$ system and the measurements for 15 and 20°C in the KCl system were not obtained due to malfunctions in the agitator. The extractable P versus time curves were remarkably parallel among temperatures. Thus extraction at any one time would reflect the differences among the various temperatures.

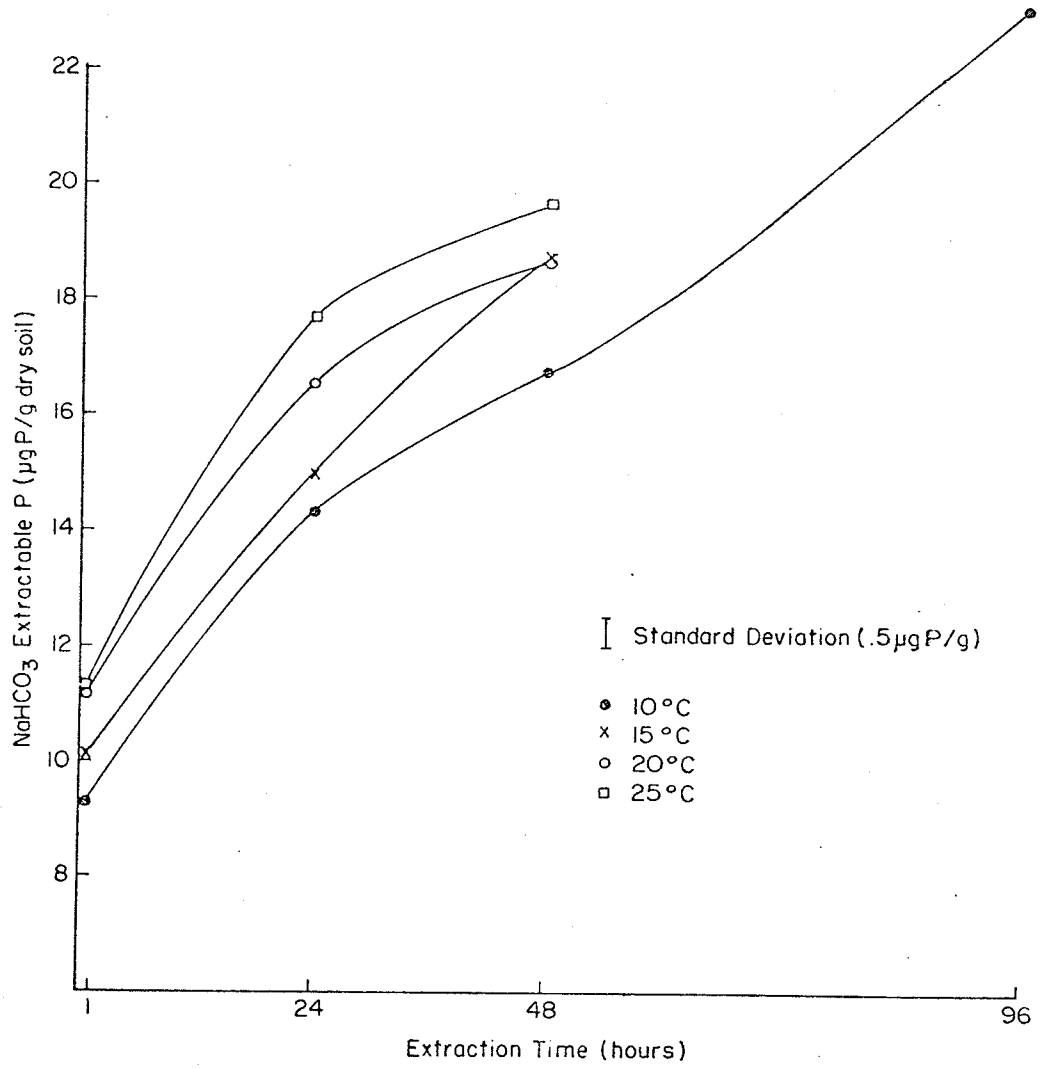


Figure 36: $NaHCO_3$- extractable ^{31}P in response to extraction time and temperature.

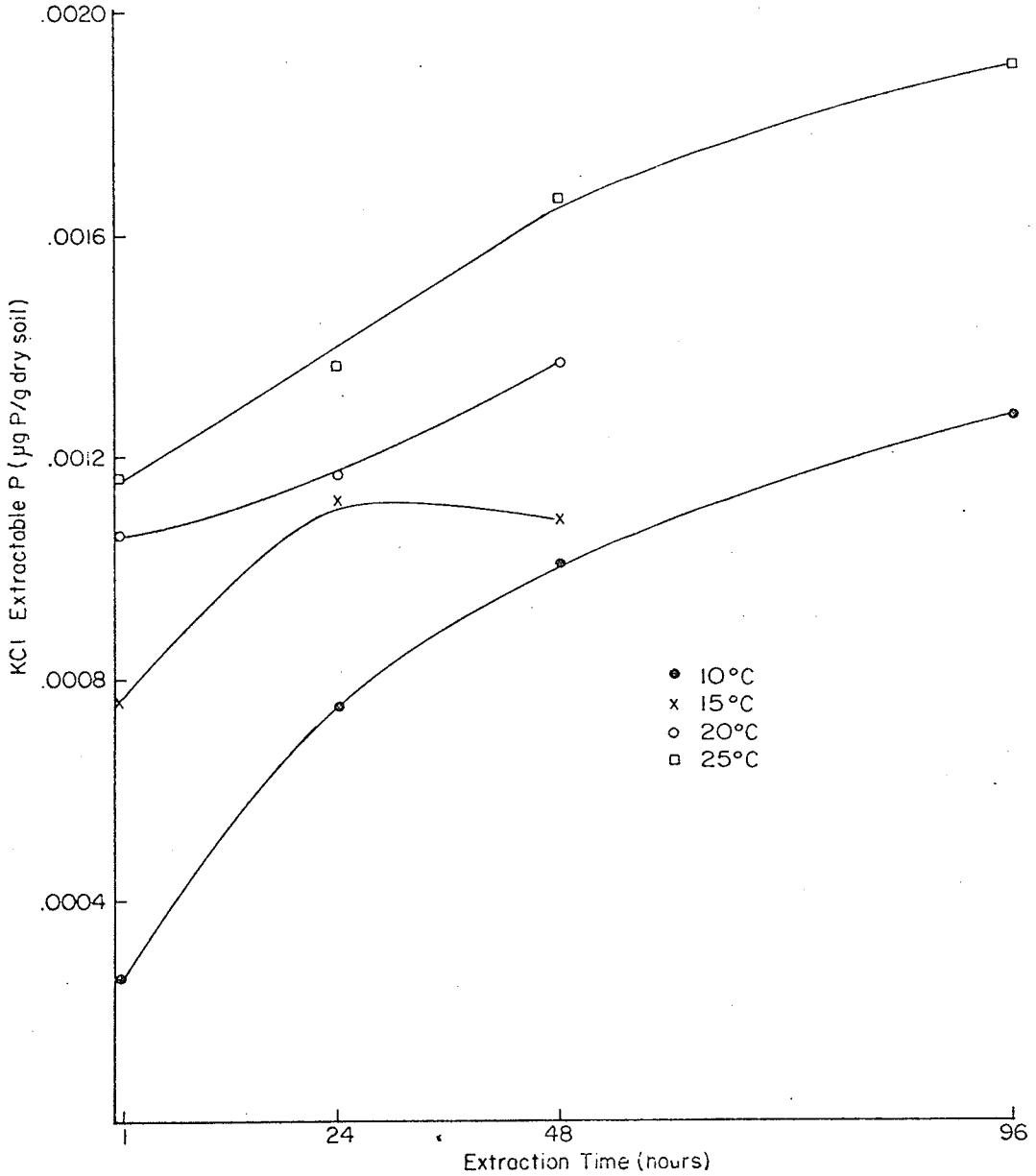


Figure 37: KCl-extractable ^{31}P in response to extraction time and temperature.

An extraction time of 24 hours was chosen for use in further studies.

The dominant sources of extractable P in a soil are the inorganic P forms. It was expected that these forms would reach an equilibrium with the extracting solutions within 96 hours. However, equilibrium was not attained within 96 hours. Thus there was concern that a mechanism other than inorganic desorption may have been operative in this first experiment. Soil dispersion with time was discounted because the Na from the $NaHCO_3$ was a very effective dispersant. Other possible mechanisms included direct effects of microorganisms and indirect effects due to CO_2 accumulation. This latter effect would result in a lowering of the pH of the extraction suspension.

A second experiment was conducted to investigate the effect of inhibiting microbial activity with toluene, aeration to reduce effects of anaerobiosis, and variations in pH due to CO_2 accumulation on the amounts of P extracted. The pH measurements did not change with extraction time and thus variations in pH were not responsible for the lack of equilibrium.

The amounts of P extracted (Figure 38) were similar for all treatments. Addition of toluene and aeration of the samples appeared to increase the amounts of P extracted by the $NaHCO_3$ at 18.48 and 96 hours. The hypothesis of this experiment was that continued release of P up to 96 hours may have been the result of biological processes releasing soil P. The toluene and aeration treatments, if the biological processes they controlled were important, should have reduced the amounts of extractable P. Since this did not occur, it was concluded that the increase in extractable P up to 96 hours in the previous study was not perpetuated by microbial activity. The continued release was therefore most likely due to a slow, inorganic reaction system. The increased desorption when toluene was

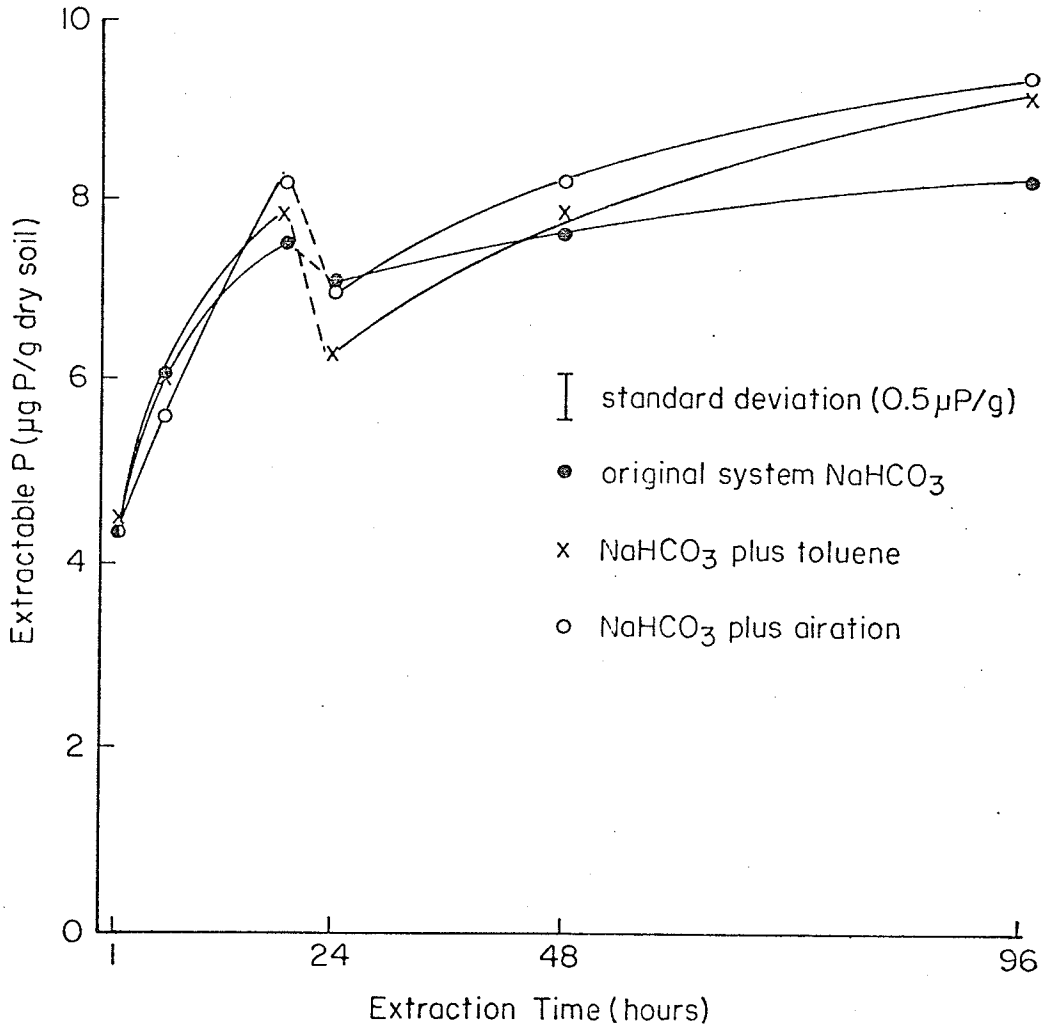


Figure 38: The effect of toluene and aeration on the $NaHCO_3$-extractable ^{31}P with time.

added, if significant, may have been due to solubilization of organic P complexes. It was not clear how aeration increased P solubility.

The apparent discrepancy in Figure 3 between the 1 to 18 hour extractions and the 24 to 96 hour extractions was due to variations in room temperature. When the later three extraction times were conducted, the building temperature decreased 6 to 12°C below the temperature which existed for the 1 to 18 hour extraction. Thus, less P was extracted than expected at 24 and 96 hours.

Conclusions

The most important conclusion of these preliminary experiments was that a common extraction time for all four temperatures was appropriate and therefore 24 hours was deemed suitable for use in further studies. Soil dispersion with time or microbial activity in the extraction suspensions did not significantly alter the amounts of extractable P.

Greater amounts of P were extracted at higher temperatures and this effect was also apparent in extractions conducted at room temperature when the room temperature was not stable.

Appendix G

DISTRIBUTION OF SEED P IN THE TRANSPLANT SYSTEM

Introduction

A portion of the P in young seedlings is derived from the seed. Although the amounts are relatively small, seed-derived P can comprise a substantial portion of the P in seedling leaves. Since the P content of seedling leaves was used to measure plant available P (Experiment B, Chapter 4), an experiment was conducted to characterize the distribution of seed-derived P in seedlings. Previous experiments (Appendix J) showed that biological dilution and translocation of the plant P were important processes. Therefore, plants were harvested over a range of ages to characterize the effect of plant size on P distribution. It was assumed that the distribution of seed-derived P in these plants would be the same as in the plants grown on soil in Chapter 4.

Methods and Materials

The seeds were pregerminated and planted as described in Experiment B of Chapter 4. After one week of growth, 48 uniform transplant pots were selected and were placed into 1 L containers containing 160 cm^3 of acid-washed, phosphate-free granitic gravel (2-5 mm diameter). Four additional transplant pots were retained for analysis at that time to provide a measure of pre-transplant P distribution.

The transplant-gravel units were placed into the four temperature controlled water baths. These units were maintained at their original moist-weights by the addition of water every second day (see Chapter 4).

Temperatures were recorded at various points in the transplant units using copper-constantan thermocouples and a portable reference-voltmeter.

Replicates were harvested from each bath 3, 5, 6, 7, 8 and 10 days after transplanting. At harvest, the number of plants per unit was recorded and the plants from each unit were separated into three components. These were (1) leaves above the Perlite surface, (2) pseudo stems, residual seed and roots within the Perlite, and (3) roots below the Perlite (in the gravel). The root samples (3) were washed free of gravel but the tissues in the Perlite (2) could not be cleaned completely of the Perlite.

The samples were dried (24 hours at 85°C) and the leaf (1) and root (3) samples were weighed. All samples were analysed for P as outlined previously (Appendix B). The residual Perlite on the middle tissues (2) contributed negligible P to the digestion solutions and was removed by filtration prior to analysis.

All of the results were adjusted arithmetically to 20 plants per transplant pot. Statistical analysis was conducted using the GLM and STEPWISE procedures of SAS (1979).

Results and Discussion

Plant Growth and P Redistribution Within the Plant

Seedling growth and development was comparable to those reported in Experiment B, Chapter 4. Leaf weights (per 20 plants) increased from 0.37 to 0.84 g dry weight during the three to ten day growth period. Leaf growth was reduced slightly by increases in root temperature, probably due to higher moisture stress. The low moisture holding capacity of the gravel made irrigation intervals more critical in this experiment. The root growth (Figure 39) was highly dependent on both time and temperature.

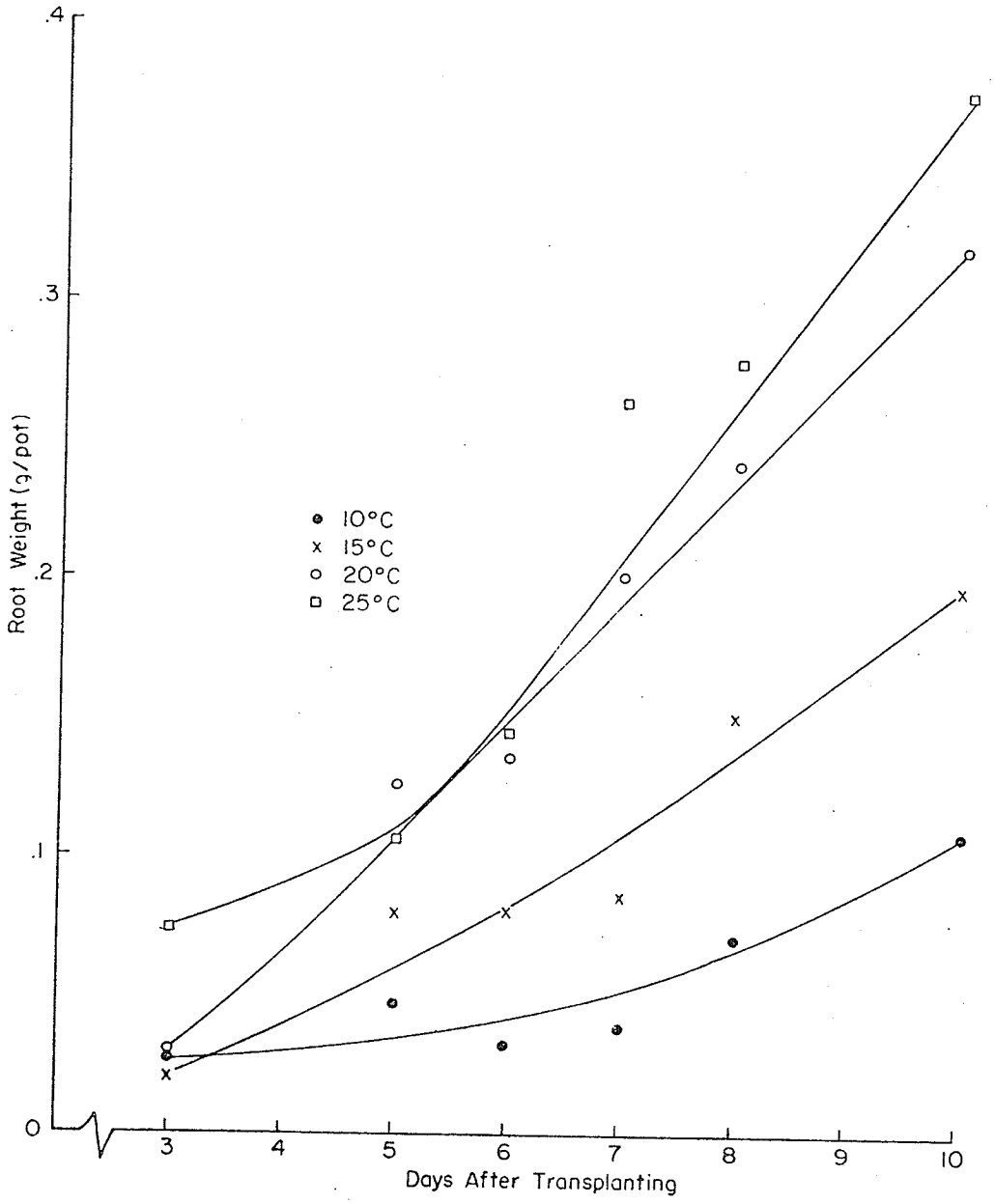


Figure 39: Root growth in response to time and temperature.

The total P content per 20 plants (the sum of the three tissue types) decreased significantly with time from 2.1 mg P on day 3 to 1.9 mg P on day 10. This may be attributed to efflux and leaching of P from the plant or to less efficient recovery of finer roots which were more prevalent on the older plants. This loss of 9.5% of the plant P was concomitant with a gain in leaf plus root weight of 169.0% and thus the effect of loss on plant P redistribution was negligible relative to biological dilution.

The loss of total plant P and the large gain in weight resulted in a rapid decrease in tissue P concentrations with time. Tissue P concentrations decreased in the leaves from 0.21 to 0.08% and in the roots from 0.34 to 0.17% during the three to ten day growth period. The decreased concentration in the leaves was parallel to the weight gain such that the total P content of the leaves was generally constant with time. However, the total leaf P content increased with temperature from 678, 704, 745 to 796 µg P/20 plants at 10, 15, 20 and 25°C, respectively.

The very large gain in root weight (over 500% in the seven-day interval) predominated over the decrease in tissue P and thus the total root P content increased with both time and temperature. The correlation of total root P content to root weight was highly significant (single $r = 0.96$, $P < 0.0001$). Thus root growth appeared to be a substantial sink for plant P.

The fraction of total plant P in each plant component was computed (Figure 40) and showed that the fraction of P in the leaves did not change significantly with time. However, the fraction of P in the leaves increased with temperature due to increased tissue P concentration and hence total P content. This coincided with a decrease in the fraction of P associated with the "middle" tissues which contained the seed. A gradient of temper-

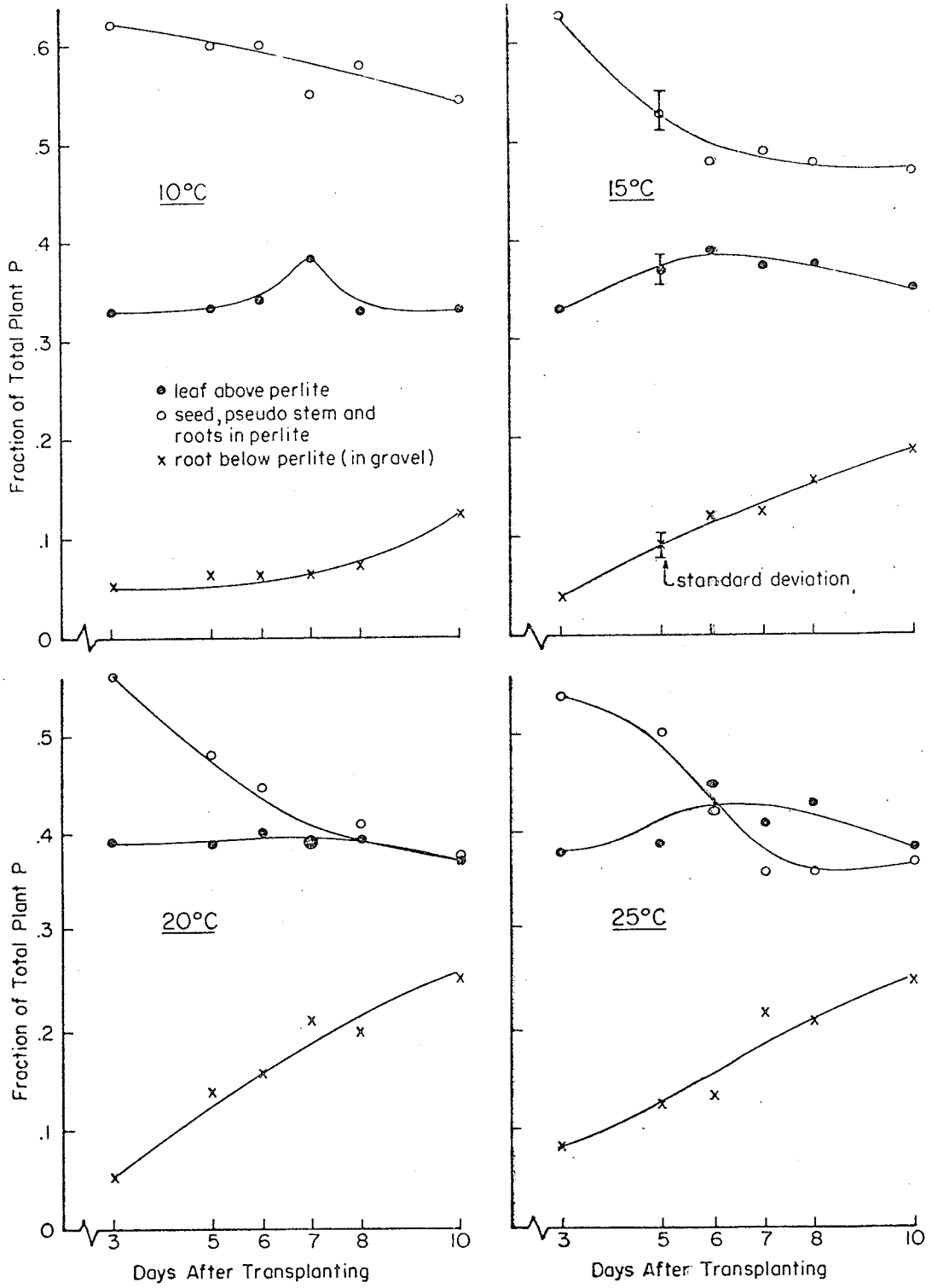


Figure 40: Fractions of total plant P in each of three plant components with time.

ature existed between the root zone and the surface of the Perlite (which was warmed above air temperature by the absorption of radiant energy) with the result that the temperatures at the seed were 27, 25, 22 and 19°C in the 25, 20, 15 and 10°C treatments, respectively. A higher temperature at the seed probably resulted in more rapid release of seed P and thus more P was translocated from the seed to the growing tissues in the plant.

The fraction of total plant P in the root also increased with temperature. A larger change in the fraction of plant P in the root was due to time with a significant interaction between time and temperature. These responses closely parallel the root growth response to temperature and time (Figure 39) and confirm that the root was the major cause for redistribution of P within the plant.

Prediction of Leaf P Content

The major purpose of this experiment was to develop a method to predict the seed P content of leaves in the transplant system. There was no consistent effect of time (and hence leaf weight) on the total P content of the leaves (Table 32) but increases in temperature significantly increased the leaf P content.

The overall standard deviation as a fraction of the mean leaf P content was 10.5% whereas the residual error term of the analysis of variance was 9.0% of the mean. Thus, there was little change in leaf P content in this study and much of this variance was attributed to random error. However, significant equations to predict the leaf P content were found by backward stepwise regression. Two sets of starting variables were defined. The first set included root temperature, leaf weight and day after transplanting including their squares and interactions. This model was devel-

TABLE 32

Total Leaf P Content in the Transplant System

Days after Transplanting	ug P/20 plants			
	10°C	15°C	20°C	25°C
3	676 ab	699 a	786 a	882 a
5	743 a	743 a	773 a	750 a
6	686 ab	736 a	751 a	770 a
7	736 ab	678 a	728 a	819 a
8	633 ab	686 a	743 a	801 a
10	594 b	681 a	690 a	757 a
mean	678	704	745	797

Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) tested by use of single degree of freedom contrasts

oped on all of the data and resulted in equation (11), with an R^2 of 0.61.

$$\text{leaf P content} = 1032 + 0.3347\ (\text{temp.})^2 + 520.9\ (\text{leaf weight}) - 51.99\ (\text{day}) \qquad [11]$$

The second set of variables included root temperature and leaf weight, their squares and interactions, and was developed using only the data for seven days after transplanting. The final use of the equation was to predict leaf P contents at that time. This yielded equation (12) with an R^2 of 0.85.

$$\text{leaf P content} = 607.4 + 12.29\ (\text{temp.})\ (\text{leaf weight}) \qquad [12]$$

The equations were compared in their ability to predict the observed leaf P contents at seven days and equation (12) was notably superior. Thus, equation (12) was used to predict the seed P component of the leaf P contents measured in Experiment B, Chapter 4.

Conclusions

Rapid decrease in tissue P concentrations of seedlings were found to occur when grown in a P-free medium. Although some plant P was lost from the larger plants, the primary cause of the decreased concentration was biological dilution. Root growth was the dominate sink for P.

There was some evidence that increased seed temperature increased the release of seed P and subsequent translocation to the rest of the plant.

The total leaf P content did not vary with time but increased by 17.6% from 10 to 25°C. A regression equation was developed to predict the leaf P content at seven days after transplanting. Predictions of seed-derived P by this equation were used to represent the content of seed-derived P in the leaves of seedling plants used in other studies. Thus, predictions of

seed-derived P based on this equation were subtracted from the leaf P contents obtained in Experiment B, Chapter 4 (where the plants were grown on soil) and the differences were assumed to be soil-derived P.

Appendix H

DATA AND STATISTICS FOR CHAPTER 4

This appendix is a more extensive and complete presentation of the data and statistics discussed in Chapter 4. Complete details on methods and experimental design are given in Chapter 4 but a few notes are included relative to these tables.

Experiment A and the plant uptake studies of Experiment B were randomized complete blocks, split-plot designs. Replication was generally a significant factor although not included in these tables. The coefficient of variability (CV) was computed using the residual error term in each case. If the residual error term had less than 16 degrees of freedom and was homogenous with the replicate x temperature interaction, then these terms were pooled for use as the error term.

The soil extraction sub-experiment of Experiment B was a regression-design experiment and the lack of fit to a linear model for temperature effects was used as the error term.

Contrasts of treatment means, unless otherwise stated, were conducted by partitioning the treatment sum of squares into single degree of freedom comparisons. The notation $P > F$ was used to identify the calculated probability of a larger F-ratio although $P \leq 0.05$ was used as the criteria for significance throughout.

Contrasts of regression coefficients were conducted by t-tests generally using a standard error of estimate pooled across regressions from other, similar treatments (as specified in each case).

All ^{32}P data were expressed as disintegrations per minute (dpm) and were corrected for decay to the day the P treatments were applied to the respective experiment, unless otherwise stated.

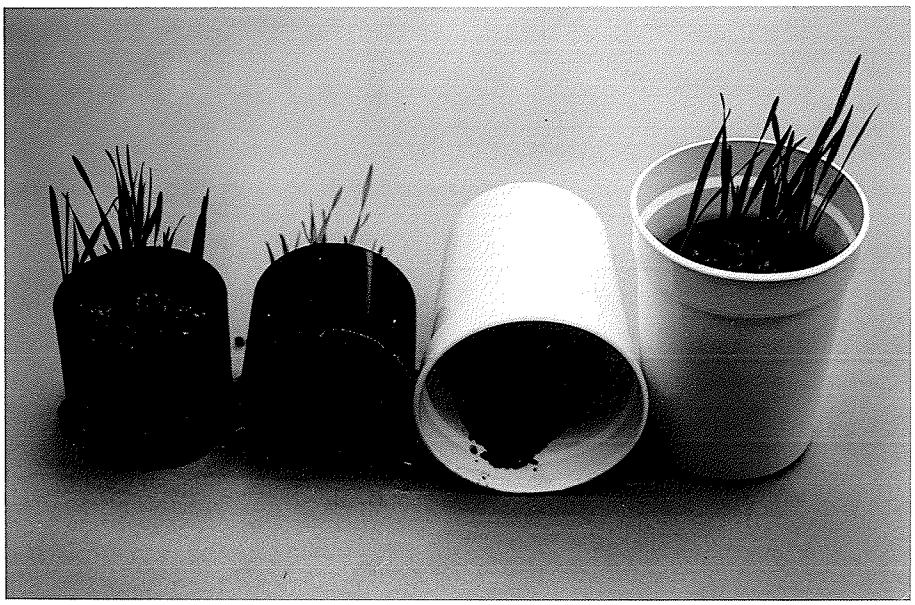


Plate 5: Preparation of short term plant uptake units: (left to right) screen-bottom tubes with transplants, 1 L container with treatment soil, and assembled unit

Plate 6: Water baths in growth chamber showing floating transplant-soil units (and end-over-end agitator used for chemical extractions)

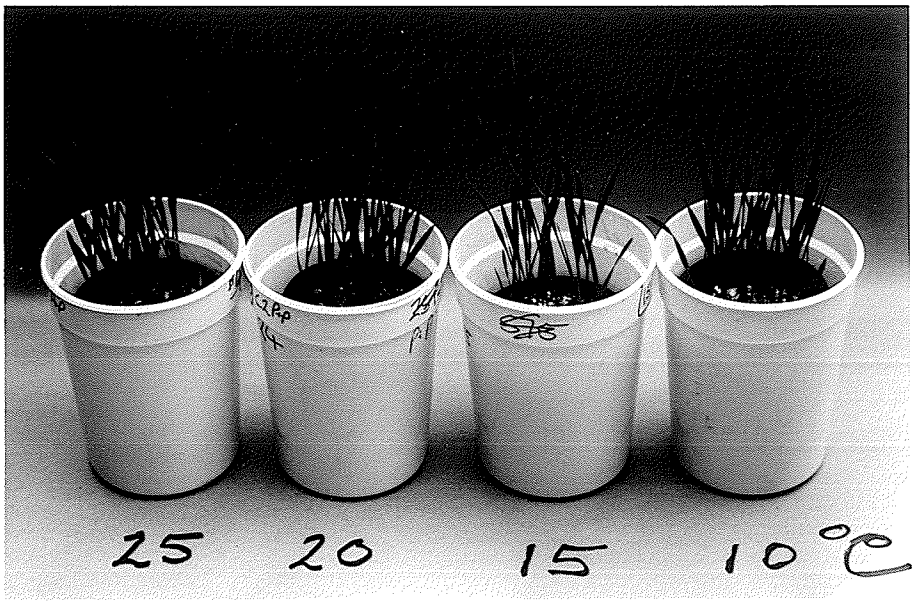


Plate 7: Transplant-soil units after one week of growth at the treatment temperatures

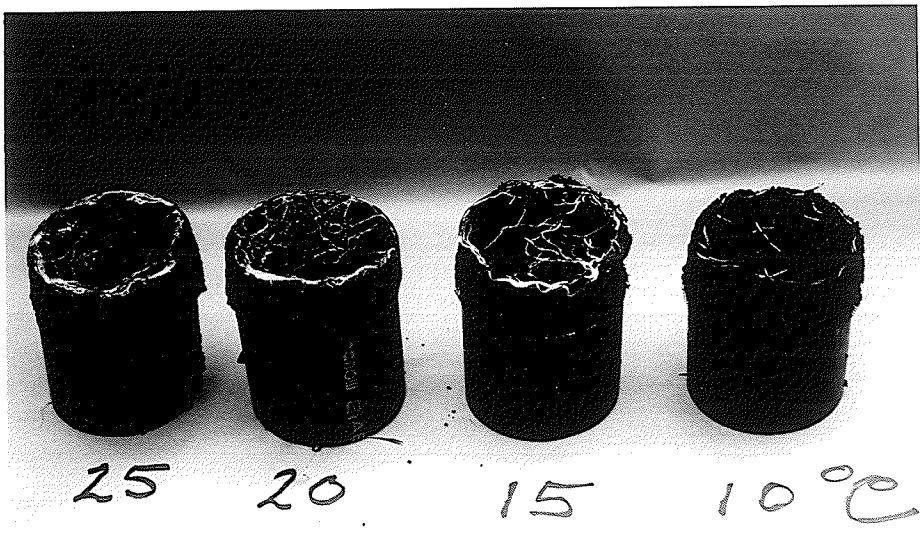


Plate 8: Inverted transplant-soil units with 1 L containers removed to show root growth after one week at the treatment temperatures

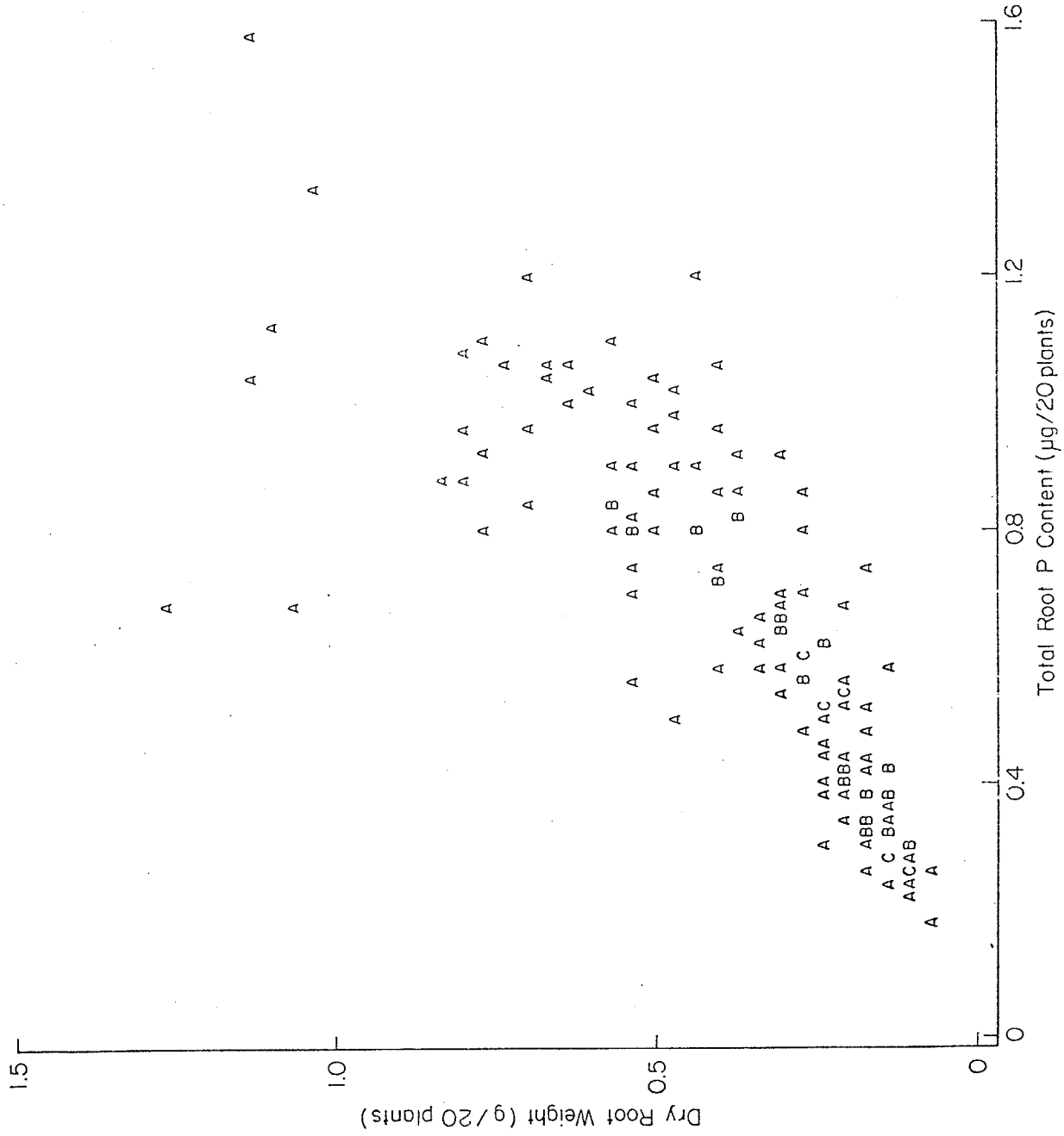


Figure 41: Root dry weight as a function of total root P content.

TABLE 33

$NaHCO_3$ Extractable ^{31}P and ^{32}P, Experiment A

Temperature (°C)	Start of the Incubation		End of the Incubation	
	Almasippi	Elm River	Almasippi	Elm River
	^{31}P from Fertilized Soils (µg/g)			
10	54.4 a	43.6 a	46.2 a	38.5 b
15	52.0 a	43.7 a	45.7 a	38.5 b
20	52.1 a	41.6 a	48.1 a	34.5 b
25	55.9 a	43.8 a	37.0 b	49.2 a
	^{31}P from Unfertilized Soils (µg/g)			
10	13.2 a	10.2 a	13.0 a	9.1 a
15	13.2 a	10.6 a	13.4 a	11.3 a
20	15.1 a	11.3 a	15.9 a	10.0 a
25	16.6 a	12.2 a	17.2 a	13.6 a
	^{32}P from Fertilized Soils ($x10^4$ dpm/g)			
10	4.68 a	3.84 a	3.49 a	3.08 a
15	4.70 a	3.85 a	3.47 a	2.91 a
20	4.40 a	3.86 a	3.13 a	2.48 a
25	5.31 a	3.75 a	2.64 a	4.67 a
	^{32}P from Unfertilized Soils ($x10^4$ dpm/g)			
10	4.03 a	3.75 a	2.46 a	2.08 a
15	4.52 a	3.60 a	2.66 a	2.31 a
20	4.17 a	3.93 a	2.54 a	1.78 a
25	4.74 a	4.12 a	2.66 a	2.32 a

- Means followed by the same letter in a column of 4 not significantly different ($P \leq 0.05$).

TABLE 34

Multiple Regression of the Desorption Curve Data, Experiment A

Coefficient	Start of the Incubation		End of the Incubation		Pooled Error
	Almasippi Soil	Elm River Soil	Almasippi Soil	Elm River Soil	
			^{32}P from Fertilized Soils		
Intercept	8.647 *	8.711 *	7.684 *	7.509 *	0.4796
^{32}P desorbed	-5.477×10^{-5} *	-6.053×10^{-5} *	-9.228×10^{-5} *	-1.017×10^{-4} *	2.1×10^{-5}
Temperature	-0.02312 NS	-0.03912 *	-0.0243 *	-0.05188 *	0.0139
Interaction	3.6629×10^{-8} NS	5.2665×10^{-7} NS	-2.549×10^{-7} NS	1.992×10^{-6} *	6.544×10^{-7}
r^2 (%)	76	79	97	92	
			^{32}P from Unfertilized Soils		
Intercept	5.607 *	5.784 *	5.111 *	4.554 *	0.1936
^{32}P desorbed	-2.782×10^{-5} NS	-2.448×10^{-5} NS	-6.269×10^{-5} *	3.170×10^{-5} NS	1.73×10^{-5}
Temperature	0.02249 *	-0.01164 NS	0.0021 NS	-0.0087 NS	0.01046
Interaction	-6.953×10^{-7} NS	7.976×10^{-7} NS	2.315×10^{-6} *	2.646×10^{-6} *	8.828×10^{-7}
r^2 (%)	39	40	28	56	
			^{31}P from Fertilized Soils		
Intercept	0.3062 NS	0.6630 NS	0.8207 NS	-5.040 *	1.1504
^{32}P desorbed	-0.0336 NS	-0.0746 NS	-0.1795 NS	0.6744 *	0.1624
Temperature	0.0175 NS	0.00386 NS	-0.1180 NS	0.1516 *	0.06375
Interaction	-0.000618 NS	0.000739 NS	0.01166 NS	-0.0311 *	0.00876
r^2 (%)	24	50	4	13	

* Significantly different from zero by a t-test using the error of estimate pooled across times and soils ($P \leq 0.05$), NS - not significant.

TABLE 35

Coefficients of the Desorption Curve Data Fitted to the Model: Log_e (P desorbed) = a + b Log_e (Ratio)[1], Experiment A

Parameter	Start of the Incubation				Pooled	End of the Incubation				Pool
	10	15	20	25°C	Error	10	15	20	25°C	Erro
Fertilized Almasippi Soil - ^{32}P [2]										
a	7.07 b	6.88 ab	6.68 a	6.99 b	0.142	4.87 b	4.72 b	4.55 a	4.48 a	0.0
b	0.384 a	0.424 ab	0.483 b	0.355 a	0.041	0.513 a	0.537 a	0.537 a	0.547 a	0.0
r^2 (%)	94	92	96	84		97	98	98	97	
Fertilized Elm River Soil - ^{32}P										
a	6.97 b	6.74 ab	6.53 a	6.48 a	0.160	4.45 c	4.30 b	3.85 a	3.74 a	0.0
b	0.402 a	0.438 a	0.491 a	0.488 a	0.046	0.596 a	0.615 a	0.712 b	0.717 b	0.0
r^2 (%)	91	90	94	95		97	98	99	99	
Fertilized Almasippi Soil - ^{31}P										
a	1.63	1.46	1.74	1.52		1.21	1.75	1.13	1.23	
b	0.350	0.439	0.310	0.413		0.259	-0.088	0.100	0.033	
r^2 (%)	55	87	38	81		65	2	6	0	
Fertilized Elm River Soil - ^{31}P										
a	1.53	1.31	1.23	1.11		0.471	0.400	-0.308	-0.123	
b	0.356	0.428	0.449	0.520		0.046	-0.017	0.287	0.486	
r^2 (%)	67	78	82	93		0	0	19	75	
Unfertilized Almasippi Soil - ^{32}P										
a	4.50 a	4.73 ab	4.68 ab	4.90 b	0.144	2.70 ab	2.81 ab	2.85 b	2.58 a	0.1
b	0.761 a	0.686 a	0.716 a	0.692 a	0.041	0.813 b	0.721 a	0.735 ab	0.930 c	0.0
r^2 (%)	97	96	99	98		99	99	95	98	
Unfertilized Elm River Soil - ^{32}P										
a	4.17 b	3.97 ab	3.89 ab	3.84 a	0.139	1.66 a	1.55 a	-	-	0.1
b	0.847 a	0.931 b	0.913 ab	0.959 b	0.040	1.00 a	1.02 a			0.0
r^2 (%)	98	98	98	99		99	99			

1. (P desorbed) as dpm/g or µg/g, (Ratio) as mL/g, coefficients followed by the same letter within a row of 4 were not significantly different ($P \leq 0.05$) by a t-test using a standard error of estimate pooled across 4 temperature treatments.
2. ^{32}P corrected for decay to 36 days (for data from start of incubation) and 58 days (for data from end of incubation) from the date the ^{32}P was applied.

TABLE. 36a

Analysis of Covariance of Desorption Curve Data, Experiment A

Source	Start of the Incubation						End of the Incubation					
	Fertilized			Unfertilized			Fertilized			Unfertilized		
	df	MS	P>F[1]	df	MS	P>F	df	MS	P>F	df	MS	P>F
						Log_e (^{32}P concentration)[2]						
Temperature	3	0.139	0.07	3	0.061	0.50	3	0.660	0.0001	3	0.027	0.62
Soil	1	0.180	0.08	1	0.325	0.04	1	1.58	0.001	1	2.12	0.0001
T & S	3	0.006	0.95	3	0.069	0.44	3	0.008	0.87	3	0.099	0.15
Log_e (Ratio)[3]	1	75.5		1	7.98		1	43.1		1	2.66	
Error	66	0.056		66	0.076		102	0.032		38	0.052	
CV (%)		3.5			5.0			2.9			4.7	
						Log_e (^{31}P concentration)						
Temperature	3	0.039	0.91				3	0.912	0.41			
Soil	1	0.250	0.30				1	22.6	0.0001			
T & S	3	0.009	0.99				3	1.73	0.14			
Log_e (Ratio)	1	82.4					1	234.				
Error	66	0.228					106	0.944				
CV (%)		138.3						58.1				

1. P>F is the probability of a larger F ratio, 0.05 was chosen as the minimum for significant difference.

2. After the model of Sharpley et al. (1981), ^{32}P conc. as dpm/ml corrected for the decay to the day the P was applied, ^{31}P conc. as µg/ml

3. Ratio as the solution:soil ratio.

- Temperature and Soil were class variables, Log_e (Ratio) was the continuous variable.

TABLE 36b

Adjusted Mean P Desorption Data from Analysis of Covariance, Table 36a

Soil and Isotope	Start or End of Incubation	Temperature 10	15	20	25°C
Fertilized ^{32}P	Start	6.93[1]	6.84	6.80	6.73
	End	6.32	6.21	6.06	5.96
Unfertilized ^{32}P	Start	5.47	5.52	5.46	5.58
	End	4.72	–	4.69	4.68
Fertilized ^{31}P	Start	-0.38	-0.33	-0.39	-0.30
	End	-1.48	-1.76	-1.80	-1.46

1. Adjusted Log_e (P concentration) as dpm/mL or µg/mL.

– Means joined by a line within a row were not significantly different ($P \leq 0.05$).

TABLE 37a

Coefficients Describing the Rate of ^{32}P Desorption Using a Segmented Polynomial Model[1], Fertilized Soils

Incubation Temperature (°C)	Extraction Temperature (°C)	a (dpm/mL)[2]	b	c	d (dpm/mL)	e	Time° (hours)	R^2 (%)
				Almasippi Soil				
10	10	673 e[3]	45.3 b	-2.16 a	888	2.20 a	10.0	100.0
10	20	810 f	35.9 a	-1.34 a	1010	3.11 a	12.2	99.9
15	15	623 de	33.5 a	-1.28 a	810	2.61 a	12.1	99.8
15	25	675 e	59.0 b	-3.30 a	918	2.37 a	8.6	99.9
20	20	567 cd	22.9 a	-0.54 a	780	1.48 a	19.9	99.9
20	10	499 ab	28.7 a	-1.44 a	624	1.91 a	9.3	99.9
25	25	545 bc	24.3 a	-0.74 a	721	1.57 a	15.4	99.9
25	15	469 a	32.5 a	-1.95 a	587	2.11 a	7.8	99.8
Pooled errors:[3]		30.52	10.30	5.384		1.00		
				Elm River Soil				
10	10	525 c	32.6 ab	1.28 a	696	2.98 c	11.6	99.8
10	20	613 d	20.7 ab	-0.51 a	802	1.08 ab	19.4	99.9
15	15	490 c	17.3 a	-0.64 a	581	2.03 abc	12.0	99.9
15	25	352 b	220. c	-43.6 a	623	2.64 c	2.5	99.9
20	20	351 b	23.9 ab	-1.12 a	451	2.74 c	9.5	99.9
20	10	324 ab	34.2 ab	-3.50 a	394	2.80 c	4.5	99.6
25	25	276 a	68.9 b	-7.83 a	417	2.32 bc	4.3	99.8
25	15	319 ab	20.3 ab	-1.05 a	407	1.05 a	9.2	99.7
Pooled errors:		34.17	25.84			0.636		

TABLE 37b

Coefficients Describing the Rate of ^{32}P Desorption Using a Segmented Polynomial Model[1], Unfertilized Soils

Incubation Temperature (°C)	Extraction Temperature (°C)	a (dpm/mL)	b	c	d (dpm/mL)	e	Time° (hours)	R^2 (%)
				Almasippi Soil				
10	10	228	-92.0	24.3	139	0.594	1.9	99.5
10	20	172	0.42	-3.72	172	0.699	-0.0	99.6
15	15	93.6	61.3	-16.7	150	0.127	1.8	99.6
15	25	241	-107	26.0	130	0.561	2.1	97.4
20	20	136	27.5	-12.5	151	0.464	1.1	96.3
20	10	119	-0.41	0.30	118	0.683	1.8	98.9
25	25	162	-3.91	0.80	156	0.672	2.9	99.1
25	15	137	0.66	0.05	132	1.71	10.0	96.7
				Elm River Soil				
10	10	17.5	78.0	-19.0	96.9	0.310	2.0	95.3
10	10	65.3	13.1	-1.51	92.6	0.281	4.2	98.5
15	15	95.5	-19.5	4.80	75.3	0.252	2.1	98.5
15	25	104	-11.4	1.61	82.7	0.164	3.6	98.0
20	20	66.6	1.52	0.21	64.8	0.281	-2.9	98.5
20	10	-22.5	81.8	-18.2	69.9	-0.195	2.3	97.2
25	25	75.4	0.78	0.29	75.1	0.165	-1.1	99.0
25	15	60.3	-2.09	0.38	55.9	0.488	3.4	99.0

1. ^{32}P concentration = $a + b(t) + c(t)^2$ up to t^o, after which ^{32}P concentration = $d + e(t)$; the curves joined such that $t^o = (e-b)/2c$ and $d = a + t^o(b-e+c\,t^o)$.

2. ^{32}P concentration as dpm/mL corrected for decay to the day the P was applied.

3. Coefficients within a column of 8 followed by the same letter not significantly different ($P \leq 0.05$) by a t-test computed using the respective pooled errors of estimate, pooled across the 8 temperature treatments.

TABLE 38a

Coefficients Describing the Rate of ^{32}P Desorption Using the Equation Log_e (^{32}P desorbed[1]) = f + g Log_e (time[2])

Intercept (f)			Slope (g)		
Inc T=Ext T[3]	Test Ext T[4]	Test Inc T[5]	Inc T=Ext T	Test Ext T	Test Inc T
		Fertilized Almasippi Soil			
8.73 a[6]	8.88 *	8.42 *	0.0834 a	0.0815	0.0767
8.63 b	8.76 *	8.37 *	0.0917 a	0.0861	0.0785
8.54 c	8.42 *	8.88 *	0.0918 a	0.0767	0.0815
8.48 d	8.37 *	8.76 *	0.0910 a	0.0785	0.0861
Pooled error:	0.02179[7]			0.008862	
		Fertilized Elm River Soil			
8.47 a	8.59 *	8.02 *	0.0992 a	0.0694 *	0.0774
8.36 b	8.48 *	7.98 *	0.0784 a	0.0720	0.0768
8.09 c	8.02 *	8.59 *	0.1020 a	0.0774 *	0.0694 *
8.01 d	7.98	8.48 *	0.1008 a	0.0768 *	0.0720
Pooled error:	0.02862			0.011648	
		Unfertilized Almasippi Soil			
7.13 a	7.22	6.90 *	0.0355 a	0.0619	0.0359
7.11 a	7.07	7.08	0.0102 a	0.0710	0.0439
7.14 a	6.90 *	7.22	-0.0053 a	0.0359	0.0619 *
7.21 a	7.08 *	7.07 *	0.0329 a	0.0439	0.0710
Pooled error:	0.0600			0.02416	
		Unfertilized Elm River Soil			
6.55 a	6.52	6.18 *	0.0196 a	0.0743	0.0393
6.47 a	6.61	6.13 *	0.0337 a	0.0201	0.0829
6.26 b	6.18	6.52 *	0.0589 a	0.0393	0.0743
6.42 a	6.13 *	6.61 *	0.0366 a	0.0829	0.0201
Pooled error:	0.07400			0.03011	

TABLE 38b

Coefficients Describing the Rate of ^{31}P Desorption Using the Equation Log_e (^{31}P desorbed[8]) = f + g Log_e (time)

Intercept (f)			Slope (g)		
Inc T=Ext T	Test Ext T	Test Inc T	Inc T=Ext T	Test Ext T	Test Inc T
		Fertilized Almasippi Soil			
1.63 a	1.87 *	1.32 *	0.0539 a	0.0779	0.0754
1.61 a	1.75	1.48	0.0823 a	0.0854	0.0188
1.68 a	1.32 *	1.87	0.0318 a	0.0754	0.0779
1.64 a	1.48	1.75	0.0252 a	0.0188	0.0854
Pooled error:	0.09886			0.04012	
		Fertilized Elm River Soil			
1.10 c	1.28	0.51	0.1270 a	0.0938	0.0605
0.61 c	0.98	-0.30 *	0.1605 a	0.1765	0.4303 *
0.25 b	0.51	1.28 *	0.2045 ab	0.0605	0.0938
-0.54 a	-0.30	0.98 *	0.4442 b	0.4303	0.1765 *
Pooled error:	0.3215			0.1307	

1. ^{32}P desorbed as dpm/g corrected for decay to the day the P was applied.

2. Time as hours from 1 to 48.

3. Inc T=Ext T describes the treatments where extraction occurred at the incubation temperatures; 10, 15, 20 and 25 (down the column).

4. Test Inc T describes the treatments 10/20, 15/25, 20/10 and 25/15 incubation/extraction temperatures such that, in contrast to the respective value in the Inc T=Ext T column, the effect of incubation temperature was tested (* indicates significant difference, $P \leq 0.05$).

5. Test Ext T describes the treatments 20/10, 25/15, 10/20 and 15/25 incubation/extraction temperatures such that, in contrast to the respective value in the Inc T=Ext T column, the effect of extraction temperature was tested (* indicates significant difference, $P \leq 0.05$).

6. Means followed by the same letter within a column of 4 were not significantly different $P \leq 0.05$.

7. Pooled standard error of estimate for the respective coefficient, pooled across temperature treatments.

8. ^{31}P desorbed as µg/g.

TABLE 39a

Coefficients Describing the Rate of ^{32}P Desorption Using the Diffusion - First Order Reaction Model: P Concentration = h $(time)^{\frac{1}{2}}$ + i $(1- e^{j(time)})$

Coefficient								
h			i			j		
Inc T= Ext T	Test Ext T	Test Inc T	Inc T= Ext T	Test Ext T	Test Inc T	Inc T= Ext T	Test Ext T	Test Inc T
Fertilized Almasippi Soil								
40.3 a	47.5	28.3 *	735 a	841 *	528 *	-2.5	-2.7	-3.0
44.3 a	38.1	27.0 *	643 b	780 *	506 *	(-3.4)[9]	-2.1	-2.7
43.5 a	28.3 *	47.4	574 c	528	841 *	(-4.9)	-3.0	-2.7
39.9 a	27.0 *	38.1	541 c	506	780 *	(-8.0)	-2.7	-2.1
Pooled error: 5.77				25.6			0.713	
Fertilized Elm River Soil								
41.9 a	32.4 *	20.5 *	545 a	617 *	351 *	-3.5	(-3.4)	(-3.4)
25.8 b	21.5	16.8	504 a	590 *	347 *	-2.5	-2.0	-2.5
28.8 b	20.5	32.4	379 b	351	617 *	-2.7	(-3.4)	(-3.4)
21.5 b	16.8	21.5	374 b	347	590 *	-1.8	-2.5	-2.0
Pooled error: 4.80				21.9			0.734	
Unfertilized Almasippi Soil[10]								
4.2 a	6.5	2.8	143 a	160	116			
1.0 a	6.7 *	6.2	143 a	137	132			
2.0 a	2.8	6.5 *	142 a	116	160 *			
4.0 a	6.2	6.7	155 a	132	137			
Pooled error: 218				8.91				
Unfertilized Elm River Soil								
1.3 a	3.6	0.5	82 a	81	61 *			
1.8 a	1.2	3.7	75 a	87 *	52 *			
2.3 a	0.5	3.6	61 b	61	81 *			
1.5 a	3.7	1.2	73 a	52 *	87 *			
Pooled error: 1.42				5.77				

- Footnotes same as Tables 38a and 38b except P concentration as dpm/mL.

9. Coefficients in parenthesis inaccurate (and not significantly different from zero) due to relatively instable iteration scheme, error estimates from these not included in pooled error term.

10. Coefficient j for unfertilized soil assumed to be less than -25 and therefore the model was abbreviated to P con entration = h $(time)^{\frac{1}{2}}$ + i.

TABLE 39 b

Coefficients Describing the Rate of ^{31}P Desorption from Fertilized Soils Using the Diffusion - First Order Reaction Model: P concentration = h $(time)^{\frac{1}{2}}$ + i

Coefficient					
h			i		
Inc T= Ext T	Test Ext T	Test Inc T	Inc T= Ext T	Test Ext T	Test Inc T
Almasippi Soil					
0.028 ab	0.047	0.027 *	0.595 a	0.739 *	0.442 *
0.039 a	0.047	0.005 *	0.563 a	0.649	0.535
0.017 ab	0.027	0.047 *	0.623 a	0.442 *	0.739 *
0.010 b	0.005	0.047 *	0.611 a	0.535	0.649
Pooled error:	0.0143			0.0580	
Elm River Soil					
0.034 a	0.031	0.022	0.363 a	0.423	0.192 *
0.019 a	0.052 *	0.045	0.299 a	0.296	0.122 *
0.032 a	0.022	0.031	0.165 b	0.192	0.423 *
0.030 a	0.045	0.052	0.137 b	0.122	0.296 *
Pooled error:	0.0154			0.0627	

- Footnotes same as Tables 38a and 38b except ^{31}P concentration as µg/mL.

TABLE 40a

Analysis of the Variance of Desorption of ^{32}P after 24 Hours of Extraction, a Subset of the Desorption Rate Data

Source	df	Dependent Variable			
		^{32}P Desorbed[1]		^{31}P Desorbed[2]	
		MS	P>F[3]	MS	P>F
		Fertilized Soils			
Temperature	7	5.789×10^{6}	0.0001	3.275	0.0022
Soil	1	3.976×10^{7}	0.0001	97.87	0.0001
T+S	7	2.943×10^{4}	0.345	1.018	0.161
Error	15	2.387×10^{5}		0.5679	
CV (%)		8.1		14.2	
		Unfertilized Soils			
Temperature	7	1.262×10^{5}	0.033		---
Soil	1	3.403×10^{6}	0.0001		---
T+S	7	2.683×10^{4}	0.711		---
Error	15	4.138×10^{4}			---
CV (%)		20.4			---

1. ^{32}P desorbed as dpm/g soil corrected for decay to the day the P was applied.

2. ^{31}P desorbed as µg/soil.

3. P>F is the probability of a larger F ratio, 0.05 was chosen as the maximum for significant difference.

TABLE 40b

Analysis of Covariance of the Desorption of ^{32}P from Unfertilized Soils, Adjusting Each Treatment to a Mean Desorption time by an Independent Desorption Rate Curve

Source	df	^{32}P Desorbed	
		MS	P>F
Temperature	7	1.4888×10^5	0.014
Soil	1	1.0694×10^7	0.0001
T+S	7	3.1143×10^4	0.0721
Time (T,S)	16	5.9258×10^4	
Error a	7	4.265×10^4	
Error b	182	1.645×10^4	
CV (%)		12.9	

- Where temperature and soil were class variables and time (in hours) was a continuous variable nested within temperature and soil.

- Footnotes as in Table 39a.

TABLE 41

Analysis of Covariance of Short Term Plant Uptake of ^{32}P and ^{31}P, Experiment A

Source	df	Dependent Variable: Log_e (^{32}P Uptake)		Log_e (^{31}P Content)		Log_e (Specific Activity)^2	
		MS	P>F[1]	MS	P>F	MS	P>F
Temperature and Time	11	3570.4	0.0001	0.03528	0.076	1918.	0.031
Soil	1	17723	0.0001	0.11685	0.017	1684.	0.147
S+T	11	120.06	0.748	0.016911	0.521	464.	0.774
P Treatment	1	2071.8	0.0020	0.000036	0.965	651.	0.357
P+T	11	133.16	0.684	0.065148	0.003	864.	0.363
S+P	1	7031.6	0.0001	0.088168	0.036	5294.	0.015
S+P+T	11	114.30	0.776	0.011450	0.785	352.	0.866
Root Weight	2	130.46		0.053238		432.	0.563
(Root Weight)^2	2	73.014		0.040996		242.	0.721
Error a	11	137.30		0.0204711		589.51	
Error b	27	177.59		0.0180232		727.77	(df=17)
CV (%)		3.2		16.1		5.7	

- Temperature and time were main plot class variables, soil and P treatment were sub-plot class variables, and root weight and root weight squared were continuous variables nested within P treatment.

- The natural logarithm of the dependent variable reduced inhomogeneity of variance which resulted from a very wide range in values, the covariable was nested within P treatment so that separate P uptake - root weight relationships existed for each P treatment.

1. P>F is the probability of a larger F ratio, 0.05 was chosen as the maximum for significant difference.

2. Specific activity of the soil P was computed as (total plant ^{32}P content)/(total plant ^{31}P content -2.19) where 2.19 mg/pot was the mean seed ^{31}P content (20 seeds per pot).

TABLE 42

Unadjusted Variables from Short Term Plant Uptake Studies at the Start of the Incubation Period, Experiment A

Temperature (°C)	Unadjusted ^{32}P Uptake ($\times 10^5$ dpm/pot)	Unadjusted ^{31}P Content (mg/pot)	Root Weight (g/pot)	Leaf P Concentration (%)
	Fertilized Almasippi Soil			
10	13.1	1.46	0.064	0.192
15	33.6	1.79	0.115	0.226
20	81.6	2.69	0.272	0.356
25	72.3	2.98	0.335	0.366
	Fertilized Elm River Soil			
10	18.1	1.80	0.112	0.210
15	35.0	1.98	0.150	0.234
20	79.6	2.76	0.330	0.282
25	74.4	2.75	0.245	0.360
	Unfertilized Almasippi Soil			
10	8.0	1.16	0.107	0.153
15	21.2	1.02	0.280	0.134
20	30.4	1.14	0.331	0.145
25	26.9	1.25	0.235	0.157
	Unfertilized Elm River Soil			
10	2.5	1.30	0.092	0.162
15	7.3	1.20	0.258	0.155
20	9.5	1.05	0.297	0.132
25	12.4	1.25	0.757	0.179
CV (%)	17.8	9.7	67.7	10.9
Probability of a large F, temp.	0.005	0.025	0.122	0.005
MS error b	3.4147×10^{11}	0.027793	0.028349	0.000555
df (error b)	12	12	12	12

1. dpm corrected for decay to the day the P was applied.

TABLE 43

Unadjusted Variables from Short Term Plant Uptake Studies at the End of the Incubation Period, Experiment A

Incubation Temperature (°C)	Growth Temperature (°C)	Unadjusted ^{32}P Uptake ($x10^4$ dpm/pot)	Unadjusted ^{31}P Content (mg/pot)	Root weight (g/pot)	Leaf P Concentration (%)
		Fertilized Almasippi Soil			
10	10	11.1	2.23	0.072	0.253
10	20	57.3	2.63	0.423	0.304
15	15	25.5	1.88	0.113	0.274
15	25	52.2	3.25	0.381	0.385
20	20	54.7	2.80	0.334	0.355
20	10	8.46	2.28	0.084	0.262
25	25	58.8	2.73	0.351	0.389
25	25	25.8	2.33	0.226	0.300
		Fertilized Elm River Soil			
10	10	8.92	1.98	0.091	0.256
10	20	50.7	2.50	0.514	0.314
15	15	19.9	2.18	0.190	0.265
15	25	70.4	2.18	0.269	0.337
20	20	24.9	2.05	0.807	0.266
20	10	4.98	1.65	0.124	0.277
25	25	37.6	2.20	0.605	0.315
25	15	23.8	2.20	0.289	0.239
		Unfertilized Almasippi Soil			
10	10	4.74	1.65	0.053	0.203
10	20	19.8	1.63	0.253	0.218
15	15	13.7	1.73	0.101	0.222
15	25	22.8	2.13	0.327	0.300
20	20	17.0	1.73	0.346	0.182
20	10	5.20	1.58	0.090	0.221
25	25	27.3	1.93	0.591	0.238
25	15	12.6	1.68	0.257	0.197
		Unfertilized Elm River Soil			
10	10	2.19	2.03	0.086	0.227
10	20	7.78	1.58	0.442	0.241
15	15	5.05	1.55	0.199	0.171
15	25	11.9	1.74	0.452	0.216
20	20	5.67	1.45	0.390	0.194
20	10	1.50	-	0.074	-
25	25	8.00	1.55	0.434	0.211
25	15	2.69	1.63	0.231	0.231
CV (%)		27.7	18.3	38.0	
Probability of a larger F, temp.		0.0004	0.006	0.017	
MS error b		$3.531x10^9$	0.13214	0.011516	
df (error b)		19	20	20	

TABLE 44

Regressions of $NaHCO_3$ and Resin Extractable ^{31}P versus Temperature, Experiment B

Soil Name	$NaHCO_3$ Intercept (µg/g)	Slope	Resin Intercept (µg/g)	Slope
Balmoral	18.5 *	0.159 *	14.4	-0.013
Almasippi	7.18	0.245 *	15.3	0.631
Inwood	22.6 *	0.037	21.3 *	- 0.180
Manitou	25.4 *	-0.076	12.3	0.552
Lakeland	20.0 *	0.107	25.7 *	-0.499
Lundar	22.9 *	0.014	21.6 *	0.105
Newdale	39.9 *	-0.088	-11.0	4.46 *
Pine Ridge	18.9 *	0.104	30.8 *	- 0.293
Elm River	3.45	0.257 *	12.4	0.438
Snowflake	9.05 *	0.202 *	8.42	0.089
Stockton	9.10 *	0.277 *	-10.3	4.92 *
Wellwood	13.9 *	0.305 *	13.7	0.161
Pooled error	3.749	0.0668	10.197	0.4242
df (pooled error)	24		24	
Overall R^2	99%		95%	

- Coefficients followed by '*' were significantly different than zero ($P \leq 0.05$) by t-test using the error of estimate pooled across soils.

TABLE 45

Standard Errors of Estimate for Desorption Curve Coefficients, Experiment B

Soil Name	Standard Errors of Estimate				df(error)
	Intercept	P desorbed	Temp.	P des. x temp.	
			^{32}P [1]		
Balmoral	0.1320	6.27×10^{-5}	6.90×10^{-3}	3.17×10^{-6}	14
Almasippi	0.2235	9.51×10^{-5}	1.24×10^{-2}	5.02×10^{-6}	16
Inwood	0.2661	1.36×10^{-4}	1.41×10^{-2}	6.50×10^{-6}	15
Manitou	0.5037	2.86×10^{-4}	2.65×10^{-2}	1.25×10^{-5}	16
Lakeland	0.1931	6.74×10^{-5}	1.06×10^{-2}	4.16×10^{-6}	14
Lundar	0.2695	1.24×10^{-4}	1.41×10^{-2}	5.69×10^{-6}	15
Newdale	0.4007	1.45×10^{-4}	2.21×10^{-2}	7.52×10^{-6}	16
Pine Ridge	0.2866	7.74×10^{-5}	1.50×10^{-2}	4.00×10^{-6}	14
Elm River	0.1316	7.97×10^{-5}	7.19×10^{-3}	4.20×10^{-6}	16
Snowflake	0.4110	1.39×10^{-4}	2.22×10^{-2}	7.67×10^{-6}	15
Stockton	0.2029	1.21×10^{-4}	1.08×10^{-2}	5.99×10^{-6}	14
Wellwood	0.2927	1.97×10^{-4}	1.57×10^{-2}	1.07×10^{-5}	15
			^{31}P		
Inwood	1.048	1.102	0.0570	0.0587	13
Manitou	2.378	2.087	0.1395	0.1175	10
Lundar	2.020	1.354	0.1148	0.0720	13
Newdale	0.938	0.072	0.0489	0.0035	14
Pine Ridge	1.808	1.134	0.0872	0.0465	11

1. ^{32}P data adjusted for decay to 53 days after the ^{32}P was applied

TABLE 46

Analysis of Covariance of Desorption Curve Data, Experiment B

Source	df	Adjusted SS	P>F
Soil	11	44.361	0.0001
Temperature	3	2.6231	0.0001
Soil + Temp.	33	1.9567	0.985
Log_e (ratio)	1	56.989	0.0001
Error	180	15.791	- CV=8.7%

where Log_e (^{32}P concentration) was the dependent variable, soil and temperature were class variables, and Log_e (solution:soil ratio) was the continuous variable

Soil Name	Temperature				Effect of Soils
	10	15	20	25°C	
Balmoral	3.03[1]	3.09	3.17	3.28	b
Almasippi	3.04	3.14	3.41	3.66	bc
Inwood	3.17	3.53	3.51	3.68	c
Manitou	3.31	3.35	3.33	3.82	c
Lakeland	3.28	3.33	3.35	3.42	c
Lundar	3.66	3.69	3.77	3.95	d
Newdale	3.88	3.97	4.23	4.32	e
Pine Ridge	4.28	4.18	4.32	4.35	e
Elm River	2.79	2.82	2.98	3.01	a
Snowflake	3.38	3.44	3.39	3.66	c
Stockton	2.91	2.83	2.99	3.00	a
Wellwood	2.71	3.00	2.66	2.81	a
Mean	3.29	3.37	3.43	3.58	

1. Mean Log_e (^{32}P concentration) as dpm/mL, adjusted for decay to 56 days after P applied and adjusted by the covariance to a mean extraction ratio.

- Means in a row joined by a line were not significantly different ($P \leq 0.05$).

- Rows followed by the same letter indicate that means for those respective soils were not significantly different ($P \leq 0.05$), tests of significance by single degree of freedom contrasts.

TABLE 47

Mean Effect of Temperature and Tests of Significance for Several Variables of the Short Term Plant Uptake Study, Experiment B

Variable	Temperature				CV	Probability of Larger F			Mean Square		df
	10	15	20	25°C	(%)	T	S	S+T	Error a	Error b	Error b
Leaf weight (g/20 plants)	0.74	0.76	0.69	0.65	11.4	0.048	0.0001	0.662	1.75×10^{-2}	6.57×10^{-3}	88
Leaf ^{31}P concentration (%)	0.137	0.157	0.217	0.267	19.0	0.0001	0.0001	0.0001	1.74×10^{-3}	1.35×10^{-3}	88
Root weight (g/20 plants)	0.149	0.286	0.493	0.569	36.9	0.009	0.0001	0.281	1.26×10^{-1}	1.92×10^{-2}	87
Root ^{31}P concentration (%)	0.231	0.219	0.192	0.185	21.2	0.439	0.0001	0.737	1.33×10^{-2}	1.93×10^{-3}	84
Leaf/root weight	5.38	3.10	1.74	1.45	32.6	0.0003	0.0001	0.324	2.95	0.891	87
Adjusted ^{32}P uptake ($\times 10^5$ dpm/20 plants)	1.58	1.88	2.59	3.66	48.7	0.0977	0.0001	0.0001	3.32×10^{10}	1.40×10^{10}	85
Adjusted ^{31}P content (mg/20 plants)	1.15	1.16	1.34	1.56	15.1	0.069	0.0001	0.0001	0.127	0.0385	85
Adjusted soil-derived ^{31}P content (mg/20 plants)	0.445	0.414	0.566	0.751	35.5	0.091	0.0001	0.0001	0.111	0.0373	85
Specific activity ($\times 10^5$ dpm/mg)	4.64	6.56	3.72	3.86	266.4	0.62	0.26	0.86	7.96×10^{11}	1.56×10^{12}	85

TABLE 48

Coefficients[1] Describing the Leaf ^{31}P Content and Soil ^{32}P Specific Activities of Short Term Plant Uptake Studies on 12 Soils at 4 Temperatures

Soil Name	Temperature					Correlation[2]
	10	15	20	25	Mean	
	Coefficient b_2 ($\times 10^{-3}$ µg ^{31}P/dpm ^{32}P)					
Balmoral	2.07	3.54	0.36	0.18	1.54	-0.72
Almasippi	5.80	0.99	0.75	-0.58	1.74	-0.90
Inwood	3.21	3.47	0.96	0.63	2.07	-0.90
Manitou	1.27	1.72	0.53	0.82	1.09	-0.62
Lakeland	1.31	1.56	0.30	0.30	0.87	-0.84
Lundar	1.77	1.62	0.75	0.88	1.25	-0.89
Newdale	2.19	2.75	1.66	1.75	2.09	-0.62
Pine Ridge	0.89	0.16	0.03	0.68	0.44	-0.25
Elm River	2.52	9.08	-2.44	-2.70	1.62	-0.64
Snowflake	-0.89	-0.05	-0.48	-0.89	-0.58	+0.05
Stockton	0.77	1.24	0.16	-0.16	0.50	-0.80
Wellwood	6.18	4.43	-2.20	0.66	2.27	-0.80
Mean	2.26	2.54	0.03	0.13	1.24	
	Coefficient b_1 (mg ^{31}P/plant)					
	0.0409	0.0411	0.0607	0.0673		

1. From the equation leaf ^{31}P content = b_1 (number of plants/pot) + b_2 (leaf ^{32}P content) where b_1 was nested within temperatures and b_2 within soil and temperature.

2. Correlation (r) between b_2 and temperature.

3. dpm corrected for decay to the day the P was applied overall r^2 = 98%, MS_{error} = 0.031558 df_{error} = 92.

TABLE 49

Unadjusted[1] Leaf ^{32}P and Soil-Derived ^{31}P[2] Contents from Short Term Plant Uptake Study on 12 Soils at 4 Temperatures, Experiment B

Soil	^{32}P Uptake				^{31}P Uptake			
	10	15	20	25°C	10	15	20	25°C
	x 10^4 dpm[3]/pot				mg P/pot			
Balmoral	4.86	8.43	24.1	24.2	0.224	0.446	0.401	0.411
Almasippi	3.19	2.97	5.20	7.65	0.362	0.170	0.350	0.227
Inwood	3.29	12.1	27.4	54.6	0.224	0.459	0.560	0.665
Manitou	9.62	17.0	34.8	53.0	0.254	0.442	0.515	0.738
Lakeland	5.21	11.8	25.9	36.3	0.210	0.316	0.431	0.460
Lundar	6.96	17.4	53.2	86.5	0.274	0.371	0.754	1.07
Newdale	11.3	33.9	76.8	90.5	0.452	1.14	1.52	1.86
Pine Ridge	6.00	9.51	20.8	23.4	0.247	0.166	0.385	0.480
Elm River	1.40	1.40	4.09	4.34	0.165	0.250	0.200	0.154
Stockton	5.58	8.02	19.5	20.1	0.221	0.215	0.346	0.262
Snowflake	3.02	26.5	12.4	15.5	0.111	0.098	0.304	0.161
Wellwood	2.06	1.43	4.74	10.5	0.226	0.168	0.234	0.348
CV	65.0%				44.9			
df (error b)	88				88			
$MS_{e(b)}$	1.6511×10^{10}				0.0351363			

1. Unadjusted for number of plants or root weight.

2. Calculated by subtracting estimated seed-derived ^{31}P (Appendix G) from unadjusted measured total leaf ^{31}P content.

3. dpm corrected for decay to the day the P was added.

Appendix I

MODIFIED HOAGLAND NUTRIENT SOLUTIONS

Macronutrient Stock Solutions

- 0.5 M KNO_3
  0.25 M $Ca(NO_3)_2$
  0.5 M Mg SO_4
  0.1 M KCl
  0.02 M KH_2PO_4

prepared each in a separate 10 L carboy

Micronutrient Stock Solutions (minus Fe)

- 0.5 u M $MnSO_4$
  0.02 u M $CuSO_4$
  0.04 u M $ZnSO_4$
  0.3 u M H_3BO_4
  0.03 u M H_2MoO_4

prepared together in one 10 L carboy

Fe Stock Solution

- 0.1 M Fe-EDTA

prepared in a 1 L darkened bottle and refrigerated

Growth Solution (Chapter 5)

Macronutrients at 1/5 and micronutrients at 1/2 the concentrations give by Hoagland and Arnon (1950)

Basic Solution	Treatment KH_2PO_4 (μM)	Corresponding KCl (μM)
1.0 m M KNO_3	0	200
1.0 m M $Ca(NO_3)_2$		
0.4 m M $MgSO_4$	5	195
	10	190
2.5 μM $MnSO_4$	20	180
0.1 μM $CuSO_4$	30	170
0.008 μM $ZnSO_4$	40	160
0.06 μM H_3BO_4	50	150
0.006 μM H_2MoO_4	100	100
	200	0
0.05 m M Fe-EDTA		

adjusted to pH 5.5 using H_2SO_4

Appendix J

PRELIMINARY SOLUTION CULTURE EXPERIMENT

Introduction

This experiment had the same overall objective as those reported in Chapter 5 but with several differences in methodology. As such, this experiment served as a preliminary.

The major difference was that in this experiment, it was assumed that the relationship between yield and plant tissue P concentration would be exact if a steady state of P supply was imposed. Thus no alternate method was used to confirm P status as was employed in Chapter 5.

An additional objective was to measure the P uptake rate at harvest by exposing the plants to a ^{32}P labelled solution just prior to harvest.

Methods and Materials

The solution culture containers consisted of five pairs of polyethylene bags (one inside the other) suspended in each of the four temperature-controlled water baths. The bags (60 cm long x 60cm flat width) were suspended from a rectangular wood frame (43 x 18 cm) and six aerator tubes were placed in each bag (see Appendix A).

Each bag held 20 L of nutrient solution which was modified from that of Hoagland and Arnon (1950) by reducing the macronutrient concentration to one tenth and the micronutrient concentration to one half. The Fe was supplied as Fe-EDTA and P levels of 0.1, 1.0, 10, 50 and 100 µMP/L were established. The P levels were located randomly within each water bath.

Seeds of Neepawa wheat were germinated as described in Chapter 5.

After germination, eighteen seedlings were placed into each of the solutions and were held in place by small foam-rubber plugs in holes in a styrofoam board. One board was attached to each solution-bag frame (Plate 9).

The nutrient solutions were replenished daily by adding 1 to 3 L of freshly prepared nutrient solution, allowing the excess solution to overflow the bags and drain into the water baths.

The number of leaves on the main shoot of each plant was recorded every 2-3 days up to the 6th leaf stage. At this stage, tiller development made identifying the main-shoot leaves difficult. Plants were harvested at several intervals (based on growth stage) throughout the experiment by randomly chosing plants from each solution P concentration and temperature. The plants were removed from the solution culture with care to disentangle the roots from the remaining plants. Each plant was transferred to an aerated, 500 mL bottle of nutrient solution at the same P level and temperature at which the plant had grown. This solution was labelled with 1.8×10^7 dpm ^{32}P/500 mL. After 1, 2, 3 or 4 hours of adsorption at 25, 20, 15 or 10°C respectively, the plant was transferred to a new bottle held at the same P concentration and temperature conditions but not labelled with ^{32}P. After the same interval, the plant was harvested by separating the roots and shoot and rinsing the roots with distilled water.

The shoot and root tissues were placed between polyethylene films and photographed. Then autoradiographs were made by pressing the plant tissues onto X-ray film for 5 to 18 hours (depending on the radioactivity). The plants were refrigerated at 2°C during this time.

Leaf areas were determined by separating each leaf from the shoot and passing it through a scanning leaf area meter (average of three measurements, (Li-Cor Portable Area Meter, Model L1-3000, Lambda Inst. Corp.). Root

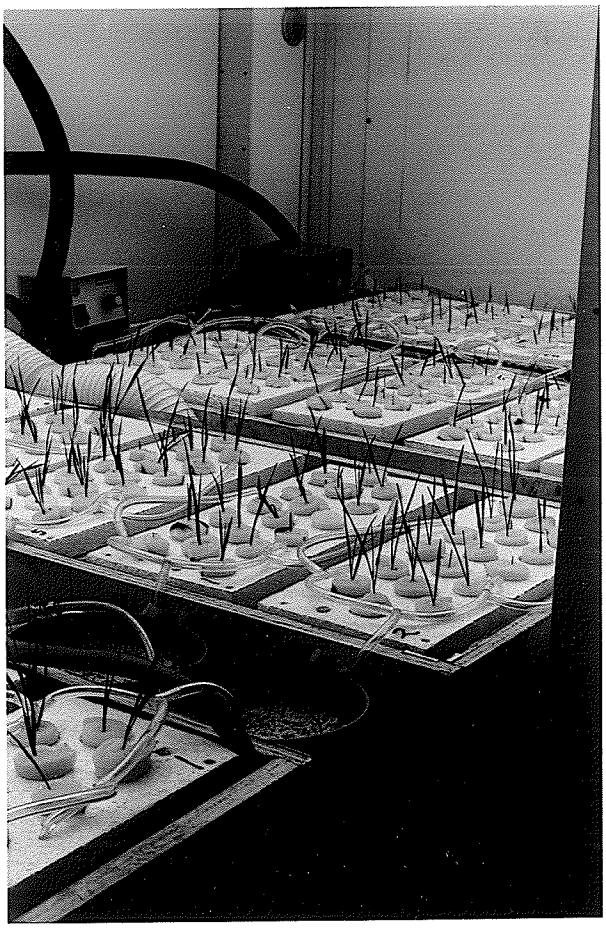


Plate 9: Apparatus used for the preliminary solution culture experiment (Appendix J)

lengths were determined using the line intersect method of Newman (1966) (50 fields of a 3 cm line). The physiological stage was estimated by dissecting the apical meristems of selected plants (Williams, 1965).

After all other fresh tissue measurements were made, the samples were weighed, dried at 105°C and reweighed. The dried samples were wet-ashed using H_2SO_4-H_2O_2 (Thomas et al., 1967), analysed for ^{32}P using Cerenkov counting (on a Searle Mark III scintillation counter, Appendix C), and for ^{31}P (Appendix B).

Growth curves of the natural logarithm of the shoot dry weight versus time were fitted using polynomial regressions for each P concentration and temperature. Thus, the first differential of these equations was the relative growth rate (RGR, g/day g).

Treatment comparisons across temperatures were based on 1) calendar date, 2) estimated day of floral initiation (double ridge formation) and 3) estimated day of attainment of a common dry weight. The values of yield and tissue P concentration were interpolated for these days from the fitted growth curves and from plots of tissue P concentration with time, respectively.

Results

Plant Growth, Leaf Areas and Root Lengths

Plant growth response to P concentration and root temperature was evident within four days after transplanting. There was some variability among the plants in each bag and thus they were divided into two groups, based on a visual ranking of size. These groups were treated as replicates for all harvests.

The most rapid growth occurred at 25°C. However, growth at 0.1 and

1 μMP/L was almost negligible (Figure 42). The plants at 0.1 and 1 μMP/L continued to develop to head-emergence but the heads had only two florets. As each new leaf emerged on these plants, the next oldest leaf senesced, suggesting that translocation of seed P was the sole source of P in these plants. Root growth ceased during the vegetative growth phase and the roots became stained (probably with Fe) and the root tips became club-like.

The plants at 10, 50 and 100 μMP/L grew and developed fairly normally. Little difference in growth between 50 and 100 μMP/L indicated that the optimal P supply was at or below 50 μMP/L but above 10 μMP/L. The plants were unique relative to field-grown wheat in the very large number tillers (up to 31) associated with each plant.

The growth curves (Figure 43 for 10, 50 and 100 μMP/L) were described reasonably well by polynomial equations. When the fit of the equation was especially poor, the required parameters (yield and RGR) were estimated manually. The primary effect of temperature on the growth curves (within each P level) seemed to be a delay in the start of the grand phase of growth. The slopes of the curves after rapid growth had begun were very similar across temperatures. Because these curves are plots of $\log_e$ (yield), the slopes represent the RGR (relative growth rates). There was no evidence that P supply compensated for temperature effects.

Temperature has such a profound effect on biological systems that response to temperature is almost inevitable. The observed similarity in growth rates among temperatures was therefore difficult to reconcile. It was concluded that this lack of response may have been the consequence of the experimental system. The plants were positioned such that the roots, the shoot meristem (until stem elongation occurred) and the lower pseudo-stem tissue of the plants was immersed in or very close to the temperature-

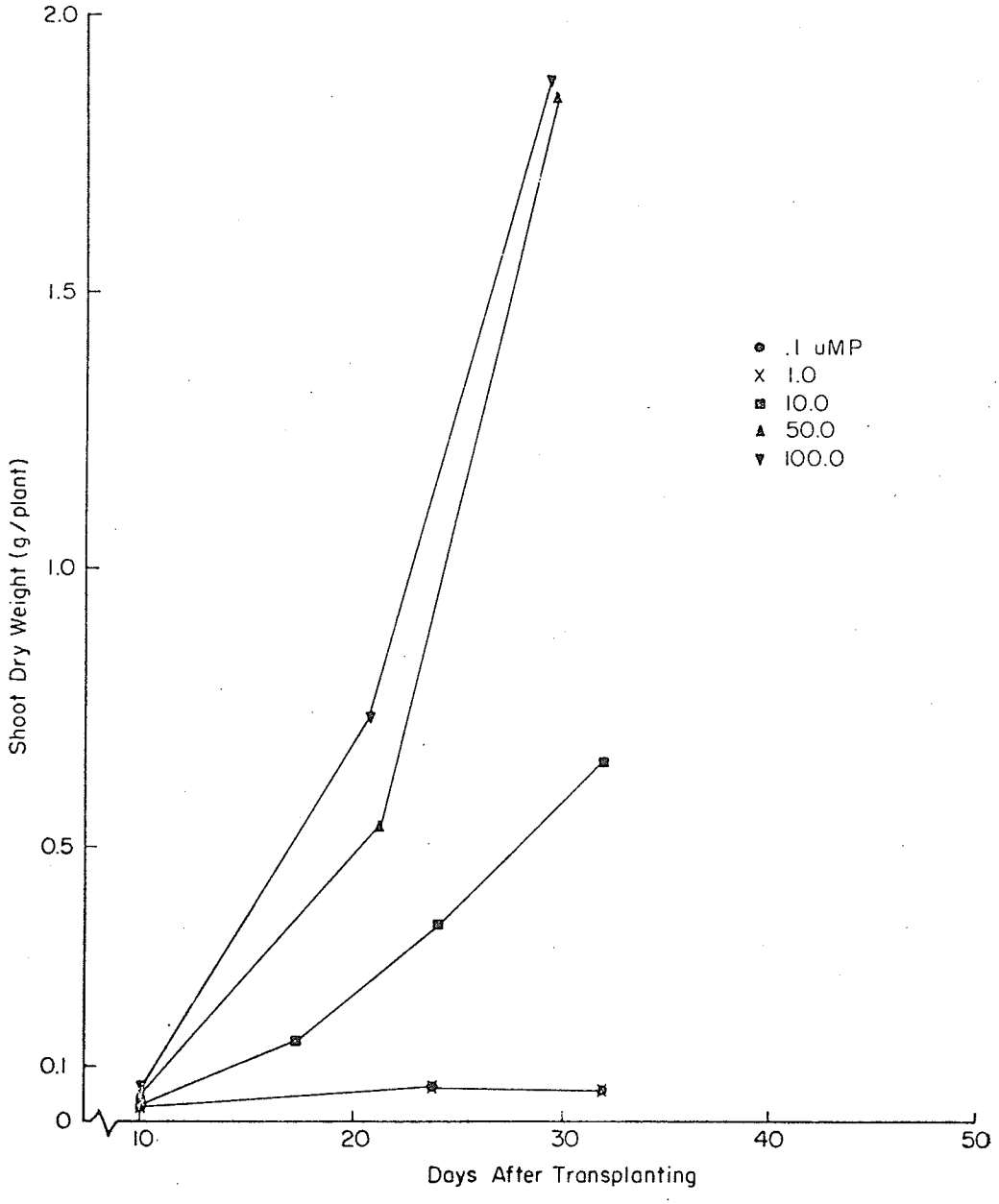


Figure 42: Shoot growth curves at 25°C root temperatures and five nutrient solution P concentrations.

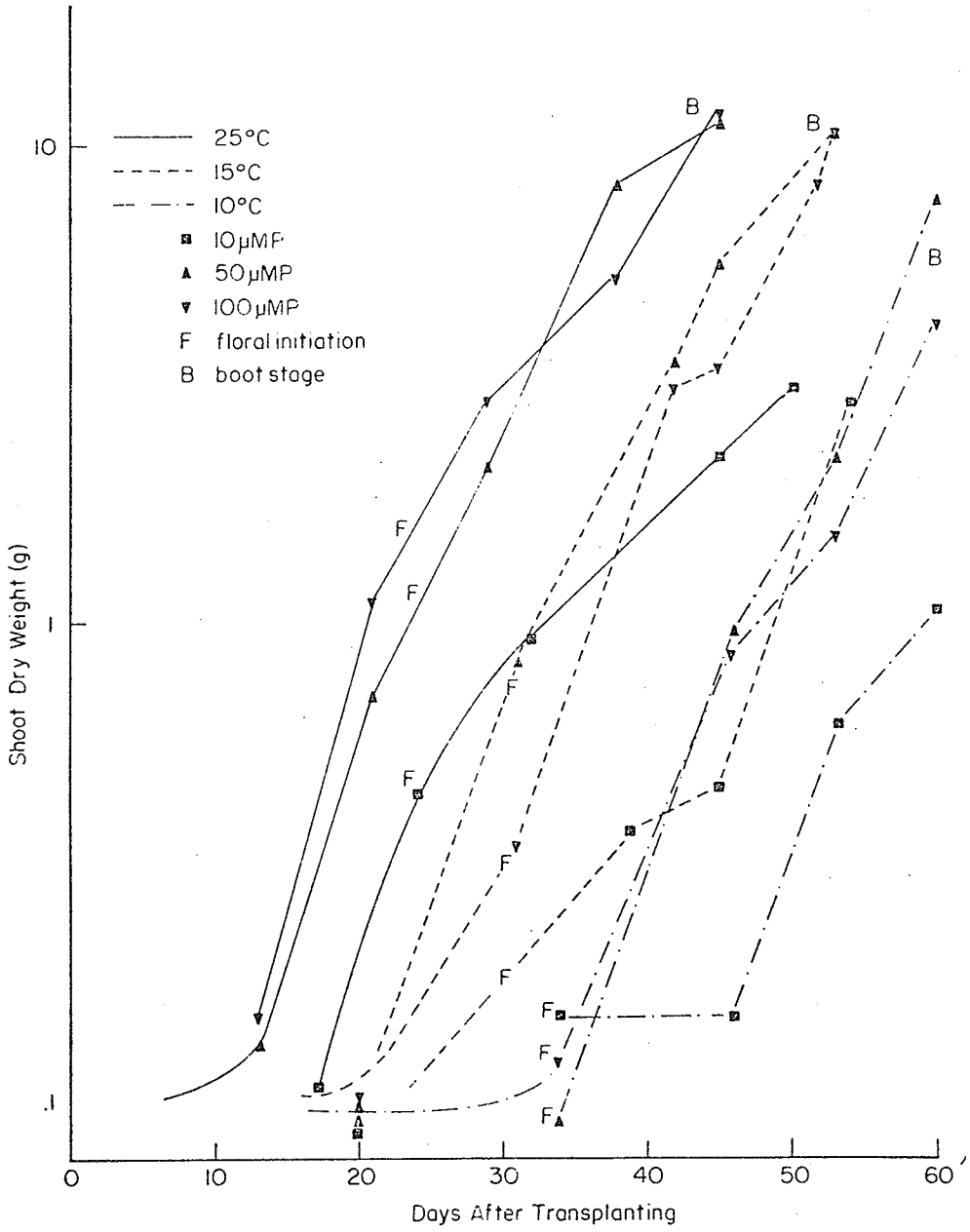


Figure 43: Shoot growth curves at three root temperatures and three nutrient solution P concentrations.

regulated nutrient solutions. The leaf blades were exposed to the uniform temperature of the growth chamber air. Thus it may be argued that the observed lag in the start of the grand phase of growth may reflect the effect of the solution temperature on leaf development and initial expansion. These temperatures may also have influenced the overall expansion rates of the first few leaves. However, when the expansion of the large fourth and later leaves become responsible for the weight gains, the uniform air temperature probably controlled the rate of weight gain regardless of the solution temperature. This would lead to parallel growth curves among solution temperatures after an initial lag, as observed.

Day of flower initiation and day of boot stage are also shown along each growth curve in Figure 43. These stages varied up to five days among P treatments with the 0.1 and 1 μMP treatments more advanced than the 10, 50 and 100 μMP treatments. These stages also varied distinctly with temperature and yet were not related to the size of plant. Thus, floral initiation occurred with 1 g of shoot dry weight at 25°C and with 0.15 g dry weight at 10°C. This weight differential was exaggerated by the number of tillers which was generally greater at higher temperatures.

The growth curves emphasized the complexity of comparing treatments across temperatures. Three time scales or stage criteria (chronologic, developmental and dry matter accumulation) were considered, but each was confounded by the other. Comparisons on a chronological time scale ignored physiological age which is an important determinate of plant nutrient status. Comparisons on a developmental time scale contrasted plants almost 10 fold different in size with the ramification of large differences in absolute growth rate. Comparisons on a dry matter accumulation time scale contrasted plants (e.g., at 1 g dry weight) varying from floral initiation to

the boot stage. Because the dry weights in this study were biased by the number of tillers, it was concluded that a developmental time scale was most appropriate. This was considered most valid when comparing intensity terms such as tissue P concentration and relative growth rate.

The leaf area curves (Figure 44 for 25 and 10°C, 0.1, 10 and 100 µMP/L) show very similar trends to the growth curves. The loss in leaf area at the 0.1 µMP level was due to extensive senscence of older leaves. The plants at 1 and 50 µMP/L responded very similarly to plants at 0.1 and 100 µMP/L, respectively. The delay in leaf area expansion due to temperature corresponded very closely to the delay in rapid dry matter accumulation.

The root length measurements were collected primarily as a basis for interpreting the ^{32}P uptake data. An attempt was made to differentiate seminal main axis, seminal laterals and nodal roots. However, by the time the nodal roots were abundant enough to be accurately measured, the seminal roots had proliferated dense mats of lateral roots which were very difficult to spread thinly enough to measure. Furthermore, the seminal lateral roots appeared to be growing less actively as the development of the nodal system progressed. However, on a length basis, the seminal laterals were still the major component of the root system. It was concluded that the root length measurements were reliable indicators of root activity only at an early stage when the roots were almost all associated with the seminal root system. Furthermore, the tangling of roots between plants made a quantitative recovery above about 5 g fresh weight almost impossible.

^{32}P Uptake Measurements and Nutrient Depletion

Autoradiographs were used as an exploratory tool to locate recently absorbed P. The time period during which the ^{32}P could translocate within

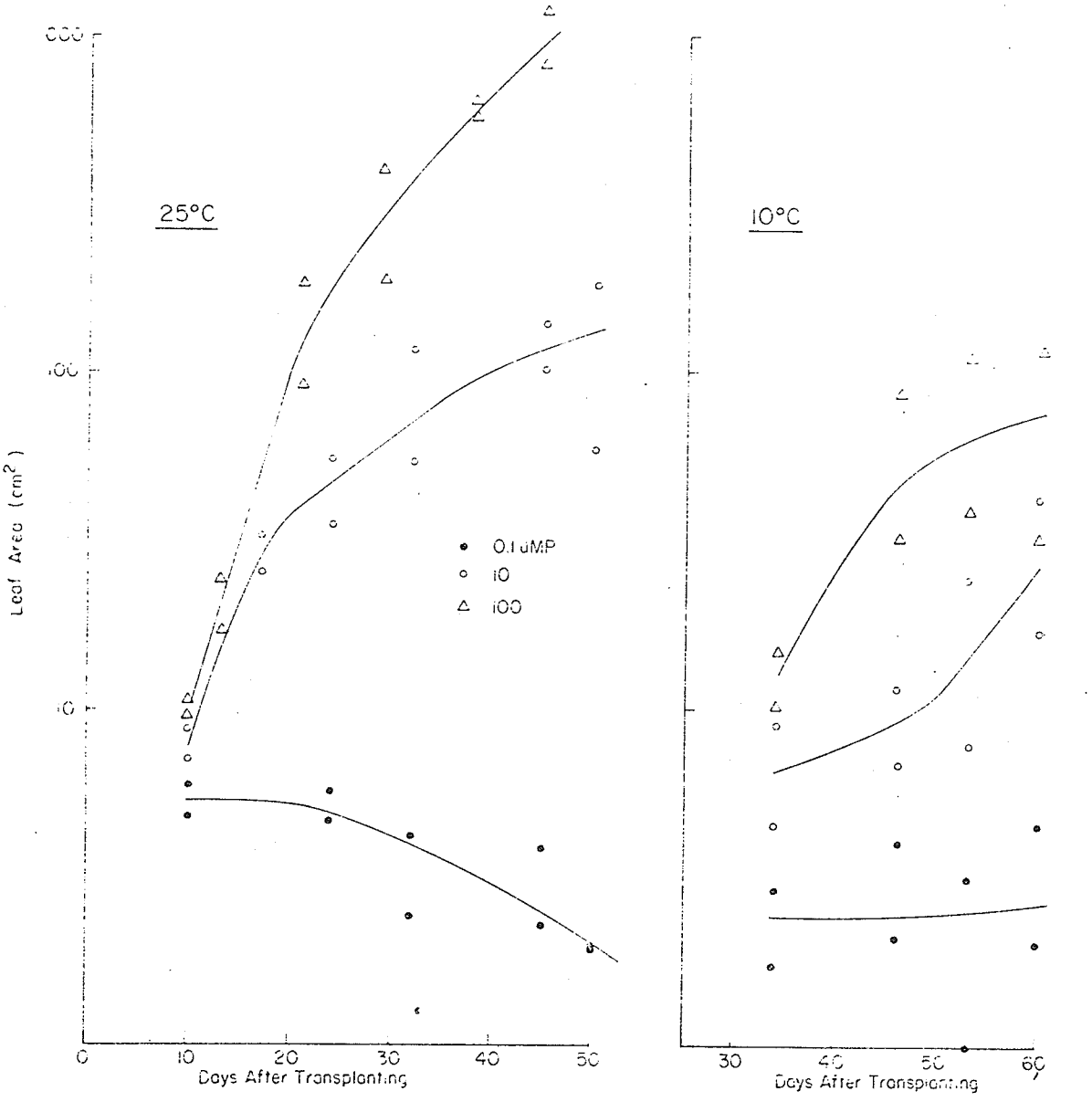


Figure 44: Leaf areas with time at 25 and 10°C root temperatures and three nutrient solution P concentrations.

the plant varied due to treatment. The absorption procedure lasted one to four hours followed by an equivalent length of time in an unlabelled solution. After this, there was up to a 24 hour delay between harvest and the end of the exposure of the X-ray film, depending on the activity of ^{32}P in the plants. Thus considerable time was available for the ^{32}P to translocate throughout the plant and isotopically exchange with plant ^{31}P. The autoradiographs indicated that this was generally sufficient time for a uniform dispersion of the ^{32}P in the plant.

Exceptions to this trend were noted. Leaves that were partially yellow due to progressive senescence received no ^{32}P, indicative that the translocation was out of the leaf only (Plate 10). There was some indication that newer leaves received a higher concentration of the ^{32}P. Finally, the roots had numerous points of ^{32}P accumulation which included the root tips but were also distributed throughout the root system (Plate 11). Dissection of the roots showed that at least some of these points were associated with lateral root primordia.

The total ^{32}P uptake rate increased with time (Figure 45 for 10 μMP/L) such that a maximum rate was achieved at 24, 28, 32 and 53 days for 25, 20, 15 and 10°C, respectively. However, the maximum rates coincided with total depletion of the ^{32}P uptake solutions. Thus these plants, at the times listed above, were capable of absorbing all of the P from the 500 mL absorption systems within four hours. Extrapolated to the 20 L solution culture bags, it was calculated that these solutions would have been totally depleted within 48 hours and the daily addition of 1 to 3 L of new solution was negligible relative to plant demand for P. Analysis of the nutrient solutions confirmed that total depletion of P had occurred but these analyses were completed too late to allow correction of the experimental system.

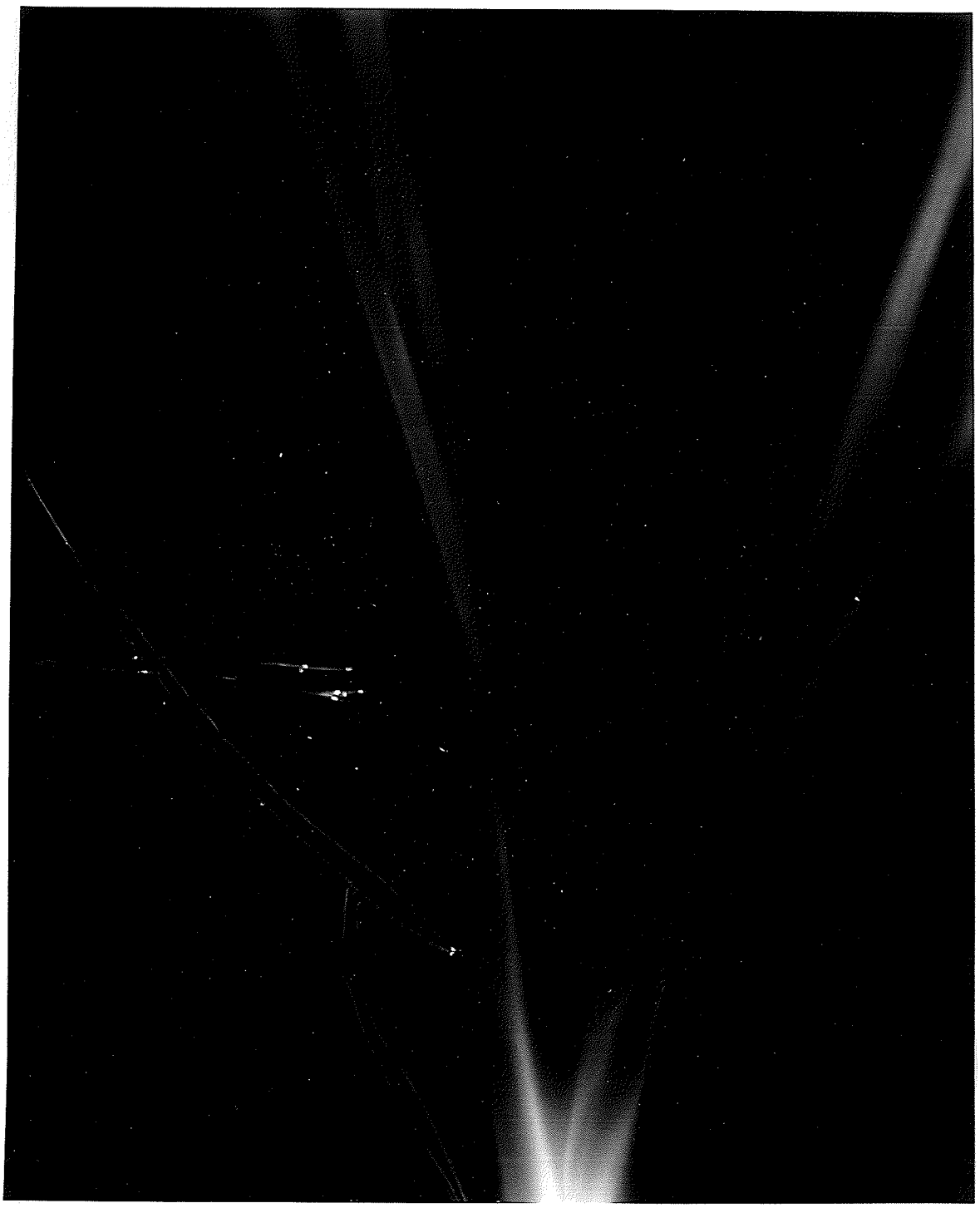


Plate 10: Autoradiograph of a plant shoot after uptake of ^{32}P-labelled solutions, leaves that contained no ^{32}P (drawn in) were senescing

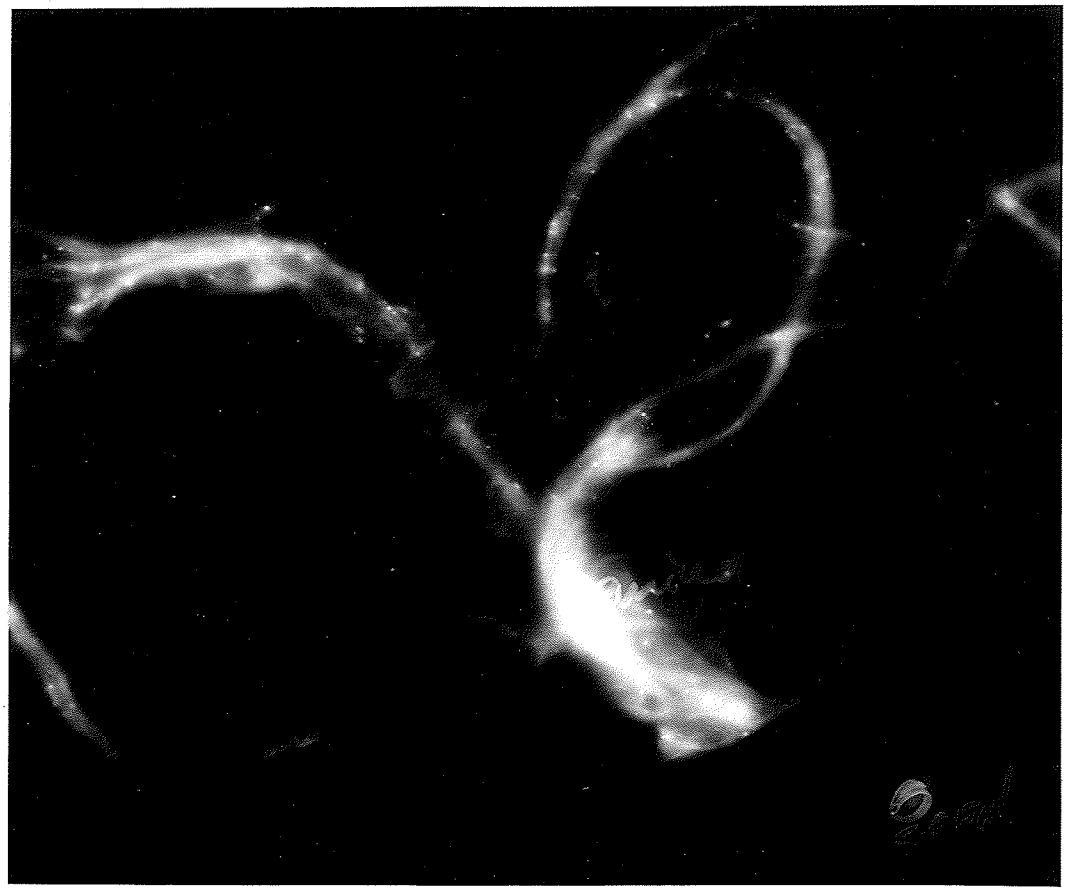


Plate 11: Autoradiograph of roots after uptake of ^{32}P-labelled solutions showing points of ^{32}P concentration corresponding to lateral root primordia

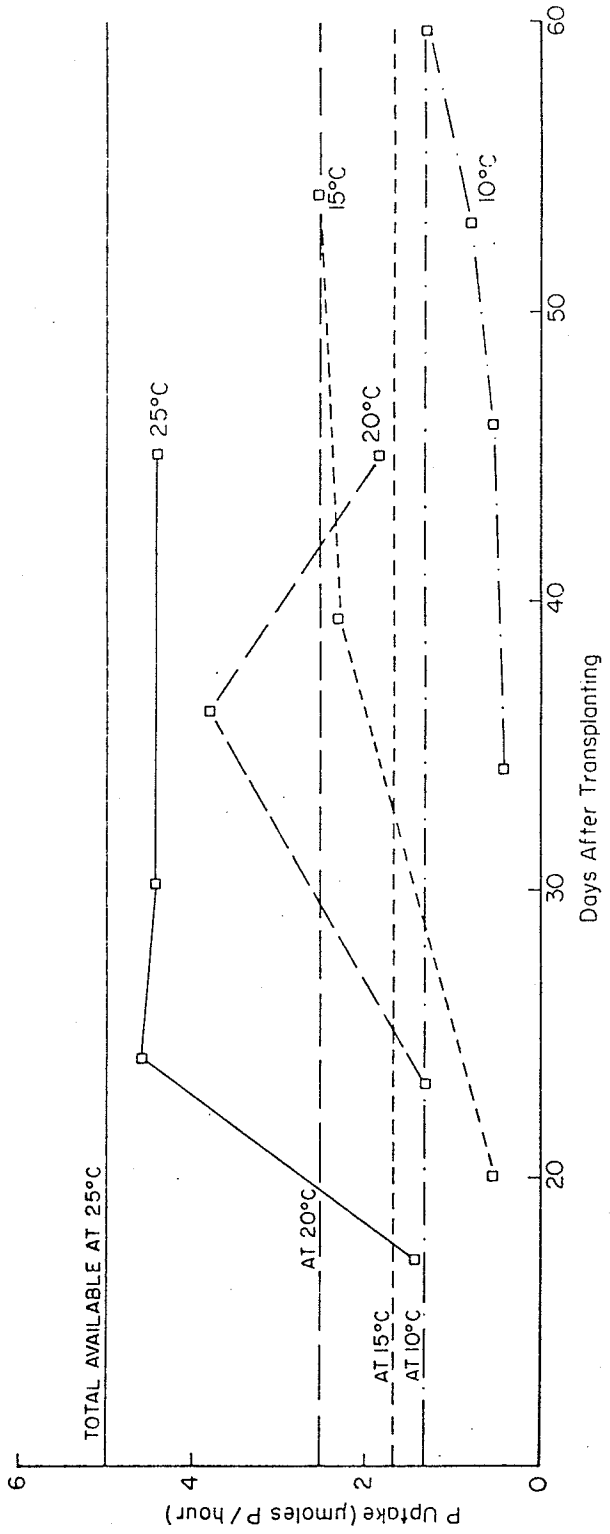


Figure 45: P uptake rates from 10 μM P solutions labelled with ^{32}P at four root temperatures, also showing the total P content/hour in the respective uptake bottles.

The implications of this nutrient depletion to the experimental design were several. The use of the ^{32}P uptake data was restricted to notes on translocation and an indication that the uptake mechanisms were probably maximized. The total ^{31}P uptake data was solely a function of the number of plants per bag and the frequency of additon of new solution.

Plant growth was obviously a function of P supply early in the experiment (because of the response to P observed) but after the nutrients were depleted, all of the plants could have been deficient. However, the plants at 50 and 100 μMP/L did not differ in growth rate throughout the experiment. Thus, despite a two-fold difference in total P supply (and hence total P content), the P nutritional status of the plants must have been similar. Since depletion of solution P occurred in both cases, this must indicate a substantial luxury consumption of P early in the plant's growth. Presumably this reserve of P in the plant (plus the relatively small amounts added daily) sustained growth throughout the experiment and since the growth did not differ significantly between these two P supply levels, it can be concluded that neither were deficient in P. The response curves shown in Figure 46 confirms this suggestion.

Plant growth at 10 μMP/L was consistently at a lower rate and thus these plants were probably deficient in P throughout the experiment. Therefore, only at this P supply level was there a direct relationship between tissue P concentration and growth. The assumption of a steady-state relationship between growth rate and P supply (one of the bases of this experimental design) was true only for this P supply level.

Tissue ^{31}P Concentrations

The tissue ^{31}P concentrations changed rapidly with time (Figure 47

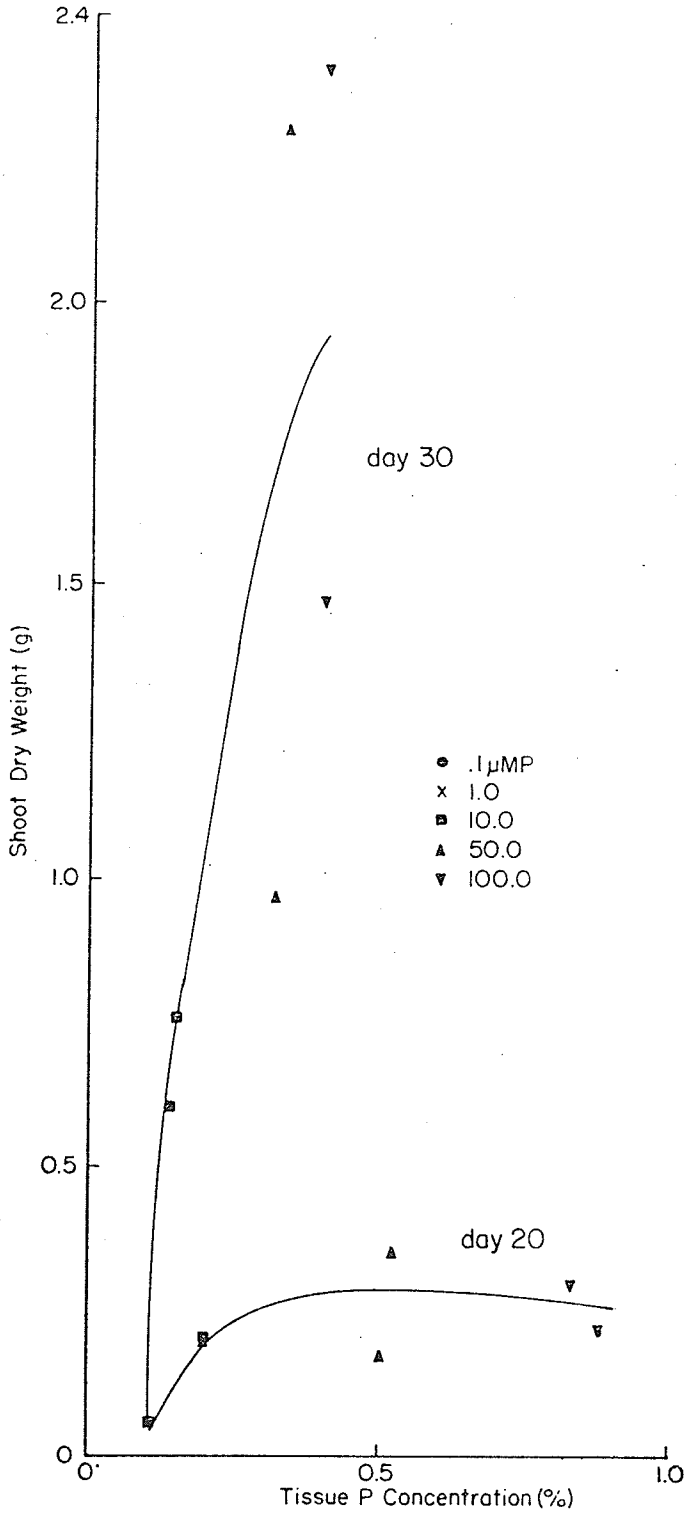


Figure 46: Shoot yield versus tissue P concentration on days 20 and 30 at 25°C.

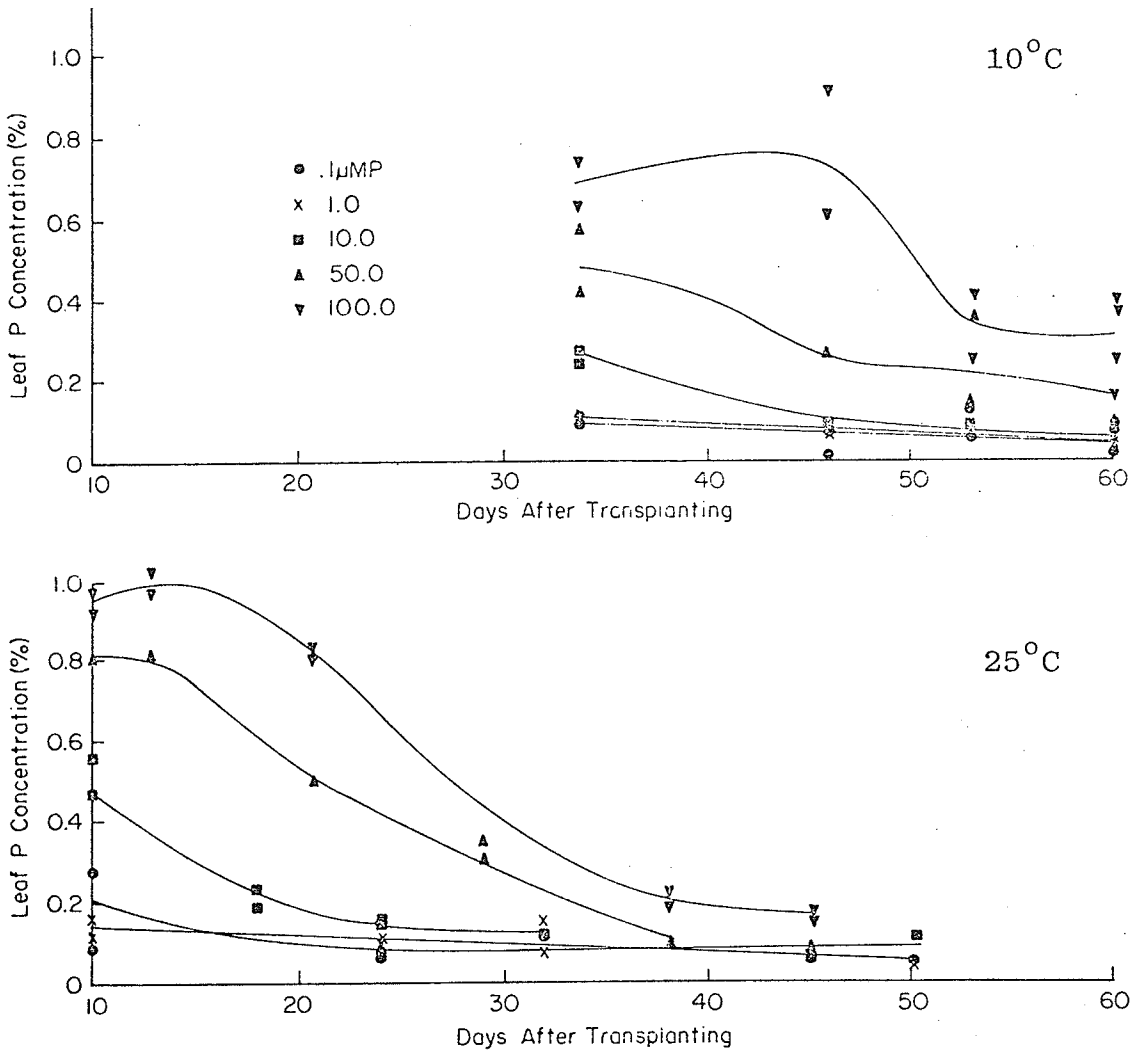


Figure 47: Tissue P concentration with time at two root temperatures and five nutrient solution P concentrations.

for 25 and 10°C) with concentrations as high as 1.17% P less than 20 days after transplanting and most plants with 0.1% P at the end of the experiment. The decrease in tissue P concentrations appeared to coincide with the start of the grand phase of growth and thus was attributed to biological dilution. This dilution was delayed at lower root temperatures and was also delayed at the higher P supply levels.

The tissue concentrations at the 50 μMP level decreased to the level of the 10 μMP treatment near the boot stage of growth and thus this treatment may have become deficient in P but at too late a stage to be evident in growth response.

The seed P content was about 0.48% and thus the very high P concentrations early in the experiment indicated a rapid initial uptake of P. This probably exceeded plant requirements in the 50 and 100 μMP levels and was stored as a buffer against changes in P supply. The phenomenon of high tissue concentrations in seedling plants is commonly observed but there is some question about whether it is essential for plant growth. In this study, the 10 μMP level was shown to be insufficient for optimal plant growth as early as ten days after transplanting (Figure 42) and yet had a tissue P concentration at that stage of 0.5% P. This tissue concentration was more than two-fold above the concentration generally considered adequate for growth (usually measured at a later stage) and was equal to that thought to be adequate in this experiment at 20 days after transplanting. Thus, it would appear that the high tissue P concentations in the seedling plants was required for early growth.

The implication of this conclusion was that plant P requirements, in terms of tissue concentration, changed very rapidly from the one leaf stage through to the floral initiation stage. This period corresponded to a

rapid change in the relative growth rate and subsequently to a change in the ratio of actively expanding to mature plant tissue. Therefore, the initial high P concentration requirements may be typical of meristematic and expanding tissues whereas the lower P concentrations required later in development may reflect the mature, fully expanded tissues.

Summary Statistics of Plant Parameters

The various measurements and variables in this experiment allow the opportunity to examine ratios and correlations which may be useful beyond these results. To examine these relationships, the data were separated into three general plant age classes (uniform across temperatures) with each class consisting of one to three sequential harvests. Ratios of shoot/root (dry weight basis), dry matter content (dry/fresh weights for both shoot and root), and leaf / shoot dry weight were computed (Table 50). Correlations between various parameters were tested (Table 51).

Disussion

In this study, five supply levels of P were imposed on plants growing at four root temperatures. Two of the supply levels (0.1 and 1.0 μMP) were extremely deficient such that growth was negligible, one supply level (10 μMP) was deficient but growth continued at a reduced rate, and two supply levels (50 and 100 μMP) were probably sufficient for optimal growth throughout most of the experiment. The effect of temperature on growth was primarily a delay in the grand phase of growth which, when it began, progressed at a similar rate regardless of temperature.

The tissue P concentrations increased rapidly shortly after trans-

TABLE 50

Means and Ranges of Several Plant Parameters Measured on Plants Supplied with 100 µMP/L in Three Age Classes

Parameter	Age Class			Range[1]
	1 (seedling)	2 (vegetative)	3 (boot stage)	
Shoot/root ratio	1.76	2.72	4.39	0.39-14.0[2]
Dry matter content				
Shoot (%)	15.2	20.4	24.6	12.0 -41.0
Root (%)	7.2	8.3	12.1	5.0 -17.0
Green leaf area/shoot dry weight (cm^2/g)	150.0	88.0	73.0	0 -241.0
Shoot P concentration (%)	0.86	0.44	0.21	0.007-1.14
Relative growth rate (g/g.day)	0.185	0.145	0.061	0 -0.362

1. Range over all data.

2. Recovery of roots was inefficient in large plants and therefore, data from all but age class 1 should be interpreted with caution.

TABLE 51

Simple Correlations Between Several Plant and Controlled Parameters

Parameter		Parameter Number											
		1	2	3	4	5	6	7	8	9	10	11	12
Solution Concentration	1				***	***	***	***	***	***	***	-	***
Plant Age Class	2				***	***	-	***	***	***	***	***	***
Temperature	3				-	-	-	***	***	*	***	***	*
Shoot Dry Weight	4	0.46	0.21	0.10		***	***	*	*	-	-	**	*
Root Dry Weight	5	0.29	0.21	0.05	0.67		-	*	-	-	-	**	*
Shoot/Root Ratio	6	0.38	0.09	0.10	0.62	-0.03		-	**	-	-	**	-
Dry Matter Content: Shoot	7	-0.42	0.72	-0.30	0.15	0.15	0.03		*	***	***	**	***
Dry Matter Content: Root	8	-0.39	0.64	-0.27	0.15	-0.08	0.23	0.15		***	***	**	***
Relative Growth Rate	9	0.46	-0.51	0.15	-0.04	-0.10	0.11	-0.66	-0.54		***	*	***
Green Leaf Area/Shoot Dry Weight	10	0.39	-0.83	0.42	-0.11	-0.11	-0.01	-0.88	-0.74	0.69		***	***
Root Length/Root Dry Weight	11	-0.09	-0.31	0.35	-0.25	-0.25	-0.26	-0.30	-0.26	0.23	0.41		**
Shoot P Concentration	12	0.59	-0.63	0.18	-0.15	-0.15	-0.03	-0.76	-0.68	0.67	0.77	0.25	

\- No significant correlation.

* Significant $0.05 \geq P > 0.001$.

** Significant $0.001 \geq P > 0.0001$.

*** Significant $0.0001 \geq P$.

planting but declined progressively to approximately the floral initiation stage. There was evidence that the optimal P concentration also changed rapidly with time.

The major premise of this experiment was that the optimal tissue P concentration varied with growth conditions such as root temperature. The rapid change in tissue P concentrations with time and the effect temperature had on physiological development made it difficult to define an optimal P concentration that could be compared across temperatures. This was further limited by the few P supply levels and the loss of some data due to contaminated reagents. The data available (Table 52) show up to a 3.7 fold range in tissue P concentrations between the deficient and sufficient plants and in no case was the range narrow enough to speculate on the effect of temperature on the optimal P concentration.

This data does provide an opportunity to contrast the three time scales or stage criteria. On a chronologic time scale, tissue P concentrations increased at lower temperatures. This was due to biological dilution since on day 30, the plants at 25°C were 30-fold larger than those at 10°C. On a developmental time scale, the effect of temperature was variable. On a uniform-weight time scale, the concentrations decreased as temperature decreased. This latter comparison may be most valid since biological dilution was a major determinant of tissue P concentration. These values could be interpreted as the total P uptake (concentration X yield) of plants all the same size. This would imply that P uptake and/or translocation to the shoot was lower at lower root temperatures.

The second premise of this experiment was that the optimal tissue P concentration would be related to the relative growth rate. It was not possible to define the optimal P concentration but the relationship may

TABLE 52

Tissue P Concentrations Shown to be Deficient and Sufficient for Growth, Compared at Three Stage Criteria

Criteria	Temperature	Deficient (10 µMP treatment) % P	Sufficient (50 µMP treatment) % P
Chronologic (day 30)	25	0.14	0.30
	20	0.10	0.32
	15	-	0.45
	10	0.25	0.48
Developmental (floral initiation)	25	0.17	0.50
	20	-	0.35
	15	-	0.46
	10	0.25	0.50
Uniform Weight (1 g dry shoot)	25	0.14	0.52
	20	0.10	0.40
	15	0.09	0.28
	10	0.06	0.26

still hold between tissue P concentration and growth rate, particularly for deficient plants.

Both relative growth rates and tissue P concentrations decreased with time and thus were positively correlated when data from several times were plotted together (Figure 48). The deficient plants (at 10 μMP/L) had lower growth rates and tissue P concentrations than the sufficient plants throughout the experiment. Despite this, when several times were plotted together, as in Figure 47, the deficient plants were not differentiated from the sufficient plants. Thus, if a % P versus growth rate relationship does hold, it may have to be considered within a time or developmental stage.

The relationships of yield and RGR versus tissue P concentrations for three-stage criteria (Figure 49) indicate the importance of the choice of stage. In each of these curves, the optimal P concentration probably lies between the 10 and 50 μMP treatments.

Thirty days after transplanting, very large differences in growth between temperatures were observed and the growth rate - % P relationships were unique to each temperature.

At floral initiation, large growth differences were evident but, in P-sufficient plants, a uniform RGR of about 0.18 g/day g occurred across all temperatures. Among the deficient plants there was a much reduced RGR. At 25°C, the RGR of the deficient plants was above that at 10°C and coincided with a lower tissue P concentration. This may imply a lower optimal P concentration at higher temperatures.

When the plants achieved 1 g dry weight, the relationship between growth rate and % P became similar across temperatures. At low tissue P concentrations (in deficient plants) the RGR increased very sharply from temperature to temperature, almost independently of the tissue % P. When

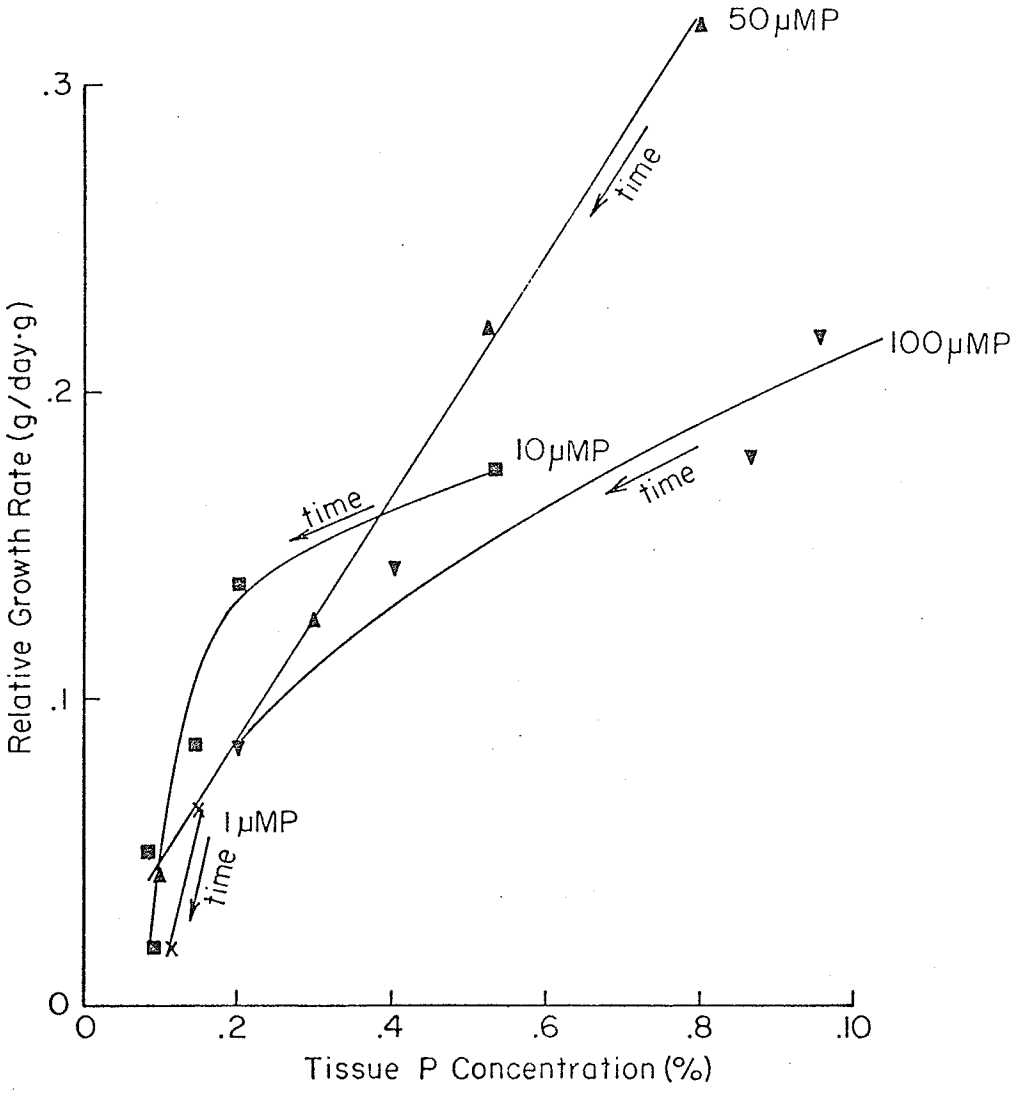


Figure 48: Shoot relative growth rate versus tissue P concentration from several dates at 25°C.

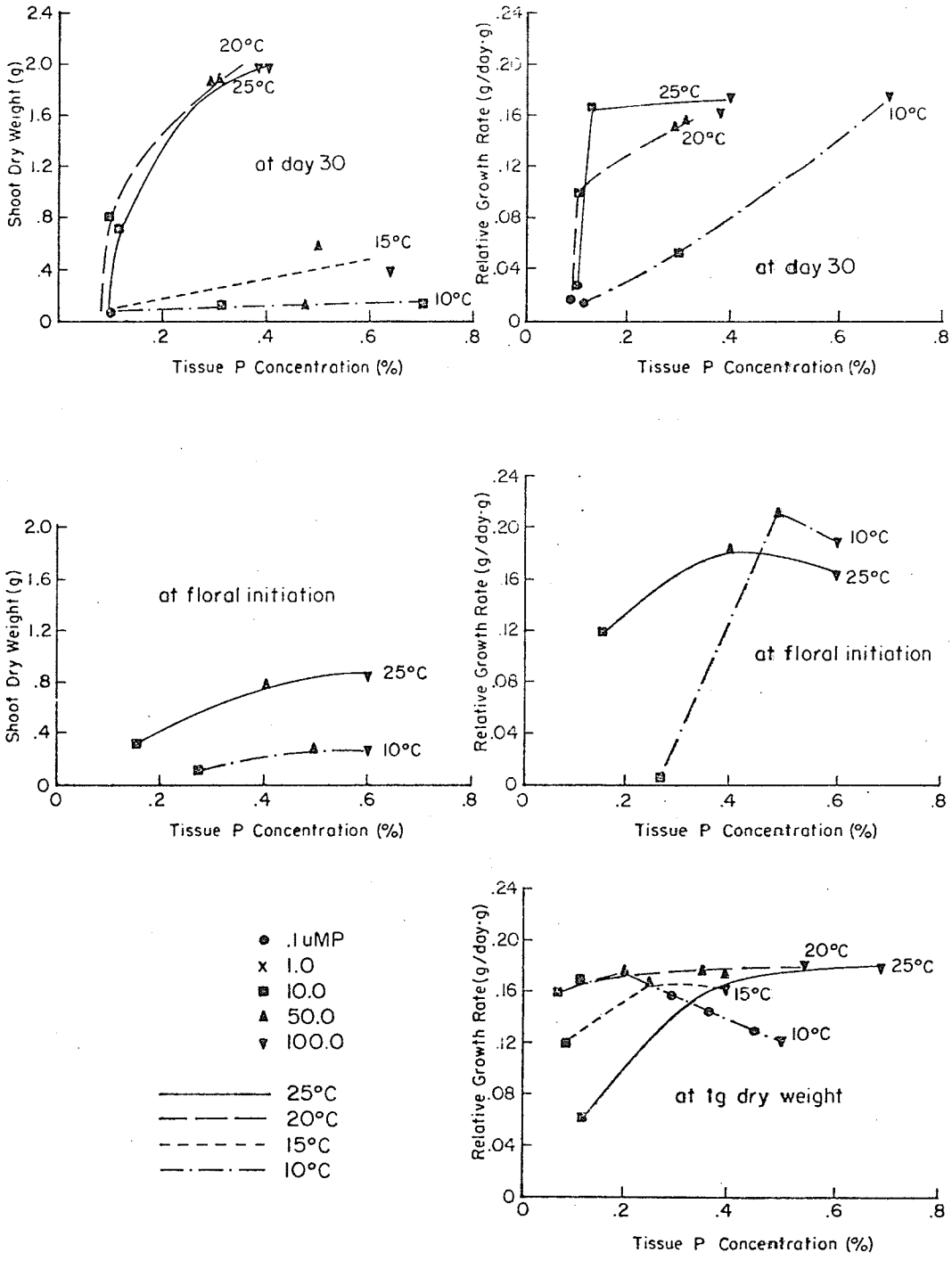


Figure 49: Shoot yield and relative growth rate in response to tissue P concentrations, plotted at three different stage criteria.

the tissue P concentration was adequate, the RGR changed very little and again was almost independent of the tissue % P. Because a constant plant dry weight was used, this curve could also be interpreted as an absolute growth rate versus total P uptake relationship.

Considerably different conclusions would be drawn from each of these three-stage criteria. The present data were not complete enough and fraught with too many design difficulties to pursue these conclusions.

Conclusions

The most significant finding of this experiment was the extent to which luxury consumption of P can occur. Very rapid early uptake of P caused depletion of the nutrient solutions and very high tissue P concentrations. This accumulation of P exceeded the demands imposed by immediate growth and probably served as a buffer to allow growth to continue despite depleted external P supplies. Further evidence from autoradiographs and from the continued development of plants supplied with virtually no P showed that translocation and reutilization of plant P could extend the resistance to P deficiency stress.

The implication of this finding was that the relationship between growth rate and tissue P concentration changed dramatically within a very short time. Therefore a response curve alone was not sufficient criteria to define the optimal P concentration because yield could not respond as quickly.

This study also showed that weight gain and physiological development were not necessarily related. Thus at 25°C, floral initials appeared when the plant shoots were over 1 g dry weight whereas at 10°C, the same physiological stage occurred at 0.15 g dry weight. Comparison of responses

across temperatures was complex due to this variation in plant development.

Finally, the shoot growth rates were unrelated to root temperature once the plants were in the grand phase of growth. It was suggested that leaf expansion was responsible for the observed shoot weight gains and that this was controlled by the uniform air temperature. This implied that root-mediated processes such as nutrient supply which would have been modified by root temperature were not limiting to shoot growth.

Several modifications in design and methods were indicated by this experiment.

1. The nutrient solutions required complete change on a regular basis.
2. Higher P supply levels and more levels in the marginally deficient range were required.
3. A second criteria, such as response to additional P, was necessary to accurately define which plants were deficient.
4. More data around discrete harvest criteria, probably physiological stage and dry matter accumulation, would have been beneficial.
5. Several changes in method including: a) individual solution culture bags for each plant to eliminate root tangling and improve treatment randomization, b) plastic film coating the foam rubber plugs to prevent roots and tiller-shoots from penetrating the foam.

Appendix K

MEANS OF DATA FOR CHAPTER 5

Plate 12: Apparatus used for the solution culture experiment (Chapter 5)

TABLE 53

Means of Plant Parameters Averaging 8 Solution P Concentrations and 2 Replicates

Temperature	'A" Plants		'B' Plants		'C' Plants	
(°C)	Leaf[1]	Weight[1]	Leaf	Weight	Leaf	Weight
			Shoot Dry Weight (g/plant)			
10	0.275	0.247	1.05	0.721	1.23	0.821
15	0.407	0.222	1.92	0.936	1.76	0.890
20	0.453	0.187	1.47	0.845	1.47	0.726
25	0.369	0.164	1.09	0.658	1.33	0.702
			Shoot/Total Fresh Weight Ratio			
10	0.398	0.386	0.376	0.382	0.424	0.413
15	0.354	0.365	0.335	0.355	0.384	0.421
20	0.331	0.322	0.326	0.326	0.362	0.396
25	0.356	0.369	0.329	0.339	0.396	0.400
			Shoot Dry Matter Content (dry/wet) Ratio			
10	0.171	0.173	0.186	0.177	0.180	0.164
15	0.174	0.162	0.193	0.171	0.157	0.148
20	0.157	0.162	0.169	0.162	0.158	0.143
25	0.150	0.152	0.156	0.152	0.140	0.134
			Relative Growth Rate (g/g.day)			
10	0.089	0.159	0.108	0.109	0.110	0.121
15	0.131	0.138	0.092	0.146	0.130	0.158
20	0.144	0.197	0.088	0.167	0.101	0.177
25	0.204	0.168	0.135	0.206	0.155	0.219
			Tissue P Concentration (%)			
10	0.535	0.446	0.338	0.432	0.857	1.005
15	0.407	0.533	0.243	0.499	0.605	1.081
20	0.601	0.503	0.372	0.457	0.677	0.877
25	0.616	0.656	0.328	0.561	0.686	0.890
			Total Shoot P Content (mg/plant)			
10	1.44	1.15	3.62	2.96	8.59	6.85
15	1.26	1.20	3.63	4.43	8.00	8.70
20	2.68	0.98	5.53	3.75	7.72	5.51
25	2.47	1.00	3.52	3.69	7.67	5.92

1. 6th leaf and 4-g fresh weight stages, respectively.

TABLE 54

Means of Parameters for 'A' Plants, Averaging 2 Replicates and 2 Harvest Stage Criteria

Temperature (°C)	Solution P Concentration (ppm)	Shoot Dry Weight (g/plant)	Shoot/Total Fresh Weight Ratio	Shoot Dry Matter Content Ratio	Relative Growth Rate (g/g day)	Tissue P Concentration (%)	Total Shoot P Content (mg/plant)
10	5	0.123	0.317	0.192	0.071	0.210	0.29
	10	0.249	0.343	0.197	0.081	0.266	0.58
	20	0.142	0.376	0.166	0.111	0.573	0.76
	30	0.335	0.393	0.173	0.155	0.476	1.41
	40	0.398	0.404	0.162	0.155	0.552	1.98
	50	0.166	0.436	0.154	0.173	0.625	1.02
	100	0.247	0.449	0.167	0.114	0.493	1.09
	200	0.404	0.423	0.154	0.154	0.752	3.12
15	5	0.143	0.290	0.213	0.057	0.262	0.23
	10	0.244	0.309	0.189	0.142	0.337	0.59
	20	0.289	0.284	0.189	0.056	0.425	0.64
	30	0.418	0.351	0.163	0.138	0.354	1.06
	40	0.464	0.397	0.153	0.160	0.613	2.32
	50	0.289	0.383	0.149	0.166	0.721	1.79
	100	0.371	0.408	0.148	0.192	0.322	0.61
	200	0.257	0.442	0.145	0.167	0.678	2.00
20	5	0.165	0.252	0.176	0.074	0.218	0.31
	10	0.182	0.276	0.170	0.141	0.368	0.58
	20	0.256	0.305	0.160	0.178	0.397	0.88
	30	0.432	0.386	0.153	0.195	0.567	1.90
	40	0.274	0.299	0.163	0.148	0.470	1.45
	50	0.507	0.352	0.156	0.214	0.771	3.47
	100	0.304	0.369	0.143	0.201	0.846	2.49
	200	0.519	0.378	0.149	0.214	0.842	4.19
25	5	0.160	0.316	0.172	0.058	0.350	0.42
	10	0.166	0.392	0.148	0.165	0.423	0.69
	20	0.242	0.338	0.147	0.186	0.704	2.13
	30	0.369	0.345	0.147	0.272	0.432	1.43
	40	0.409	0.345	0.147	0.282	0.582	2.38
	50	0.279	0.373	0.153	0.201	0.880	2.33
	100	0.338	0.420	0.150	0.186	0.803	2.76
	200	0.264	0.399	0.143	0.166	0.921	2.49

TABLE 55

Means of Parameters for 'B' Plants, Averaging 2 Replicates and 2 Harvest Stage Criteria

Temperature (°C)	Solution P Concentration (ppm)	Shoot Dry Weight (g/plant)	Shoot/Total Fresh Weight Ratio	Shoot Dry Matter Content Ratio	Relative Growth Rate (g/g day)	Tissue P Concentration (%)	Total Shoot P Content (mg/plant)
10	5	0.261	0.361	0.182	0.081	0.187	0.43
	10	0.472	0.359	0.199	0.094	0.189	0.85
	20	0.762	0.355	0.191	0.085	0.239	1.73
	30	1.115	0.392	0.190	0.119	0.241	1.66
	40	1.058	0.336	0.179	0.119	0.369	3.34
	50	1.329	0.383	0.181	0.096	0.380	4.16
	100	1.046	0.401	0.168	0.146	0.693	6.81
	200	1.009	0.444	0.162	0.132	0.771	7.21
15	5	0.320	0.263	0.199	0.083	0.148	0.56
	10	0.602	0.304	0.204	0.052	0.222	0.78
	20	1.058	0.318	0.196	0.094	0.293	1.38
	30	1.420	0.314	0.177	0.135	0.337	2.47
	40	1.868	0.362	0.183	0.109	0.372	3.27
	50	1.851	0.376	0.179	0.144	0.377	3.03
	100	1.944	0.388	0.174	0.139	0.409	4.54
	200	1.890	0.407	0.157	0.171	0.644	10.4
20	5	0.298	0.259	0.195	0.082	0.403	0.88
	10	0.648	0.253	0.187	0.054	0.481	3.21
	20	0.952	0.308	0.177	0.099	0.392	4.36
	30	1.177	0.323	0.170	0.135	0.269	2.98
	40	1.066	0.358	0.150	0.136	0.355	3.48
	50	1.600	0.303	0.166	0.172	0.209	3.34
	100	1.658	0.378	0.146	0.153	0.587	7.99
	200	1.847	0.406	0.140	0.163	0.625	10.7
25	5	0.416	0.258	0.182	0.100	0.149	0.59
	10	0.605	0.276	0.170	0.109	0.538	2.98
	20	0.679	0.291	0.154	0.161	0.235	1.62
	30	0.839	0.352	0.152	0.194	0.584	3.74
	40	0.982	0.337	0.144	0.213	0.437	3.77
	50	1.349	0.350	0.149	0.187	0.305	3.67
	100	1.154	0.412	0.141	0.230	0.720	7.43
	200	1.002	0.397	0.141	0.172	0.587	5.01

TABLE 56

Means of Parameters for 'C' Plants, Averaging 2 Replicates and 2 Harvest Stage Criteria

Temperature (°C)	Solution P Concentration (ppm)	Shoot Dry Weight (g/plant)	Shoot/Total Fresh Weight Ratio	Shoot Dry Matter Content Ratio	Relative Growth Rate (g/g day)	Tissue P Concentration (%)	Total Shoot P Content (mg/plant)
10	5	0.329	0.383	0.160	0.090	1.851	6.10
	10	0.345	0.374	0.158	0.123	1.376	4.54
	20	0.660	0.381	0.176	0.107	0.947	6.19
	30	0.850	0.412	0.169	0.115	0.867	7.36
	40	1.478	0.427	0.170	0.131	0.785	9.97
	50	1.049	0.445	0.176	0.102	0.774	8.47
	100	1.390	0.428	0.171	0.121	0.744	7.94
	200	1.565	0.465	0.183	0.131	0.573	8.97
15	5	0.295	0.427	0.142	0.106	1.829	5.57
	10	1.470	0.326	0.156	0.135	0.638	9.39
	20	1.207	0.413	0.153	0.162	0.956	9.83
	30	1.419	0.409	0.147	0.153	0.918	10.9
	40	0.958	0.372	0.160	0.134	0.514	7.46
	50	1.796	0.396	0.160	0.133	0.698	10.0
	100	2.024	0.389	0.161	0.155	0.416	5.79
	200	1.299	0.436	0.141	0.159	0.483	5.66
20	5	0.412	0.352	0.154	0.057	1.938	8.03
	10	0.723	0.407	0.147	0.140	0.902	5.12
	20	1.103	0.338	0.160	0.121	0.846	8.18
	30	1.115	0.401	0.152	0.161	0.761	7.68
	40	1.190	0.374	0.145	0.160	0.557	5.88
	50	1.422	0.361	0.146	0.143	0.572	7.89
	100	1.682	0.398	0.144	0.171	0.317	5.33
	200	1.074	0.387	0.160	0.132	0.588	5.47
25	5	0.488	0.376	0.137	0.143	1.084	5.56
	10	0.682	0.402	0.132	0.174	1.110	7.45
	20	0.905	0.377	0.136	0.200	0.835	7.03
	30	1.005	0.415	0.131	0.194	0.519	3.59
	40	1.343	0.406	0.143	0.184	0.654	8.68
	50	1.252	0.401	0.135	0.180	0.681	6.47
	100	1.370	0.389	0.141	0.212	0.767	9.19
	200	1.121	0.416	0.140	0.209	0.654	6.38