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I am submitting herewith a thesis written by Steven Duane Hardin entitled "Effect of Rates of Applied Phosphorus and Soil Storage Temperature on P availability of a Low-P Loess-Derived Alfisol". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

Donald D. Howard, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

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and Dean of The Graduate School

# Effect of Rates of Applied Phosphorus and Soil Storage Temperature on P Availability of a Low-P Loess-Derived Alfisol

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ABSTRACT

Overwintering soil temperature may influence crop response to phosphorus (P) and indices of P availability in the humid temperate transitional climate of Tennessee. The effects of P fertilization and soil incubation temperature upon sorghum-sudangrass (Sorghum bicolor x S.sudanese) grown on a Typic Hapludalf was investigated in two greenhouse experiments at Knoxville, Tennessee. Soils were incubated for 9 months at a constant temperature of 6°C or a diurnal fluctuation of 24 and 36°C. Reagent grade $Ca(H_2PO_4)_2 \cdot H_2O$ was used as the fertilizer source and applied at rates of: 0, 10, 20, and 30 mg P $kg^{-1}$ for the first test and 0, 20, 40, 60, and 80 mg P $kg^{-1}$ for the second test. Critical P concentration in the shoots for optimum yield was 1.25 $\mu g$ $g^{-1}$, corresponding to soil solution and labile P concentrations of 2.4 $\mu mol$ $L^{-1}$ and 90 $\mu g$ $g^{-1}$, respectively. Optimum yield occurred for applications of >40 mg P $kg^{-1}$ and was unaffected by soil incubation temperature. Phosphorus fertilization rates affected the amounts of P extracted by five chemical extractants(Bray I, Bray II, Mehlich I, Mehlich III, and Mississippi), but soil incubation temperature had no affect. These extractants however, were poorly correlated to plant P uptake and no one extractant was superior to the others as an indicator of P availability. Indices of P availability to plants in general were not affected by soil incubation temperature.

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# CHAPTER I

## INTRODUCTION

Phosphorus (P) is considered to be one of the essential elements for plant growth. Phosphorus is found in three separate pools in the soil, these being: 1) soil solution P which is readily available for plant uptake, 2) labile P which is held in rapid equilibrium with soil solution P, and 3) solid phase P which is held in forms unavailable for plant growth but is in equilibrium with labile P. Phosphorus availability is dependent upon the capability of the soil to maintain sufficient levels of P in the soil solution for plant uptake, which is, in turn, dependent upon such factors as: pH, cation exchange capacity, soil texture, and the ability of the soil to fix P.

The mineralization of organic P also plays an important role in P availability. The rate of organic P mineralization is affected by temperature, with soil organic P content being the highest during the winter months and the lowest during summer months (Sharpley, 1985). In unfertilized soils organic P content has been found to be significantly related to "available" P. However, "available" P has not been shown to be significantly related to organic P in fertilized soils (Sharpley, 1985).

There are several ways in which P availability can be assessed; these can be placed into two main categories: 1) intensive measurements and 2) quantitative measurements. Intensive measurements include both labile P and soil solution P. Quantitative measurements include the actual plant growth responses along with P removed by chemical extractants. "Critical" P levels for each one of these

measurements for optimum plant growth can be established through experimentation.

Research has been conducted to establish critical P levels for various crops (Dotzenka, et al. 1966; Asher and Longeragan, 1967; Soltanpour et al., 1974; Cope and Kirkland, 1975); however, these critical P levels may not be accurate when applied to different soils. Phosphorus fertilization research on crops (soybeans, corn, grain sorghum) grown on the loess-derived soils of West Tennessee has shown little yield response to P application. Several factors may be responsible for the effect: 1) forms of soil P may affect P availability, 2) mineralization of organic P may affect P availability, and 3) the Mehlich I extractant may not adequately measure P availability on these soils.

This research project was conducted to evaluate the effect of P fertilization and soil storage temperature on P availability of a low-P Alfisol using sorghum-sudangrass (Sorghum bicolor x S. sudanese) as an indicator crop. The null hypotheses (Ho) tested were:

1. Sorghum-sudangrass growth is not affected by different levels of P fertilization.
2. Soil P fractions are not affected by fertilization rates or soil storage temperature.
3. There is no correlation between sorghum-sudangrass growth and soil P fractions.

The objectives established for this research project were:

1. To determine the effects of P fertilization on sorghum-sudangrass grown on a Typic Hapludalf.

2. To determine soil solution P, extractable P, and organic P levels as affected by P fertilization rates and soil storage temperature.
3. To correlate sorghum-sudangrass growth and P uptake with soil solution P and extractable P.

# CHAPTER II

# LITERATURE REVIEW

## 1. FACTORS AFFECTING PHOSPHORUS AVAILABILITY TO PLANTS

The availability of phosphorus (P) for plant growth depends mainly on two factors: 1) the ability of a soil to maintain a sufficient P supply to the roots which depends on the level of P in soil solution, and 2) the ability of the soil solid phase to supply P to the solution (Schenk and Barber, 1979). These factors are in turn affected by: pH, cation exchange capacity, soil texture, and the ability of the soil to fix P. These factors need to be considered when determining plant P requirements.

Soil pH has a highly significant effect on P availability. Phosphorus forms insoluble Fe and Al compounds at low pH, while more soluble Ca and Mg compounds are formed at pH 6 and above (Bohn et al., 1985). Raising the pH on acid soils by liming is believed to increase P availability by inhibiting the absorption-precipitation reactions between P and Fe and Al. Liming also accelerates organic matter decomposition and thus, P mineralization (Adams and Odom, 1985).

Phosphorus fixation is a pH-dependent process which plays a major role in P availability. Fixation is a term used to collectively describe both adsorption and precipitation reactions of P. Fixation of P is high in acid soils having high $Fe^{3+}$ and $Al^{3+}$ activities. Fixation of P occurs as Ca phosphate in basic soils, where $Ca^{2+}$ activity is high (Bohn et al., 1985). Phosphorus availability is maximized at approximately pH 6.5 for most mineral soils.

Cation exchange capacity and texture also effect P availability. Fine-textured soils that have a high CEC will produce higher yields at lower soil test P levels than soils that are coarser-textured with a lower CEC (Lins et al., 1985). Therefore, P fertilization requirements maybe less on fine-textured soils having high CEC than on coarser-textured soils having a lower CEC.

## 2. PHOSPHORUS IN THE SOIL SOLID PHASE

Phosphorus from fertilizers and from indigenous soil sources reacts with soil constituents to create less soluble forms. Phosphorus thus removed is said to be retained or fixed (Tisdale et al., 1985). Phosphorus is fixed in acid soils in the form of Fe and Al phosphates, which may be present as forms of variscite ($AlPO_4 \cdot 2H_2O$) or strengite ($FePO_4 \cdot 2H_2O$) (Fried and Shapiro, 1960). In alkaline soils, P is fixed in the form of Ca phosphates (Cho and Caldwell, 1959).

Phosphorus is adsorbed on surfaces with two types of charges in the soil: 1) surfaces with a constant charge, such as the crystalline clay minerals and 2) variably charged surfaces including Fe and Al oxides and organic matter (Sample et al., 1980). Crystalline clay minerals form a constant charge as a result of occurrence $Ca^{2+}$ and $Al^{3+}$ close to the surface. Hydrated iron and aluminum oxides are the most important surfaces of variable charge in most soils except peats and highly calcareous soils. The variable charge reaction is highly dependent upon pH with the adsorption reaction increasing as pH decreases.

In addition to adsorption, precipitation of Ca, Al, and Fe phosphates is also important for P fixation. Precipitates of these various compounds form when their solubilities are exceeded. This is also a pH-dependent process with Ca-P precipitates forming in high pH soils where Ca concentrations are high. The same type of reaction will occur in low pH soils which also have high Al and Fe concentrations (Sample et al., 1980).

Organic matter also has a substantial effect on the amount of soil P, with organic P fractions ranging between 20 and 80% of the total P in most mineral soils (Mengel and Kirkby, 1982). Most naturally occurring forms of P are esters of orthophosphoric acid and numerous mono- and diesters have been characterized. The organic P esters have been grouped into five classes of compounds: inositol phosphates, nucleic acids, nucleotides, and sugar phosphates, with the first three being the dominant forms (Tisdale et al., 1985).

## 3. ASSESSMENT OF P AVAILABILITY

There are several ways to measure the availability of P for plant growth. These can be grouped into two general categories: 1) intensive measurements, and 2) quantitative measurements. Intensive measurements include both labile P and soil solution P which are the forms of P most readily available for plant growth. Quantitative measurements include the actual plant responses which can be measured in both greenhouse and field experiments along with chemical extractants which are used to estimate soil P availability.

## A. DETERMINATION OF SOIL SOLUTION P

One of the most accurate methods for assessment of plant available P is the determination of soil solution P. However, because of the low quantities of P found in soil solution, routine determination is usually very difficult. Several procedures have proven to be reliable, but these procedures are usually very lengthy.

One such procedure is the use of isobutyl alcohol to concentrate the phosphomolybdate complex into an aliquot which can be easily determined by colorimetric procedures (Watanabe and Olsen, 1962). This procedure has proven to be very accurate and reliable in determining soil solution P. An advantage to the isobutyl alcohol method is the elimination of most the interferences of other ions; however dissolved organic matter coloration in the aqueous phase may interfere with accurate determination of P (Watanabe and Olsen, 1962). A modification of this procedure was proposed by Helyar and Brown (1976), which uses 1-octanol in place of isobutyl alcohol. One octanol is a highly insoluble alcohol in water and enables the concentration of P from the aqueous phase to the organic phase, thus making possible the determination of P at low aqueous concentrations (Helyar and Brown, 1976). This procedure has been reported to have a detection limit of 0.1 $\mu$g $PO_4$ and can be used to accurately measure P at solution concentrations >0.01 $\mu$mol $L^{-1}$ (Helyar and Brown, 1976).

Other determinations of soil solution P have been conducted using larger quantities of soil solution (Mclean et al., 1983; Watanabe and Olsen, 1965). These methods proved to be quite accurate when sufficient amounts of P where present in soil solution. Watanabe and

Olsen (1965) showed that P determined by the absorbic acid method was essentially the same as those obtained by the isobutyl alcohol method. However, for very low P soils or where larger aliquots cannot be obtained these types of methods may not be as accurate.

B. DETERMINATION OF LABILE P

Labile P is the quantity of soil P in rapid equilibrium with soil solution P. The most commonly used method of measuring labile P is by isotopic dilution with the radioisotope $^{32}P$. This determination is based on the exchange reaction: $^{31}P$-surface + $^{32}P$-solution $\rightleftharpoons$ $^{32}P$-surface + $^{31}P$-solution, that occurs when the radioactive isotope $^{32}P$ is added to the soil solution system (Russell et al., 1954). The $^{31}P$-surface represents that portion of the soil P that is readily exchangeable with $^{32}P$. Labile P is equal to the isotopically exchangeable soil P plus P in the solution and the formula for determining labile P is labile P $= {}^{31}P \frac{\text{solution}}{f}$ where f is the fraction of $^{32}P$ remaining in solution after equilibrium has been achieved (Olsen and Sommers, 1982).

The primary difficulty with this procedure involves determining labile P in soils that have either a high P-fixation capacity or low solution P levels. In soils that have a high P-fixation capacity, some proportion of $^{32}P$ added to the soil can be fixed irreversibility and prevented from undergoing isotopic exchange with $^{31}P$ on the surface (Amer et al., 1969; Dahal and Hallsworth, 1977). As a result, the equilibrium is not achieved and the calculated labile P value will be an overestimate of the actual exchangeable P (Wolf et al., 1986). In soils where the solution P concentration is low, the difficulty in

making labile P measurements lies in the decreased accuracy for solution P measurements (Russell et al., 1954; Olsen and Sommers, 1982).

To overcome these difficulties, the addition of $^{31}P$ carrier with $^{32}P$ has been recommended (Russell et al., 1954; Olsen and Sommers, 1982). The addition of the carrier increases the solution P concentration allowing for a more accurate measurement and decreases $^{32}P$ fixation by the soil (Wolf et al., 1986).

A second method used for the determination of labile P is the use of an anion exchange resin. The strong base anion exchange resin will remove anions from solution over a wide pH range, can be added in neutral form, and is only slightly buffered over the pH range of interest in soils (Amer et al., 1955).

Research conducted by Wolf et al. (1986) showed that P measurements performed by resin extraction and isotopic dilution were comparable. This research, performed on medium to high P Alfisols and Mollisols, indicated that when difficulties are not encountered with the isotopic dilution technique, the isotopic and resin techniques will measure the same or nearly the same P pool. On soils with high P fixing capacity, comparisons of isotopic and resin P values showed that the similarity of results depended upon the amorphous Fe and Al content of the soils. These results demonstrate that for soils with high P fixation capacity the isotopic exchange technique should not be used to measure labile P. Instead the resin extraction, which appears to measure all or nearly all of the labile pool, should be used (Wolf et al., 1986).

## C. DETERMINATION OF SOIL TEST P

The selection of a chemical extractant for use in a soil test is dependent upon its ability to extract nutrients which correlate well with actual plant uptake. In the case of P, the extractant needs to estimate the water soluble P and portions of Ca, Al, and Fe phosphates. Several chemical extractants have been developed to evaluate plant-available P on both acid and calcareous soils. Five such extractants used on acid soils are: Bray I (0.03 M $NH_4F$ + 0.025 M HCl), Bray II (0.03 M $NH_4F$ + 0.01 M HCl), Mehlich I (0.0125 M $H_2SO_4$ + 0.05 M HCl), Mehlich III (0.025 M $NH_4NO_3$ + 0.2 M HOAc + 0.015 M $NH_4F$ + 0.01 M $HNO_3$ + 0.001 M EDTA), and the Mississippi method (0.05 M HCl + buffered acidic $AlF_3$ solution at pH 4.0).

The Bray I procedure has been shown to be well-correlated with yield response on most acid and neutral soils. It can also be used for soils which contain small amounts of dolomite or calcium carbonate (less than 2%). It is not however, recommended for soils containing large amounts of lime since P may be precipitated during the extraction process resulting in very low test values (Knudsen, 1980). The Bray I procedure extracts mainly Al-P from soils containing this inorganic P fraction, however, it will extract some P from the Fe-P fraction in the soil (Martens et al., 1969). The Bray I procedure has not been found to be well-correlated with labile P. Fluxes of phosphates in the soil such as additions through fertilizer applications and losses by plant uptake affect the amount of labile P. These changes in labile P were found not to be correlated with the changes in extractable P (Probert and Willet, 1983). Additional research has

shown that the degree of soil weathering affects the correlation between labile P and Bray I-extractable P. Sharpley et al. (1987) showed that in slightly weathered soils the Bray I procedure was a good indicator of labile P, with resin-extractable P accounting for 91% of the variability in the Bray I procedure. Bray I also gave the best indication for labile P in highly weathered soils (Sharpley et al., 1987).

The Bray II soil test was adopted in Missouri when rock phosphate was the major P source used in fertilization of low P soils. The higher acid concentration in the Bray II vs the Bray I extractant recovered a higher quantity of P from rock phosphate and was superior for interpreting fertilizer P requirements for soils previously fertilized with rock phosphate. Water-soluble P fertilizers are predominantly used today and consequently the Bray I extractant may be a more suitable method to measure available P (Aquino and Hanson, 1984). Aquino and Hanson (1984) conducted research to correlate cumulative P removal by consective harvest of grain sorghum with extractable P. They concluded that without knowing the previous P fertilizer materials applied the Bray II extractant appeared to show more promise than the Bray I extractant as a measure of labile P.

The Mehlich I extractant was developed for use in the Pedimont and Mountain regions of North Carolina on soils which strongly fix P. The mixed acids are more effective than HCl alone as a P extractant on these soils, and the results correlate better with plant response (Olsen and Sommers, 1982). Mehlich I extractable P seems to be more closely related to Ca-P than to Fe-P or Al-P, especially in soils that

have been fertilized with rock phosphate (Martens et al., 1969). The Mehlich I extractant does not correlate well to plant growth on neutral to alkaline soils where apatite is the predominant source of plant available P (Mehlich, 1984).

The Mehlich III method was also developed in North Carolina as a multi-element extractant to evaluate the status of P, K, Mg, Mn, Zn, and Cu (Mehlich, 1984). Mehlich (1984) found that this extractant is more effective at evaluating the "available" P status on neutral to alkaline soils than the Mehlich I extractant.

The Mississippi method was developed to be a multi-element extractant for the assessment of P, K, Mg, Ca, Zn and Mn. Since many investigators have shown Al-P to be highly correlated to available P this extractant was developed to extract mainly this form, while extracting only small amounts of Fe-P and Ca-P forms (J. D. Lancaster, 1980, personal communication). The main advantages of this method over the Bray I are: 1) the Ca-P fraction is extracted with greater intensity, especially in neutral and acid soils and 2) Al-P is extracted with greater and more uniform intensity in alkaline soils. The quantity of Ca-P extracted is considered to be minimal but can be increased or decreased, respectively, by increasing or decreasing the concentration of HCl in the first stage of extraction or by the addition of malic or malonic acids (J. D. Lancaster, 1980, personal communication). Mississippi has adopted this method because of its ability to extract reasonable amounts of P along with the extraction of reliable amounts of K, Ca, Mg, Mn and Zn.

Research has been conducted to correlate various P extractants to plant growth factors. The Bray I and Mehlich I procedures have been shown to be weakly correlated to P uptake. Mehlich (1978) showed that the Mehlich I (r=88) and Bray I (r=83) were correlated to plant P uptake by millet grown on six different Ultisols in North Carolina. Along with being correlated to plant uptake these extractants plus the Mehlich III extractant have been shown to be related to P application rates. Research conducted by Alley and Bertsch (1983) on two sandy loam soils in Virginia indicated a linear increase in extractable P with broadcast P applications resulting in $R^2$ values of 0.97, 0.98, 0.82 for the Bray I, Mehlich I and Mehlich III extractants, respectively. Although the Mehlich III method extracted considerably more P than did the Bray I or Mehlich I, the critical soil test level as determined by this extractant was also higher. Therefore, none of these extractants seemed to be superior over the others for these types of soils (Alley and Bertsch, 1983).

The Bray I and Mehlich III chemical extractants have also been shown to be highly correlated ($R^2$=0.97), with both extracting similar quantities of P (Wolf and Baker, 1985). Conflicting results, however, have been reported. The Mehlich III extractant was reported to extract and average of 66% more P than the Bray I extractant for volcanic ash soils and 12% more for loess soils in Alaska (Michaelson et al., 1987). Mehlich (1984) reported that the Mehlich III test extracted 3 to 4% more P than did the Bray I for 105 soils from the Southern and mid-Atlantic states; however, the amount of P extracted by these two extractants was highly correlated (r=0.96). The Mehlich

I and Bray I extractants have also been shown to be weakly correlated ($R^2$=0.68) (Wolf and Baker, 1985). Even though these tests differ in the amount of P extracted, any one of them can be used reliably on acid soils for calibrating soil test data with plant uptake.

## D. USE OF PLANT ANALYSIS TO ASSESS P AVAILABILITY

The use of plants to measure P availability is the most reliable assessment method primarily because: 1) the plant is the ultimate measure of truly available P, and 2) it will indicate the availability regardless of soil type (Kamprath and Watson, 1980). To utilize a chemical extractant, the objective is to evaluate the "available" soil P so that an amount of fertilizer P can be recommended which will increase the existing available level to one which is sufficient for near maximum yields (Mclean et al., 1979; Mclean et al., 1983). Plant yields will increase to a maximum then level off with increased P fertilization rates, while concentration of P in plants will increase markedly without leveling off (Mclean et al., 1983). Mclean et al. (1983) reported a linear relationship for plant response components (yield, concentration of P, or uptake of P) when regressed on available P as estimated by various chemical extraction methods. Phosphorus concentration and P uptake were better regressed on extractable P than was yield. Similar results where found by Sutton et al. (1983) where maximum wheat yields where obtained when P was supplied to the plant up to the first node stage, with no significant increase in mature root and top yields associated with additional weeks of P uptake. With the developing roots and tops continuing P uptake beyond the first node stage there were no increases in mature

dry matter content with increasing P concentrations in the plants.

Plant analysis can be used to evaluate the critical level of P for plant growth. As P availability is increased, plant growth rate and P nutrient content also increases until the critical level is obtained (Mengel and Kirby, 1982). This level has been defined as the elemental concentration at 90% of the maximum yield (Marten and Matocha, 1973).

Critical P values in the shoot have been established for many crops. Research conducted by Melsted et al. (1969) established critical P levels for various crops, two of these being corn (0.25% P) and wheat (0.30% P). Comparable critical P values have been established for various forage grasses such as tall fescue (0.26 to 0.30%), bluegrass (0.24 to 0.30%), and orchardgrass (0.20 to 0.24%) (Martin and Matocha, 1973). Lower critical P values have been established for millet and sorghum-sudangrass with values of 0.16 to 0.20% and 0.14 to 0.18%, respectively (Dotzenka et al., 1966).

The determination of critical P values is very important in the determination of actual P availability. Once the critical level has been established it is possible to determine other parameters such as soil solution P, labile P, or soil test P levels which are necessary to obtain the critical P level. This will allow for accurate fertilizer recommendation to be made to obtain optimum yields.

## E. MINERALIZATION OF ORGANIC P

Organic P also plays an important role in plant nutrition. The rate of organic P mineralization is affected by temperature, with soil organic P content being the highest during the winter months and

lowest during summer months (Sharpley, 1985). This increase in organic P content during the fall and winter months results, in part, from the addition of fresh organic residues. In the warm, moist spring and summer months, organic P can be removed from the surface soil by plant uptake (Sharpley, 1985; Dormaar, 1972;).

In unfertilized soils the available P content has been found to be significantly related to the organic P content. However, available P is not significantly related to organic P in fertilized soils (Sharpley, 1985). Cultivation and P fertilization have also been observed to increase total amounts of inorganic P and decrease organic P in surface horizons (Sharpley and Smith, 1983).

## 4. JUSTIFICATION OF RESEARCH

Phosphorus fertilization research on crops (soybeans, corn, grain sorghum) grown on the loess-derived soils of West Tennessee has shown little yield response to P application. Several factors may be responsible for the effect: 1) forms of soil P may affect P availability, 2) mineralization of organic P may affect P availability, and 3) the Mehlich I extractant may not adequately measure P availability on these soils. Therefore, consideration of P fertilization along with overwintering soil temperature effects on available P may provide information as to why crops have shown little response to applied P. This research was conducted on a Memphis silt loam (Typic Hapludalf), which is representative of many West Tennessee soils, to evaluate rates of P application and soil storage temperature (a model of overwintering soil temperature) on P availability as determined by

plant response and various soil indices of "available" P.

# CHAPTER III

## MATERIALS AND METHODS

A cultivated Memphis silt loam (Typic Hapludalf, fine silty mixed thermic) surface soil testing <8 $\mu g\ g^{-1}$ in Mehlich I extractable phosphorus (P) was sampled at Ames Plantation, Grand Junction, Tennessee in the fall of 1986. The sample was collected from the surface 15 cm, sieved field moist to pass a 3.18 mm screen, uniformly mixed then split into two lots and placed in air-tight plastic containers. One lot of soil was maintained in a cooler at 6°C, and the other lot was placed in a greenhouse where the average daily minimum and maximum temperatures were 24°C and 36°C, respectively. Soils were incubated for nine months at these two temperature regimes until greenhouse experiments were initiated.

Incubation treatments (low temperature and high temperature) were characterized for total and organic P by the ignition method (Olsen and Sommers, 1982) using the ascorbic acid colorimetric procedure (Olsen and Sommers, 1982) modified to use 1 to 20 $\mu g$ of P in a total volume of 12 mL. The incubated soils were additionally characterized for extractable nutrients (Ca, Mg, and K) via the Mehlich I procedure (Johnson, 1984). Initial soil pH was measured with a combination electrode in a 1:1 soil to water mixture.

Exchangeable bases were determined for pH 7 1M $NH_4OA_c$ extracts and extractable Al by extraction with 1M KCl. CEC was determined by ammonia saturation and steam distillation (Soil Conservation Service Staff, U.S.D.A., 1982).

## 1. GREENHOUSE EXPERIMENT

A greenhouse experiment was conducted in the summer of 1987 at Knoxville to investigate the effect of phosphorus (P) fertilization rates and soil incubation (low temperature vs high temperature) on sorghum-sudangrass (Sorghum bicolor x S. sudanese) growth. The experimental design consisted of a split-plot arrangement of a randomized complete block having four replications with P fertilization levels as the main effect and soil incubation as the subplot. The experiment was repeated in time with varied levels of P fertilization.

Reagent grade $Ca(H_2PO_4)_2 \cdot H_2O$ was used as the P source and applied at rates of: 0, 10, 20, and 30 mg P $kg^{-1}$ for the first test and 0, 20, 40, 60, and 80 mg P $kg^{-1}$ for the second test. Phosphorus treatments were applied to field moist soil samples, weighing 4.5 kg each, which were thinly spread on a table top and the appropriate amount of $Ca(H_2PO_4)2 \cdot H_2O$ based on oven dry soil bases was added and mixed. Each treated sample was then wetted to field capacity (220 g $kg^{-1}$) mixed, and four subsamples each weighing 1 kg were placed in 15-cm diameter pots, covered with plastic and allowed to incubate for 3 days. Six seeds of Dekalb SX-17 sorghum-sudangrass hybrid were sown in each pot at a depth of 1 cm. Pots were placed in an unshaded greenhouse where the mean daily maximum and minimum temperatures were 36°C and 22°C, respectively for the first test and 31°C and 16°C for the second test. Five days after the number of plants were thinned to four plants per pot.

Seven days and 21 days after planting the first test, pots were fertilized with solutions of reagent grade $NH_4NO_3$ and $KNO_3$ to provide 67.5 mg N $kg^{-1}$ and 50 mg K $kg^{-1}$ to all pots. Solutions of reagent grade $NH_4NO_3$ and $KNO_3$ were applied weekly to provide a total of 67.5 mg N $kg^{-1}$ and 50 mg K $kg^{-1}$ in the second test. All pots were watered as needed with deionized water to maintain the moisture content at field capacity.

Plants were harvested 25 days after planting. Shoots were cut at the soil surface, placed in paper bags, and oven dried for 24 hours at 60°C. Roots were separated from the soil by hand, washed in 0.1M HCl, triple rinsed with deionized water, blotted dry, placed in paper bags, and oven dried at 60°C. The entire soil sample from each pot was placed in a plastic bag and refrigerated for future analysis.

Dry weights were recorded for both shoots and roots. Samples were ground to pass 1-mm openings in a cyclone mill. Ground samples were wet-oxidized using the tertiary acid procedure (Johnson and Ulrich, 1959) in which 0.5 g samples were first digested overnight with $HNO_3$ and next digested with a 10:3:1 mixture of $HNO_3$-$HClO_4$-$H_2SO_4$. Phosphorus was determined colorimetrically by the molybdovandate procedure (Olsen and Sommers, 1982) using a Technicon auto-analyzer. Calcium, Mg, and K were determined by atomic absorption spectroscopy of La-buffered solutions.

## 2. SOIL SOLUTION COMPOSITION

Six subsamples of soil solutions were obtained for each level of P fertilization by vacuum displacement (Wolt and Graveel, 1986) and

analyzed for pH and electrical conductivity. Samples were then composited and analyzed for P and total concentrations of Al, Ca, Mg, K, Na, NO3, Cl, and $SO_4$. A Ross combination electrode (Orion Research, Inc. Boston, MA) was used to measure pH, and EC was measured with a conductance meter utilizing a 3 mL sample cell with a 1.0 $cm^{-1}$ cell constant. Phosphorus was determined colorimetrically by the stannous chloride method using 1 octanol partitioning of the phosphomolybdic acid complex (Helyar and Brown, 1976) and a Hitachi 100-20 spectrophotometer with a 2-cm pathlength cell. Total Al was determined by the 8-hydroxyquinoline complexion and butyl acetate partitioning (James et. al., 1983). Calcium and Mg were determined by atomic absorption spectroscopy of La-buffered solutions. Potassium and Na were determined by flame emission spectroscopy. Nitrate, Cl, and $SO_4$ were determined by ion chromatography using a Dionex 2120 ion chromatograph. Soil solution composition data were entered into a chemical equilibrium model for determination of ion activities and the ion activity products for selected mineral species (Wolt, 1987).

## 3. LABILE P AND EXTRACTABLE P

Labile soil P was determined by the anion exchange resin method (Olsen and Sommers, 1982). Extractable P was determined using five different chemical extractants: Bray I (0.025M HCl + 0.03M $NH_4F$; Olsen and Sommers, 1982), Bray II (0.03M $NH_4F$ + 0.1M HCl; Johnson, 1984), Mehlich I (0.05M HCl + 0.0125M $H_2SO_4$; Olsen and Sommers, 1982), Mehlich III (0.2M HOAc + 0.25M $NH_4NO_3$ + 0.015M $NH_4F$ + 0.013M $HNO_3$ + 0.001M EDTA; Johnson, 1984), and Mississippi method (0.05M HCl,

buffered acidic $AlF_3$ solution at pH 4.0; Lancaster, 1980). Both labile and extractable P were determined by the previously described ascorbic acid procedure (Olsen and Sommers, 1982). Concentrations were determined using a Hitachi 100-20 spectrophotometer with a 2-cm pathlength cell.

## 4. STATISTICAL ANALYSIS

An analysis of variance utilizing the GLM procedure of the Statistical Analysis System (SAS) was used to determine the significance of incubation temperature and P rates on plant growth factors, soil solution P and extractable P. Data was initially analyzed across tests as an incomplete block in time. When significant effects of tests or the interactions of tests with main and subplot effects were observed at the 5% level, data was reanalyzed by test. When P rate differences were significant at the 5% level, treatment means were separated using the Waller-Ducan k-ratio (k=100).

# CHAPTER IV

# RESULTS AND DISCUSSION

Analysis of plant responses to applied P rates and soil incubation temperature for data combined across tests showed significant differences between the two tests. Therefore, plant responses where reanalyzed by test.

## 1. TEST 1

There was a >3-fold increase in shoot dry weight as rates of applied P were increased from 0 to 30 mg P $kg^{-1}$(Table 1). Shoot dry weight increased 37% as rates of applied P were increased from 20 to 30 mg P $kg^{-1}$ indicating that plant P requirements were not optimized at the highest rate of P. Shoot dry weight increased with each increment of applied P. Soil incubation temperature did not affect shoot dry weight, nor was there a significant interaction between rate of applied P and soil incubation temperature.

Increases in root dry weight with increased P fertilization differed for the two incubation temperatures as indicated by the applied P rate by soil incubation temperature interaction (Table 1). Root weight for the high incubation temperature, was increased with P additions up to 20 mg P $kg^{-1}$, where as root dry weight, for the low incubation temperature, increased with only the initial 10 mg P $kg^{-1}$ increment and was unaffected by higher P rates.

Phosphorus uptake in the shoot was not affected by P rate, incubation temperature, or the interaction between the two. Uptake of P in the root increased with increased P fertilization and differed

Table 1. Treatment means for dry weight and P uptake of sorghum-sudangrass as affected by rate of applied P and soil incubation temperature, for test 1.

| Effect | Dry Weight | | | P Uptake | | |
|---|---|---|---|---|---|---|
| | Shoot | Root | | Shoot | Root | |
| | g pot$^{-1}$ | | | µg pot$^{-1}$ | | |
| | | High | Low | | High | Low |
| P Rate (mg kg$^{-1}$) | | | | | | |
| 0 | 0.73d† | 0.96c | 1.26b | 0.6 | 0.6d | 0.9b |
| 10 | 1.21c | 1.78b | 2.78a | 0.6 | 1.2c | 1.8a |
| 20 | 1.63b | 2.98a | 2.48a | 1.2 | 1.8b | 1.8a |
| 30 | 2.23a | 3.42a | 2.78a | 1.6 | 2.5a | 2.5a |
| Incubation Temperature | | | | | | |
| High | 1.49 | 2.29 | | 1.0 | 1.5 | |
| Low | 1.41 | 2.34 | | 1.1 | 1.6 | |
| Significance | NS‡ | NS | | NS | NS | |
| PRate*Inc.Temp. | NS | ** | | NS | ** | |

† Means in a column within main treatments followed by the same letter are not significantly different (k ratio = 100).

‡ Effects significant at the 1.0(**) % level or not significant (NS).

for the two soil incubation temperatures as indicated by the applied P rate by soil incubation temperature interaction. Root P uptake from high incubation temperature soil increased with each increment of added P. As with root dry weight, only the 10 mg P $kg^{-1}$ increment of P affected P uptake by the roots in the low incubation temperature soil even though the trend in root P uptake was similar to that for the high incubation temperature treatment. The relatively greater P uptake and yield of roots relative to shoots for any increment of applied P would tend to indicate that plant P requirement had not been attained in this test.

Concentration of P in the shoots is also an important measure of plant response to applied P. Critical levels of P in leaf tissue have been established for many plants and are good indicators of P availability in the soil. Phosphorus concentrations in the shoot in test 1 (Appendix C) were below the critical P level of 0.14 to 0.18% P reported by Dotzenka et al. (1966) for sorghum-sudangrass growth. This confirms that plant P requirements were not satisfied in test 1 and higher rates of applied P were needed in test 2 if critical levels were to be attained.

## 2. TEST 2

Both plant yields and P uptake by the shoots and roots were considerably lower in test 2 than in test 1 (compare Table 2 with 1), probably because low night temperatures limited the magnitude of the observed P response. Shoot dry weight was affected by applied P rate, soil incubation temperature and their interaction (Table 2). Shoot

Table 2. Treatment means for dry weight and P uptake of sorghum-sudangrass as affected by rate of applied P and soil incubation temperature for test 2.

| Effect | Dry Weight | | | P Uptake | | |
|---|---|---|---|---|---|---|
| | Shoot | | Root | Shoot | | Root |
| | g pot$^{-1}$ | | | μg pot$^{-1}$ | | |
| | High | Low | | High | Low | |
| P Rate (mg kg$^{-1}$) | | | | | | |
| 0 | 0.17d† | 0.18b | 0.38 | 0.2c | 0.2c | 0.3b |
| 20 | 0.23c | 0.20b | 0.51 | 0.2c | 0.2c | 0.4b |
| 40 | 0.32b | 0.21b | 0.56 | 0.6b | 0.3bc | 0.6a |
| 60 | 0.38a | 0.23b | 0.63 | 1.0a | 0.4ab | 0.6a |
| 80 | 0.34ab | 0.28a | 0.56 | 0.8ab | 0.5a | 0.6a |
| Incubation Temperature | | | | | | |
| High | 0.29 | | 0.53 | 0.6 | | 0.5 |
| Low | 0.22 | | 0.52 | 0.3 | | 0.5 |
| Significance | *‡ | | NS | *** | | NS |
| PRate*Inc.Temp. | ** | | NS | * | | NS |

† Means in a column within main treatments followed by the same letter are not significantly different (k ratio=100).

‡ Effects significant at the 0.1(***), 1.0(**), or 5.0(*) % level or not significant (NS).

weight was significantly increased by applied P rates up to 60 mg P $kg^{-1}$ when incubated at the high temperature, a higher P rate (80 mg $kg^{-1}$) was required to significantly increase shoot dry weight when incubated at the low temperature. High incubation temperature resulted in higher shoot dry weight when compared with low incubation temperature soil (0.29 and 0.22 g $pot^{-1}$, respectively). The fact that shoot dry weight was not increased with P fertilization rates above 60 mg P $kg^{-1}$ at high incubation temperature tends to indicate that plant P requirements were met in test 2.

Phosphorus uptake by the shoot was affected by P rates, soil incubation temperature, and their interaction. Uptake of P was significantly increased by applied P rates up to 60 mg P $kg^{-1}$ for both incubation temperatures. Phosphorus uptake by the shoot was affected by soil incubation temperature, with greater P uptake occurring in the high incubation temperature soil. Although, shoot uptake was affected by incubation temperature, P uptake indicates that plant P needs were achieved, at least in high incubation temperature soils.

Root dry weights were unaffected by applied P rates and soil incubation temperature. Root P uptake was increased by P fertilization up to 40 mg P $kg^{-1}$. The lack of dry weight differences due to applied P rates may be have been caused by the low night temperatures and the fact that P rates were higher in test 2 than test 1, allowing more P to be available for shoot growth. Root P uptake also helps to confirm that plant P requirements were satisfied in this test.

Plant response to applied P as affected by soil incubation temperatures in test 2 and the interactive effects of applied P rates

by soil incubation temperature in both tests is not clear. It may be due to the different rates of applied P used in each test and the interaction may be specific to certain rates of P. In the first test the interaction was observed in the shoot dry weights under the lower P rates. However, in the second experiment the interaction was observed with root dry weight, therefore the higher rates of P may have had an affect, causing the interaction to occur in the roots.

These interactions may also involve the nature of the labile P pool in the unamended soil and the effect of temperature regime during the time of plant growth on P availability. Under conditions of suboptimal P fertilization (<40 mg P $kg^{-1}$) and high temperatures during the period of plant growth (test 1), soils stored at a constant low temperature resulted in greater root dry weight and root uptake of P than soils stored under a high temperature regime in which temperature fluctuated diurnally. This seems to indicate greater residual P availability from the low temperature soil but this was not evident in organic P content (132 $\mu g$ P $g^{-1}$ and 131 $\mu g$ P $g^{-1}$ for high and low incubation temperature, respectively). However, P cycling under high temperature incubation may have resulted in P fixation in more stable organic forms without affecting the total organic P pool as determined by the ignition method. The ignition method does not differentiate between P held in organic matter and that held in the microbial biomass which may also effect P availability (Brookes et al., 1984). Under conditions of greater P availability and lower temperatures for plant growth (Test 2), low incubation temperature soils tended to supply less P to plants (as evidenced by low shoot yields and shoot P

uptake in comparison to high incubation temperature soil). The soil incubated at the low temperature may have contained less stable organic matter which when decomposed microbially resulted in P incorporation into the microbial biomass at the expense of plant growth. This effect may have been intensified by the limited plant growth occurring in test 2.

### 3. RELATIONSHIP OF RELATIVE YIELD TO P CONCENTRATION IN THE SHOOT

Due to differing environmental conditions between the two tests, dry weights and P uptake are not directly comparable. In test 1 the average daily maximum temperature of 36°C was above the optimum of 25 to 30°C (Fribroug,1985) for sorghum-sudangrass growth. Average daily minimum temperature of 16°C during test 2 was very close to the minimum temperature of 15°C (Fribourg,1985) for sorghum-sudangrass growth. Therefore, optimal environmental conditions were not present during either test. Because of these differing environmental conditions relative yields may better express the plant response to applied P allowing for comparisons between tests.

Relative yields (average mean percentages of the maximum yield for each test) are plotted against percent P in the shoot in Figure 1. By using the partitioning method developed by Cate and Nelson (1971) a clear break point between deficient and sufficient shoot P can be seen. The critical shoot P level for sorghum-sudangrass growth determined from this graph is 0.125% P (1.25 mg P $g^{-1}$), which is very

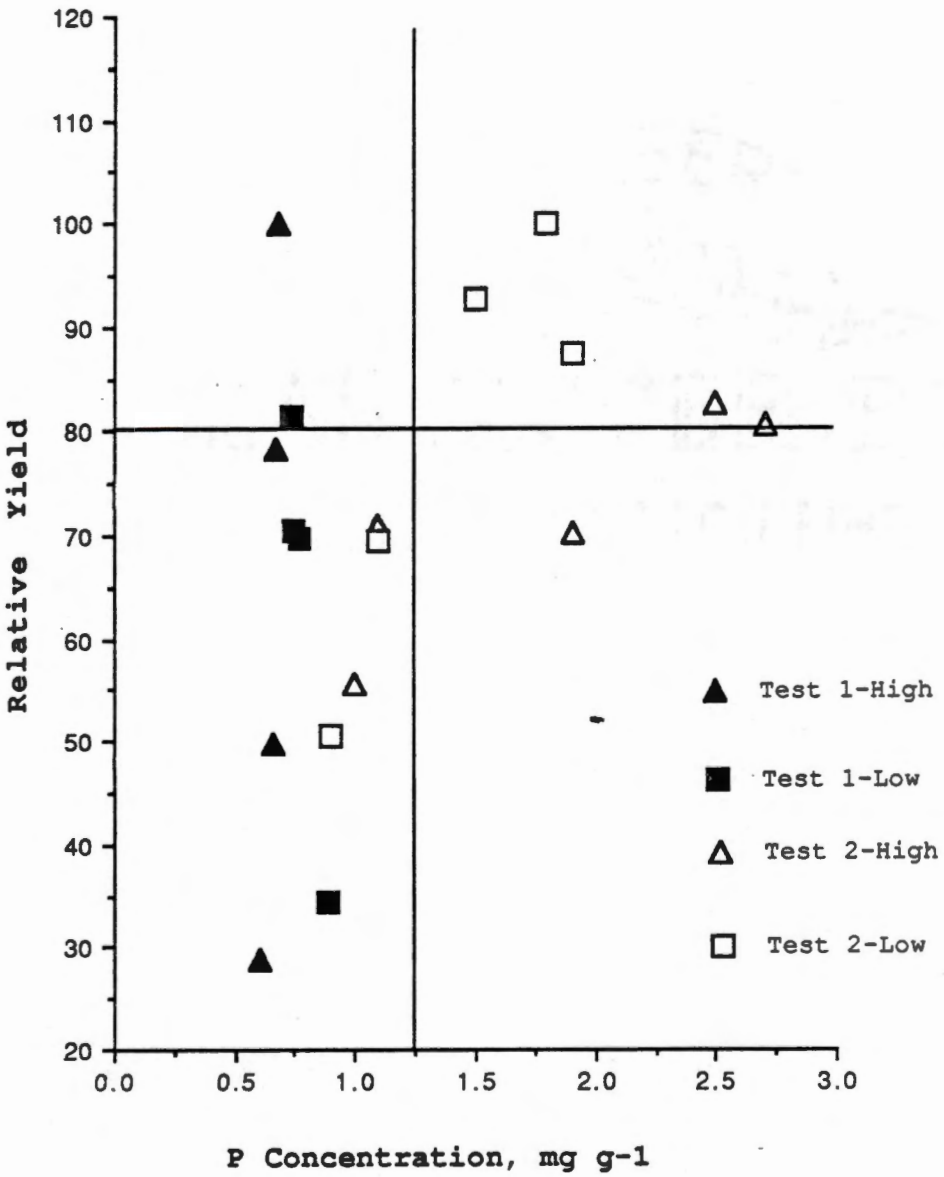



Figure 1. Quadrant partitioning of relative sorghum-sudangrass yield versus P concentration in the shoot (Critical P = 1.25 mg $g^{-1}$).

close to the critical level for forage sorghum of 0.14 to 0.18% P reported by Dotzenka et al. (1966). Despite the temperature extremes under which the greenhouse experiment was conducted, the agreement of critical P level with literature values indicates that physiological response of the test crop was not adversely affected by temperature.

## 4. RELATIONSHIP OF RELATIVE YIELD TO FORMS OF SOIL P

Relative yield can also be plotted against forms of soil P such as labile P or soil solution P to estimate critical levels of these P forms necessary for optimum growth. When relative yield is plotted against labile P, as determined by the resin method, a highly significant ($R^2$=0.995) quadratic response is observed (Fig.2), with maximum yield occurring at 140 $\mu$g P $g^{-1}$ of resin-extractable P. The critical level is reported to occur at 90% of the maximum yield (Martens and Motcha,1983) or 90 $\mu$g P $g^{-1}$ in this instance. This is considerably higher than the critical level of 50 $\mu$g P $g^{-1}$ reported by Soltanpour et al. (1974) for sorghum-sudangrass as determined by isotopic dilution with $P^{32}$. A quadratic response ($R^2$=0.780) is also observed when soil solution P is plotted against relative yield (Fig. 3), with maximum yield occurring at 4.5 $\mu$mol P $L^{-1}$ and the critical level at approximately 2.4 $\mu$mol P $L^{-1}$. This soil solution concentration is lower than the value reported by Soltanpour et al.(1974) of 22 $\mu$mol P $L^{-1}$ for maximum sorghum-sudangrass growth. However, Asher and Loneragan (1967) reported solution P levels ranging between 5 to 25 $\mu$mol $L^{-1}$ for maximum growth of several plant species grown in flowing nutrient solutions. The lower critical threshold of soil solution P

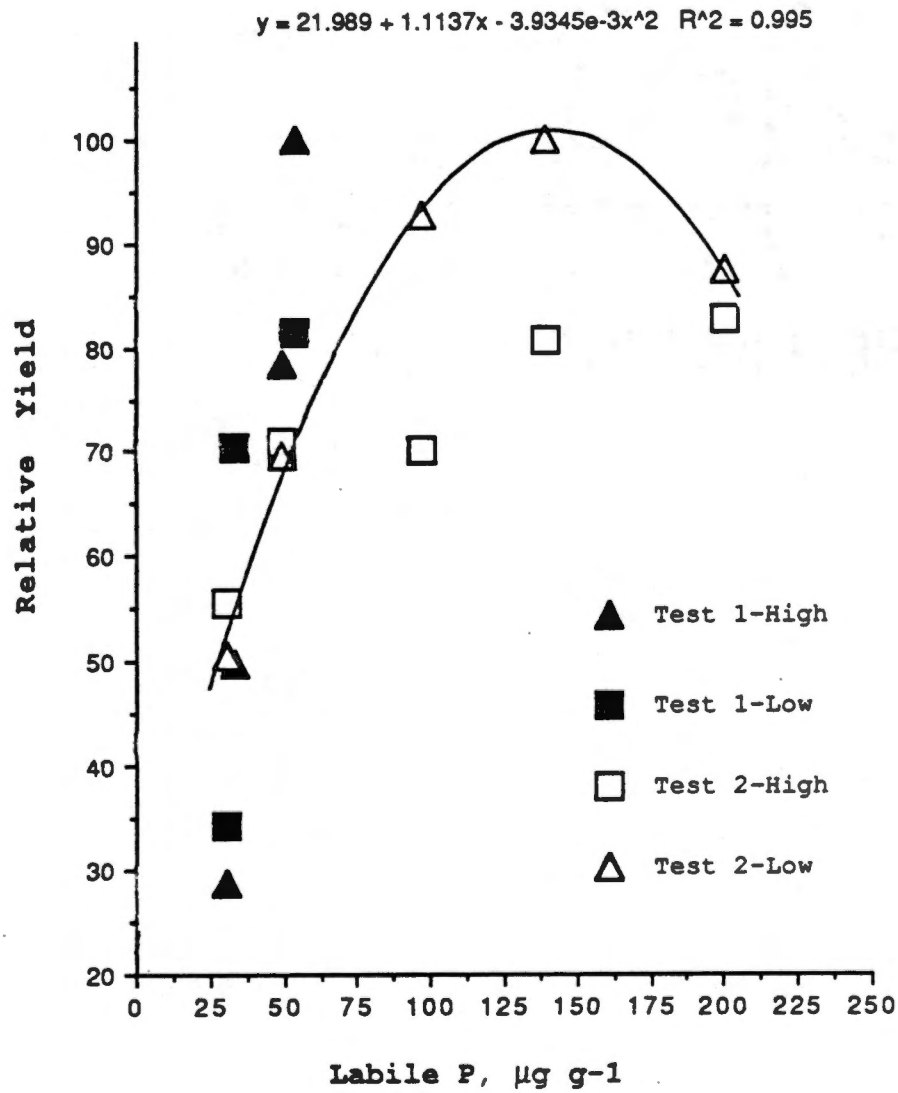



Figure 2. Relative sorghum-sudangrass yield as a function of resin extractable P (labile P).

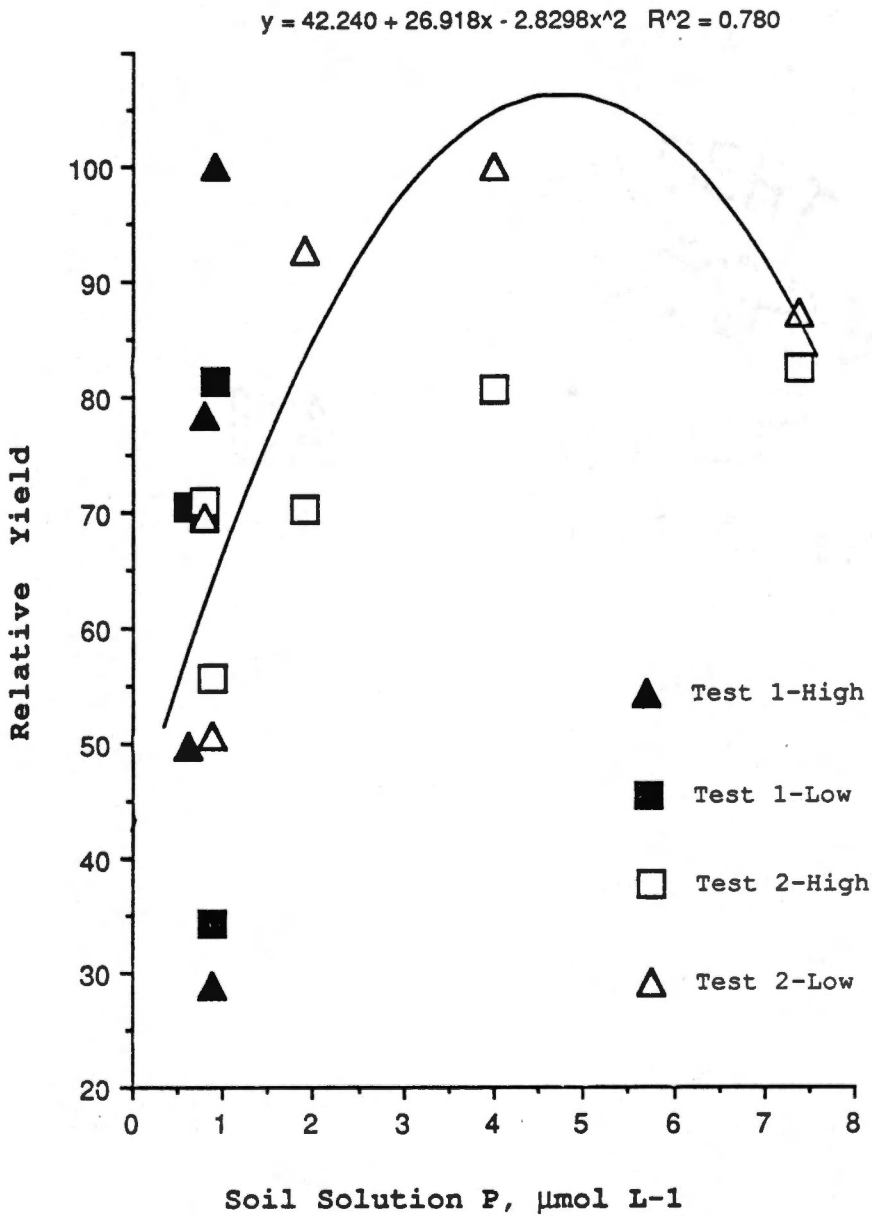



Figure 3. Relative sorghum-sudangrass yield as a function of soil solution P.

for optimum crop response observed here may explain why P differences are seldom reported on these soils despite their relatively low P status. Such an observation, however, must be tempered by the some what weak response which was modeled and the difficulties inherent in the detection of these low levels of P in soil solution. Fertilizer recommendations can also be made by plotting relative yield against applied P rates as shown in Figure 4. A quadratic equation depicts the relationship between relative yields and applied P ($R^2$=0.96), with maximum yield occurring at 60 mg P $kg^{-1}$ and the critical level at 40 mg P $kg^{-1}$ (90 Kg P $ha^{-1}$). This critical level was considerably higher than the current rate of 60 kg P $ha^{-1}$ (26 mg P $kg^{-1}$) recommended for summer annual grasses in the southeast (Cope and Kirland,1975; Dr. John Jared,1988,personal communication). This difference is not unexpected since nutrient demands in greenhouse environments are frequently higher than for field environments.

## 5. USE OF SOIL TESTS TO ASSESS P AVAILABILITY

Main effect for soil P chemical extractants, resin P, and soil solution P are shown in Table 3. The Mississippi method was the only extractant to show a significant affect of test. Extractable P as determined by the Bray I, Bray II, Mehlich I and Mehlich III methods increased with each increment of applied P. The Mississippi method varied similarly with applied P, but the data indicated that this method was less sensitive at low rates of P addition than the other methods of P extraction. The Bray

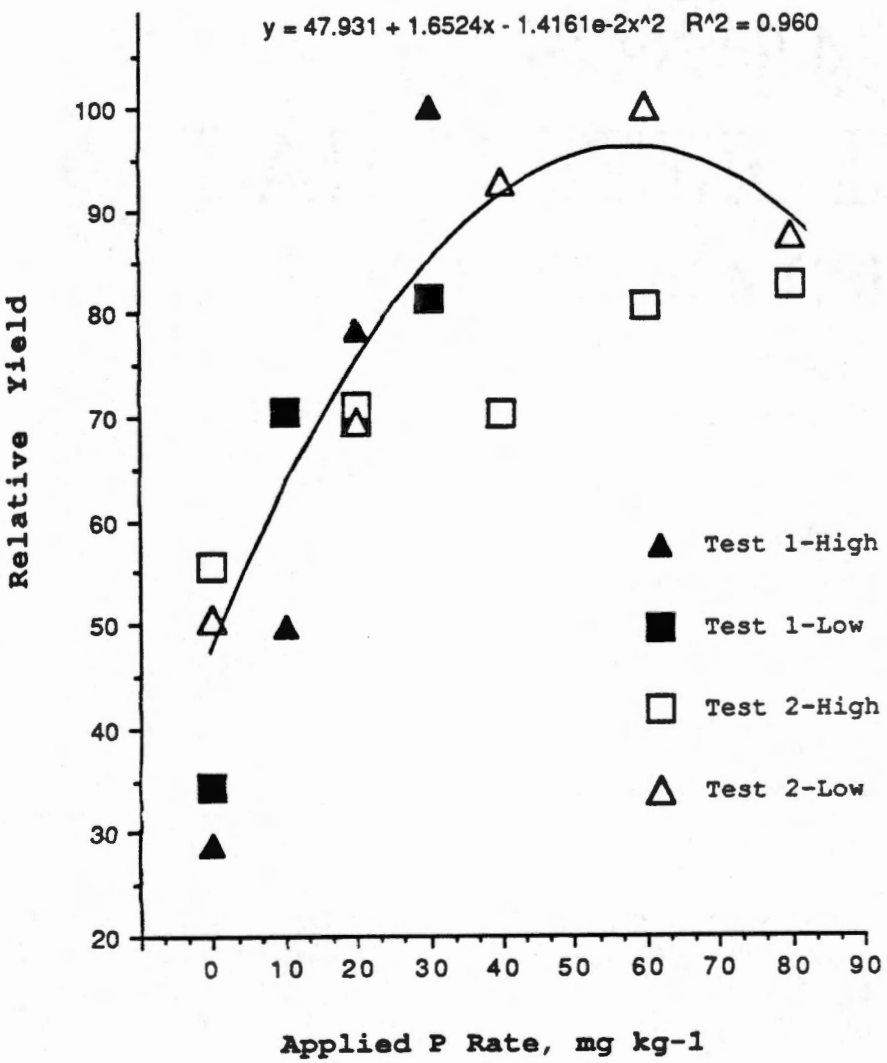



Figure 4. Relative sorghum sudangrass yield as a function of applied P rate.

Table 3. Treatment means for soil P extractants, resin P, and soil solution P as affected by test, rate of applied P, and soil incubation temperature.

| | Bray I | Bray II | Mehlich I | Mehlich III | Mississippi | Resin P | Solution P |
|---|---|---|---|---|---|---|---|
| Effect | ---------- | ---------- | ---------- | $\mu g\ g^{-1}$ | ---------- | ---------- | $\mu mol\ L^{-1}$ |
| Exp | | | | | | | |
| 1 | 6.9 | 11.9 | 8.5 | 10.7 | 24.9 | 39.9 | -- |
| 2 | 15.1 | 23.6 | 13.9 | 20.3 | 33.5 | 104.7 | -- |
| Significance | NS† | NS | NS | NS | ** | NS | -- |
| P Rate ($mg\ kg^{-1}$) | | | | | | | |
| 0 | 4.7g‡ | 9.3g | 5.9g | 7.6g | 16.4e | 30.3e | 0.9cd |
| 10 | 5.9f | 10.4f | 7.6f | 9.4f | 22.3d | 33.3e | 0.6d |
| 20 | 7.9e | 13.5e | 9.0e | 11.6e | 23.8d | 49.2d | 0.8cd |
| 30 | 9.6d | 15.4d | 10.9d | 13.8d | 31.3c | 53.7d | 0.9cd |
| 40 | 14.0c | 21.9c | 14.0c | 18.1c | 31.3c | 96.9c | 1.9c |
| 60 | 20.3b | 30.3b | 18.2b | 27.2b | 43.3b | 139.3b | 4.0b |
| 80 | 28.5a | 42.9a | 23.3a | 37.3a | 57.8a | 200.8a | 7.4a |
| Incubation Temperature | | | | | | | |
| High | 11.8 | 18.6 | 11.5 | 11.3 | 29.8 | 74.6 | 2.2 |
| Low | 11.2 | 18.2 | 11.6 | 15.8 | 29.5 | 77.3 | 2.6 |
| Significance | * | NS | NS | ** | NS | NS | NS |

† Effects significant at the 1.0(**) % level or not significant (NS).
‡ Means in a column within subplot treatments followed by the same letter are not significantly different (k ratio = 100)

I and Mehlich III extractants were affected by soil incubation temperature, with the Bray I method extracting <1 $\mu g\ g^{-1}$ more P from the high incubation soil and the Mehlich III method extracting >4 $\mu g\ g^{-1}$ more P from the low incubation soil. Although soil incubation temperature affected Bray I extractable P, the difference was of little diagnostic usefulness. The difference between incubation temperature was greater with the Mehlich III extractant perhaps indicating that forms of soil P in the low incubation soil where more readily extracted by the Mehlich III method.

The Bray I, Bray II, Mehlich I, and Mehlich III were closely related to labile P as determined by the resin method, yielding $R^2$ values of 0.93, 0.93, 0.94, and 0.93, respectively. The Mississippi method was not as closely related to labile P ($R^2$=0.85). Although the Bray I, Bray II, Mehlich I, and Mehlich III methods extracted varying amounts of "available P", they seem to be equally responsive to rates of P fertilization and are closely related to labile P. The Mississippi method was not as responsive to P fertilization and was not as closely related to labile P.

## 6. LABILE P AND SOIL SOLUTION P

Labile P as determined by the resin method was not affected by test 1 vs test 2 (Table 3). There was a significant increase in labile P with P fertilization, however, the data indicates that this method was less sensitive at lower applied P rates (<40 mg P $kg^{-1}$) than were most of the soil test extractants. Incubation temperature did not affect labile P determination.

Since soil solution P was only determined at the higher P rates (40 to 80 mg P $kg^{-1}$) in the second test, soil solution P was analyzed as a split plot design with applied P rates as the main effect and soil incubation temperature as the subplot (Table 3). Soil solution P was increased by applied P, however the data indicates that it was less sensitive to lower P rates (<40 mg P $kg^{-1}$). This was because at these rates soil solution P was at or below the detection limit for the analytical technique employed. Incubation temperature had no affect on soil solution P.

A phosphorus mineral stability diagram (Figure 5) was constructed from the ion activities calculated from soil solution compositional data (Appendix D) using an ion speciation model (Wolt,1987). Variscite ($AlPO_4$ $2H_2O$) and hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$) were assumed to be the mineral phases responsible for equilibrium P distribution in soil solution. Points which fall on either the variscite or hydroxyapatite stability line are said to be at equilibrium with respect to that solid phase component. Points falling below the line are understaturated with respect to solid phase minerals and points lying above the line are supersaturated and would be expected to precipitate from solution. Low P rates are represented by those points which lie closest to the variscite line, points farther from the variscite line are for higher rates of applied P (>30 mg P $kg^{-1}$) where soil solution P is a consequence of the dissolution of $Ca(H_2PO_4)_2 \cdot H_2O$ applied as the P fertilzer source. Variscite appears to control soil solution P in the soil recieving lower levels of applied P.

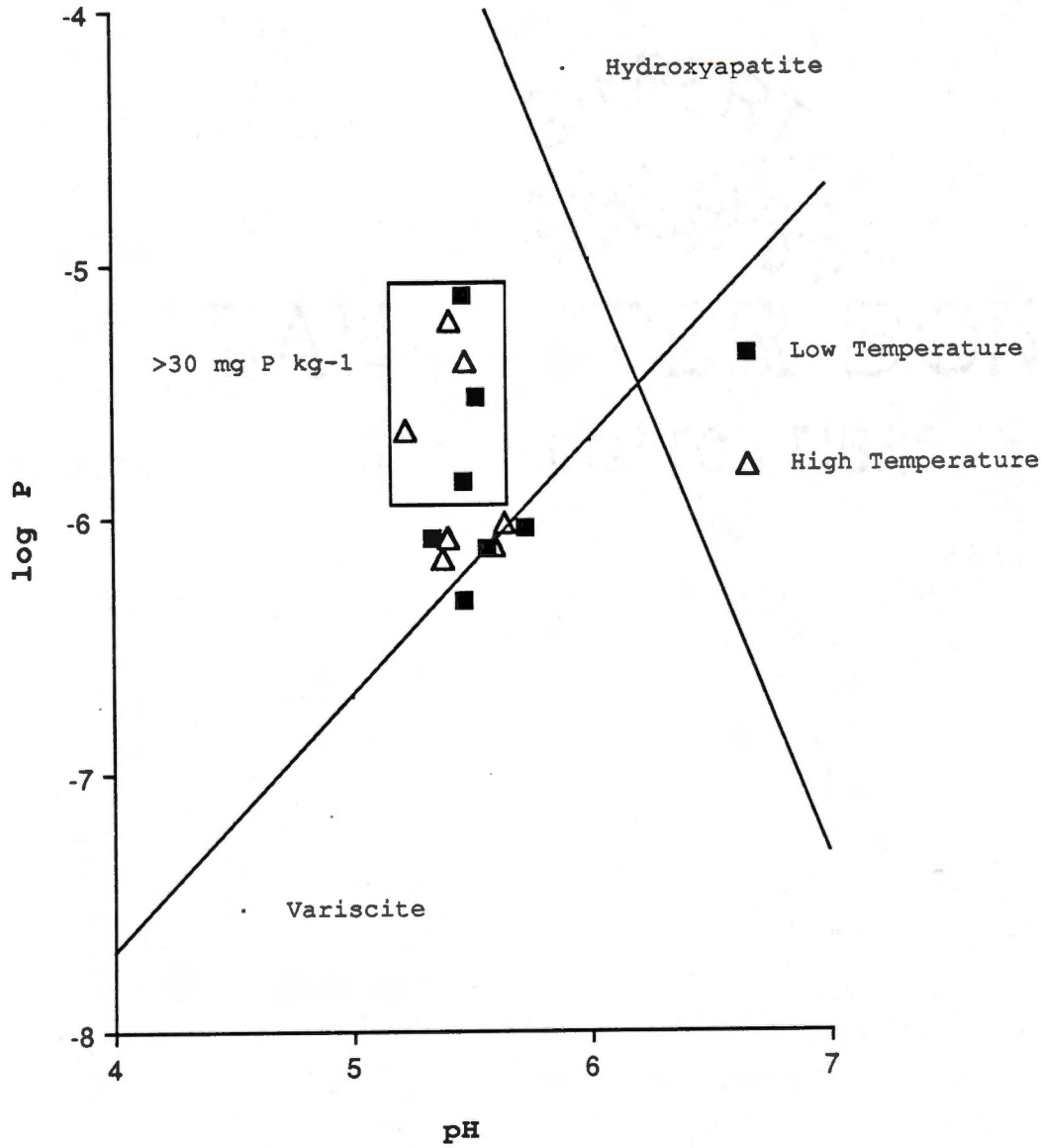



Figure 5. Soil solution P as compared to variscite and hydroxyapatite, where log P = log $[(H_2PO_4^-)+(HPO_4^{2-})]$ (after Lindsay, 1979).

## 7. REGRESSION OF CHEMICAL EXTRACTANTS ON P UPTAKE

Regression of the shoot and root P uptake on the various soil extractants showed differences between tests and soil incubation temperatures (Tables 4 and 5). These differences were probably due to different responses relating to both soil incubation temperature and temperature during plant growth. The highest regression was seen in test 1 for regression of root P uptake at high incubation temperature where $R^2$ values ranged from 0.83 to 0.91. However, the other combinations of incubation temperature and P uptake were poorly regressed on these extractants, with the extractants only accounting for approximately 50 to 70% of the variation in shoot P uptake and 30 to 40% of the variation in root P uptake. Therefore, regression of P uptake on these extractants shows that no one extractant stands out from the rest as an indicator of plant "available" P. In order for any one of these extractants to be used reliably to assess P availability on this soil, their relationship to crop response must be better correlated.

Table 4. Regression of root and shoot P uptake on extractable P by soil incubation temperature for test 1.

| Effect | Bray I | Bray II | Mehlich I | Mehlich III | Mississippi |
|---|---|---|---|---|---|
| | | | $R^2$ | | |
| | | | High Temperature | | |
| Shoot | 0.66 | 0.52 | 0.56 | 0.62 | 0.74 |
| Root | 0.83 | 0.83 | 0.91 | 0.86 | 0.89 |
| | | | Low Temperature | | |
| Shoot | 0.50 | 0.35 | 0.56 | 0.65 | 0.54 |
| Root | 0.35 | 0.36 | 0.34 | 0.50 | 0.39 |

Table 5. Regression of root and shoot P uptake on extractable P by soil incubation temperature for test 2 .

| Effect | Bray I | Bray II | Mehlich I | Mehlich III | Mississippi |
|---|---|---|---|---|---|
| | | | $R^2$ | | |
| | | | High Temperature | | |
| Shoot | 0.54 | 0.56 | 0.63 | 0.55 | 0.60 |
| Root | 0.30 | 0.36 | 0.39 | 0.37 | 0.35 |
| | | | Low Temperature | | |
| Shoot | 0.65 | 0.67 | 0.65 | 0.66 | 0.60 |
| Root | 0.31 | 0.31 | 0.31 | 0.27 | 0.33 |

# CHAPTER V

## SUMMARY AND CONCLUSIONS

A greenhouse experiment was conducted to investigate the effect of phosphorus (P) fertilization rates and soil incubation temperature (low vs high temperature) on sorghum-sudangrass (Sorghum sudangrass x S. sudanese) growth. A soil sample was collected from the top 15 cm of soil, uniformly mixed and split into two lots. One lot was stored in a cooler at a constant temperature of 6°C, and the other was placed in a greenhouse where the temperature fluctuated diurnally (24 and 36°C). Soils where incubated at these two temperature regimes for nine months.

Reagent grade $Ca(H_2PO_4)_2 \cdot H_2O$ was used as the P fertilization source and applied at rates of: 0, 10, 20 and 30 mg P $kg^{-1}$ for the first test and 0, 20, 40, 60 and 80 mg P $kg^{-1}$ for the second test. Factors used to evaluate the effects of P fertilization rates and soil incubation temperature were: 1) yield response to applied P rates and soil incubation temperature, 2) efficiency of P uptake by plants, and 3) soil test results using five chemical extractants along with labile and soil solution P. The following conclusions were drawn from the results of this study:

1. Soil incubation temperature had little effect on plant dry weights, P uptake, extractable P, or organic P.
2. Plant and soil indices of P availability were affected by rates of applied P.

3. Relative yields indicate that maximum yield for both tests occurred at 60 mg P $kg^{-1}$ with the critical level at 40 mg P $kg^{-1}$.

4. Relative yields indicate a critical level of 90 $\mu g$ P $g^{-1}$ for labile P and 2.4 $\mu mol$ P $L^{-1}$ for soil solution P and critical plant P of 1.25 mg $g^{-1}$ to obtain maximum yield on this soil.

Additional variables evaluated were the effect of P fertilization rates and soil incubation temperature on levels of P extracted by Bray I, Bray II, Mehlich I, Mehlich III, and Mississippi chemical P extractants along with resin extractable P and soil solution P. Phosphorus fertilization rates affected levels of P extracted by all the chemical extractants but soil incubation temperature did not. Although, these methods extracted varying amounts of P, all of them except the Mississippi method seemed to be equally responsive to rates of P fertilization. These extractants were poorly regressed on root and shoot P uptake and no one extractant stands out from the rest as an indicator of P availability. Labile P as determined by the resin method was affected by P application rates, however it was less sensitive at lower P rates (<40 mg P $kg^{-1}$). Soil solution P was also affected by P rates but it to was less sensitive to lower P rates (<40 mg P $kg^{-1}$), in part, because of to the analytical difficulty in determining soil solution P at low levels. Neither labile P or soil solution P was affected by soil incubation temperature.

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## LITERATURE CITED


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# APPENDICES

APPENDIX A

1 Octanol Extraction for Determination

of P in Soil Solutions

Reagents and Materials:

1. Molybdate Solution. -- Dissolve 40g of $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ in 500 mL water and add 400 mL of 5 m $H_2SO_4$ and dilute to 1 liter.
2. Stannous Chloride Solution. -- Dissolve 10g $SnCl_2 \cdot 2H_2O$ in 25 mL conc. HCl. Store in a refrigerator. Dilute 0.5 ml to 200 mL with 1 M $H_2SO_4$ just before use.
3. Standard Phosphate Solution. -- Prepare 1 liter of a 50$\mu$g P $mL^{-1}$ solution containing 0.2197 g of $KH_2PO_4$. Prepare working phosphate solutions from this stock with the desired concentration of phosphate.
4. 1 - Octanol -- Organic reagent grade.
5. Spectrophotometer -- double beam, UV-VIS.

Procedure:

1. Pipette an aliquot of soil solution containing between 1 and 5 $\mu$g $PO_4$-P into a 125 mL separatory funnel and dilute to 50 mL with deionized water.
2. Add 25 mL of molybdate reagent, shake briefly, and let stand for 5 min.
3. Add precisely 5 mL of 1-octanol and shake vigorously for 3 minutes. Let the mixture separate long enough to ensure

that sufficient octanol is recovered for use in the spectrophotometer before discarding aqueous phase.

4. Add 15 mL of dilute stannous chloride reagent and shake for 1 minute; allow mixture to separate and discard aqueous phase. Add an additional 15 mL of $SnCl_2$. Shake for 1 minute and discard aqueous phase.
5. Collect 1-octanol in a centrifuge tube and centrifuge at a relative centrifugal force of 3000 x gravity for 30 seconds to clarify any emulsion which may have developed.
6. Read the absorbance at a wavelength of 730 nm using a 2-cm pathlength cell.

Notes:

1. The practice of standard additions may need to be used to determine P at low concentrations.
2. The sample may need to be centrifuged for a longer period of time to clarify emulsion.

Reference:

Helyar, K. R. and A. L. Brown. 1976. Octan-I-ol extraction of molybdophosphoric acid in colorimetric determination of ortho-phosphate. Soil Sci. Soc. Am. J. 40:43-46.

## APPENDIX B

### Anion Resin - Extractable Phosphorus

Reagent and Materials:

1. Anion exchange resin, strong base (e.g., Dowex 2), particle size > 20 mesh and < 50 mesh, Cl saturated.
2. Sodium chloride (NaCl) solution, 10%.

Procedure:

1. Weigh 1g of 100-mesh soil and 1g of resin into a 250-mL Erlenmeyer flask. Add 10 mL of distilled water. Shake the contents for 16 hours.
2. Separate the resin from the soil by washing the soil through a screen that retains only the resin.
3. Transfer resin from the screen into a 50-mL beaker with 25 mL of 10% NaCl solution. Place beaker on a steam plate for 45 minutes.
4. Cool the mixture. Transfer the solution to a 100-mL volumetric flask. Leach the resin with additional 10% NaCl solution until 100 mL of filtrate is obtained.
5. Determine P concentration of filtrate by the ascorbic acid method (Olsen and Sommer, 1982) modified to use a total volume of 12 mL.

Notes:

1. Use 3-5 mL aliquots of deionized water to separate resin from the soil.

reagent using double beam spectrophotometer, UV-VIS, with a 2-cm pathlength cell.

Reference:

Olsen, R. S., and L. E. Sommers. 1982. Phosphorus In A. L. Page et al (eds.). Methods of Soil Analysis Part 2 2nd edition. Agronomy 9:4033-446.

APPENDIX C

Table C-1. Treatment means for percent P in the shoot and root and total P uptake for test 1.

| | Shoot | | Root | | Total P Uptake | |
|---|---|---|---|---|---|---|
| | % P concentration | | | | $\mu g\ pot^{-1}$ | |
| Effect | High | Low | High | Low | High | Low |
| P Rate (mg $kg^{-1}$) | | | | | | |
| 0 | 0.060a† | 0.089a | 0.064a | 0.072a | 1.0d | 1.6c |
| 10 | 0.066a | 0.075ab | 0.069a | 0.064a | 2.0c | 2.8bc |
| 20 | 0.067a | 0.077ab | 0.062a | 0.072a | 2.9b | 3.0ab |
| 30 | 0.068a | 0.073b | 0.074a | 0.072a | 4.2a | 3.4a |
| Incubation Temperature | | | | | | |
| High | 0.065 | | 0.067 | | 2.6 | |
| Low | 0.078 | | 0.070 | | 2.7 | |
| Significance | ***‡ | | NS | | NS | |
| P Rate*Temp. | ** | | * | | * | |

† Means in a column within main treatments followed by the same letter are not significantly different (k ratio=100).

‡ Effects significant at the 0.1(***), 1.0(***), or 5.0(*) % level or not significant (NS).

Table C-2. Treatment means for percent P in the shoot and root and total P uptake for test 2.

| | Shoot | Root | Total P Uptake | |
|---|---|---|---|---|
| | ----% P concentration---- | | $\mu g\ pot^{-1}$ | |
| Effect | Shoot | Root | High | Low |
| P Rate ($mg\ kg^{-1}$) | | | | |
| 0 | 0.099d† | 0.089a | 0.5c | 0.5c |
| 20 | 0.111cd | 0.089a | 0.7c | 0.7c |
| 40 | 0.165bc | 0.113a | 1.1b | 0.9b |
| 60 | 0.224a | 0.103a | 1.6a | 0.9b |
| 80 | 0.218ab | 0.111a | 1.5ab | 1.2a |
| Incubation Temperature | | | | |
| High | 0.183 | 0.100 | 1.0 | |
| Low | 0.145 | 0.102 | 0.8 | |
| Significance | *‡ | NS | *** | |
| P Rate*Temp. | NS | NS | ** | |

† Means in a column within main treatments followed by the same letter are not significantly different (k ratio=100).
‡ Effects significant at the 0.1(***), 1.0(**), or 5.0(*) % level or not significant (NS).

Table C-3. Regression of percent P in the shoots and roots and total P uptake on extractable P by soil incubation temperature for test 1.

| Effect | Bray I | Bray II | Melhich I | Mehlich III | Mississippi |
|---|---|---|---|---|---|
| | | | $R^2$ | | |
| | | | High Temperature | | |
| Shoot | 0.19 | 0.07 | 0.13 | 0.12 | 0.27* |
| Root | 0.23 | 0.20 | 0.18 | 0.15 | 0.05 |
| Total | 0.85***† | 0.77*** | 0.84*** | 0.84*** | 0.92*** |
| | | | Low Temperature | | |
| Shoot | 0.29* | 0.37* | 0.24 | 0.30* | 0.20 |
| Root | 0.10 | 0.02 | 0.14 | 0.04 | 0.01 |
| Total | 0.49** | 0.44** | 0.52** | 0.68*** | 0.55*** |

† Effects significant at the 0.1(***), 1.0(**), or 5(*)% level.

Table C-4. Regression of to percent P in the shoots and roots and total P uptake on extractable P by soil incubation temperature for test 2.

| Effect | Bray I | Bray II | Melhich I | Mehlich III | Mississippi |
|---|---|---|---|---|---|
| | | | $R^2$ | | |
| | | | High Temperature | | |
| Shoot | 0.53***† | 0.55*** | 0.61*** | 0.55*** | 0.59*** |
| Root | 0.17 | 0.20 | 0.18 | 0.18 | 0.16 |
| Total | 0.56*** | 0.62*** | 0.68*** | 0.62*** | 0.65*** |
| | | | Low Temperature | | |
| Shoot | 0.46*** | 0.46*** | 0.46*** | 0.47*** | 0.40** |
| Root | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 |
| Total | 0.72*** | 0.74*** | 0.73*** | 0.69*** | 0.72*** |

† Effects significant at the 0.1(***), 1.0(**), or 5(*)% level.

## APPENDIX D

Table D-1. Soil solution composition data by P fertilization rate for high incubation temperature soil.

| P Rate | pH | EC | P | Ca | Al | $NO_3$ | Cl | $SO_4$ | Mg | K | Na |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mg $kg^{-1}$ | | | | | | mmol $L^{-1}$ | | | | | |
| 0 | 5.41 | 2.71 | 0.0009 | 8.95 | 0.554 | 27.47 | 0.467 | 0.068 | 3.01 | 0.407 | 0.767 |
| 10 | 5.38 | 2.43 | 0.0007 | 8.15 | 0.456 | 26.20 | 0.246 | 0.139 | 2.67 | 0.253 | 0.670 |
| 20 | 5.60 | 1.14 | 0.0008 | 3.48 | 0.475 | 12.58 | 0.359 | 0.092 | 1.37 | 0.135 | 0.520 |
| 30 | 5.64 | 0.94 | 0.0010 | 3.31 | 0.339 | 10.08 | 0.317 | 0.135 | 1.17 | 0.093 | 0.418 |
| 40 | 5.53 | 1.75 | 0.0024 | 7.99 | 0.698 | 27.66 | 2.010 | 0.528 | 2.09 | 0.212 | 0.637 |
| 60 | 5.48 | 1.49 | 0.0049 | 6.60 | 0.625 | 22.36 | 1.590 | 0.445 | 1.77 | 0.538 | 0.864 |
| 80 | 5.41 | 1.67 | 0.0067 | 7.41 | 0.785 | 22.06 | 1.621 | 0.426 | 1.83 | 0.190 | 0.572 |

Table D-2. Soil solution composition data by P fertilization rate for low incubation temperature soil.

| P Rate | pH | EC | P | Ca | Al | $NO_3$ | Cl | $SO_4$ | Mg | K | Na |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mg $kg^{-1}$ | | | | | | | mmol $L^{-1}$ | | | | |
| 0 | 5.34 | 2.41 | 0.0009 | 6.96 | 0.411 | 27.36 | 0.178 | 0.089 | 2.69 | 0.353 | 0.623 |
| 10 | 5.47 | 1.36 | 0.0005 | 3.55 | 0.354 | 14.40 | 0.332 | 0.052 | 1.42 | 0.155 | 0.508 |
| 20 | 5.56 | 0.97 | 0.0008 | 2.49 | 0.590 | 10.28 | 0.402 | 0.078 | 0.96 | 0.078 | 0.355 |
| 30 | 5.73 | 0.73 | 0.0008 | 2.15 | 0.141 | 6.94 | 0.397 | 0.128 | 1.29 | 0.125 | 0.388 |
| 40 | 5.47 | 1.77 | 0.0015 | 7.71 | 0.971 | 21.80 | 1.590 | 0.356 | 2.07 | 0.208 | 0.584 |
| 60 | 5.52 | 1.55 | 0.0032 | 7.05 | 0.721 | 19.51 | 1.790 | 0.474 | 1.87 | 0.179 | 0.507 |
| 80 | 5.47 | 1.54 | 0.0081 | 6.57 | 0.534 | 19.20 | 1.731 | 0.527 | 1.75 | 0.492 | 0.673 |

## APPENDIX E

Table E-1. Analysis of variance with means squares for analysis as an incomplete block in time (test 1 and 2).

| Effect | df | % P Concentration | | Dry Weight g pot$^{-1}$ | | | P Uptake µg pot$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Total | Shoot | Root | Total |
| Rep | 3 | 85937† | 29288 | 18619721 | 10475351 | 5939503 | 25.94 | 1.491 | 27.56 |
| Exp | 1 | 819200*‡ | 378450 | 775362665** | 1755977506** | 4865023440** | 345.46** | 640.244*** | 1926.26*** |
| Rep*Exp | 3 | 25266 | 51118 | 14489049*** | 37295022** | 125045794*** | 8.80 | 2.047 | 11.58 |
| P Rate | 6 | 1790778*** | 78059 | 1339721471*** | 230181363** | 2113633266*** | 96.15*** | 137.827*** | 438.66*** |
| Exp*PRate | 1 | 43512 | 50 | 145432248*** | 437895019** | 1088041276*** | 61.16*** | 176.354*** | 445.23*** |
| Rep*Exp*PRate | 21 | 103981 | 65767 | 2833047 | 21464349 | 14980435 | 3.21 | 4.586 | 6.19 |
| Inc.Temp | 1 | 645532 | 1444 | 12150221* | 1499833 | 5112300 | 34.82** | 0.410 | 18.18 |
| PRate*Inc.Temp | 6 | 339647 | 78320 | 7289638** | 59060158** | 97945681*** | 19.82*** | 25.292*** | 61.40*** |
| Exp*PRate* Inc.Temp | 2 | 89781 | 15362 | 1074995 | 18217946 | 18373759 | 5.11 | 2.375 | 12.76 |

† Actual values were multiplied by $10^6$.
‡ Significance at the 0.1(***), 1.0(**), or 5.0(*) % level.

Table E-2. Analysis of variance with mean squares of dry weights and P uptake from test 1.

| Effect | df | Dry Weight | | | P Uptake | | |
|---|---|---|---|---|---|---|---|
| | | Shoot | Root | | Shoot | Root | |
| | | | High | Low | | High | Low |
| Rep | 3 | 3008845*†‡ | 1699667 | 42095624 | 28.17** | 4.38 | 9.41 |
| P Rate | 3 | 3244250312*** | 503205000*** | 22125624*** | 144.43*** | 280.87*** | 95.87*** |
| Rep*P Rate | 9 | 6487812 | -- | -- | 3.75 | -- | -- |
| Inc. Temp | 1 | 4132812 | -- | -- | 3.95 | -- | -- |
| P Rate*Inc. Temp | 3 | 13443646 | -- | -- | 8.81 | -- | -- |

† Actual values were multiplied by $10^6$.
‡ Significance at the 0.1(***), 1.0(**), or 5.0(*) % level.

Table E-3. Analysis of variance with mean squares of dry weights and P uptake from test 2.

| Effect | df | Dry Weight | | | P Uptake | | |
|---|---|---|---|---|---|---|---|
| | | Shoot | | Root | Shoot | | Root |
| | | High | Low | | High | Low | |
| Rep | 3 | 162205† | 196010 | 4873786* | 6.90 | 1.91 | 0.49 |
| P Rate | 4 | 3009546***‡ | 579780 | 7460566** | 55.72*** | 8.93** | 12.91* |
| Rep*P Rate | 12 | -- | -- | 1226960 | -- | -- | 3.18 |
| Inc. Temp | 1 | -- | -- | 196140 | -- | -- | 0.16 |
| P Rate*Inc. Temp | 4 | -- | -- | 936374 | -- | -- | 1.37 |

† Actual values were multiplied by $10^6$.
‡ Significance at the 0.1(***), 1.0(**), or 5.0(*) % level.

Table E-4. Analysis of variance of soil extractants with mean squares for analysis as an incomplete block in time (test 1 and 2).

| Source | df | Bray I | Bray II | Mehlich I | Mehlich III | Mississippi | Resin P | Solution P |
|---|---|---|---|---|---|---|---|---|
| Rep | 3 | 1.066 | 1.937 | 0.470 | 0.283 | 18.203 | 309.974 | 240‡ |
| Exp | 1 | 1.638 | 9.461 | 3.348 | 0.763 | 258.326** | 391.230 | -- |
| Rep*Exp | 3 | 0.800 | 1.757 | 0.478 | 1.146 | 2.283 | 174.256 | -- |
| P Rate | 6 | 500.750***† | 921.383*** | 286.486*** | 807.083*** | 1767.538*** | 2486.755*** | 5063*** |
| Exp*Rate | 1 | 4.620 | 9.245* | 0.506 | 3.163 | 0.826 | 142.087 | -- |
| Rep*Exp*PRate | 21 | 1.442 | 2.097** | 0.468 | 0.954 | 2.624 | 184.005 | -- |
| Inc.Temp | 1 | 8.417* | 1.046 | 0.008 | 6.040** | 0.436 | 101.246 | 97 |
| PRate*Inc.Temp | 6 | 2.137 | 1.701 | 0.199 | 1.088 | 2.195 | 172.742 | 183 |
| Exp*P Rate* Inc.Temp. | 2 | 0.0005 | 0.918 | 0.065 | 1.210 | 3.032 | 60.738 | -- |
| Rep*Inc.Temp | 3 | -- | -- | -- | -- | -- | -- | 125 |

† Significance at the 0.1(***), 1.0(**), or 5.0(*) % level.
‡ Actual values were multiplied by $10^6$.

APPENDIX F

Table F-1. Physical and chemical properties of Memphis Silt Loam (Typic Hapludalf) surface soil (1-15cm).

| Property | | |
|---|---|---|
| **Physical** | | |
| Mechanical Analysis, g $kg^{-1}$† | | |
| Sand | 15.00 | |
| Silt | 645.00 | |
| Coarse Silt | 140.00 | |
| Clay | 200.00 | |
| **Chemical** | | |
| pH (1:1 soil/water) | 5.50 | |
| Exchangeable Cations, mmol(+)kg‡ | | |
| Ca | 3.73 | |
| Mg | 0.62 | |
| K | 0.14 | |
| Na | 0.02 | |
| Al | <0.01 | |
| CEC | 4.20 | |
| Extractable Nutrients, mg $kg^{-1}$# | | |
| Ca | 828.00 | |
| Ma | 127.00 | |
| K | 46.00 | |
| P | 8.00 | |
| P, Total $\mu g\ g^{-1}$## | 275.00 ±30 | |
| | Incubation Temperature | |
| | High | Low |
| C, Total g $kg^{-1}$ | 7.3 ±0.2 | 7.8 ±0.1 |
| Easily oxidizable organic matter g $kg^{-1}$### | 11.0 | 10.6 |
| P, Organic $\mu g\ g^{-1}$ | 131.0 ±18 | 132.0 ±16 |
| P, Labile $\mu g\ g^{-1}$¶ | 28.0 ±3 | 30.0 ±2 |

† Pipette Method (Soil Conservation Survey Staff, 1982).
‡ Bases exchanged with pH 7.0 1M $NH_4OAc$, Al extracted with 1M KCl, CEC determined by ammonia stauration and steam distillation (Soil Conservation Survey Staff, 1982).
# Mehlich I (Johnson, 1982).
## Total and organic P by ignition method (Olsen and Sommers, 1982).
### Walkley-Black Method (Nelson and Sommers, 1982).
¶ Anion Exchange Resin method (Olsen and Sommers, 1982).

# VITA

Steven Duane Hardin was born in West Union, Ohio an April 25, 1964. In June 1982, he graduated from Georgetown High School in Georgetown, Ohio. In May 1986, he received his Bachelor of Science degree for Berea College, Berea, Kentucky with a major in Agriculture. In September 1986, he enrolled in The University of Tennessee, to pursue a Master of Science degree in Plant and Soil Science, which was awarded to him in December 1988.