



Effect of withholding phosphorus on its distribution in wheat, (*Triticum aestivum* L.)
by Charles Kong-Wu Wang

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Agricultural Products Utilization
Montana State University
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Abstract:

The spring wheats (*Triticum aestivum* 'Thatcher') were grown in the green house on vermiculite in pots. They were subjected to different P treatments by initial withholding of P supply for various periods of time. The yields and types of P were determined at selected growth stages.

The effect of the degree of P deficiency on total P uptake and changes of types of P by spring wheat plants were studied. Initial withholding of P supply resulted in a significant decrease in both yield and total P uptake by the plants.

The leaves and roots both showed a general decrease in total P concentration upon increasing the period for withholding P. After the milk stage, P deficiency resulted in a sharp decrease in nucleic acid P and an increase in acid-soluble and phytic acid P in the kernels, as in percentage of total P. Lipid P was found only to increase slightly in kernels at maturity.

When the plants were beyond the milk stage, the inorganic P became predominant in both the roots and leaves. Organic types of P existed only in very small amounts and the various types of P remained relatively constant regardless of the effect of the early deprivation of P.

For some unknown reasons wheat plants tend to increase total P uptake when under a P stress during the early growth stages. At maturity, the kernel showed a significant decrease in total and nucleic acid P and an increase in acid-soluble and lipid P under the influence of P deficiency. The changes in types of P may decrease the protein content of the flour as was shown by a previous study on the relationship of types of P in flour to flour quality. Therefore, changes in types of P may be expected to decrease the baking quality of wheat flour by lowering the protein content. Early P deficiency for three weeks seems to be most critical to flour quality, since maximum changes in types of P in kernels occurred at this time.

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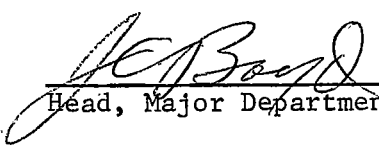
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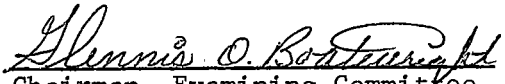
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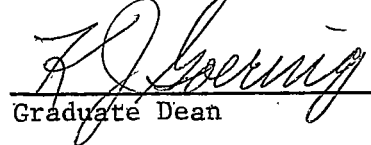
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Approved:


Head, Major Department


Chairman, Examining Committee


Graduate Dean

MONTANA STATE UNIVERSITY
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ABSTRACT

The spring wheats (Triticum aestivum 'Thatcher') were grown in the green house on vermiculite in pots. They were subjected to different P treatments by initial withholding of P supply for various periods of time. The yields and types of P were determined at selected growth stages.

The effect of the degree of P deficiency on total P uptake and changes of types of P by spring wheat plants were studied. Initial withholding of P supply resulted in a significant decrease in both yield and total P uptake by the plants.

The leaves and roots both showed a general decrease in total P concentration upon increasing the period for withholding P. After the milk stage, P deficiency resulted in a sharp decrease in nucleic acid P and an increase in acid-soluble and phytic acid P in the kernels, as in percentage of total P. Lipid P was found only to increase slightly in kernels at maturity.

When the plants were beyond the milk stage, the inorganic P became predominant in both the roots and leaves. Organic types of P existed only in very small amounts and the various types of P remained relatively constant regardless of the effect of the early deprivation of P.

For some unknown reasons wheat plants tend to increase total P uptake when under a P stress during the early growth stages. At maturity, the kernel showed a significant decrease in total and nucleic acid P and an increase in acid-soluble and lipid P under the influence of P deficiency. The changes in types of P may decrease the protein content of the flour as was shown by a previous study on the relationship of types of P in flour to flour quality. Therefore, changes in types of P may be expected to decrease the baking quality of wheat flour by lowering the protein content. Early P deficiency for three weeks seems to be most critical to flour quality, since maximum changes in types of P in kernels occurred at this time.

INTRODUCTION

Phosphorus is one of the major elements in plant nutrition and its compounds of P have important functions in many phases of cellular metabolism and cell division.

Initial withholding of P not only significantly affects the yield of wheat but may also affect the quality of wheat flour as a result of changes in types of P under the influence of P deficiency (2,16).

The objectives of this study were mainly to elucidate the changes of total P uptake in various morphological fractions of wheat plants as well as the changes of P as influenced by initial withholding of P. These findings may enable an improved understanding of the nutritional significance of P and the possible effect of P fertilization on wheat quality.

LITERATURE REVIEW

Wheat contains approximately 1.5 to 2 percent mineral matter. Phosphorus, one of the main mineral constituents, is present in high concentration in more than one form in the ash of the flour of the wheat (8).

Phosphorus compounds may be separated according to their chemical and physical properties into several different types (7): (1) acid soluble, which consists of phytin, inorganic, and ester type P; (2) acid labile or ester type P; (3) acid non-labile (acid-stable) in the form of phytic acid or its salts; (4) inorganic P; (5) nucleic acid P, consisting of DNA and RNA; and (6) lipid P in lecithins, cephalins, and phosphatidyl inositol.

Phytic acid is a phosphorylated myo-inositol, (hexaphosphate of myo-inositol). Phytin is the magnesium or calcium salt of phytic acid. Phytic acid and phytin occur in various plants (15). A considerable portion of the P in wheat kernels exists in the phytin form (7,13), mainly located in the bran and germ. These are stable in an acid solution.

Ester type P are considered to be the carbohydrate ester of phosphoric acid, which are acid-labile.

Pons (13) found nucleic acid P comprises an average 10.8 percent of the total P in wheat plants and found little ester type P. Andrews and Bailey (1) showed that a large proportion of the total P in flour existed in the nucleic acid forms. Analysis of plant phosphatides have revealed the presence of several additional long-chain bases in addition to the common phospholipids (13). These include phytosphyngosine, a saturated C₁₈ dihydroxysphyngosine, with the additional secondary alcohol group adjacent to that in sphingosine, and two homologues of phytosphyngosine containing 20 carbon atoms. Unsaturated sphingosines (dehydrosphyngosines) are also present in plants (18). Small quantities of phosphatidic acids and tri-phosphate are present in plant as well as animal tissues (4). Plasmalogens represent an unusual type of glycerolphosphatide which has been isolated from animal and plant tissues (4,18). Hydrolysis of these materials yields long-chain aldehydes plus monacylated 2-glycerophosphate. The distribution of phosphorus and nitrogen between the straw and the ear in wheat during growth was studied by Knowles and Watkin (9). They found a low proportion of phytin P in the ear soon after its emergence and a

relatively high proportion of inorganic P at harvest. The distribution of P was such that three-quarters of phytin and four-fifths of the inorganic P was in the ear, while four-fifths of the protein and non-protein nitrogen were transferred to the ear.

Brenchley (3) studied the phosphate requirement of barley at different periods of growth. The P supply had a high nutritional value at the early growth stage. Providing phosphate for the first six weeks, or longer, followed by the omission of P, permitted normal growth. Growth was seriously depressed in all respects with shorter initial periods of phosphate supply. Growth was steadily depressed, with longer periods of initial deprivation of P for barley. The amount of phosphate absorbed by the plant increased steadily in more or less direct proportion to the length of time it was given at the beginning of growth. However, sufficient P was taken up in the first six weeks to enable the plants to attain their maximum dry weight. The percentage of phosphate in the plants increased rapidly after six weeks. The absence of phosphate supply up to the first six weeks of growth caused an extremely rapid drop in the amount of phosphate ultimately taken up by the plant, after which a more gradual decrease occurred with lengthening periods of phosphate deprivation. Maximum dry weight was obtained when the wheat plants were grown in complete nutrient solutions for four weeks, then transferred to solutions devoid of phosphorus (3,5).

McKee and Robertson (11) found that total P increased steadily in the pea seeds over the period from 14 to 40 days from blossom, while the

hull showed a downward trend. Inorganic phosphate, which increased at a lower rate than total P, decreased slightly between the 20th and 23rd days following blossoming. This was accompanied by a corresponding increase in ester phosphate. This could have an important bearing on the synthesis of carbohydrates.

Power (14) showed that the fraction of the wheat material derived from fertilizer to be markedly influenced by moisture conditions. Uptake of soil P and total P by spring wheat was a linear function of the moisture, but the uptake of the fertilizer P was not influenced by moisture conditions once the moisture content of the surface soil had been brought to field capacity for a few days. They also found that absorption of fertilizer P by plants may be reduced considerably, unless the soil is sufficiently moist during the early stages of plant growth to keep an appreciable amount of it in solution.

Mihailovi and Hadzijev (12) investigated the P content of DNA and RNA and the ratio of the two acids in the gluten of various low-yielding and high-yielding varieties of wheat. They reported that ribonucleic acid content of wheat grain depended not only on the variety, but also on the use of nitrogen, P, and potassium fertilizers. The ratio of the nucleic acids in the grain was not specific for a wheat variety, since it was not constant.

Boatwright and Viets (2) used solution cultures to study the importance of P absorption during various growth stages of spring wheat (Triticum vulgare) and intermediate wheat grass (Agropyron intermedium).

They reported that maximum dry matter and grain production was attained when the supply of P was adequate during the first five weeks of growth.

Initially, withholding P for two weeks or more would hinder the yield of wheat and wheat grass tops and wheat grain. Maximum accumulation of P occurred when P was initially withheld, but supplied later until harvest. Watson (16) studied the relationship of P types in wheat flour and gluten to flour quality and reported that the types of P in wheat flour and gluten was influenced by the location of growth and to a lesser extent by variety. He also found that if the effect of protein content on loaf volume was removed, the total acid-soluble, inorganic, and ester-type P in the gluten and the flour showed significant relationships to the loaf volume. This relationship did not exist if the effect of protein on loaf volume was not removed. Several types of P and total P in the flour associated with the gluten were significantly related to protein content of flour.

Jennings and Morton (7) studied P types and changes of nucleic acid in the developing wheat grain under field conditions. They found the relative concentration of total P in the endosperm to decline rapidly between day 5 and 19 after flowering, remaining constant thereafter. A large portion of the total P existed in the embryo. The concentration of total P (as percent of dry weight) declined slightly in the embryo during maturation, while in the testa-pericarp, total P was fairly constant. The relative concentration of inorganic and acid-labile P declined throughout the development of the endosperm. But the relative concentration of acid stable organic P (phytic acid) declined to day 26 after flowering

then increased markedly. Similar changes were found in the testa-pericarp fraction, except for a decrease in amount of inorganic and an increase of acid-stable organic P at day 12 after flowering. Jennings and Morton (7) suggested that an enzyme system may catalyze the conversion of inorganic P to phytic acid. The relative concentration of lipid P in the endosperm declined between day 8 and 26 after flowering. The decline in the testa-pericarp started after day 20. The amount of DNA per grain in the testa-pericarp was nearly constant throughout the development after day 5, while it increased in the endosperm between day 8 and 19 then remained constant. Jennings and Morton (7) claimed that the number of cells present in the testa-pericarp were essentially constant throughout the period of development. But, if the DNA content was expressed as percent of the dry weight; then it declined until about day 26. The RNA content of the endosperm per grain increased rapidly during cell division and thereafter remained constant. If expressed as percent of the dry weight, the RNA content declined in both the testa-pericarp and the endosperm until day 20.

MATERIALS AND METHODS

Spring wheat (Triticum aestivum 'Thatcher') was grown under green house conditions in a vermiculite media placed in plastic pots of 3,000 ml. capacity. Twenty-four seeds were evenly spread on top of the vermiculite of each pot and covered with another inch of vermiculite. Seven hundred ml. of distilled water were added to initiate germination. When the shoots were about 2 inches tall, each pot was adjusted to 22 plants by pulling out the extra plants. The plants were then subjected to a series of P treatments randomized within each of three replications. The following treatments were imposed by use of a hydroponic nutrient solution (Appendix B, Table I, page 61).

- (1) Adequate P continually (complete nutrient solution).
- (2) Initial withholding of P for two weeks, followed by adequate P thereafter.
- (3) Initial withholding of P for three weeks, followed by adequate P thereafter.
- (4) Initial withholding of P for four weeks, followed by adequate P thereafter.
- (5) Initial withholding of P for six weeks, followed by adequate P thereafter.

The pots were flushed with water to wash out all residual nutrients. Nutrient solutions were added when the plants were thinned and every four weeks thereafter. Nutrient solutions A, B, C, D, and E, described in Appendix Table I, were added at a rate of 80 ml. each. Solution C was applied only to the pots receiving phosphorus.

Plant sampling (or harvest) periods were two, three, four and six weeks after the first addition of the nutrients and at the milk, soft dough,

and maturity stages. These were separated into tops, roots, and kernels at each sampling date, then were immediately placed in a drying oven at 65°C for 48 hours to obtain the dry weights. The dried samples were ground in a Wiley Mill and analyzed for various types of P.

Total P and total acid-soluble P were determined by the reduced molybdate colorimetric method described by Pons, et. al. (13).

Inorganic P was determined colorimetrically by the modified isobutyl alcohol procedure as described by Martin and Doty (10).

Lipid P was determined by extracting the samples with a benzene-alcohol azeotropic mixture, followed by ashing of the extracted lipids.

Inorganic P was determined in the ash as described above.

Acid-labile P was determined by hydrolyzing the material in 4 ml. of 1 N HCl for seven minutes at 100°C. After cooling, 6 ml. isobutanol, 1 ml. silicotungstic acid and 1 ml. neutral molybdate reagent (17) were added, and the inorganic P was estimated (7).

Acid non-labile P (phytic acid or phytin) was estimated by difference (7), i.e., acid non-labile P equals (total acid soluble P) minus (the inorganic P plus the acid-labile P).

Nucleic acid P was estimated by the difference, i.e., nucleic acid P equals (total P) minus (total acid-soluble P plus lipid P) (13). All of these procedures are detailed in Appendix A, page 51.

Statistical analyses were made and P treatments were significantly correlated with the changes of types of P in every growth stage in wheat roots, leaves and kernels.

RESULTS AND DISCUSSION

Influence of P and Stage of Growth on Dry Matter Yield and Total P Percentage

Total dry matter yield of the tops was significantly affected by P stress during the early stages of plants development, (see Figures 1 and 2). Withholding of P did not cause a yield decrease when the plants were harvested at the two-week stage. However, when the plants were three weeks old, the P stress during the first two weeks resulted in a decrease in the total dry matter production. This indicates that P stress was detrimental even though growth was not influenced during the first two weeks. As the period of initial P stress increased, the dry weight of the tops decreased irrespective of growth stage. Thus, it can be concluded that a P stress during the early stages of growth decreases the yield potential of spring wheat and this detrimental effect becomes greater as the stress period increases.

Grain yield of spring wheat decreased significantly as the period for withholding initial P increased (Figure 2). Grain yields at maturity were about 10 percent less when the plants were deprived of P for the first two weeks than when they received P continuously. Grain yields decreased as the period of withholding P increased.

Root yields decreased as the period for withholding initial P increased, as shown in Figures 1 and 2. However, the magnitude of this decrease was not as great as the decrease in the yield of the tops.

Maximum root growth was achieved at the milk stage of growth. Root weights decreased as the plants approached maturity. This indicates that the translocation of carbohydrates and other nutrients from the roots to other parts of the plant may have occurred at this time.

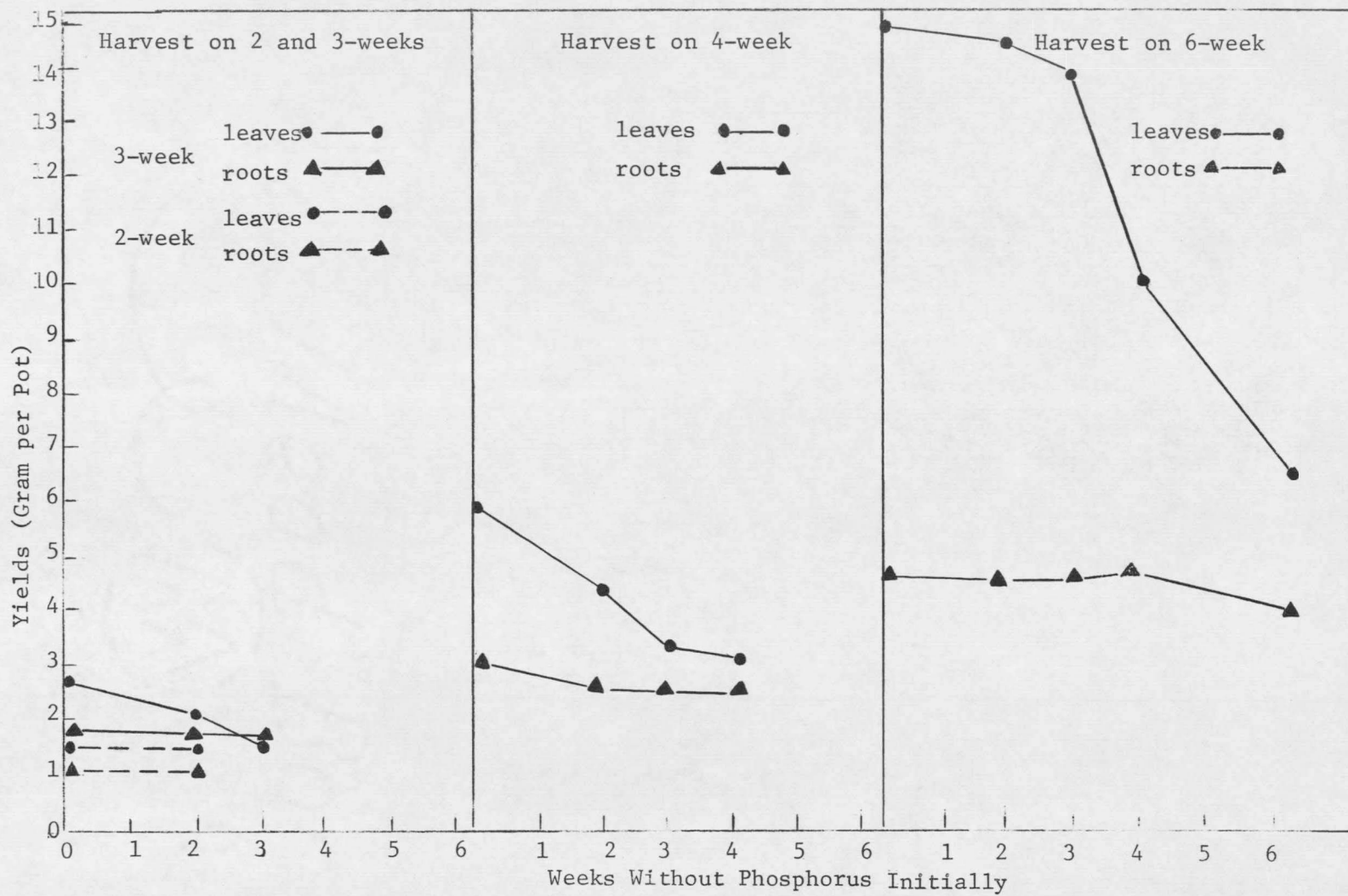


Figure 1. The Yields of Wheat Tops and Roots as Influenced by P Applications at Different Growth Stages. (up to six weeks)

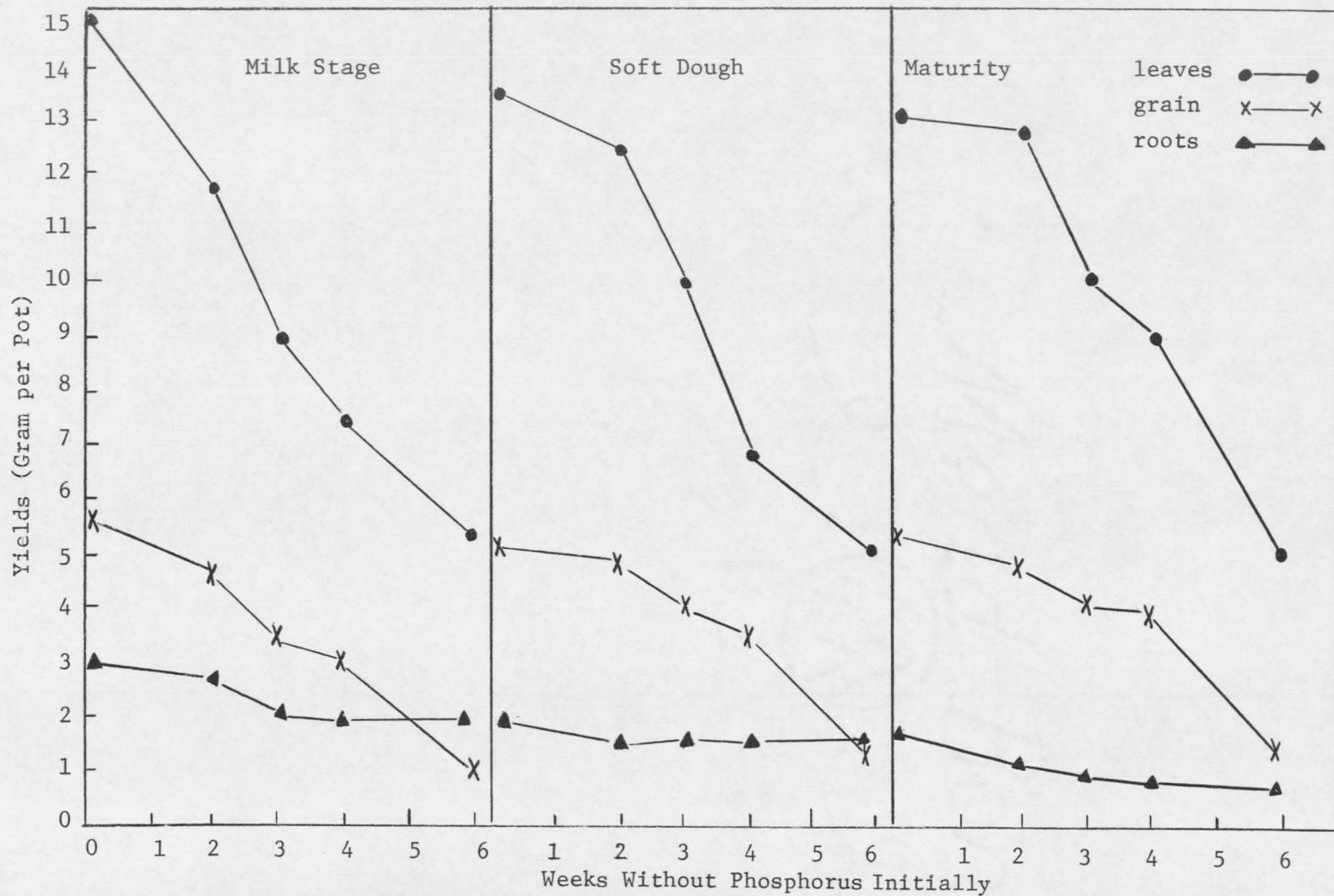


Figure 2. The Yields of Wheat Tops, Roots and Grain as Influenced by P Applications at Different Growth Stages. (milk stage to maturity)

Since the yield of all component parts of the wheat plant decreased when P was initially withheld, nucleic acid P may be involved. As the period for withholding initial P increased, nucleic acid P decreased (see pages 30-34). Reitz and Quisenberry (15) reported that P deficiency frequently resulted in an increased uptake of nitrogen by wheat plants which in turn resulted in increased accumulation of free amino acids. But the formation of protein was slowed down because of lack of P-containing enzymes. They suggested that synthesis of RNA and DNA probably would be decreased, thus restricting nuclear division and impaired the formation of protein and grain yields.

Total phosphorus concentration in the leaves of spring wheat plants as influenced by P treatments and stage of growth is illustrated in Figure 3. Total P concentration decreased in the leaves of the plants receiving no P, irrespective of the growth stage. When plants received P continuously, P concentration in the leaves decreased as the plants matured. When the plants were less than six weeks old, addition of P to plants initially deprived of it resulted in higher P concentration in the leaves than occurred when P was continuously supplied. This confirms the results of Boatwright and Viets (2). This may indicate that some mechanism for increasing uptake of P is triggered when plants that were initially deprived of P receive it thereafter. Addition of P to plants initially deprived of P for less than six weeks showed no significant decrease in total P concentration as the plants approached maturity. However, when P was withheld for six weeks, total P concentration in the leaves, at maturity, decreased.

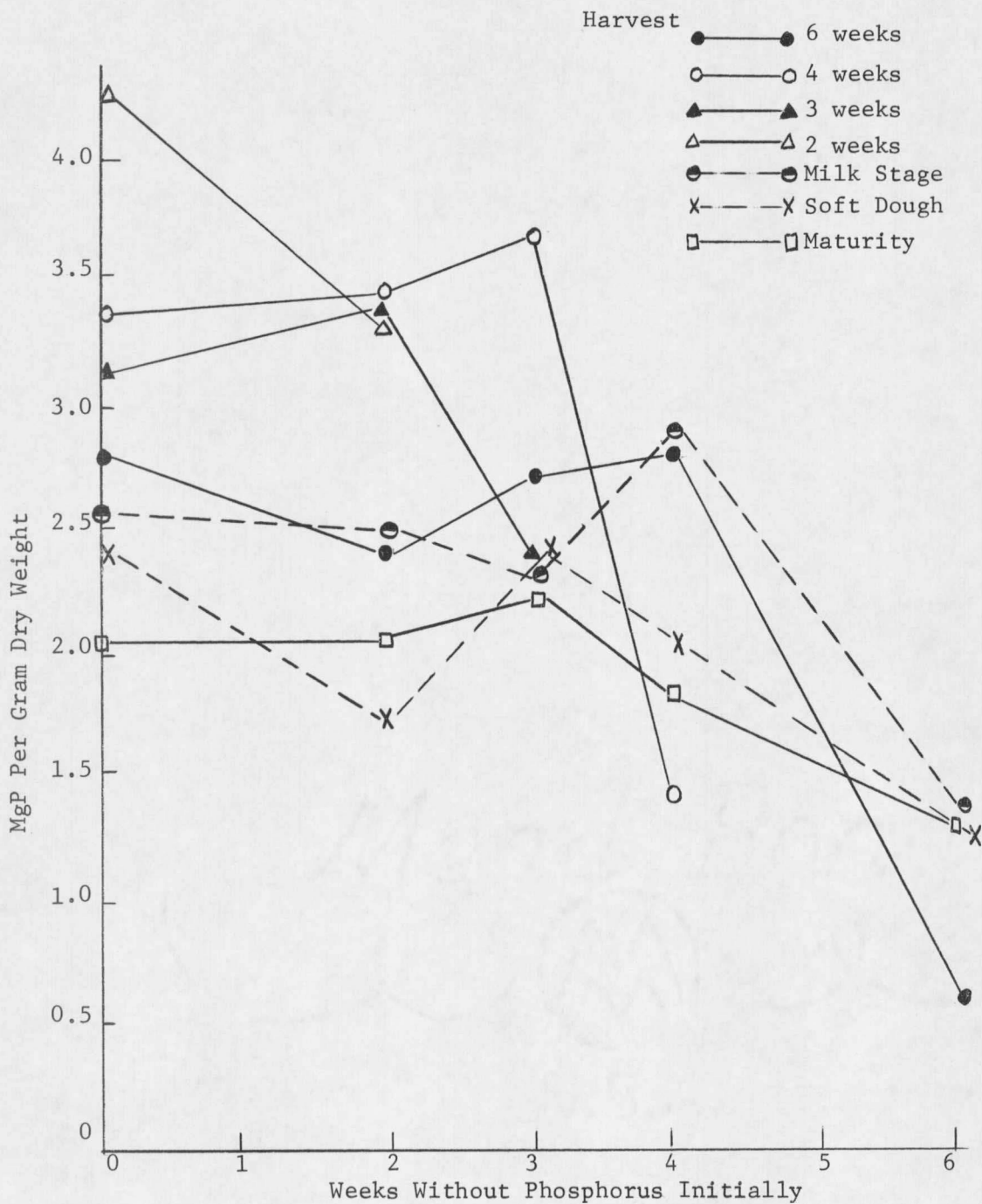


Figure 3. The Influence of Growth Stages and Time of P Applications on the Total P Concentration Present in Spring Wheat Leaves.

The total phosphorus concentration in the grain as influenced by P stress and stage of growth is shown in Figure 4. Total P concentration in the grain decreased at maturity as the period for withholding P increased from two weeks to six weeks. Initial withholding of P for two weeks did not lower the total P concentration at any stage of the grain development. Total P concentration at the soft dough and maturity stages was slightly higher in the grain when P was withheld for periods up to four weeks after emergence provided P was supplied during the later stages of growth. In all cases, plants deprived of P for the first six weeks yielded grain which was lower in total P concentration than plants which received P throughout the growth period.

The total phosphorus concentration in the roots of spring wheat plants as influenced by time of P application and stage of growth is illustrated in Figure 5. Total P concentration in the roots decreased at all stages of plant growth except the three-week stage as the period for withholding initial P supply increased, even after supplying adequate P thereafter. Plants harvested at the three-week stage yielded roots which was higher in total P content when P was omitted for the first two weeks than plants receiving P for the initial three-week period. This again indicates that some mechanism for increasing uptake of P may be triggered when the young plants are initially deprived of P but receive it thereafter. This stimulation effect of increasing of P uptake ceased when the plants grew older.

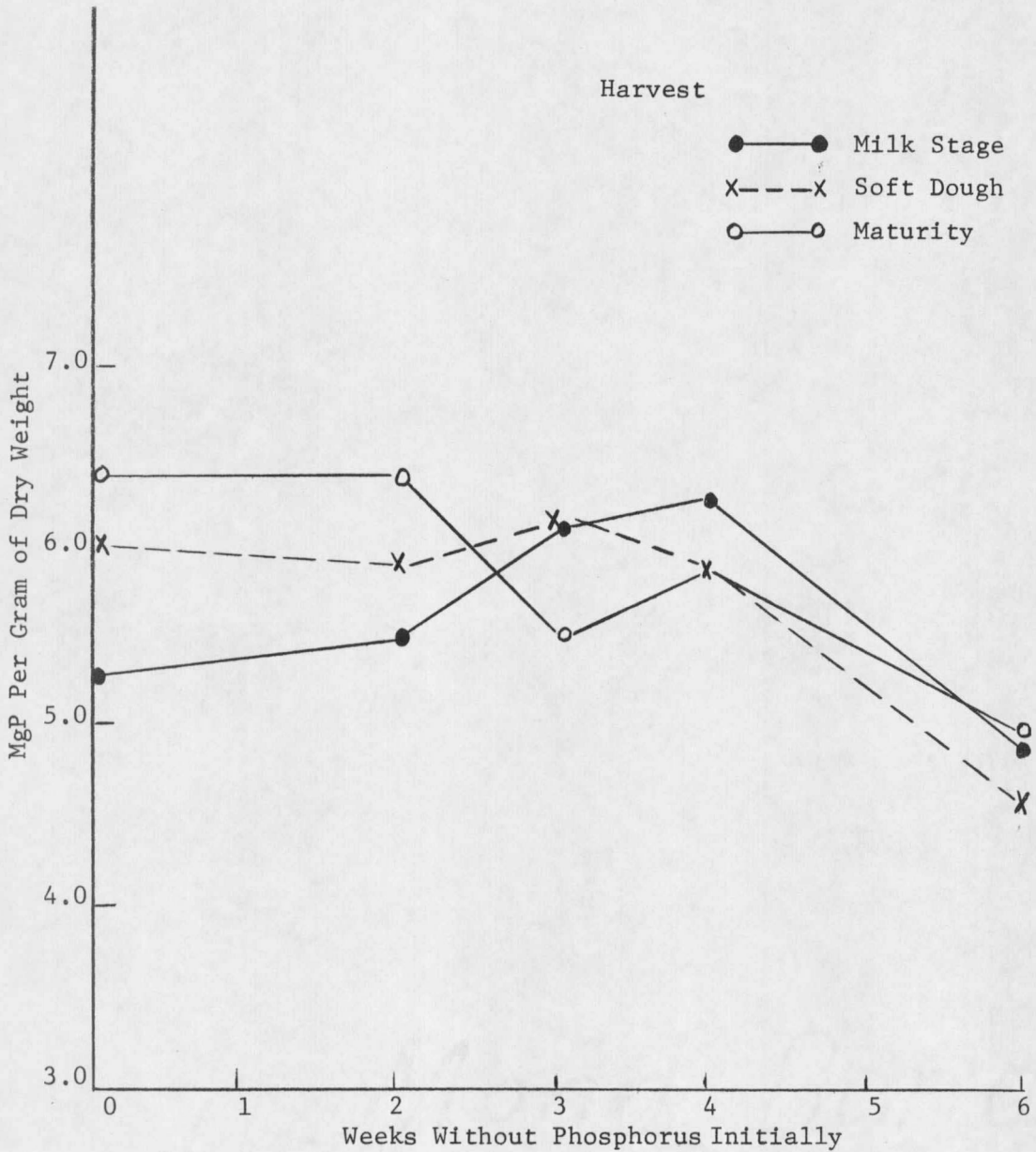


Figure 4. The Influence of Growth Stages and Time of P Applications on the Total P Concentrations Present in Spring Wheat Grains.

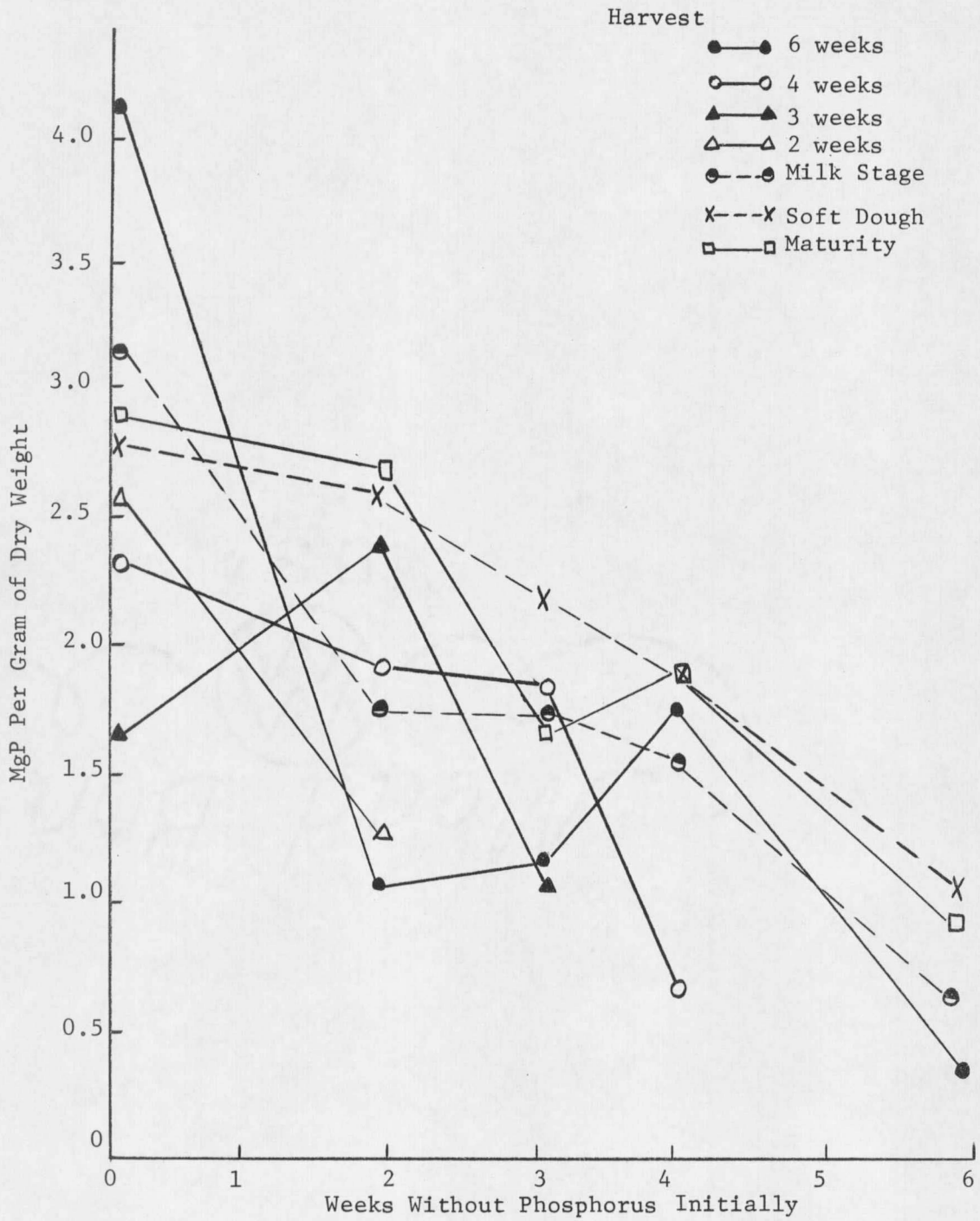


Figure 5. The Influence of Growth Stages and Time of P Applications on the Total P Concentrations Present in Spring Wheat Roots.

The total phosphorus uptake by wheat plants as influenced by initial P deficiency and growth stage is shown in Figure 6. Omitting P initially caused a significant decrease in total P uptake by plants as expressed by mg. of total P uptake per pot (one pot contains 22 plants) at various growth stages. The P uptake decreased significantly as the period for omitting initial P increased. This is due mainly to the effect of P deficiency on the yields of wheat plants as previously discussed. Secondly, when the plants were less than six weeks old, P uptake was apparently affected by the stage of growth. When the plants were harvested before the milk stage, the total P uptake increased, irrespective of the time of P application. When the plants grew older, beyond the milk stage, P uptake was not significantly affected by the stage of growth.

INFLUENCE OF PHOSPHORUS AND STAGE OF GROWTH
ON TYPES OF PHOSPHORUS IN SPRING WHEAT LEAVES

At the two-week growth stage (Figure 7), the percentage of total P in nucleic acid and lipid P fractions decreased when P was omitted. Whereas the percentage of acid-soluble, inorganic, phytic acid, and acid-labile P increased slightly when P was omitted.

At the three-week growth stage (Figure 8), plants which received no P contained a greater percentage of the total P as nucleic acid P. This appears to be at the expense of acid-soluble, inorganic, and phytic acid P. These data are interesting since a complete reversal in the predominant types of P occurred between the second and third week. This indicates that P stress affected the metabolic activity as the plants

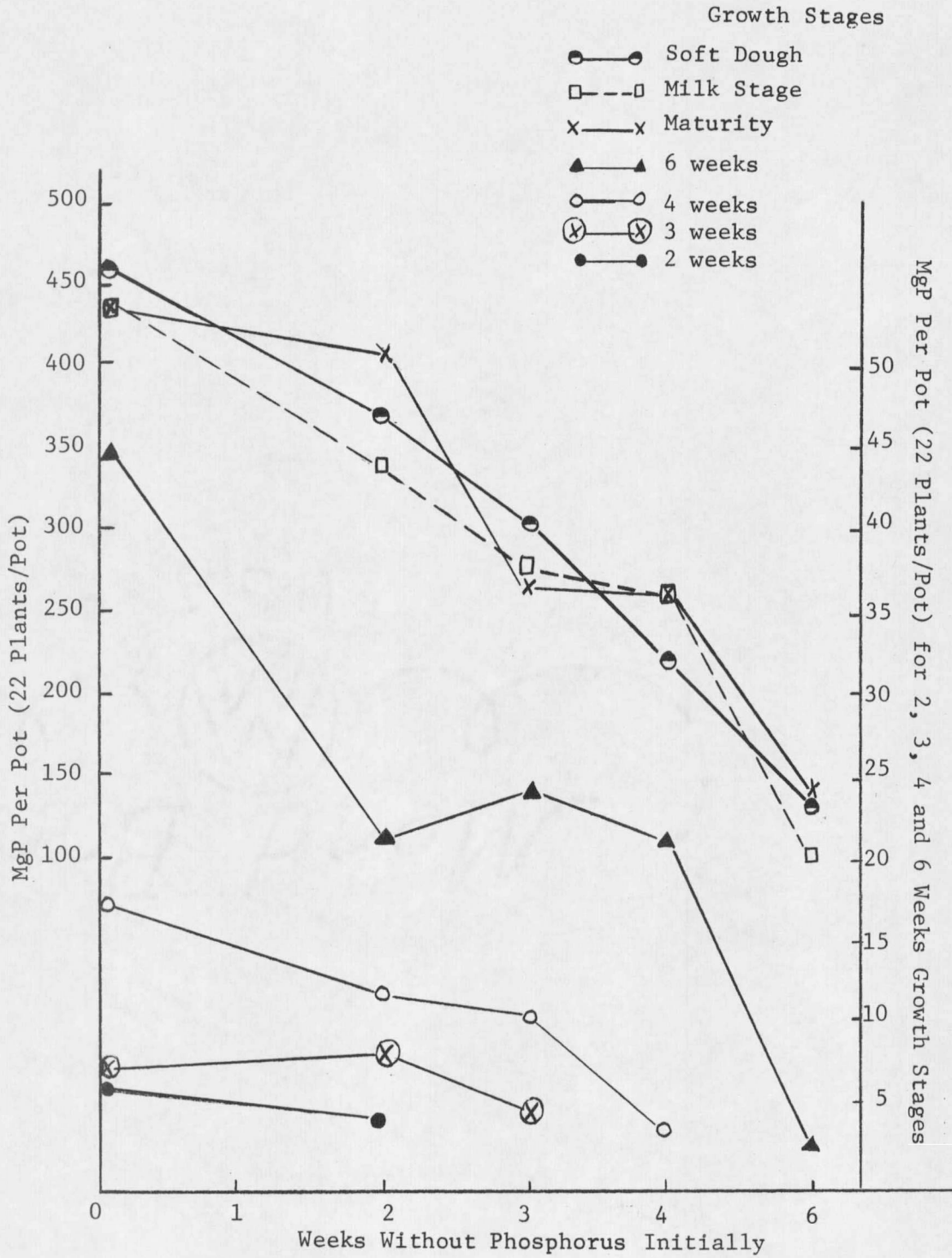


Figure 6. The Influence of Time of P Applications on Total P Uptake by the Spring Wheat at Various Growth Stages.

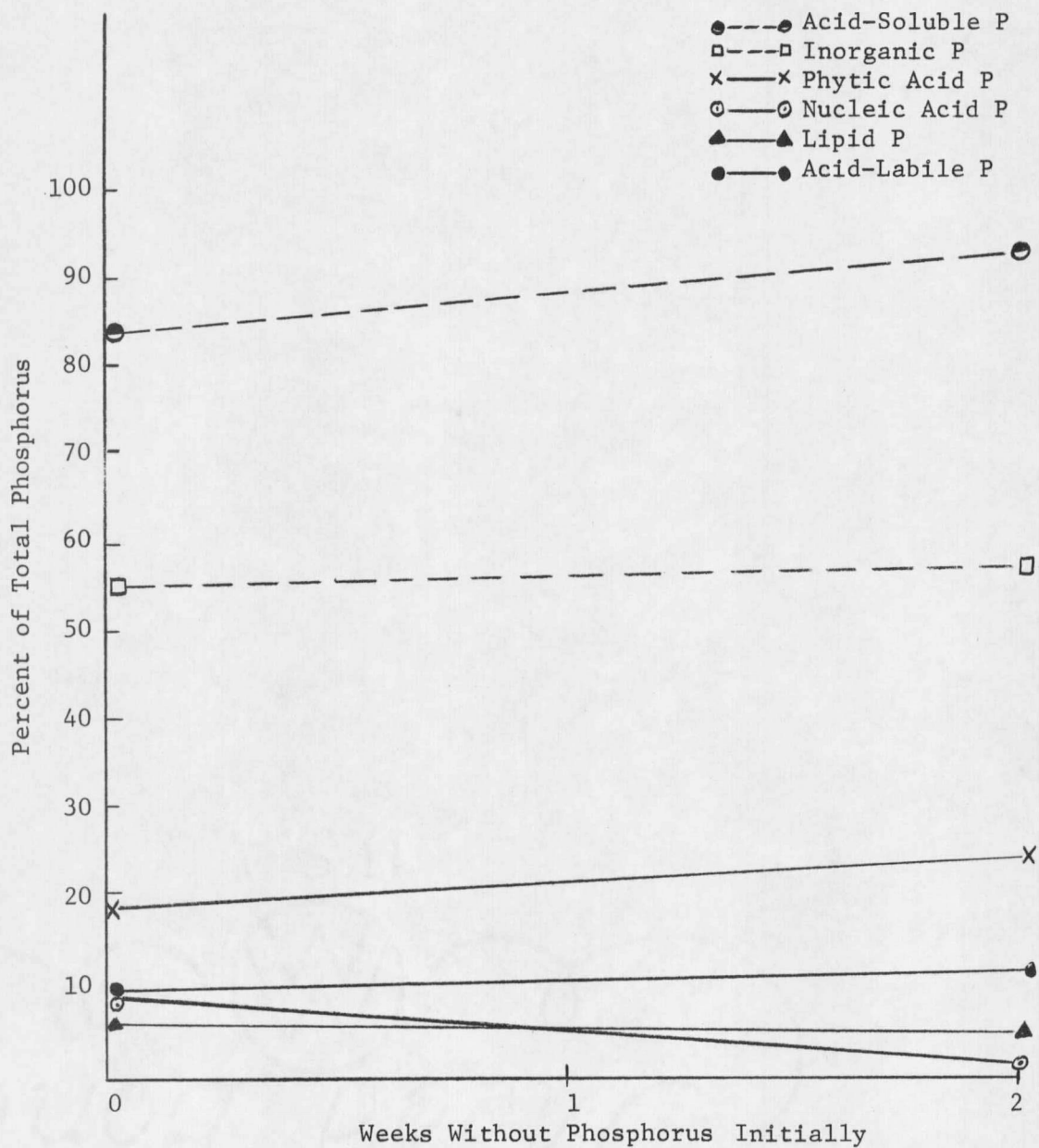


Figure 7. The Influence of Time of P Application on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at 2-week Growth Stage.

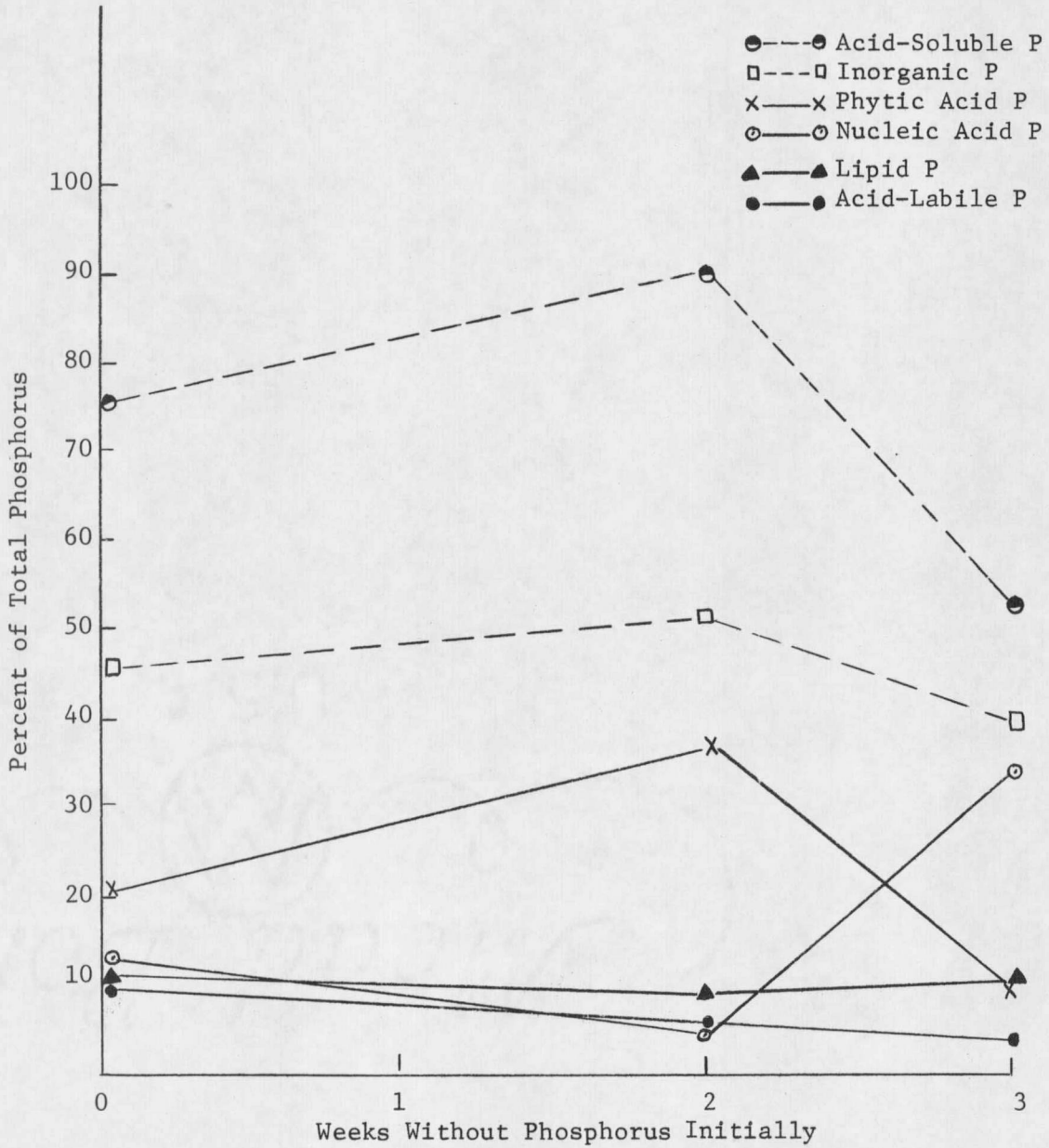


Figure 8. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at 3-week Growth Stage.

increased in age. When P was initially omitted for two weeks, the addition of adequate P for one week resulted in a decline in the percentage of nucleic, lipid, and acid-labile P. This was accompanied by an increase in the percentage of the total P in the form of acid-soluble, inorganic, and phytic P. Nucleic acid P was not synthesized although the plants were supplied with inorganic P between the second and third weeks. This indicates that in this early stage of plant growth, a P stress altered the mechanism by which inorganic P is converted to nucleic acid P. The data also suggest that inorganic P was converted to phytic acid P.

At the four-week growth stage (Figure 9), plants that were deprived of P for the first two weeks but received P thereafter produced leaves with higher concentration of acid-soluble, inorganic, and phytic acid P but lower in nucleic acid P than plants that received P continuously. When P was applied at three weeks or entirely omitted, there was no change in acid-soluble, inorganic, and nucleic acid P in terms of percentage of total P. The data indicate that a P stress during the first two weeks causes a decrease in nucleic acid P synthesized in the leaves. However, when P was omitted for longer periods of time the plants apparently adjusted to the low P level and were able to synthesize nucleic acid when supplied with inorganic P. The low rate of nucleic acid P synthesis at the early stage of growth could be due to the enzyme system which may be responsible for this synthesis being less active at that time. The slower rate of nucleic acid P synthesis resulted in more available P for synthesis of phytic acid P.

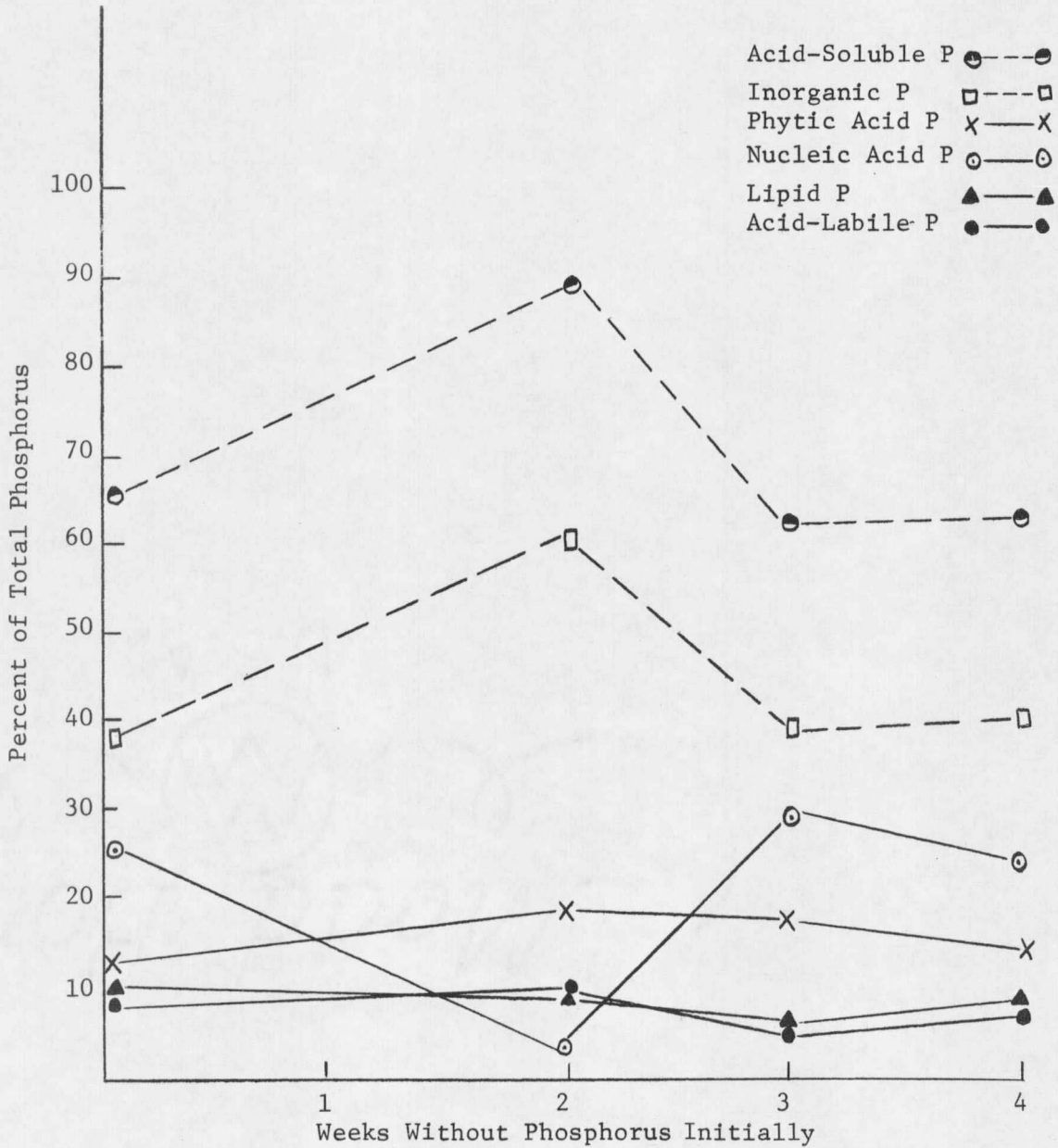


Figure 9. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at 4-week Growth Stage.

At the six-week growth stage (Figure 10), plants that were deprived of P for the first two weeks, but received P thereafter, produced leaves that showed an increase in acid-soluble P and phytic acid P accompanied by a sharp decrease in nucleic acid P. The same suggestion which explained the sharp decrease of nucleic acid P at the four-week growth stage could be again applied here. It is interesting to note that acid-labile P, which contains ATP, decreased when P was omitted for the first two weeks. This may be responsible for the synthesis of phytic acid P. Since inorganic P is required for synthesis of ATP (adenosine 5-triphosphate), the decrease in inorganic P may indirectly account for the increased conversion of phytic acid P. When plants were initially deprived of P for three and four weeks, the leaves harvested at six weeks showed an increase in phytic acid P. All other types of P either decreased or remained about equal in amount to those when P was always present. Inorganic and acid-labile P were the two P compounds showing the greatest decrease due to a P deprivation for the first three or four weeks. These data indicate that a P stress during the period of maximum growth resulted in an increase in the synthesis of phytic acid P. Phosphorus stress during the very early stage of growth, increased the synthesis of phytic acid P and this appears to be at the expense of nucleic acid and inorganic P. Phosphorus stress during the later stages of growth resulted in increased synthesis of phytic acid P at the expense of acid-labile and inorganic P.

When P was withheld for the entire growth period, the percentage of the total P in the form of acid-soluble, nucleic acid, acid-labile,

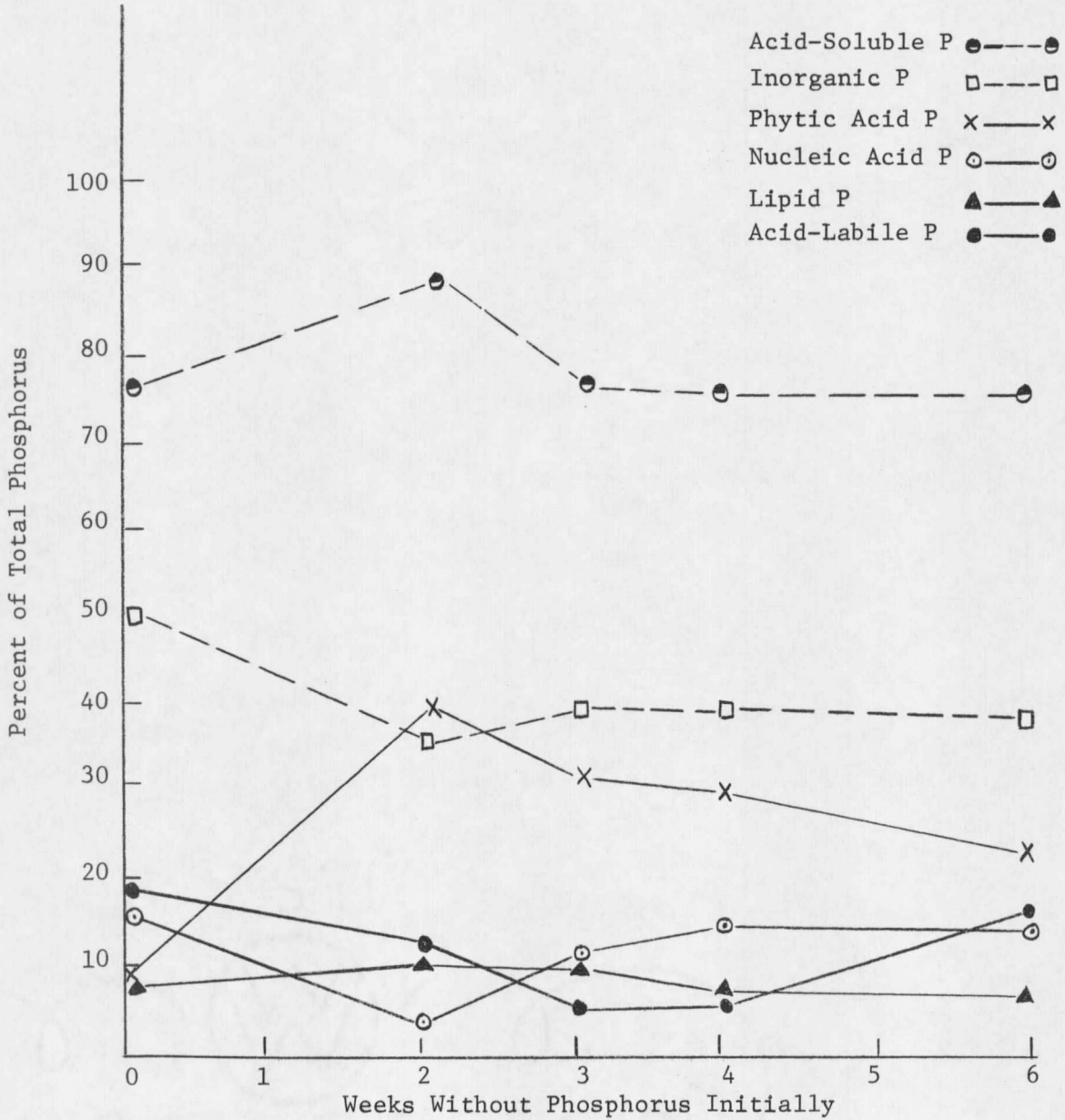


Figure 10. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at 6-week Growth Stage.

and lipid P fractions were about equal to those when P was continuously supplied. However, the percentage of phytic acid P increased whereas inorganic P decreased.

At the milk stage or beyond (Figures 11-14), over 90 percent of the total P was in the inorganic type. Phosphorus stress for various periods of time did not significantly influence P types in the leaves at later growth stages. These data indicate that P translocation from the leaves was not influenced by the kind of P present in young leaves, because young leaves contained different amounts of the various forms of P. At the later stages of growth only total P uptake was influenced by early P stress. This indicates that the synthesis rates of the organic types of P from inorganic P were very low when the plants were old. It is suggested that the plants were unable to convert inorganic P into organic types because the enzyme systems which catalyze these conversions became inactive as the plants matured.

The influence of growth stage on the synthesis of various types of P, when it was continuously supplied, is presented in Figure 14. When the plants increased in age from two to four weeks, inorganic P decreased in the leaves. From the fourth week until the milk stage, inorganic P increased sharply up to 90 percent of the total P. From milk stage to maturity, the inorganic P remained constant.

The stage of growth influenced acid-soluble P and inorganic P similarly. The amount of P in the acid-soluble fraction was greater at all growth stages than the amount of inorganic P. This is expected because acid-soluble P includes inorganic, phytic acid and acid-labile P.

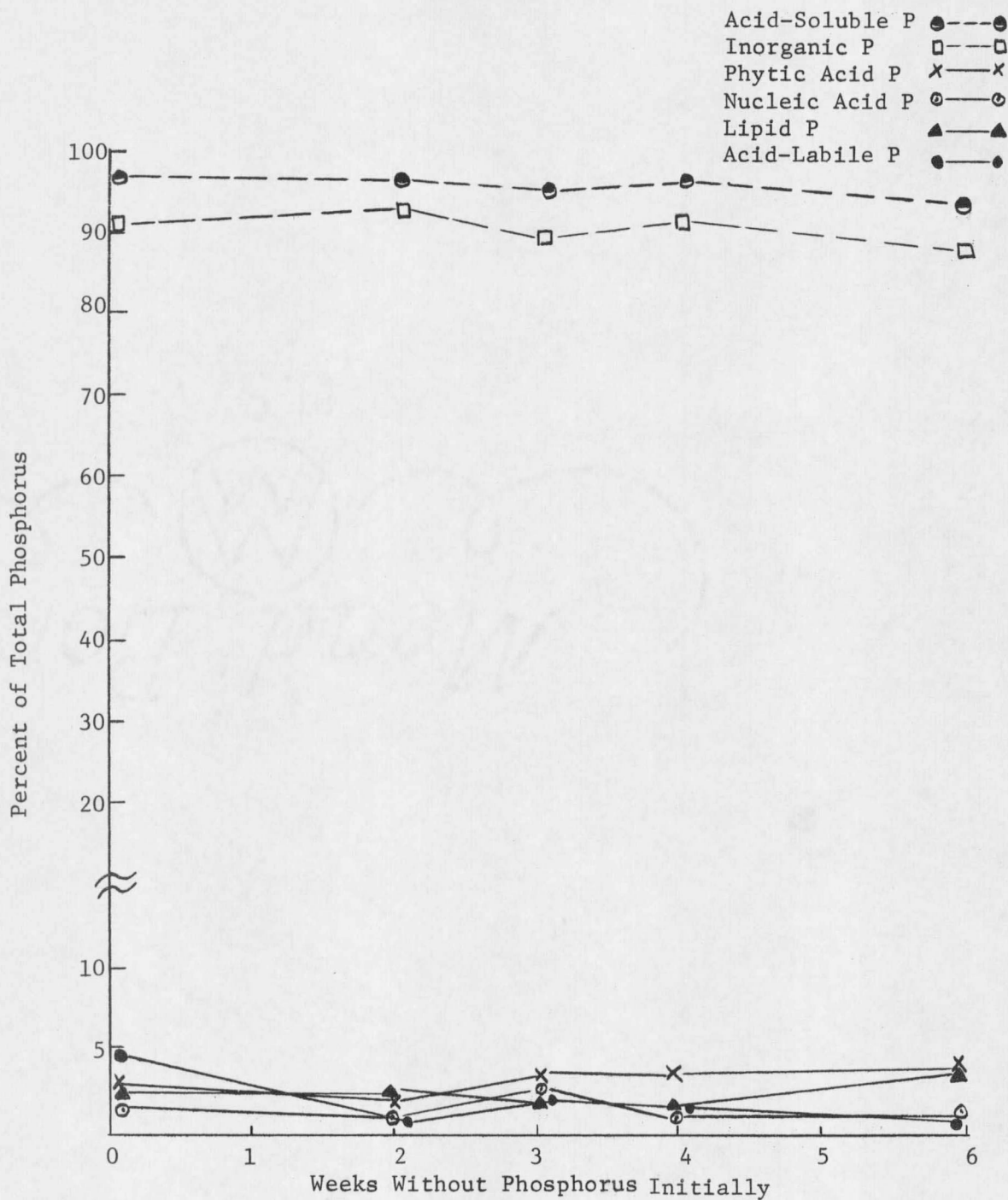


Figure 11. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at Milk Stage.

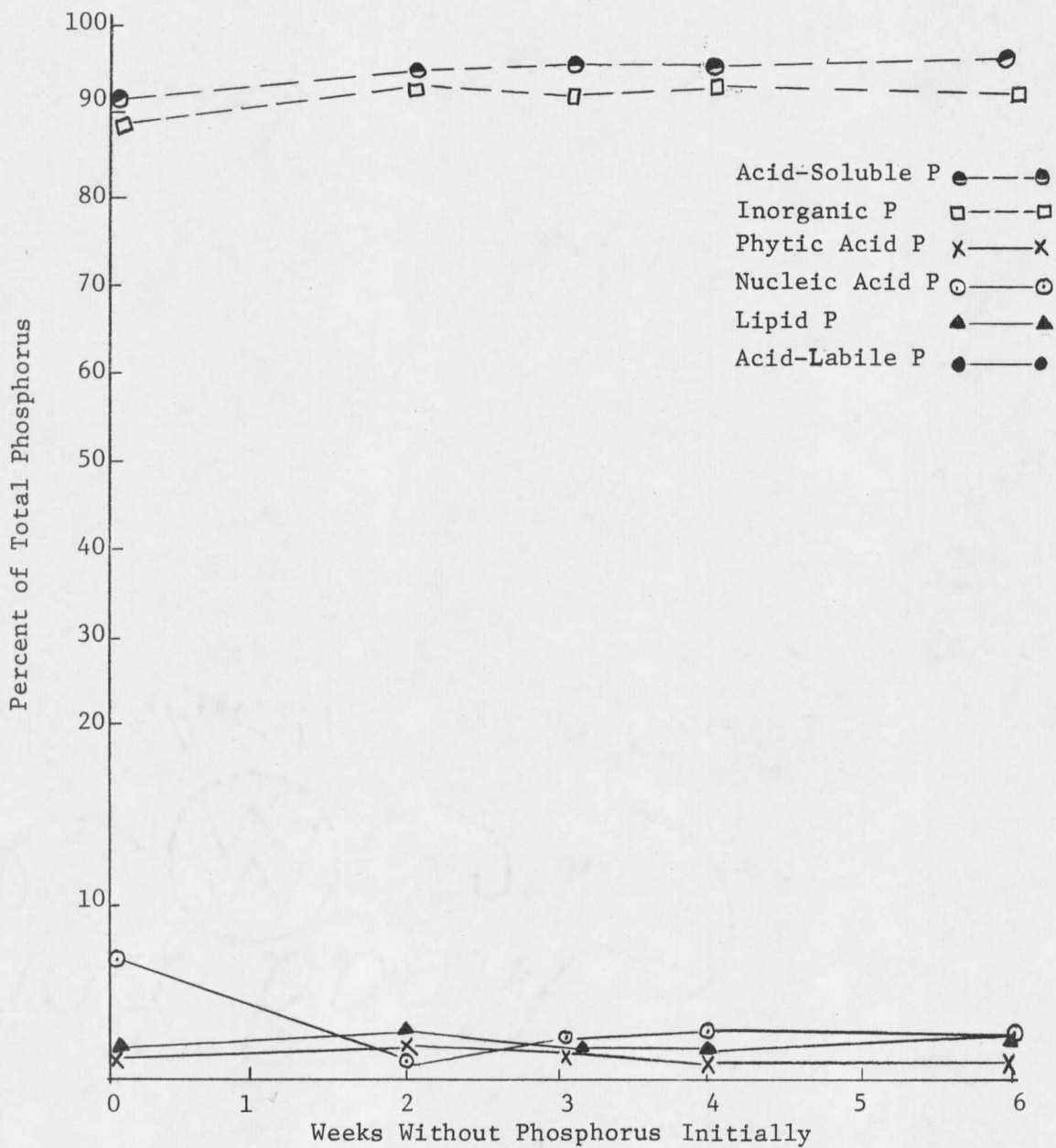


Figure 12. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at Soft Dough Stage.

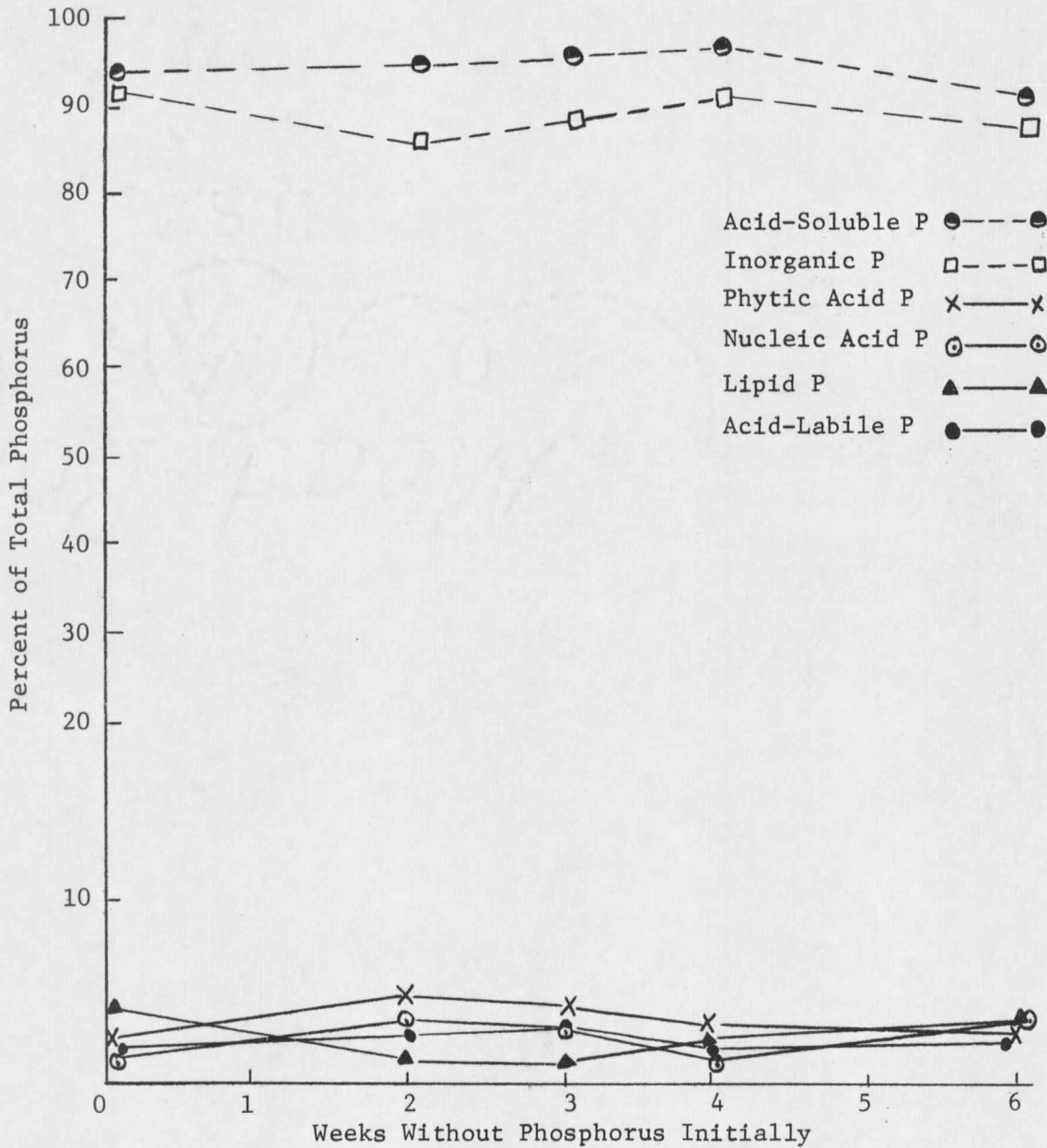


Figure 13. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves at Maturity.

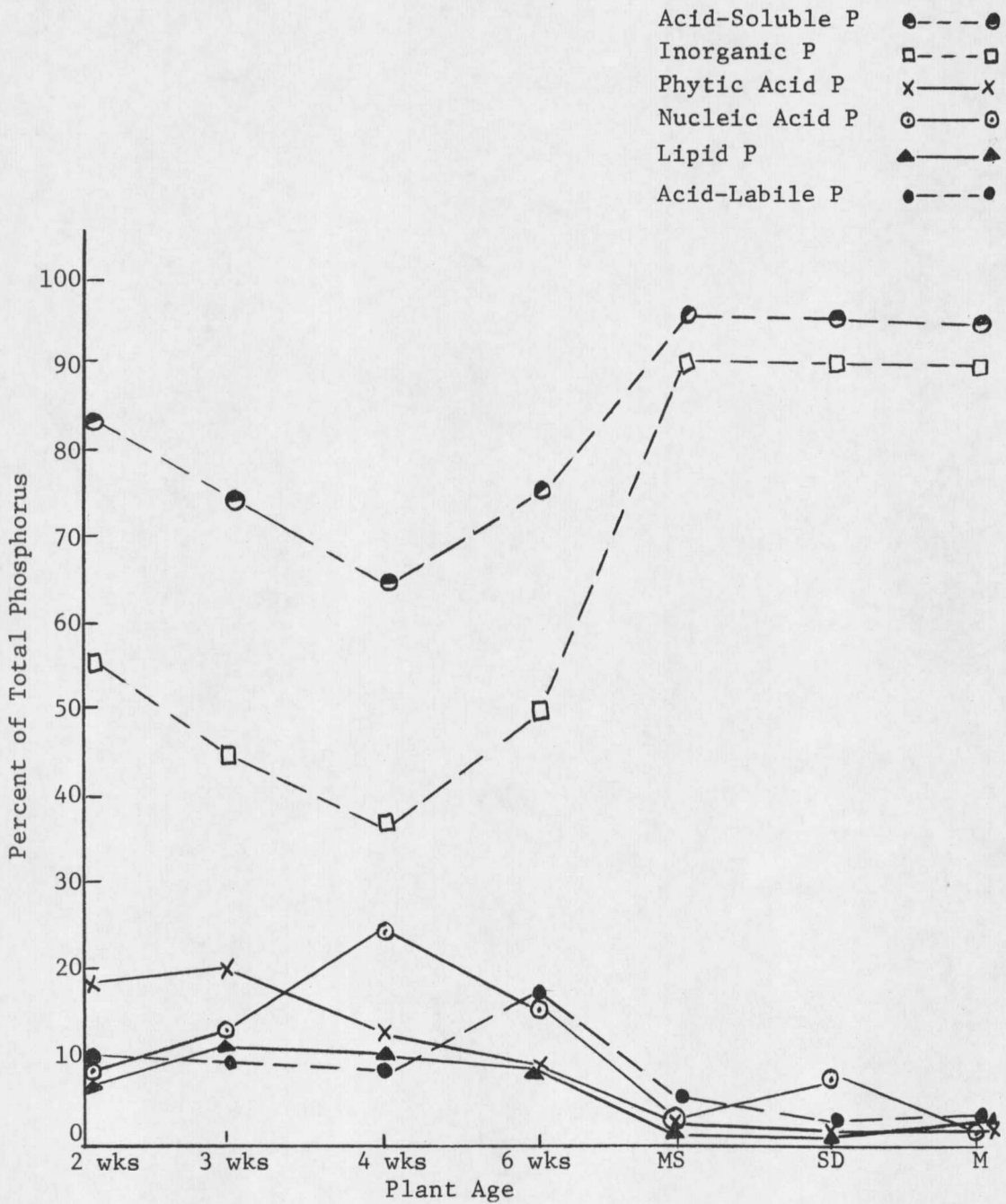


Figure 14. The Influence of Growth Stages on Different Types of P (Percentage of the Total P) in Spring Wheat Leaves when P Supply is Adequate.

The percentage of P in the nucleic acid fraction increased as the plant increased in age from two to four weeks. From four weeks to the milk stage of growth, nucleic acid P decreased. It is interesting to note that at the four-week growth stage, nucleic acid P made up 25 percent of the total P. In contrast, when plants were deprived of P for the first two weeks, then supplied with it thereafter, the plants contained only about 1 percent nucleic acid P. This indicates that a P stress for the first two weeks may restrict synthesis of nucleic acid which may be very critical for the proper development of wheat plants.

Phytic acid P represented about 20 percent of the total P up to the time of the third week in growth. From the third week until the milk stage phytic acid P decreased. In general, when P was supplied to plants initially deprived of it, phytic acid P increased in the leaves. This indicates that phytic acid P decreases in normal plants with age. However, an inverse relationship occurred when plants were initially subjected to a P stress.

CHANGES IN TYPES OF PHOSPHORUS IN THE WHEAT PLANT KERNELS AS INFLUENCED BY P STRESS AND GROWTH STAGES

In contrast to tops and roots (pg. 36), when plant age was between the milk and maturity stages (Figures 15-17), the kernels contained a higher percentage of organic types of P than inorganic types. As the plants approached maturity the percentage of the total P in inorganic fractions decreased. Time of P application did not significantly influence the amount of P in the inorganic fraction. The average inorganic P (for

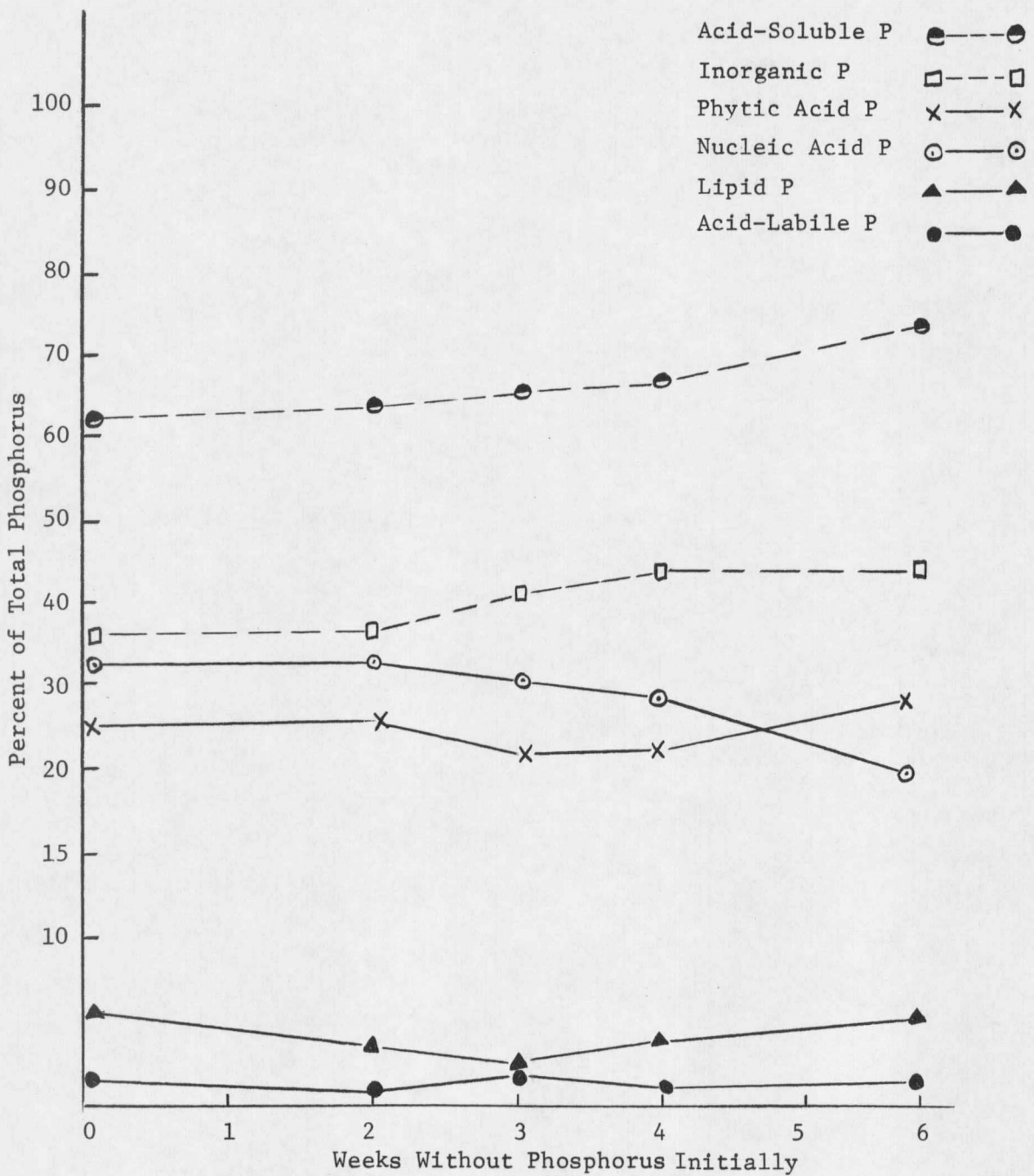


Figure 15. The Influence of Time of P Application on Different Types of P (Percentage of the Total P) in Spring Wheat Kernels at Milk Stage.

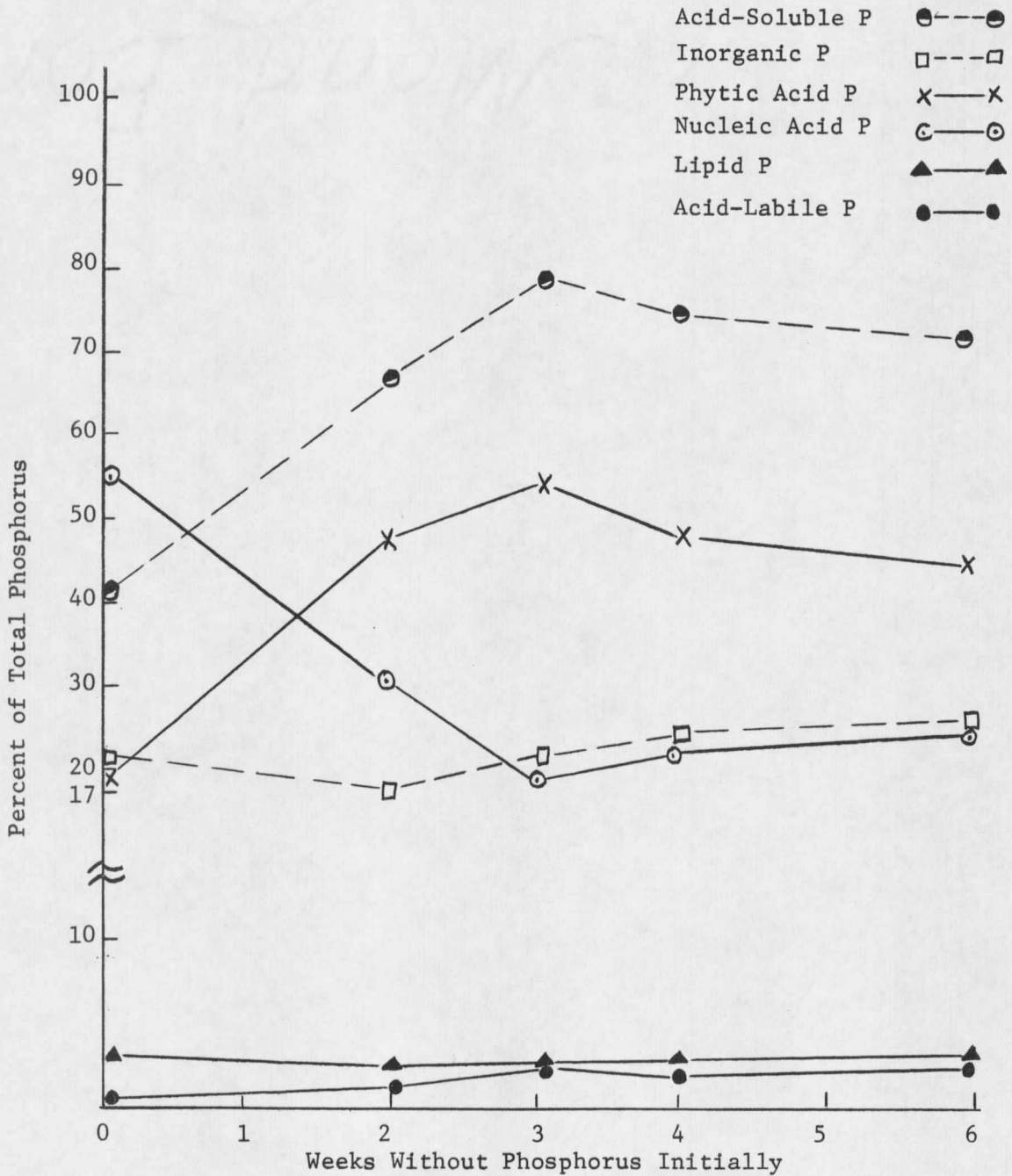


Figure 16. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Kernels at Soft Dough Stage.

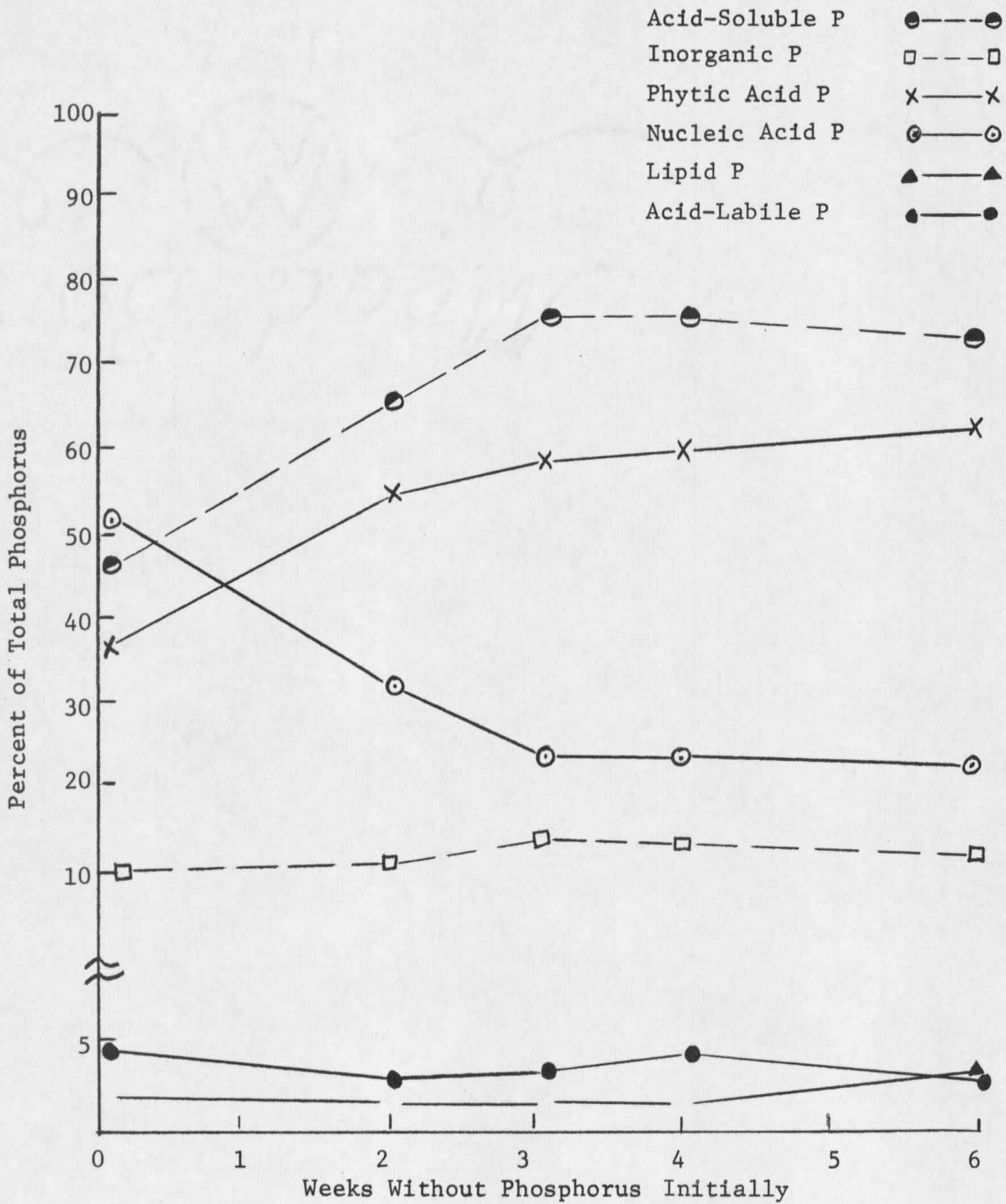


Figure 17. The Influence of Time of P Applications on Different Types of P (Percentage of the Total P) in Spring Wheat Kernels at Maturity.

every P treatment) at the milk stage was about 42 percent of the total P or 2.64 mg/g sample. When the plants approached the soft dough stage, inorganic P decreased to 21 percent of the total P or 1.28 mgP/g sample. At maturity, the inorganic P decreased further to an average of 12 percent of the total P or 0.65 mg/g sample.

Phytic acid P showed an inverse trend. The percentage of the total P in the phytic acid fraction increased with increase in plant age, irrespective of P application. This finding is in agreement with the early investigations of Jennings and Morton (7). They showed that the inorganic P sharply decreased in both the testa-pericarp and endosperm of wheat kernels as the age of plants increased. Jennings and Morton suggested that the existence of an active enzyme system in both the endosperm and the testa-pericarp of the kernels which would catalyze the conversion of inorganic P into organic acid-non-labile P (phytic acid).

Hoffman, Ostenhof, Jungwirth, and David (7) showed that crude preparation of yeast kexokinase contains an enzyme which catalyzes the phosphorylation of inositol from ATP. However, little information is available concerning the enzymatic synthesis of phytic acid (7).

Nucleic acid P increased in the grain from adequately fertilized plants from about 32 percent of the total P at the milk stage to about 52 percent at the soft dough stage. Further increases in percentage of nucleic acid P did not occur between the soft dough and maturity stages. Plants that were initially deprived of P yielded grains with 30 percent

or less of the total P in the nucleic acid fraction in the period from the milk stage to maturity.

At the milk stage of growth (Figure 17), the percentage of total P, in the form of inorganic and acid-soluble P, in the grain, increased slightly as the period of withholding initial P increased. At this stage of growth the rate of synthesis of phytic acid P was relatively slow since inorganic and nucleic acid P remained relatively constant.

At the soft dough and maturity stages (Figures 18 and 19), the percentage of total P increased significantly in the acid-soluble and phytic acid P fractions as the period for withholding P increased. The maximum rate of increase occurred when P was initially withheld for the first three weeks. Longer periods for withholding P did not further increase the percentage of total P in the form of acid-soluble P. At maturity, the percentage of total P in the phytic acid form increased as the period of withholding P increased. These data indicate that synthesis of phytic acid P may continue until maturity when P was withheld for longer periods of time. The increase in the percentage of acid-soluble and phytic acid P appears to be at the expense of inorganic P. The percentage of the total P in the nucleic acid P fraction decreased sharply as the period for withholding initial P increased from zero to three weeks. Longer periods of P omission (from three to six weeks), did not result in any further decrease in the percentage of nucleic acid P. Evidently, at the soft dough stage, the conversion of inorganic to phytic acid P is very rapid. This is in agreement with the findings of Jennings (7). These data

suggest that when inorganic P is limited at the early growth stages, the metabolic processes may be altered and the synthesis of phytic acid P may be encouraged. Also, early P stress appears to restrict the amount of nucleic acid synthesized. Again, the metabolic processes may have been altered or the competition for available inorganic P may have been greater in the synthesis of phytic acid P than for the synthesis of nucleic acid P.

This data also indicate that P deficiency or decrease of P uptake bears important relation to cell division in the kernels. The P deficiency due to initial withholding of the P supply resulted in decrease in the nucleic acid P (15) and suggests a decrease in RNA and DNA contents in the kernels. The DNA is important in nuclear and cell division. RNA is important in protein synthesis; both are related to the yield of the grain. The decrease in nucleic acid may be responsible for the decrease in the yield of grain.

CHANGES IN TYPES OF P IN THE ROOTS AS INFLUENCED
BY TIME OF P APPLICATION AND GROWTH STAGES

At the two-week growth stage (Figure 18), the roots of plants receiving no P decreased in the percentage of total P in the nucleic acid P fraction, but increased slightly in all other types of P. The synthesis of the other P fractions appears to be at the expense of nucleic acid P. The lack of nucleic acid P synthesis in the roots may be an important factor because the withholding of P for the first two weeks seems to be very critical with respect to the synthesis of other P compounds in the plant system. An increase in the synthesis of nucleic acid resulted when P was initially withheld for periods longer than two weeks. This

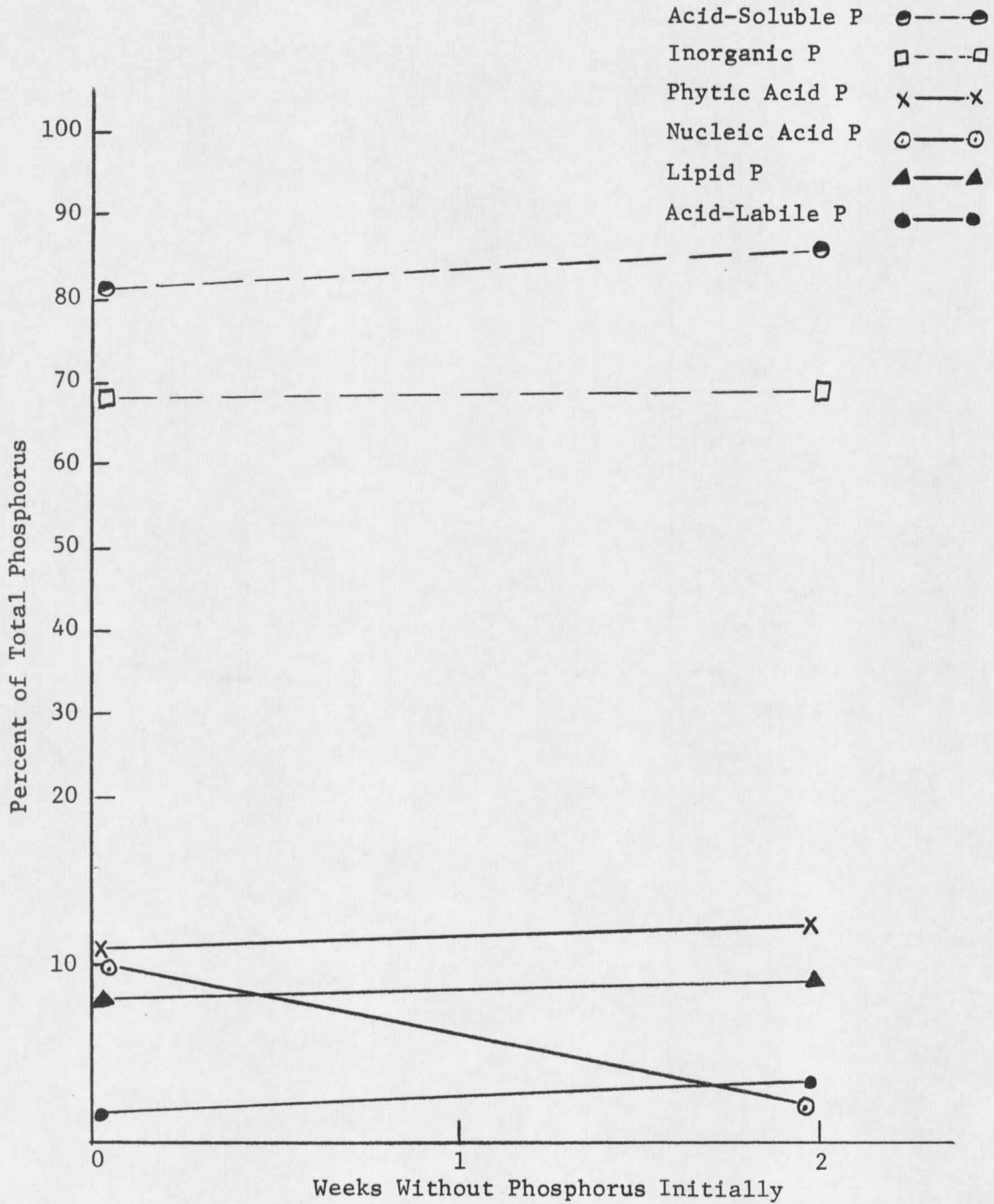


Figure 18. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at 2-week Growth Stage.

complete reversal may lead to the conclusion that changes in metabolism within the roots occurred between the second and third weeks when P was omitted at this early stage.

At the three-week growth stage (Figure 19), the roots of plants that never received P converted more of the total P to nucleic acid P than when P was continuously supplied. The increase in nucleic acid P resulted in a decrease in the percentage of total P in the inorganic and acid-soluble forms. Since the two-week P stress resulted in a slight decrease in the synthesis of nucleic acid by the roots and the three-week P stress caused an increase in synthesis of nucleic acid P, it appears that the plants may have over-adjusted themselves to the low P supply. When inorganic P was initially applied to the plants deprived of P for the first two weeks, the percentage of the total P converted to nucleic acid and lipid P decreased. On the other hand, the percentage of total P in the phytic acid and acid-soluble P forms increased. These results indicate that an early P stress followed by P fertilization results in an abnormally high concentration of phytic acid P within the root system.

At the four-week growth stage (Figure 20), the roots showed a decrease in nucleic acid P and a slight decrease in lipid P percentage of total P when P was initially omitted for two weeks then supplied thereafter. Further omission of P further decreased the nucleic acid and lipid P only slightly. Complete deprivation of P supply resulted in an increase of nucleic acid and lipid P as percent of total P. A reverse trend was observed in inorganic and phytic acid P. These increased then decreased as the withholding of P supply continued. This data indicate that

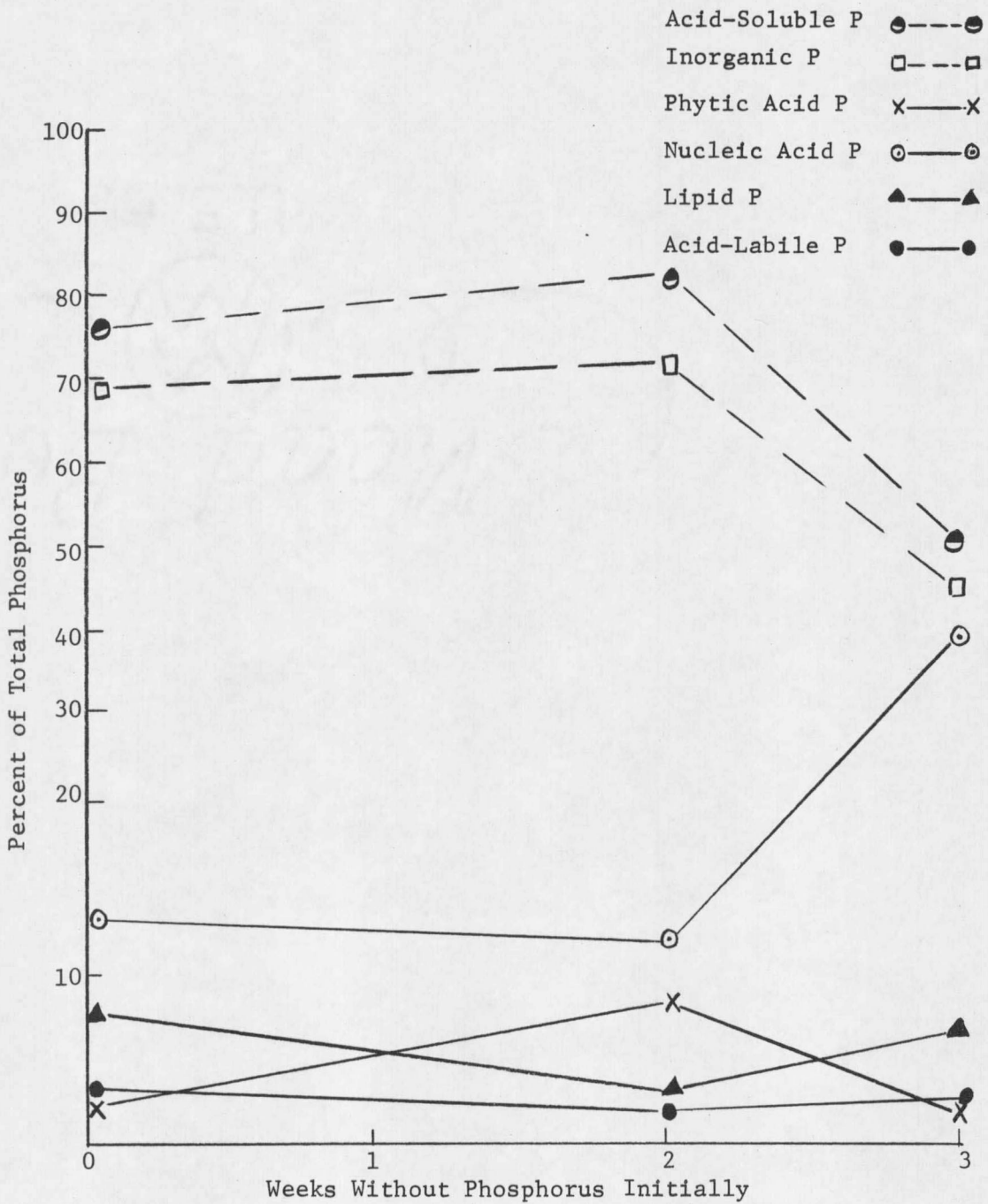


Figure 19. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at 3-week Growth Stage.

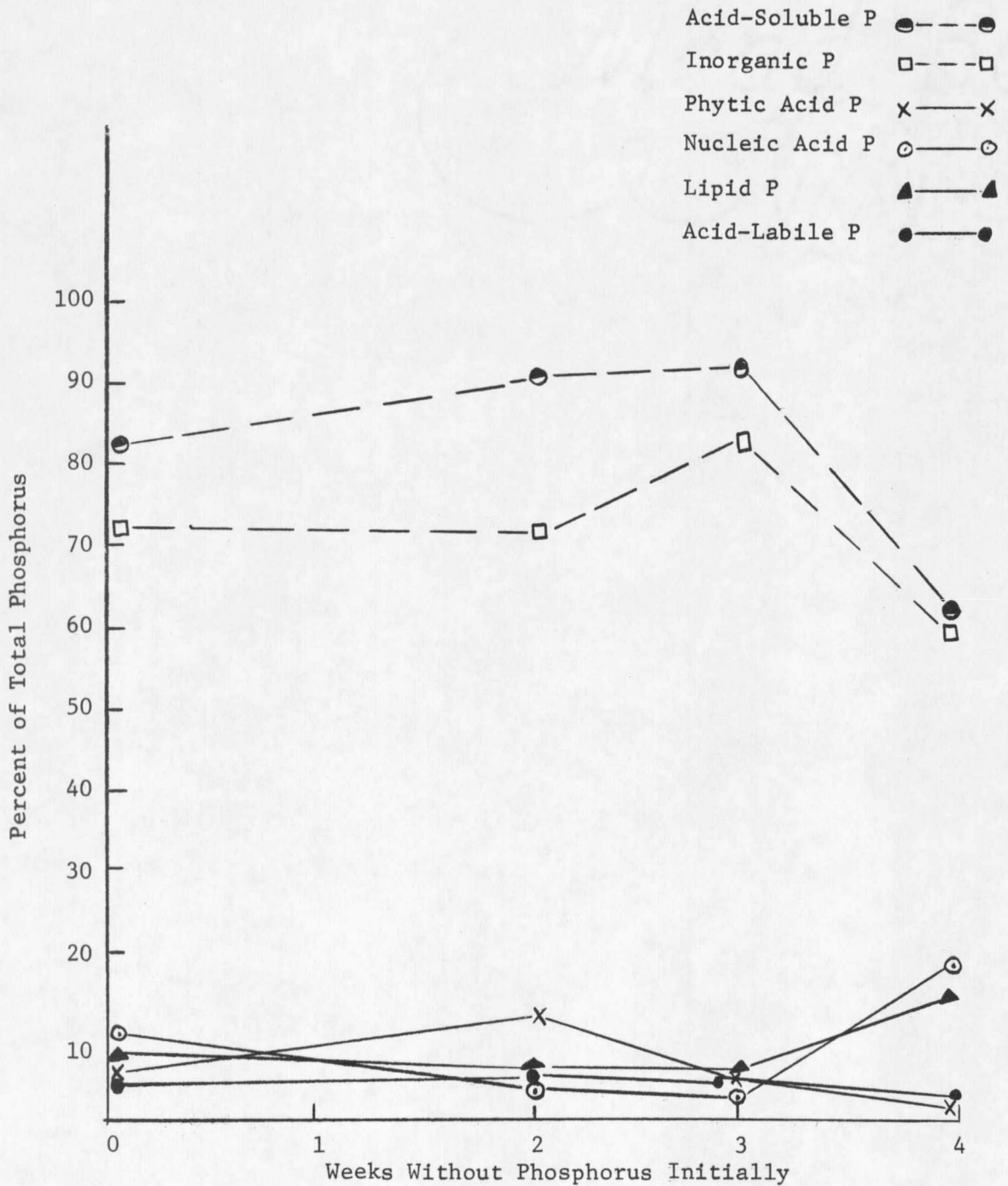


Figure 20. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at 4-week Growth Stage.

the increase in nucleic acid and lipid P takes place at the expense of inorganic and phytic acid and vice versa. The data also indicate the importance of nucleic acid P to plant nutrition. Omitting P at two weeks of growth resulted in a higher decrease in nucleic acid P causing a steeper decrease in root yield (Figures 20 and 21). Further omission of P to three weeks caused a slight decrease in nucleic acid P, and hence, a slight decrease in root yield.

At six-week growth stage (Figure 21), the roots did not show much change in types of P when the initial deprivation of P was for less than three weeks. Further initial deprivation or complete deprivation of P resulted in an increase in nucleic acid and lipid P as percent of total P but a decrease in organic, acid-soluble and phytic acid P. The increase in nucleic acid P and lipid P was at the expense of inorganic and phytic acid P when P was initially omitted for four weeks thus accounting for the slight increase in root yield. Roots receiving no P unexpectedly showed a reduction in yield even though they showed higher nucleic acid P concentration. This can be explained in that nucleic acid P and lipid P concentration increased, but total P decreased (Appendix Table IV). In other words, the roots receiving no P were still in a P deficiency condition and P deficiency would result in decreased protein synthesis (15). This could result in a reduction in root weight. The data show at all stages of growth that changes in lipid P in the roots were similar to nucleic acid P changes. This suggests that lipid P is an important constituent of root cells membrane (7). The increase in cell division

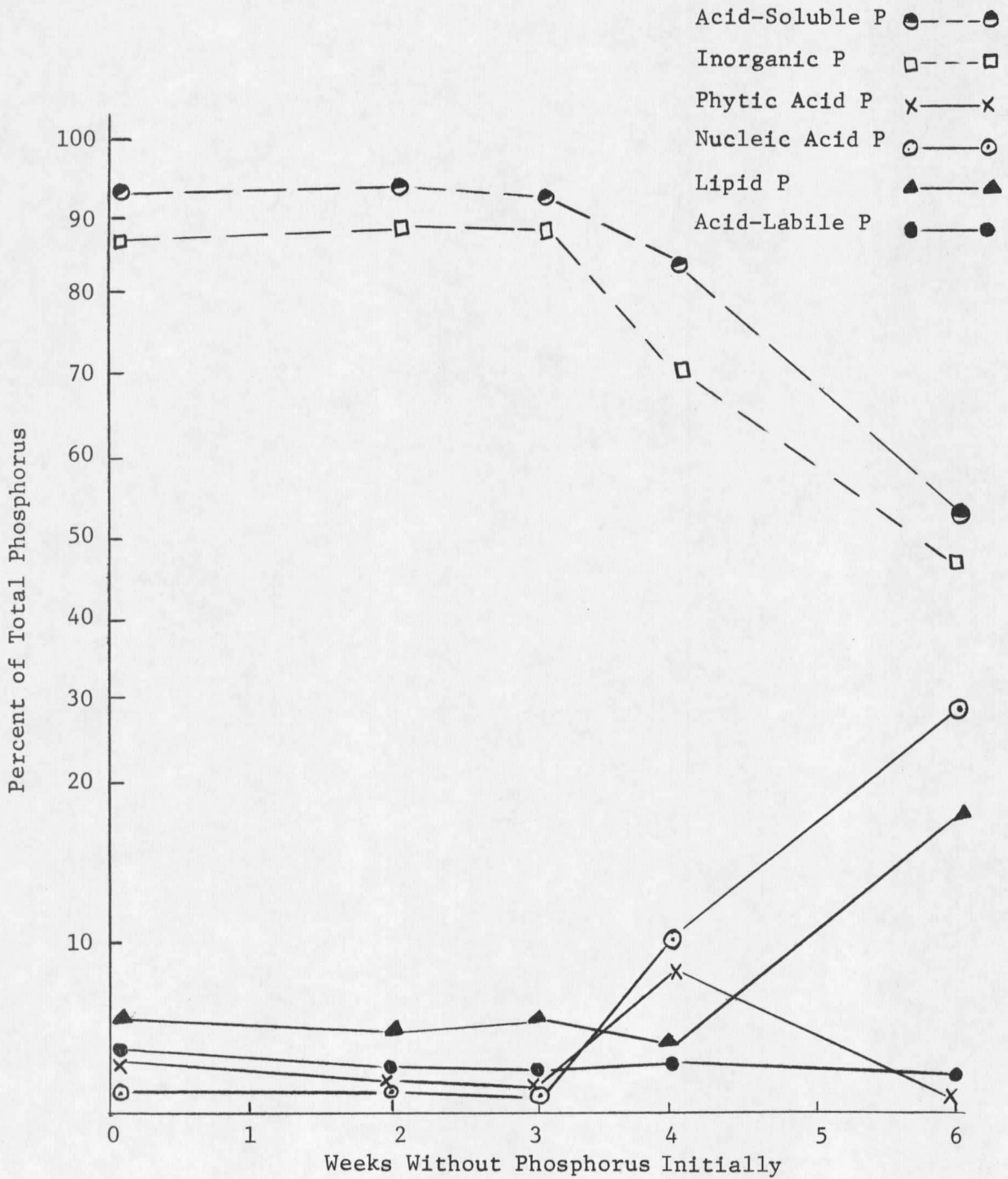


Figure 21. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at 6-week Growth Stage.

due to increase of nucleic acid P, also increased the amount of cell membrane, in turn increasing the lipid P.

When the plants were above milk stage up to maturity (Figures 21-24), inorganic P became predominant in the roots and the roots contained little of the organic types of P. The changes in P types were relatively constant regardless of P application. The changes in P types in leaves were similar at these same growth stages.

THE POSSIBLE INFLUENCE OF PHOSPHORUS FERTILIZATION ON THE BAKING QUALITY OF WHEAT FLOUR

The protein of wheat flour plays an important role in the baking quality of flour. The loaf volume of bread is mainly dependent on protein content (16). The protein complex known as gluten is one of the most important components of bread wheat flour.

The ability of a dough to expand and provide a large loaf is due to the unique properties of gluten. The variation in quality between different bread wheat flours lies to a large extent in the quantity and quality of the gluten they contain.

Watson, et al. (16) studied the statistical relationship of types of P in wheat flour and gluten to flour quality. They reported that if the effect of protein on the baking quality of flour was not cancelled out there was no significant relationships between any of the types of P in flour and loaf volume--hence, its baking quality. However, by using partial correlations which removed the effect of protein on loaf volume, negative and significant relationships were found between total acid-soluble and ester-type P. They also found significant correlations

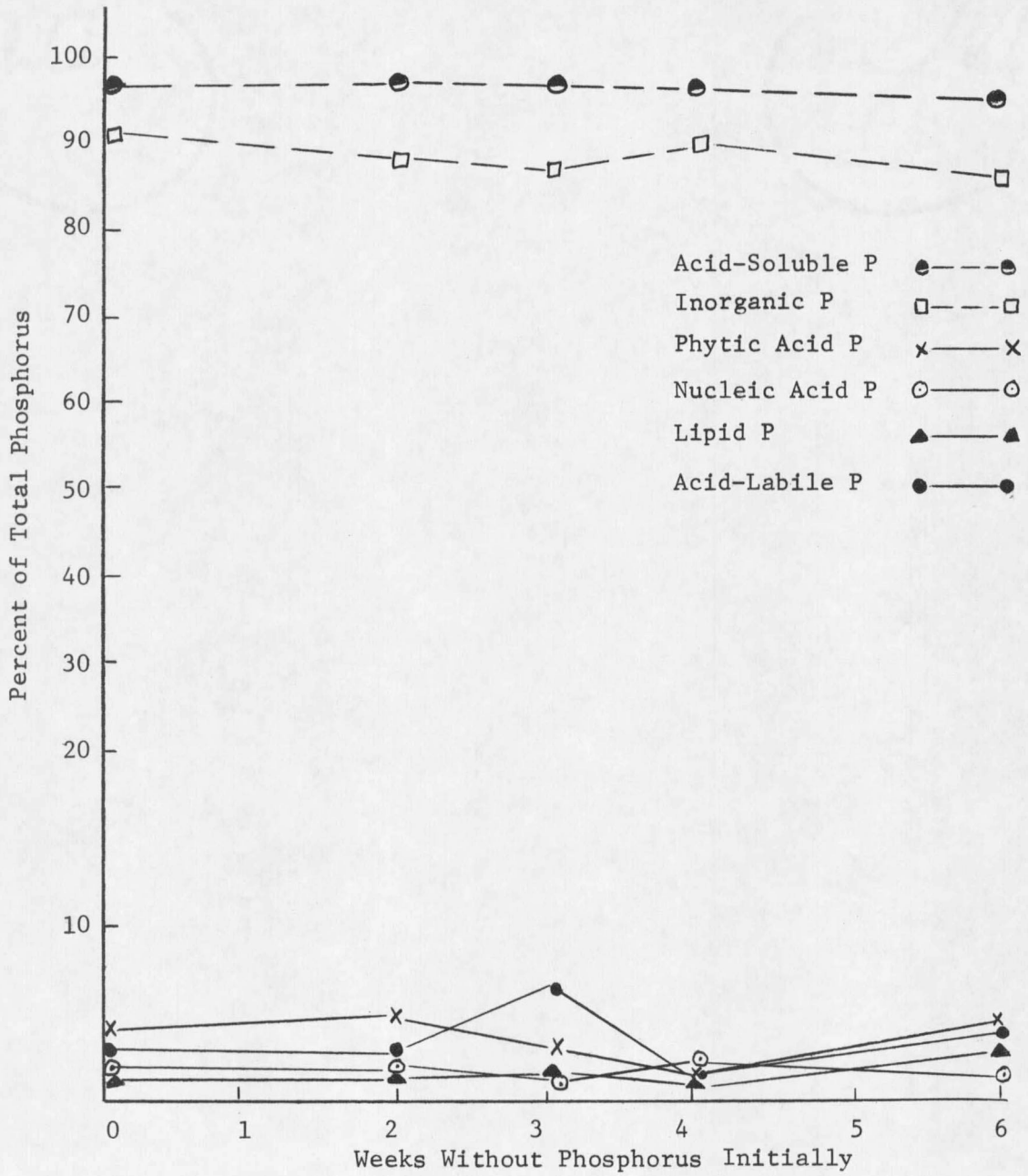


Figure 22. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at Milk Stage.

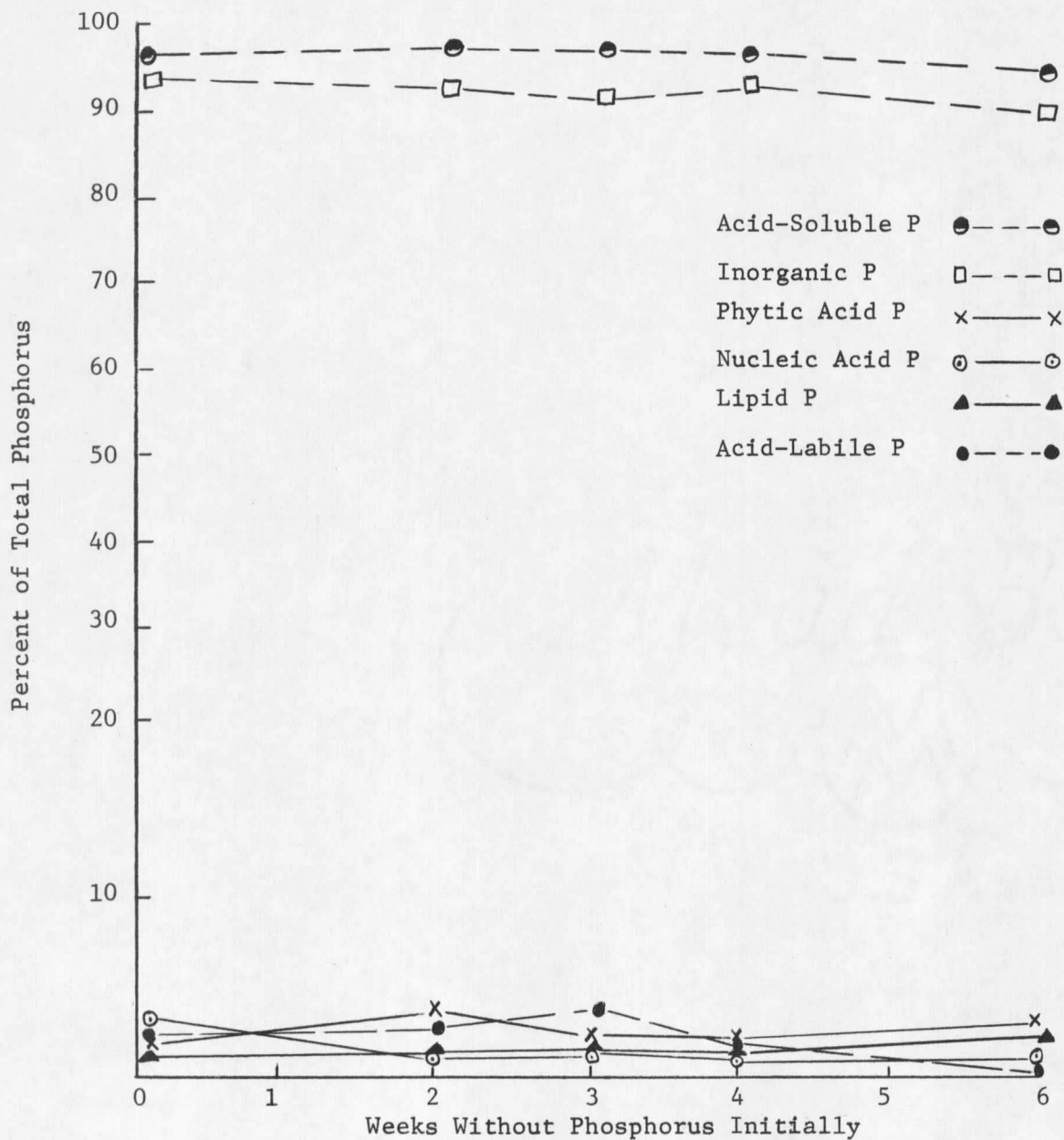


Figure 23. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at Soft Dough Stage.

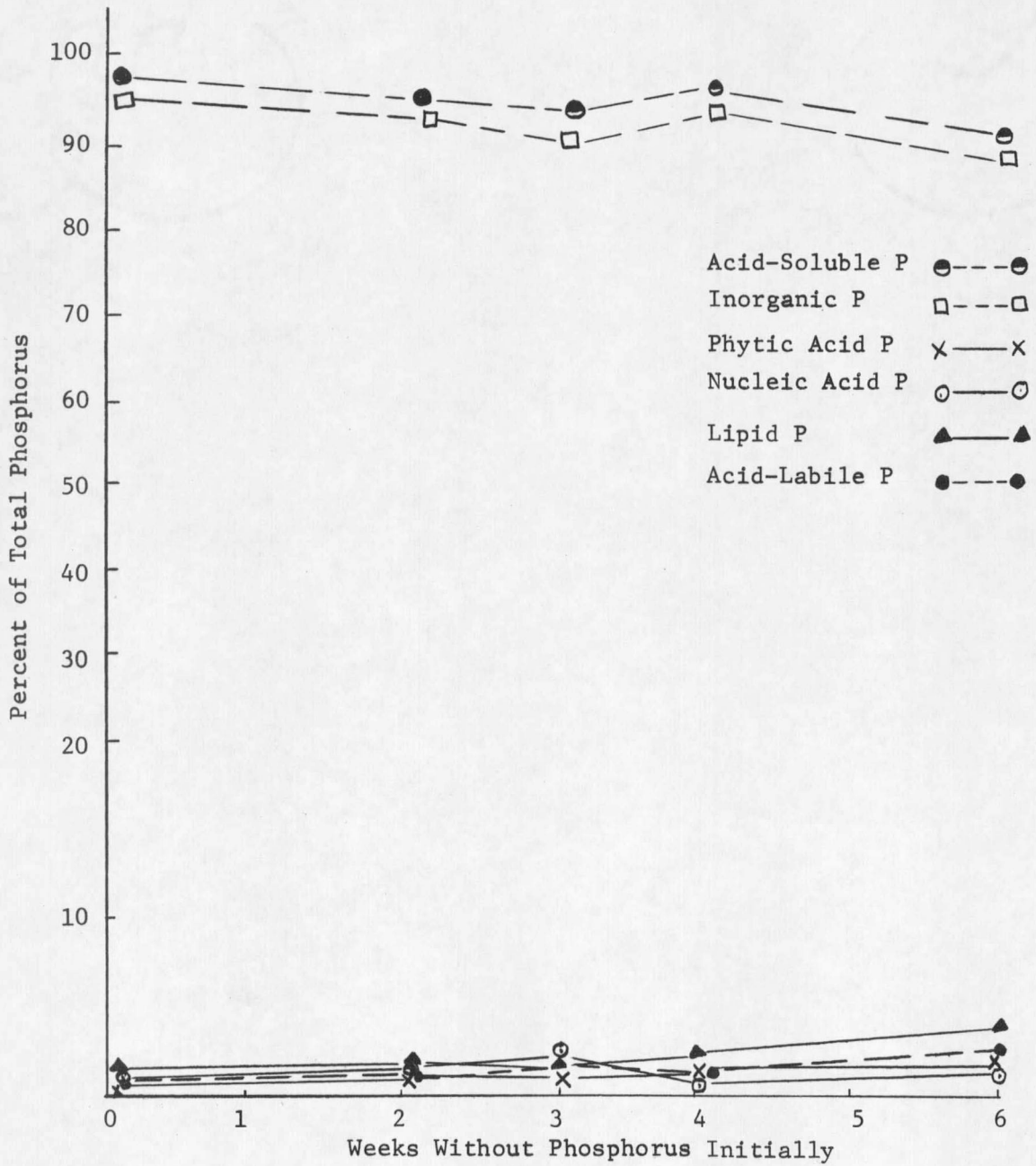


Figure 24. The Influence of Time of P Application on Different Types of P (Percentage of Total P) in Spring Wheat Roots at Maturity.

between the types of P and protein content. The total P in flour and gluten was positively and significantly correlated with protein content in flour. Acid-soluble and lipid P in gluten were negatively correlated with the protein content of the flour. Inorganic P in the gluten was positively correlated with protein content. Nucleic acid P in the flour was highly correlated with protein content of the flour. Ester-type P which is acid-labile P in flour was significantly and negatively correlated with protein content.

According to the data of this study (Figure 17, Appendix Table III), it is apparent that P deficiency can cause a decrease in total and nucleic acid P and an increase in lipid and acid-soluble P in the kernels at maturity. Watson (16) found that both changes--the decrease in total and nucleic acid P and increase in lipid and acid-soluble P, may lower the protein content in the flour. Thus, it may be concluded that P deficiency probably may influence protein levels and baking quality of wheat.

According to this study it was shown that the changes of inorganic and ester type P (which is acid-labile P) in the kernels at maturity were not significantly influenced by withholding P supply, so their effect on protein content can be neglected.

In conclusion, the direct effect of P alone on baking quality is comparatively small and non-significant (16). However, types of P may affect the protein content significantly and thus may indirectly influence baking quality. Furthermore, the data (Figure 17, Appendix Table III) also

indicate P fertilization is most important in the first three weeks of plant growth because maximum changes of types of P occurred at the third week of plant growth as affected by deprivation of P supply. Further deprivation of P up to six weeks caused fewer changes of the types of P.

APPENDICES

APPENDIX A

Procedures of P Determination

APPENDIX A

Reduced Molybdate Colorimetric Method for P Determination Preparation of Solutions (13)

The concentrated reduced molybdate reagent was prepared by weighing 39.12 g. of reagent grade molybdate anhydride (M_0O_3) into a two liter, round-bottomed pyrex flask with two necks and adding 800 ml. of concentrated sulfuric acid. A mechanical driven glass stirring rod was introduced through one neck of the flask and a thermometer through the other neck. The solution was heated, with continuous stirring on an electrically heated mantle, at 150°C until solution was complete (1.5 to 2.0 hours) as indicated by a clear, greenish color. After the quantitative addition of 2.20 g. of powdered molybdenum metal (99.5 percent M_0), heating and stirring was resumed until the solution was complete. This required about two hours. The deep blue solution was cooled, transferred quantitatively to a one liter volumetric flask, and diluted to volume with concentrated sulfuric acid.

The diluted reduced molybdate solution was prepared by pipeting 10 ml. of the concentrated reduced molybdate reagent into a 100 ml. volumetric flask containing about 50 ml. distilled water. Because of the viscosity of the reagent, the inside of the pipet was washed with distilled water into the flask. The dilute reagent was cooled to room temperature and diluted to volume with distilled water. A fresh solution of this reagent was prepared each day.

The concentrated stock phosphate solution was prepared by dissolving 4.3929 grams of ACS grade dry monobasic potassium phosphate in 300 ml. of distilled water and 200 ml. of N sulfuric acid contained in a one liter

volumetric flask. Several drops of 0.1 N potassium permanganate were added and the solution diluted to volume with distilled water. This solution contained 1.0 mg. of phosphorus per ml. and was stable.

The dilute stock phosphate solution containing 0.01 mg. of phosphorus per ml. was prepared by diluting the concentrated stock solution one to 100. A fresh solution of this solution was prepared each time before use.

Digestion of Sample. An appropriate amount of sample was weighed, or a suitable aliquot of extract pipeted, into a 100 ml. micro-Kjeldahl flask. Three ml. of concentrated sulfuric acid and two six mm. glass beads were added. The sample was heated until all organic material was charred and a homogeneous solution obtained. After cooling, four drops of 30 percent hydrogen peroxide were added and the solution was heated until colorless. It was usually necessary to add additional peroxide up to a total of 10 drops with intermittent heating and cooling. The solution was heated for 10 minutes after the last addition of peroxide. When cool, 20 ml. of distilled water was added and the solution boiled for five minutes to remove any remaining peroxide and to insure conversion of phosphorus to the ortho form. After cooling, the solution was quantitatively transferred to a 100 ml. volumetric flask and diluted to volume. This sample was used for the colorimetric determination of phosphorus.

Colorimetric Determination of Phosphorus. A suitable aliquot of the sample solution was transferred to a 100 ml. volumetric flask. Sufficient 3.60 N sodium hydroxide was added to neutralize the acid. Two drops of

indicator, 0.2 percent aqueous solution of sodium alizarin sulfonate were added and the acidity adjusted with 1 N sulfuric acid and 1 N sodium hydroxide until one drop of the acid turned the solution yellow. The solution was then diluted to approximately 70 ml. with distilled water. A reagent blank was prepared using the same amount of 3.60 N alkali as for the samples and the acidity adjusted in the same manner. A 10 ml. aliquot of the dilute reduced molybdate reagent was added to the blank and each sample solution. All flasks were mixed by swirling and placed in a boiling water bath for exactly 30 minutes. After cooling in a cold water bath, the reaction solutions were diluted to volume with distilled water. Intensity of the color was read in a Buasch and Lomb "Spectronic 20" colorimeter at 820 mu. Using the reagent blank, the instrument was set at 100 percent transmission. Milligrams of phosphorus in the sample aliquot was determined by reference to the calibration curve.

Calibration Curve. Aliquots of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, and 12.0 ml. of the diluted phosphate solution (0.01 mg. of phosphorus per ml.) were pipeted into a 100 ml. volumetric flask. Two drops of indicator and one drop of 1 N sulfuric acid were added and the solution diluted to approximately 70 ml. with distilled water. After adding 10 ml. of reduced molybdate solution, the procedures outlined above for treatment of the sample aliquot was followed. The standard containing no phosphorus was used to set the instrument at 100 percent transmission. The logarithms of the transmittance values obtained from the standards were plotted against the known phosphorus concentrations to obtain the standard calibration curve which is a straight line.

Modified Isobutyl Alcohol Colorimetric Determination of Phosphorus (10)

Preparation of Solutions. The molybdate reagent was prepared by dissolving 50 g. of ammonium molybdate in 400 ml. of 10 N sulfuric acid and was diluted to one liter with water.

The silicotungstate reagent was made by dissolving 5.7 g. of sodium silicate nonahydrate and 79.4 g. of sodium tungstate dihydrate in about 500 ml. of water, 15 ml. of concentrated sulfuric acid was added. The solution was boiled for five hours, cooled, and was diluted to one liter with distilled water.

The isobutyl alcohol-benzene solution was prepared by mixing equal volumes of isobutyl alcohol and thiophene-free benzene. The stannous chloride stock solution was made by dissolving 10 g. of stannous chloride dihydrate in 25 ml. of concentrated hydrochloric acid and stored in a small glass-stoppered brown bottle.

The stannous chloride dilute solution was made by diluting 1 ml. of the stannous chloride stock solution to 200 ml. with approximately 1 N sulfuric acid. This solution was prepared fresh daily.

The acidic ethyl alcohol or alcoholic sulfuric acid was prepared by dissolving 20 ml. of concentrated sulfuric acid in 980 ml. of 99.5 percent ethyl alcohol. (Instability of the molybdenum blue color has been attributable at times to some contaminant in 95 percent ethyl alcohol.)

The phosphate standard solution was made by dissolving 0.4950 g. of reagent grade potassium dihydrogen phosphate (dried at 110°C) in water and diluted to one liter. Dilute 50 ml. of this solution with distilled

water to 200 ml. to obtain a solution containing 28.2 micrograms of phosphorus per ml.

Colorimetric Determination of Phosphorus

A suitable aliquot of a 12.5 percent trichloroacetic extract or a digested sample was pipeted into a 25 x 200 mm. test tube. Distilled water was added to bring the volume to 15 ml. Then 25 ml. of the isobutyl alcohol-benzene solution was added and followed by adding 5 ml. of silicon-tungstate reagent and 5 ml. of the molybdate reagent. Stopper the tubes with rubber stoppers and immediately shake the tubes for 15 seconds and allow the phases to separate.

Pipet a 10 ml. sample of the isobutyl alcohol-benzene layer from the tubes into a 50 ml. volumetric flask and wash the sample from the pipets with an alcoholic sulfuric acid solution. Dilute the solution in the 50 ml. volumetric flask to about 45 ml. with the alcoholic sulfuric acid. One ml. of dilute stannous chloride solution was added and diluted with alcoholic sulfuric acid to volume. Mixed well and a blue color was developed. A sample blank was determined in the same manner as above using all reagents.

The percent transmittance of the colored solution was read in a Bausch and Lomb "Spectronic 20" colorimeter at 725 mu. using the reagent blank to set the instrument at 100 percent transmittance. Milligrams of phosphorus in the sample aliquot was determined by reference to the calibration curve.

Calibration Curve. A calibration curve was prepared by substituting aliquots of 0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ml. of dilute phosphate standard solution for the sample aliquots. The color was developed exactly as outlined above for the sample determination. The standard containing no phosphorus was used to set the instrument at 100 percent transmittance. The logarithms of the transmittance value was plotted against phosphorus concentration. A straight line should be obtained.

Method for Determining Types of Phosphorus Distribution (10,13,17)

Preparation of Solutions. The 12.5 percent of trichloroacetic acid solution was prepared by dissolving 125 g. of reagent grade acid in distilled water and diluting to one liter. This acid solution was either made as needed or stored in a refrigerator.

The benzene-alcohol azeotropic solvent was made by mixing 32.4 weight percent of absolute ethyl alcohol and 67.6 weight percent of benzene. This solvent has a boiling point of 68.2°C.

The magnesium nitrate solution used was a saturated solution of magnesium nitrate hexahydrate in 95 percent ethyl alcohol.

Total phosphorus was determined by digesting 100 mg. of samples as outlined under the reduced molybdate method. Colorimetric determination also were made by the reduced molybdate colorimetric procedure.

Acid-soluble phosphorus was determined by weighing 100-200 mg. of samples into a 11.0 cm. filter paper, Whatman No. 42. The filter paper was folded, and enclosed in a second so as to retain the sample. The second filter paper was left open at the top to form a thimble. The

sample was extracted with Skelly Solve B in a Soxhlet apparatus for four hours. After the extraction period, the sample was air dried for 24 hours and transferred quantitatively to a 125 ml. glass-stoppered Erlenmeyer flask. Fifty ml. of 12.5 percent trichloroacetic acid was pipeted into the flask and the sample extracted on a mechanical shaker for one hour. The sample was then filtered through 11 cm. filter paper, Whatman No. 42, discarding the first few drops of the filtrate. A 10 ml. aliquot of this filtrate was digested as outlined under the reduced molybdate method. The whole aliquot of the digested sample was used for the colorimetric determination of phosphorus by the reduced molybdate procedure.

Inorganic phosphorus. A 10 ml. aliquot of the trichloroacetic acid filtrate from the acid-soluble phosphorus determination was pipeted into a 25 x 200 mm. test tube. Inorganic phosphorus was determined by the modified isobutyl alcohol procedure.

Acid-labile phosphorus. The material was hydrolyzed in four ml. H₂O for seven minutes at 100°C after cooling, six ml. isobutanol, one ml. silicotungstic acid, and one ml. neutral molybdate reagent were added and inorganic phosphorus was estimated as outlined in the modified isobutyl alcohol colorimetric method procedure.

Lipid phosphorus. One-hundred mg. to 200 mg. sample was weighed into an 11 cm. filter paper, Whatman No. 42. The filter paper was folded, and enclosed in a second filter paper so as to retain the sample. The second filter paper was left open at the top to form a thimble. The sample was extracted with 75 ml. of the alcohol-benzene solvent for two hours in a lipid extract instrument. Because of the quick extraction of this instru-

ment, the time used to extract the lipids in the sample was reduced to two hours. By use of the ordinary small size Soxhlet extraction time should be four hours. Most of the solvent from the boiling flask was removed by distillation and the residue poured into a small porcelain evaporating dish, 60 ml. capacity. The boiling flask was rinsed with two 10 ml. portions of 95 percent ethyl alcohol, heating to boiling each time, and adding the washings to the evaporating dish. This was repeated, using two 10 ml. portions of 75 percent ethyl alcohol.

After removing most of the solvent in the evaporating dish on a steam bath, three ml. of magnesium nitrate solution was added. The sample was heated over the steam bath until the residue was dry and then over a hot plate until well-charred. The charred residue was ashed in a muffle furnace at 400°C until most of the carbon was burnt off (about two hours) and the temperature was raised to 600°C and the sample heated until a white ash was obtained (about two hours).

The cooled ash was dissolved in two ml. of 10 N sulfuric acid, warming if necessary, and transferred quantitatively to a 100 ml. volumetric flask with the aid of distilled water. The digest was made up to volume with distilled water and mixed well. A 10 ml. aliquot of this digest was pipeted into a 25 x 200 mm. test tube and the phosphorus determined colorimetrically as outlined in the modified isobutyl alcohol method.

Nucleic acid phosphorus. Acid-soluble phosphorus determination includes acid non-labile phosphorus which is phytic acid or phytin, acid-labile phosphorus, and inorganic-type phosphorus. Therefore, the value

for acid-soluble phosphorus plus the value for lipid phosphorus subtracted from the value for total phosphorus was made in order to estimate the amount of nucleic acid phosphorus (13).

Acid non-labile phosphorus. This is phytic acid or phytin. It was estimated by subtracting acid-labile and inorganic phosphorus from total acid-soluble phosphorus.

APPENDIX B

Tables

APPENDIX B

Tables

APPENDIX TABLE I. SALTS OR ACIDS NEEDED FOR MAKING UP STOCK NUTRIENT SOLUTIONS.

Salts or Acids	Grams of salts or acids per one liter stock solution	Ml. stock solution per 3,000 ml. capacity pot	PPM elements in the nutrition solutions	Solution
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	177.2	20	Ca - 200 N - 140	A
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	74.0	20	Mg - 49 S - 64	B
KI	37.2	20	K - 130 I - 118	B
$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	12.2	20	Ca - 13 P - 20	C
$\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	0.0029	20	Mo - 0.01	D
$\text{CuCl}_2 \cdot 4\text{H}_2\text{O}$	0.008	20	Cu - 0.02	D
ZnCl_2 (95%)	0.015	20	Zn - 0.05	D
H_3BO_3	0.422	20	B - 0.5	D
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.272	20	Mn - 0.5	D
Ferric citrate	15.9	20	Fe - 20	E

APPENDIX TABLE II. THE CONCENTRATION OF TYPES OF PHOSPHORUS IN THE LEAVES EXPRESSED IN MgP PER GRAM OF DRIED SAMPLE AND THE PERCENTAGES OF TYPES OF P IN THE TOTAL P.

Without P	Harvest	Total P		Inorganic P		Acid-Soluble P		Acid-Labile P		Lipid P		Acid Non-Labile P		Nucleic Acid P	
		Weeks	Stage	MgP/g Sample	MgP/g %TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP
0	2 wks	4.29	2.35	54.80	3.60	84.00	0.423	9.86	0.316	7.38	0.827	19.34	3.970	8.62	
2	2 wks	4.30	1.93	58.10	3.10	94.00	0.403	12.20	0.168	5.10	0.850	25.70	0.030	0.90	
0	3 wks	3.14	1.41	44.80	2.36	75.00	0.316	10.00	0.365	11.60	0.630	20.20	0.423	13.40	
2	3 wks	3.37	1.68	50.00	2.98	88.50	0.118	3.50	0.272	8.06	1.180	35.00	0.116	3.44	
3	3 wks	2.39	0.96	40.20	1.26	52.80	0.090	3.93	0.273	11.40	0.207	8.67	0.856	35.80	
0	4 wks	3.39	1.30	38.40	2.19	64.60	0.283	8.35	0.350	10.30	0.603	17.80	0.851	25.10	
2	4 wks	3.41	2.09	61.20	3.09	90.50	0.323	9.46	0.308	9.10	0.677	19.84	0.014	0.40	
3	4 wks	3.69	1.41	38.20	2.26	61.30	0.200	5.42	0.246	6.90	0.659	17.86	1.173	31.80	
4	4 wks	1.41	0.61	43.30	0.94	66.60	0.120	8.50	0.137	9.75	0.209	14.90	0.334	23.65	
0	6 wks	2.80	14.0	50.00	2.15	76.80	0.513	18.30	0.230	8.20	0.238	8.50	0.420	15.00	
2	6 wks	2.20	0.8	36.30	1.95	88.90	0.286	13.00	0.230	10.40	0.871	39.60	0.016	0.70	
3	6 wks	2.73	1.11	40.60	2.10	77.20	0.140	5.13	0.315	11.50	0.854	31.27	0.309	11.30	
4	6 wks	2.78	1.10	39.60	2.13	76.50	0.166	5.96	0.206	7.64	0.859	30.90	0.441	15.86	
6	6 wks	0.01	0.24	39.00	0.47	77.00	0.100	16.70	0.047	7.80	0.130	21.30	0.093	15.20	
0	Milk Stage	2.48	2.26	91.00	2.42	97.00	0.130	5.00	0.042	1.60	0.0496	2.00	0.0347	1.40	
2	Milk Stage	2.45	2.32	94.00	2.38	97.00	0.040	1.00	0.062	2.50	0.0490	2.00	0.0123	0.50	
3	Milk Stage	2.32	2.10	90.00	2.23	96.00	0.053	2.20	0.048	2.00	0.0882	3.80	0.0464	2.00	
4	Milk Stage	2.90	2.67	92.00	2.83	97.50	0.046	1.58	0.058	2.00	0.1130	3.90	0.0145	0.50	
6	Milk Stage	1.38	1.23	89.00	1.30	94.00	0.010	0.70	0.063	4.50	0.0593	4.30	0.0207	1.50	
0	Soft Dough	2.43	2.17	89.00	2.22	91.00	0.033	1.30	0.0357	1.46	0.0170	0.70	0.1832	7.54	
2	Soft Dough	1.72	1.65	95.00	1.67	97.00	0.010	0.50	0.0497	2.88	0.0358	1.50	0.0021	0.12	
3	Soft Dough	2.37	2.19	92.00	2.29	96.00	0.060	2.50	0.0437	1.84	0.0356	1.50	0.0512	2.16	
4	Soft Dough	2.05	1.93	94.00	1.98	96.00	0.040	1.90	0.0310	1.51	0.0021	0.10	0.0510	2.49	
6	Soft Dough	1.32	1.24	93.00	1.28	96.00	0.025	1.80	0.0286	2.16	0.0158	1.20	0.0243	1.84	
0	Maturity	2.05	1.89	92.00	1.95	95.00	0.030	1.00	0.0883	4.30	0.0410	2.00	0.0144	0.70	
2	Maturity	2.06	1.79	86.00	1.96	95.00	0.080	3.00	0.0107	0.50	0.1240	6.00	0.0927	4.50	
3	Maturity	2.27	2.02	89.00	2.18	96.00	0.080	3.00	0.0300	1.30	0.0910	4.00	0.0613	2.70	
4	Maturity	1.82	1.67	92.00	1.77	97.00	0.040	2.00	0.0417	2.30	0.0550	3.00	0.0127	0.70	
6	Maturity	1.35	1.15	85.00	1.25	92.00	0.050	3.00	0.0560	4.10	0.0540	4.00	0.0527	3.90	

APPENDIX TABLE III. THE CONCENTRATION OF TYPES OF PHOSPHORUS IN THE KERNELS EXPRESSED IN MgP PER GRAM OF DRIED SAMPLE AND THE PERCENTAGES OF TYPES OF P IN THE TOTAL P.

Without P	Harvest	Total P		Inorganic P		Acid-Soluble P		Acid-Labile P		Lipid P		Acid Non-Labile P		Nucleic Acid P	
		Weeks	Stage	MgP/g Sample	MgP/g %TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP
0	Milk Stage	5.30	1.93	36.40	3.30	62.20	0.07	1.32	0.277	5.23	1.316	24.48	17.26	32.57	
2	Milk Stage	5.70	2.11	37.00	3.63	63.60	0.05	0.86	0.222	3.90	1.410	24.74	1.853	32.50	
3	Milk Stage	6.23	2.64	42.40	4.10	65.80	0.15	2.30	0.167	2.68	1.320	21.20	1.964	31.52	
4	Milk Stage	6.26	2.79	44.60	4.19	67.00	0.05	0.78	0.260	4.20	0.727	21.62	1.803	28.80	
6	Milk Stage	4.90	2.20	44.90	3.67	74.90	0.08	1.63	0.307	6.26	1.390	28.37	0.923	18.84	
0	Soft Dough	6.03	1.26	20.80	2.48	41.10	0.05	0.83	0.188	3.12	1.174	19.47	3.364	55.78	
2	Soft Dough	5.90	1.01	17.10	3.90	66.10	0.06	1.01	0.170	2.88	2.830	47.99	1.830	31.02	
3	Soft Dough	6.20	1.28	20.60	4.93	79.50	0.15	2.42	0.150	2.42	2.502	56.48	1.121	18.08	
4	Soft Dough	5.90	1.41	23.90	4.37	74.10	0.10	1.69	0.174	2.95	2.862	48.51	1.354	22.95	
6	Soft Dough	4.60	1.08	23.50	3.30	71.70	0.12	2.60	0.140	3.04	2.098	45.60	1.162	25.26	
0	Maturity	6.40	0.618	9.65	2.96	46.20	0.03	0.47	0.141	2.20	2.309	36.08	3.302	51.60	
2	Maturity	6.35	0.692	10.90	4.20	66.10	0.02	0.31	0.113	1.94	3.485	54.89	2.029	31.96	
3	Maturity	5.50	0.783	14.20	4.06	73.80	0.02	0.36	0.170	3.09	3.258	59.24	1.271	23.11	
4	Maturity	5.90	0.763	12.90	4.33	73.40	0.03	0.51	0.152	2.58	3.561	60.35	1.417	24.00	
6	Maturity	5.03	0.443	8.80	3.63	72.10	0.02	0.39	0.198	3.93	3.174	63.10	1.200	23.97	

APPENDIX TABLE IV. THE CONCENTRATION OF TYPES OF PHOSPHORUS IN THE ROOTS EXPRESSED IN MgP PER GRAM OF DRIED SAMPLE AND THE PERCENTAGES OF TYPES OF P IN THE TOTAL P.

Without P	Harvest	Total P		Inorganic P		Acid-Soluble P		Acid-Labile P		Lipid P		Acid Non-Labile P		Nucleic Acid P	
		Weeks	Stage	MgP/g Sample	MgP/g %TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP	MgP/g Sample	%TP
0	2 wks	2.62	1.79	68.50	2.12	81.00	0.030	1.14	0.210	8.000	0.314	12.00	0.288	11.00	
2	2 wks	1.34	0.904	70.00	1.18	88.00	0.050	3.73	0.134	10.00	0.191	14.27	0.161	2.00	
0	3 wks	1.64	1.13	69.50	1.25	76.20	0.060	3.65	0.124	7.600	0.050	3.05	0.266	16.20	
2	3 wks	2.38	1.67	70.00	1.98	83.20	0.040	1.68	0.061	2.600	0.269	11.32	10.34	14.20	
3	3 wks	1.20	0.54	45.00	0.608	51.00	0.037	3.00	0.090	7.500	0.036	3.00	0.498	41.50	
0	4 wks	2.29	1.67	72.50	1.88	82.00	0.080	3.49	0.188	8.200	0.138	6.01	0.0224	9.80	
2	4 wks	1.88	1.31	70.30	1.72	91.50	0.110	5.85	0.132	7.040	0.289	15.35	0.0274	1.46	
3	4 wks	1.79	1.50	83.90	1.65	92.50	0.060	3.35	0.117	6.550	0.094	5.25	0.0170	0.95	
4	4 wks	0.65	0.39	60.00	0.425	65.40	0.020	3.07	0.104	16.00	0.0151	2.33	0.1210	18.60	
0	6 wks	4.16	3.61	87.00	3.87	93.00	0.130	3.12	0.250	6.00	0.120	2.88	0.0416	1.00	
2	6 wks	1.13	1.01	89.50	1.06	94.00	0.030	2.65	0.057	5.00	0.021	1.85	0.0113	1.00	
3	6 wks	1.18	1.05	89.00	1.10	93.20	0.030	2.28	0.066	6.00	0.027	1.92	0.0094	0.80	
4	6 wks	1.78	1.29	72.50	1.51	85.00	0.060	3.37	0.063	3.50	0.163	9.13	0.2050	11.50	
6	6 wks	0.41	0.20	49.00	0.21	54.10	0.0123	3.00	0.078	19.00	0.009	2.10	0.1210	27.00	
0	Milk Stage	3.13	2.87	91.00	3.10	97.00	0.130	4.00	0.0373	1.20	0.125	4.00	0.0563	1.80	
2	Milk Stage	2.08	1.83	88.00	2.03	97.00	0.065	3.10	0.0293	1.40	0.123	5.90	0.0333	1.60	
3	Milk Stage	2.10	1.83	87.00	2.05	97.00	0.150	7.00	0.0350	1.60	0.063	3.00	0.0294	1.40	
4	Milk Stage	2.39	2.15	90.00	2.34	97.00	0.020	0.80	0.0300	1.30	0.005	0.20	0.0406	1.70	
6	Milk Stage	0.82	0.71	86.00	0.783	95.00	0.040	4.00	0.0306	3.70	0.041	5.00	0.0107	1.30	
0	Soft Dough	3.25	3.06	94.00	3.17	97.00	0.080	2.00	0.0320	0.98	0.0325	1.00	0.0657	2.02	
2	Soft Dough	3.03	2.84	93.00	3.00	98.00	0.010	3.00	0.0330	1.08	2.00	0.0303	1.00	1.00	
3	Soft Dough	2.53	2.33	92.00	2.50	98.00	0.110	4.00	0.0207	0.81	0.0506	2.00	0.0301	1.19	
4	Soft Dough	2.01	1.92	95.00	1.97	98.00	0.030	1.00	0.0200	0.99	0.0402	2.00	0.0203	1.01	
6	Soft Dough	1.06	0.96	91.00	1.03	95.00	0.020	1.00	0.0277	2.60	0.0318	3.00	0.0254	2.40	
0	Maturity	3.52	3.43	97.00	3.46	98.00	0.020	0.56	0.0360	1.02	0.0155	0.44	0.0345	0.98	
2	Maturity	3.31	3.16	95.00	3.22	97.00	0.040	1.20	0.0453	1.36	0.0265	0.80	0.0543	1.64	
3	Maturity	1.86	1.69	91.00	1.77	95.00	0.060	3.22	0.0320	1.72	0.078	0.78	0.0610	3.28	
4	Maturity	1.91	1.84	96.00	1.88	98.00	0.020	1.04	0.0530	1.00	0.0183	0.96	1.00	1.00	
6	Maturity	0.98	0.87	88.00	0.916	93.00	0.030	3.06	0.0407	4.15	0.0190	1.94	0.0279	2.85	

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