EVALUATION OF DIAGNOSTIC TECHNIQUES, FOR DETERMINING

AVAILABLE SOIL PHOSPHORUS

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By

ROBERT LEE WESTERMAN It Bachelor of Science Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Thesis Advisar

Dean of the Graduate School

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I. INTRODUCTION

Determination of the so called "available" soil phosphorus in soils has been the object of intense investigations in soil science for many years. Early investigators determined total phosphorus of soils and tried to apply this information to the needs of the soil, but the results did not correlate well with field responses. Later investigators tried to imitate root absorption by use of various acid extractions but these procedures also failed. However, Bray and Kurtz (2)¹ obtained success by extracting all or a proportinate part of the form or forms of soil phosphorus that are available to the plant.

In spite of these investigations and the many methods that have been proposed for assessing "available" soil phosphorus, it is not always possible to predict response of winter wheat to phosphorus fertilizer on some soils in Oklahoma. It is important to know when a given soil cannot supply adequate phosphorus to a crop for maximum yield.

Experiments on several key soils in Oklahoma have established the need for phosphate fertilization for economical yields of wheat. These studies have shown exigency for better diagnostic tools to evaluate the need for phosphorus. Therefore, it was an objective of this study to evaluate procedures for diagnosing the need for phosphorus fertilization.

In order to accomplish this objective, three improved procedures for assessing "available" soil phosphorus were evaluated. Improved techniques evaluated included a short term nutrient absorption method under greenhouse conditions, leaf analyses of winter wheat at a definite growth

¹Figures in parenthesis refer to Literature Cited, page 41.

stage and acid extractions of soils.

Other objectives of this study were a study of the fate of added phosphorus to a key soil in Oklahoma by fractionating known soil phosphorus forms; and the determination of the influence of high rates of phosphorus fertilization on the yield and chemical composition of winter wheat.

II. LITERATURE REVIEW

In recent decades, many new methods to aid in diagnosing soil fertility requirements for plants have been devised. Soil tests and plant tissue tests have been used extensively in research. Some significant factors of plant behavior with respect to soil phosphorus have been established from these tests. Some of these significant factors are the relatively high needs for phosphorus in the early stages of plant growth and the degree of water solubility of the phosphorus present in the soil. The water solubility of the phosphorus present directly influences the availability of this element, since most of the phosphorus sorbed directly by the plant is from the soil solution.

Phosphate Equilibrium

Many soil properties influence the amount of fertilizer phosphorus that is extracted by an extractant. Olsen² has stated that soil texture influences the amount of fertilizer phosphorus that is recovered by extracting with $NaHCO_3$.

Smith, et. al. (14) found that the amount of fertilizer phosphorus recovered depended to some degree on soil reaction and the amount of calcium present. In all studies of phosphorus "fixation" the phosphorus must be allowed to equilibrate for a period of time. In studying

²Sterling R. Olsen, personal communication, December 17, 1962.

extractants and methods for assessing phosphorus availability, various extractants should be tested after equilibrating known amounts of fertilizer phosphorus with soils varying widely in chemical and physical characteristics.

Peach (12) states, "Theoretically water alone or in equilibrium with known partial pressure of CO_2 should be an ideal extracting agent for determining available soil phosphorus, since it displaces the soil solution." The displaced phosphorus in the soil solution is immediately available to the plant and therefore, correlates well with phosphorus responses. Until recently, colorimetric methods lacked sensitivity and water soluble phosphorus could not be detected in many soils due to the minute amounts present. Recently, however, a highly sensitive method has been proposed by Jackson (7) which has a range of 0.02 ppm to 0.60 ppm and enables a colorimetric determination of very low amounts of water soluble phosphorus present in soils.

Short Term Nutrient Absorption

Stanford and DeMent (16) suggested a short term nutrient absorption technique to supplement existing methods and aid in securing a better understanding of the complex relationships which control nutrient absorption from the soil-plant system. In this procedure, the major components of study with reference to fertilizer applied are phosphorus uptake, method of placement, moisture content and kind of plant. These investigators have successfully measured availability of various phosphorus compounds to wheat with this procedure. Plants were grown in cartons containing phosphorus-free sand for 11 days, then transferred to other cartons containing equilibrated soil phosphorus treatments for an absorp-

tion period of 3 days. Plant tops and roots were harvested and total phosphorus was determined on the ashed sample. This method has shown varying amounts of phosphorus taken up in the plant with varying amounts of phosphorus applied.

Stanford and DeMent have also employed a similiar technique for the evaluation of nitrogen and potassium fertilizer.

In the search for methods of supplementing or aiding in determining the availability of phosphorus to plants, many researchers have suggested that the composition of the plant should be the best indicator for nutrient needs.

Tissue Analyses

The major problems confronting this diagnostic technique for determining "available" soil phosphorus are two-fold. The first problem is to determine what stage of growth to sample the plant in order to detect maximum amounts of "available" phosphorus present. The second problem encountered is deciding which portion of the plant to analyze.

Ulrich (21) stated that the concentration of a nutrient in the plant as a whole or in part of it is a function of soil, climate, plant, time, management and possible other factors. He further stated that the amount of nutrient present in the plant can be expressed by Jenny's mathematical equation for factors of soil formation.

Lundegardh (9) suggested that leaf analysis of wheat would reflect the "available" soil phosphorus level, providing that the leaf samples were taken at a definite stage of growth, preferably about the bloom stage or shortly before the close of vegetative growth.

Thomas (19) also indicated that the leaf may serve as an index tissue in the integration of all factors that influence the availability of soil nutrients and their uptake by plants. Thomas agreed with Lundegardh that the best time to take leaves for diagnosing available nutrients is at flowering time when the vegetative parts of the plant are fully grown but still vigorous. He further states the uptake of an element is not always in direct proportion to external concentrations, a fact which accounts for the difficulties encountered in the methods of traditional Agronomy in seeking too closely for a direct relationship between applied fertilizer and yield.

According to Lundegardh (9), Spanning found that leaf phosphorus reached a maximum at the beginning of flowering and then declined rapidly.

Tissue analyses concerned with diagnosis of the nutritional status of crops as an index of the amounts of available nutrients present in the soil, have proved to be a valuable tool in supplementing many soil tests for determining "available" soil phosphorus.

Soil Extraction

In order to understand the extraction method of determining "available" soil phosphorus, the forms of phosphorus present in the soil must be con-

Olsen and Fried (11) stated that phosphorus occurs naturally in soils in the form of $Ca_x(PO_4)_y$, hydroxyapatite and fluorapatite, Fe and Al(PO_4), various primary and secondary minerals in which a phosphate group substitutes for a silicate group in a crystal lattice and as organic phosphate which may constitute as much as 75% or as little as 3% of the total soil phosphorus.

In the past few decades many extraction methods have been proposed for determining "available" soil phosphorus. Jackson (7) evaluated a number of dilute acid extraction methods that have been proposed for measuring a fraction of the "available" soil phosphorus. The degree of correlation varies according to the nature of the soil. Generally, the phosphorus extractable from acid soils by dilute solutions of strong acids can be correlated with crop yield response to phosphate fertilizers. Dilute solutions of strong acids gives a better indication of phosphate response on neutral soils or soils that have been fertilized with rock phosphate. When all soils are considered, a higher degree of correlation between soil test and crop response is obtained with dilute acids, fluoride or NaHCO₂.

As previously stated, phosphorus occurs in various forms in the soil, but the availability of these forms to the plant is not known. However, Chang and Jackson (3) have proposed a fractionation procedure for inorganic phosphorus which measures discretely the total of each chemical form. Water soluble and loosely bound phosphate, "AlPO₄", "FePO₄", occluded AlPO₄, "Ca_x(PO₄)_y" and reductant soluble FePO₄ are the forms that can be extracted. These phosphates occur in varying proportions in most neutral, alkaline and acid soils.

Knowledge of the distribution of soil phosphorus forms should prove useful in the development of extraction solutions for improved soil tests.

The fractionation system used as a device for obtaining information about the fate of fertilizer phosphorus may supplement other diagnostic techniques for the determination of "available" soil phosphorus.

Fate of Fertilizer Phosphorus

When water soluble phosphates are applied to the soil, they are rapidly converted to less soluble forms. This is due primarily to the presence of Fe and Al compounds which bind the phosphate ions to their surface or precipitate them. This change in chemical form can be empirically studied by the fractionation procedure of Chang and Jackson (3).

Yuan, Robertson and Neller (23) used the method of Chang and Jackson (3) in extracting successively three acid sandy soils to represent water soluble and loosely bound, "Al", "Fe" and " $Ca_x(PO_4)_y$, respectively. The acid soils were Red Bay fine sandy loam, Norfolk loamy fine sand, and Leon fine sand. Increments of monobasic potassium phosphate were added to the soil in various treatments. Over 80% of the added phosphorus was retained by the soils as "Fe" and "AlPO₄". Less than 10% was in the water soluble and Ca forms. The balance may have been in forms other than those mentioned.

The rates of "AlPO₄" to "FePO₄" increased with rates of applied phosphorus for the three sandy soils. In general, the water soluble and loosely bound phosphorus increased as treatments increased but the " $Ca_x(PO_4)_y$ " varied considerably.

Laverty and McLean (8) fractionated 5 different soils of varying pH treated with rates of phosphorus fertilizer. In all cases they found increased amounts of "saloid bound", "Al", "Fe" and $"Ca_x(PO_4)_y"$ even though the pH varied from 4.9 to 7.2. The total quantities of "FePO₄" in the soils fluctuated somewhat, but generally there was a tendency for them to decrease with an increase in pH. There was no definite trend that could be followed by "AlPO₄" with varying pH values. There was a

definite trend in both the treated and untreated soils for $"Ca_x(PO_4)_y"$ to increase with an increase in soil pH. The quantities of $"Ca_x(PO_4)_y"$ formed compared to total amount of phosphorus added represented 10 to 15%. "Iron" and "AlPO₄" fractions present comprised the largest portions of the four forms extracted.

The total phosphorus recovered in the four fractions of phosphate accounted for 108% of the amount applied. Laverty and McLean did not ascertain whether this tendency to exceed 100% of the added phosphate is due to random variation, or to the effect of applied phosphate on the extractability of some other phosphate fraction, such as the occluded AlPO₄ that was not determined.

Response of Wheat to Phosphate Application

Although the genetic composition of any plant species determines the chemical composition to some extent, generally this composition may be modified by nutrition or by variation in the environmental factors of climate and soil type.

Stubblefield and DeTurk (18) reported that the seeds of grain crops represent the final product of growth of a given generation under a wide variety of environmental conditions with remarkable constancy of chemical composition. He further stated that nitrogen and phosphorus are found primarily in the grain of cereal crops, potassium and calcium in the straw and magnesium and sulfur are approximately evenly distributed.

Vandecaveye (22) summarized reports of Guyon, Lefort, Murphy and Brayfield which showed definitely that the addition of phosphate fertilizer for wheat resulted in larger percentages of phosphate in the grain, while reports of Geddes and Weideman indicate this treatment resulted in

the reduction of protein content and had little or no effect upon the phosphorus content of the grain.

Murphy (10) working with winter wheat in Oklahoma showed that protein content of the grain increases with increased amounts of soil nitrogen and decreased with phosphate fertilization with or without nitrogen. He also found potassium fertilizer had little or no effect upon the protein composition of the wheat kernel.

Smith, Kapp and Potts (15) found that the application of P_2O_5 had no effect upon the percentages of nitrogen, potassium and calcium in the forage of wheat, but very significantly increased the phosphorus from 0.37% to 0.414%.

Boatwright and Hass (1) reported phosphate fertilization increased phosphorus uptake by the whole plant from emergence to maturity and as plants matured, phosphorus uptake declined. They also reported dry matter and nitrogen uptake by wheat reached a maximum at heading, soft dough and maturity on nitrogen-phosphorus, nitrogen and unfertilized plots, respectively. It was concluded that nitrogen and phosphorus in the grain were derived primarily by translocation from leaves, stems and chaff, and the amounts present were directly influenced by fertilization.

III. MATERIALS AND METHODS

Field Experiment

The field plots were located on series 1800 of the Agronomy Research Farm, Perkins, Oklahoma. The soil type is Norge loam and is described in O. A. E. S. Process Series P-315A (5).

The field soil was acid, therefore liming was desirable to obtain meaningful results. The titration method of Tisdale and Nelson (20) was employed to determine the lime requirement. The neutralization value (6) was determined and hydrated lime was applied July 24, 1961, with an E-Z flow applicator. Hydrated lime was applied in four equal increments and the land was disced after each coverage to help insure thorough coverage and mixing.

The phosphorus rates were established in the laboratory using an equilibrium technique. Rates of phosphorus in the form of monocalcium phosphate plus 0.15 gram of Ca(OH)₂ were added to 250 ml. beakers containing 200 grams of field plot soil and mixed thoroughly. Water was added to approximately field capacity and the beakers were weighed. A constant weight was maintained by adding distilled water to the beakers throughout the 8-week equilibrium period. The applied phosphorus rates were an arithmetic progression equivalent to 45 lb. P/acre with 10 treatments and 3 replications.

About 20 grams of phosphorus-free sand were poured into a leaching tube. Five grams of the previously equilibrated soil were mixed with 20 grams of the P-free sand and evenly distributed over the sand. Approximately 15 grams of sand were sprinkled on top of the soil mixture. The

leaching tube was filled with distilled water and allowed to set over night. The next day a 500 ml. Erlenmeyer flask was filled with water and mounted upside down in a holding rack to maintain a constant head for the leaching apparatus. Each equilibrated rate of phosphorus was leached with 100 ml. of distilled water. Color was developed using Jackson's number 1 method (7). The optical density was read on Baush and Lomb colorimeter with the wave length setting of 700 and a red filter.

The average number of pounds of water soluble phosphorus per acre removed from the leachings were plotted against the treatments applied. A partial plateau in the equilibrium curve was obtained in the region of 225 lb. P/acre as shown in Figure 1. From the equilibrium curve, the assumption was made that the yield possibility would result from an application of 225 lb. P/acre. In order to test this assumption, 3 lower rates and 4 higher rates were applied.

The field experiment was organized into a randomized block design with 8 treatments and 3 replications. The eight treatments were 0, 75, 150, 225, 300, 375, 450 and 525 lb. P/acre. The phosphorus was applied as concentrated superphosphate (0-53-0) with a mounted combination grain drill and fertilizer applicator on August 10, 1961. The grain box was calibrated for the 75 lb./acre rate of phosphorus and used because of its accurate distribution. Since the phosphate rates were in arithmetic sequence, each plot was covered the appropriate number of times and disced after each application to insure good field mixing.

Concho hard red winter wheat was sown October 17, 1961, at the rate of approximately 65 lb./acre across the 50' by 21' plots.

A 60-pound rate of nitrogen was applied as top dressing February 22, 1962, on all plots.

Water Soluble Phosphorus (lb./acre)



Figure 1. Average Pounds Per Acre of Water Soluble Phosphorus Removed From Equilibrated Phosphorus Applications.

The plots were harvested with a 7' self-propelled combine June 14, 1962. The grain was weighed in the field and straw samples were taken from each plot. Both the grain and the straw samples were stored for chemical analyses.

Greenhouse Experiment

A short term nutrient absorption technique as suggested by Stanford and DeMent (16) was employed to determine the availability of fertilizer phosphorus to wheat. Fhosphorus-free sand was obtained by washing with concentrated HCl several times. The chlorides were then removed by successive leachings of distilled water. Six hundred eighty grams of P-free sand were added to each wax freezer carton and thirty Concho wheat seed were planted. Fifty ml. of minus phosphorus nutrient solution was added to each culture, supplying 15 mg. of N, 15 mg. of K, as $Ca(NO_3)$, $Mg(NO_3)$ and K_2SO_4 . Traces of Zn, Fe, Mn, Cu, SO_4 and B were also supplied in the nutrient solution. In addition 40 ml. of distilled water was added, giving a total of 90 ml. of liquid per culture. During the next two weeks an additional 50 ml. of minus phosphorus nutrient solution was added to each culture. The wheat was allowed to grow in the sand for a period of 12 days.

Soil samples were taken diagonially across each field plot February 1, 1962. These soils were passed through a 20 mesh screen and 200 gms. were added to freezer cartons. The bottoms of the cartons containing the sand culture were removed and placed on top of the equilibrated soil treatments in the freezer cartons. The plants were allowed to grow in the sand plus soil culture for a period of 12 days. Distilled water was added as needed throughout the growing period. Plant tops and roots

were harvested, dried and stored for phosphorus analyses.

Leaf Analyses

Leaf samples of wheat plants were taken from the field experiment May 7, 1962, during the bloom stage. Approximately 100 plants per plot were clipped about 3" above the ground at random. The plant samples were oven dried at 100°C and the leaves were stripped from the stalk, ground and stored for chemical analyses.

Chemical Analyses of Plant Materials and Soils

Plant materials were oven dried at 100°C and ground. Nitrogen content was determined by the Kjeldhal method (6). Total phosphorus was obtained by digestion in 3:1 nitric-perchloric acid. The molybdate complex for color was used (6). The cations (Ca, Mg, Na, K) were determined from the previous extract by means of the Beckman DU Flame Spectrophotometer and photomultiplier.

Soil samples were taken from the field experiments July 26, 1962, air dried, processed and passed through a 20 mesh screen. The Beckman glass electrode pH meter was used to determine pH in a soil-water paste. Total phosphorus was obtained by perchloric acid digestion as suggested by Harper (6). The molybdate complex of sodium molybdate and hydrazine sulfate was used for color development. Cation exchange capacity was measured by the ammonium acetate and distillation method of Peech et al. (13). The cations (Ca, Mg, Na, K) were determined in the ammonium acetate leachate by use of the Beckman DU Flame Spectrophotometer with photomultiplier.

The "available" phosphorus was determined by 0.1 N acetic acid and 0.02 N H₂SO₄ using a 20:1 solution to soil ratio. Two and one-half

grams of soil plus 50 ml. of the extractant were added to a 100 ml. centrifuge tube. The samples were shaken for 30 minutes on a rotary shaker and centrifuged until a clear supernatant was obtained. An aliquot was taken from the supernatant, diluted to volume and color was developed. Optical density was obtained by the use of the Baush and Lomb colorimeter.

Bray number 2 phosphorus (2) was obtained using a 20:1 solution to soil ratio. The soils were shaken for 1 minute using a wrist-action shaker, filtered and color was developed.

Phosphorus fractionation was accomplished by the method of Chang and Jackson (3) except that the NH_4F was adjusted to pH 8.5 as later suggested by Fife (4). Fluoride interference in the molybdenum blue reaction was eliminated by addition of boric acid (7). Iron interference in the " $Ca_x(PO_4)_y$ " determination was eliminated by adding increased amounts of the reducing agent used in the molybdenum blue reaction.

Water soluble and loosely bound phosphorus was determined by a successive extraction technique using 1 N NH₄Cl. One gram of 225 lb. P/ acre soil plus 50 ml. of the extracting agent was added to a 100 ml. centrifuge tube. The soil was shaken for 30 minutes, centrifuged and the supernatant was poured off. An additional amount of the extractant was added to the same soil and this procedure was repeated successively 11 more times. Color was developed using Jackson's number 1 method (7). This technique was used to determine the number of successive extractions that would be necessary to reduce the phosphorus level of this treatment to the phosphorus level of the next lowest treatment (150 lb. P/ acre).

Analysis of variance of randomized block material were determined as suggested by Steel and Torrie (17) and significant differences between treatments were examined using the least significant difference test.

IV. RESULTS AND DISCUSSION

Field Experiment

The growing season for wheat in 1961-62 was unusually variable. Prolonged cold weather during the latter portion of November and portions of December retarded early vegetative growth of wheat. The wheat remained at this rather dormant stage until March. Rapid growth began and continued until maturity. However, shortly before maturity, moisture stress was encountered and yields were reduced. Rains during the ripening period delayed harvest until the middle of June.

Shortly after emergence and throughout the growing period, visual differences were apparent between the phosphated and check plots.

Yields

A summary of grain yields are reported in Table I. Variance due to treatment was significant at the 1% level. The differences in yield of grain between soil treatments are illustrated graphically in Figure 2. The yield increased significantly as treatments of phosphorus increased.

The phosphorus percentages of the wheat kernel increased as the applications of phosphate increased (Table II). The relationship between phosphate treatments and percent phosphorus in the grain is illustrated graphically in Figure 3. The average nitrogen percentages and test weights of wheat are represented in Table III. The percent nitrogen of the wheat kernel was higher from the check plots than from plots where phosphate was added.

TABLE I

ANALYSIS OF VARIANCE AND LEAST SIGNIFICANT DIFFERENCE TEST OF YIELD OF GRAIN (expressed as bu./acre)

Source	d.f.	M.S.	F
Total	23		
Treatments	7	118.42	63.50*
Replications	2	2.05	1.10
Error	14	1.87	

* Indicates significance at the 1% level.

	Treatmer	nts	Mean Yields
	N - P ₂ 05	(P) - K ₂ 0	(bu./acre)
1. 2. 3. 4. 5.	60 - 0 60 - 175 60 - 350 60 - 525 60 - 700 60 - 875	- 0 (75) - 0 (150) - 0 (225) - 0 (300) - 0 (375) - 0	13.6 27.2 28.1 29.3 30.7 32.2
7.	60 - 1050	(450) - 0	32.4
8.	60 - 1225	(525) - 0	32.8

LSD is 3.3 at the 1% level.



Figure 2. Average Yield of Wheat Expressed as Bushels Per Acre.

TABLE II

ANALYSIS OF VARIANCE AND LEAST SIGNIFICANT DIFFERENCE TEST OF PHOSPHORUS PERCENTAGES IN THE GRAIN

Source	d.f.	M.S.	Ţ
Total	23		
Treatments	7	0.0173	52.52*
Replications	2	0,0004	1.06
Error	14	0.0003	

* Indicates significance at the 1% level.

Treatments N - P_2O_5 (P)	- к ₂ 0	Mean Phosphorus Content percentage
1. $60 - 0$	- 0	0.28
2. $60 - 175$ (75)	- 0	0.41
3. $60 - 350$ (150)	- 0	0.47
4. $60 - 525$ (225)	- 0	0.48
5. $60 - 700$ (300)	- 0	0.49
6. $60 - 875$ (375)	- 0	0.48
7. $60 - 1050$ (450)	- 0	0.50
8. $60 - 1225$ (525)	- 0	0.50

LSD is 0.04 at the 1% level.



Figure 3. Average Phosphorus Percentages in the Wheat Grain.

The wheat straw was analyzed for phosphorus but no differences were found due to treatment (Table IV).

TABLE III

THE AVERAGE PERCENT NITROGEN AND TEST WEIGHT OF WHEAT

lb. P/acre	0	75	150	225	300	375	450	525
%N	2.93	2.47	2.50	2.51	2.46	2.41	2.48	2.44
Test Wt.	57	56	55	56	56	56	56	56

Fate of Added Phosphate

After wheat harvest, soil samples were taken for phosphorus extractions. The data of the phosphorus fractionation are listed in Table V. Figures 4, 5, 6, and 7 illustrates the average amount of the various forms of phosphate and average yield of grain per field treatment. Generally, all forms of phosphate increased as the treatments increased. The fate of added fertilizer phosphorus resulted in the formation of large portions of "AlPO4" and lesser amounts of "FePO4", "Ca_X(PO4)_y" and water soluble and loosely bound forms of phosphate. The "AlPO4" comprised 9.7 to 49.6% of the total phosphorus extracted. The water soluble and loosely bound phosphate varied from 0 to 10.8% of the total and "Ca_X(PO4)_y" remained at a rather constant level of about 9.0%. The "FePO4" varied slightly and remained about 11.0% of the total phosphorus extracted. Organic phosphorus of the check plots accounted for 54.8% of the total phosphorus originally present in the soil.

Three acid extractions were made to determine "available" soil phosphorus. The average yield and amounts of phosphorus extracted are

TABLE IV

ANALYSIS OF VARIANCE OF PHOSPHORUS PERCENETAGES OF WHEAT STRAW

Source	d.f.	M.S.	F
Total Treatments Replications Error	23 7 2 14	0.000931 0.000065 0.000638	1.46** 0.10**

** Indicate not significant at the 10% level.

Treatments Mean Phosphorus $N - P_2 O_5 (P) - K_2 O_5$ Content Percentages 0.018 1. - 0 2. - 0 0.013 0.021 3. 4. - 0 - 0 0.020 5. 6. 0.024 - 0 0.024 - 0 7. 60 -1025 (450) 8. 60 -1225 (525) - 0 - 0 0.027 0.031

Treatments	Total	H ₂ 0 sol.	AlPOL	FeP04	$Ca_x(PO_4)_y$	Organic*	
0	206.6	0.0	20.0	23.0	19.2	113.3	
75	283.3	3.0	66.7	38.0	34.2		
15 0	326.6	6.0	86.7	43.3	25.8		
225	396.6	18.0	131.7	54.8	35.8		
300	536.6	49.7	243.3	61.8	37.2		
375	533•3	42.0	239.2	55•7	50.8		
450	643.3	71.0	295.2	73.3	52.5		
525	700.0	75.7	347.5	69.8	65.0		

FORMS OF SOIL PHOSPHORUS PRESENT 11 MONTHS AFTER ADDING CONCENTRATED SUPERPHOSPHATE (expressed as 1b. P/acre)

TABLE V

* Organic phosphorus determined from the check plots only.



Yield of Grain (bu./acre)

Figure 4. Average Wheat Yield and Water Soluble and Loosely Bound Phosphorus Per Acre.





Figure 6. Average Wheat Yield and $Ca_x(PO_4)_y$ Per Acre.



shown in Figure 8. The three extractions were 0.1 N acetic acid, 0.02 N H₂SO₄ and 0.1 N HCl \neq 0.03 N NH₄F. The 0.1 N HCl \neq 0.03 N NH₄F extracted greater amounts of soil phosphorus than the other acid extractants. The dilute sulfuric acid extracted more phosphorus than acetic acid and less than the dilute ammonium fluoride in hydrochloric acid. The lowest amount of phosphorus extracted per treatment was obtained using 0.1 N acetic acid. If the fractionation procedure as proposed by Chang and Jackson (3) is correctly characterizing the forms of Phosphate present (Table V), the extractants as shown in Figure 8 are extracting a portion of the "AlPO₄" and the water soluble and loosely bound forms.

In Figure 5, "AlPO₄" and yield obtained are plotted with treatments of phosphorus applied. Large amounts of "AlPO₄" were present and the amount increased as yield increased. The significance of the large amount of "AlPO₄" present suggested that this form may be directly related to yield. If this assumption is true, and if the acid extractants used reflect yield, then a major portion of this form ("AlPO₄") was extracted by the acid extractants.

It is believed that these acid extractants also extract almost all of the water soluble and loosely bound phosphate forms.

If these forms of phosphate can be related to yield, and if the acid extractants can be related to various forms present, then a universal soil extracting agent for determining "available" soil phosphorus is possible.

Accumulated amounts of phosphorus obtained with successive extractions of NH₄Cl are shown graphically in Figure 9. The amount of phosphorus removed per extraction decreased with increasing number of extractions. Eight successive extractions were necessary to reduce the phosphorus



Figure 8. Average Wheat Yield and Pounds Per Acre of Phosphorus Removed by Each Acid Extraction.



Number of Successive Extractions

Figure 9. Average Pounds Per Acre of Water Soluble and Loosely Bound Phosphorus Successively Extracted from the 225 lb./acre Rate.

level of the 225 lb. P/acre rate to the level of the 150 lb. P/acre rate which was the next lowest treatment. A total of 118 lb. P/acre were removed in eight extractions. It may be significant that the yield response curve (Figure 2) takes on about the same shape as the successive NH_4Cl extractions. The successive extractions are related to the rate of "availability" which may govern yield responses. The rate of "availability" involves the kinetics of phosphate release and phosphorus supplying power of the soil for plant growth.

Leaf Analyses

Even though the average percent phosphorus of the leaves increased as the field treatments increased up to the 375 lb. P/acre rate, the increases were not significant due to the large variation between treatments (Table VI). Generally, there was a reduction of Ca and K for the first increment of added phosphorus and apparently constant thereafter. There were no differences in the percent Mg. The potassium content in the wheat leaves averaged approximately 1% lower in the phosphorus treatments than in the check or zero phosphate treatment (Table VII).

In this study, tissue analysis of wheat leaves taken at bloom stage were not valuable as a diagnostic technique in ascertaining phosphate levels needed for maximum yield.

Short Term Nutrient Absorption

The short term nutrient absorption technique was employed to determine the availability of phosphorus fertilizer to wheat. Table VIII indicates there were no significant differences in the phosphorus percentages of the wheat tops, but Table IX shows significance at the 1% level

TABLE VI

ANALYSIS OF VARIANCE OF PHOSPHORUS PERCENTAGES IN WHEAT LEAVES AT THE BLOOM STAGE

Source	d.f.	M.S.	F	
Total Treatments Replications Error	23 7 2 14	0.006173 0.000247 0.000293	2.10** 0.84**	

** Indicates not significant at the 10% level.

TABLE VII

AVERAGE PERCENTAGES OF PHOSPHORUS, CALCIUM, MAGNESIUM AND POTASSIUM OF WHEAT LEAVES TAKEN AT THE BLOOM STAGE

		Rat	te of Ph	osphorus	(lb./acr	<u>e)</u>		
Element	0	75	150	225	300	375	450	525
P	0.17	0.18	0.23	0.24	0.27	0.28	0.28	0.28
Ca	0.28	0.23	0.22	0.21	0.22	0.21	0.19	0.20
Mg	0.25	0.24	0.24	0.24	0.24	0.23	0.23	0.24
К	3.22	2.32	2.40	2.37	2.35	2.39	2.33	2.40

in the phosphorus percentages of the wheat roots. Visual differences of growth of the wheat tops were noted during the latter portion of the growing period. The average percent phosphorus of both the wheat tops and roots increased as the treatments increased with the exception of the highest treatment and it decreased. The dry weight yield of wheat tops and roots varied among replications and treatments did not always increase as phosphorus treatments increased. Generally, average percentages of Ca, Mg, and K decreased in the wheat tops as the phosphorus treatments increased and there were no detectable amounts of sodium present. The wheat roots showed no indications or apparent trends of Ca, Mg, K or Na percentage composition as related to phosphate additions.

With radioactive sources, the short term nutrient absorption method has proven valuable as a tool for evaluating various fertilizer forms; but as the results in Tables VIII and IX indicate, it was not sensitive enough to be used for determining "available" soil phosphorus.

TABLE VIII

ANALYSIS OF VARIANCE OF PHOSPHORUS PERCENTAGES IN WHEAT TOPS OF THE SHORT TERM NUTRIENT ABSORPTION TECHNIQUE

Source	d.f.	M.S.	F	
Total	23			
Treatments	7	0.111752	1.21**	
Replications	2	0.025350	0 .27**	
Error	1 4	0.0 92 735		

** Indicates not significant at the 10% level.

	Treatments N - P ₂ 0 ₅ (P) - K ₂ 0		Mean Phosphorus Content of wheat tops	
1. 2. 3. 5.	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	- 0 - 0 - 0 - 0 - 0	0.313 0.353 0.447 0.567 0.647 0.800	
8.	60 -1225 (525)	- 0	0.707	

ANALYSIS OF VARIANCE AND LEAST SIGNIFICANT DIFFERENCE TEST OF PHOSPHORUS PERCENTAGES IN WHEAT ROOTS OF THE SHORT TERM NUTRIENT ABSORPTION TECHNIQUE

TABLE IX

Source	d.f.	M.S.	F	
Total Treatments Replications Error	23 7 2 14	0.043380 0.045150 0.006790	6.39* 6.65*	•

Mean Phosphorus

0.226

0.300

0.380

0.387

0.387

0.533

0.567

0.500

Content of wheat roots

* Indicates significance at the 1% level.

Treatments $N - P_2 O_5 (P)$ - K20 60 **-** 0e. . 1. - 0 60 - 175 (75) - 0 2. 60 - 350 (150) - 0 3. 60 - 525 (225) - 0 60 - 700 (300) - 0 4. 5. 60 - 875 (375) - 0 6. 60 -1050 (450) - 0 7. 60 -1225 (525 - 0 8.

LSD is 0.064 at the 1% level.

V. SUMMARY AND CONCLUSIONS

A field experiment utilizing wheat as the test plant was conducted at the Agronomy Research Farm, Perkins, Oklahoma. Treatments of 0, 75, 150, 225, 300, 375, 450 and 525 pounds of phosphorus per acre were organized into a randomized block design with three replications. Leaf analyses during the bloom stage, yield of grain and phosphorus percentages in the grain were used to determine the influence of high phosphorus applications on winter wheat. The fate of added phosphorus fertilizer was determined by soil extractions and fractionation procedures.

In the greenhouse, a short term nutrient absorption technique was used to determine the availability of added phosphorus fertilizer to wheat.

From the results of this investigation, the following conclusions which seem justifiable may be stated:

- Applications of phosphorus increased the yield of wheat. The percent phosphorus in the wheat kernel increased as phosphorus applications increased, but the percent nitrogen decreased.
- The application of concentrated superphosphate resulted in the formation of large amounts of "AlPO₄". Iron, Ca, water soluble and loosely bound phosphates were formed to a lesser extent.
- The short term nutrient absorption technique was found to be inadequate for determining "available" soil phosphorus levels.
- 4. Leaf analyses during the bloom stage of winter wheat could not be used as a diagnostic tool in determining needed phosphate

levels for maximum yield in this study.

5. An equilibrium technique was investigated and it offers distinct possibilities for determining optimum phosphorus levels for field applications.

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VITA

Robert Lee Westerman

Candidate for the Degree

of

Master of Science

Thesis: EVALUATION OF DIAGNOSTIC TECHNIQUES FOR DETERMINING AVAILABLE SOIL PHOSPHORUS

Major Field: Agronomy (Soils)

Biographical:

- Personal Data: Born November 20, 1938, at Mountain Park, Oklahoma, son of Freeman and Mary Westerman.
- Education: Graduated from Snyder High School, Snyder, Oklahoma, 1956; attended Cameron State Agricultural College 1956-1958; received the Bachelor of Science degree from Oklahoma State University with a major in Agriculture Education, May 1961; graduate study at Oklahoma State University, January 1961 to 1963.
- Experience: Reared on a farm; farm labor during summer and vacations 1953-1958; service station attendant summer of 1959; research work for Agronomy Department 1960, Graduate Research Assistant, Oklahoma State University, January 1961 to March 1963.

Member: Agronomy Club, Alpha Zeta and Collegiate FFA.

Date of Final Examination: February, 1963.