



Effect of freezing and thawing on phosphorus availability  
by Hang-Tan Phung

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of  
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Abstract:

The effect of freezing and thawing on the availability of P and the subsequent growth of winter wheat was studied in a greenhouse experiment.

Soils incubated under frozen condition produced less dry matter than soils incubated at room temperature regardless of period of incubation. The cause of decreased yields was believed to be due mainly to the impaired physical properties of the frozen soils.

Plants generally absorbed lesser amounts of P from soils incubated at room temperature. Prolonged incubation resulted in reduced uptake. The significant differences due to prolonged incubation varied with soils, P rates, and temperatures of incubation.

Plants grown on unfrozen soils showed luxury consumption of P while those grown on frozen soils appeared to have response to increased P rates.

That the amounts of P absorbed per unit of available P were greater on the soils incubated at room temperature may be explained as largely due to the greater activity of roots in those soils. However, the overall uptake of P was higher for plants grown on soils incubated under frozen condition. This was believed to be largely due to greater availability of P in the soils that had been frozen.

The results, as a whole, suggest that the possibility of applying P in summer fallow is not feasible, since the availability of the fertilizer P applied will have been reduced appreciably by the time of planting in fall so that the growth of winter wheat may be affected to a certain extent.

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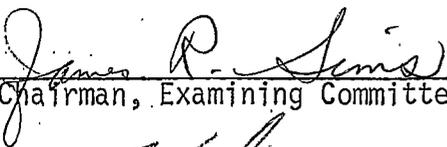
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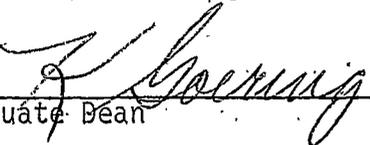
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ABSTRACT

The effect of freezing and thawing on the availability of P and the subsequent growth of winter wheat was studied in a greenhouse experiment.

Soils incubated under frozen condition produced less dry matter than soils incubated at room temperature regardless of period of incubation. The cause of decreased yields was believed to be due mainly to the impaired physical properties of the frozen soils.

Plants generally absorbed lesser amounts of P from soils incubated at room temperature. Prolonged incubation resulted in reduced uptake. The significant differences due to prolonged incubation varied with soils, P rates, and temperatures of incubation.

Plants grown on unfrozen soils showed luxury consumption of P while those grown on frozen soils appeared to have response to increased P rates.

That the amounts of P absorbed per unit of available P were greater on the soils incubated at room temperature may be explained as largely due to the greater activity of roots in those soils. However, the overall uptake of P was higher for plants grown on soils incubated under frozen condition. This was believed to be largely due to greater availability of P in the soils that had been frozen.

The results, as a whole, suggest that the possibility of applying P in summer fallow is not feasible, since the availability of the fertilizer P applied will have been reduced appreciably by the time of planting in fall so that the growth of winter wheat may be affected to a certain extent.

## INTRODUCTION

There has been little research devoted to studying the influence of freezing and thawing on the availability of phosphorus (P) to plants. This may be partly due to the fact that the processes responsible for P availability in soils are mainly chemical rather than biological. In general, biological processes are relatively unimportant as far as P fertility in soils is concerned (47, 18, 23). However, it should be expected that the effect of temperature on soil chemical processes may be a primary factor influencing P availability. This may be especially so in areas with temperature extremes such as those in the Northern Great Plains.

In recent years, the effects of freezing and thawing as well as wetting and drying on the physical properties of soils and mineralization of nitrogen have received considerable attention, particularly by workers in Canada and Russia. However, seemingly contradictory results have been reported by different investigators (13, 27, 55, 68, 70). These results may be taken as an indication of the difficulty in separating out the physical, chemical, and biological effects of freezing and thawing.

In Montana, P fertilizers are normally applied to wheat at the time of planting. Little is known about the fate of the applied P that is subjected to the processes of freezing and thawing during winter. Recently, interest has been shown in the possibility of adding P fertilizer to wheat in the off-season such as during the summer fallow period. The availability of fertilizer P added during the summer fallow period is likely to be much different than that added with the seed. The hypothesis for this study is that it would be less available to the crop than P applied at planting time.

The objectives of this study were:

- 1) To compare the availability of fertilizer P in soils incubated for different periods under the frozen and unfrozen conditions.
- 2) To investigate the influence of freezing and thawing on the subsequent growth of winter wheat and the availability of P.
- 3) To investigate possible contributing factors related to plant growth and P availability in such soils.

## REVIEW OF LITERATURE

### The effect of temperature on plant growth and P uptake

The growth of higher plants is a function of both the aerial temperature and the soil temperature. The response to temperature may vary with species of plants, age, previous environment conditions, and other factors.

Ketcheson (31) indicated the important physiological effects of lowered temperature including such things as decreased kinetic energy, fluidity of protoplasm, solubility of certain solids, and diffusion and reaction velocities. The consequence of these effects may be reduced respiration and translocation within the plant system. In roots, a reduction in these processes reduced the energy and food available for root growth, for nutrient absorption and for assimilation.

Kramer and Currier (34) consider that permeability of root cells influences absorption and that permeability is decreased by low temperature and low respiration rate. Hoagland and Broyer (26) point out that energy must be expended to transfer ions across cytoplasmic membranes against a concentration gradient. Thus, a low temperature further reduces the availability of the roots to absorb nutrients. The effects of low temperature in decreasing nutrient absorption has also been shown to be more pronounced under a low than a high nutrient concentration in the substrate. Furthermore, the absorption of anions tends to be more dependent on temperature than is the absorption of cations.

Nightingale (51) observed the morphology of fruit tree roots was influenced by temperature. More root hair appeared at temperature below 24° C. This suggests that a measure of root activity rather than root weight

may be necessary in the evaluation of temperature effect on nutrient uptake. Root activity of corn, particularly in the bottom portion of the pots, was reduced by low temperature (4).

The effects of soil temperature on the plant growth and P uptake by barley have been extensively investigated by Power and his associates (58, 59, 60, 61). They reported that (a) soil temperatures near 59° F were optimum for the growth of barley; (b) the efficiency of P utilization was highest at 59° F; (c) growth responses to P fertilization were more dependent upon soil temperatures for soils low in available P than for those medium in available P; (d) increasing the available P supply with either increased soil or fertilizer P widened the soil temperature range over which nearly maximum growth occurred; and (e) at each temperature studied dry matter production was very closely associated with water use regardless of the available P level.

Gringrich (21) planted winter wheat and showed that dry matter yield was affected little by applications of phosphate fertilizers when soil temperature was maintained at 50° F regardless of available P in the soil. However, at 65° and 80° F soil temperatures, applications of phosphate doubled the yield of dry matter on a low P soil and had a variable effect on the high P soil. He concluded, from the results, that even on a soil low in available P, early growth of winter wheat may not be increased greatly by P fertilization if soil temperatures are too low.

McKell et al. (46) showed that top growth, root growth, and P content increased with increasing temperature and P fertility levels, when legumes were grown at different soil temperatures.

Cannell et al. (10) found a curvilinear relationship between yield of tomato plants and temperature. The highest yields were at 68° F, low soil suction, and high P. Yield increased with decreasing soil suction and with increasing P. Locascio and Warren (35) using the same plants found that dry weights were similar for plants growth at 70° and 85° F, but were lower in plants grown at 55° F. Uptake of P with increased P level was correlated with growth, being linear at 55° but curvilinear at 70° and 85° F. Similar results have been obtained on beans (2), corn (31, 52), bromegrass and potatoes (52), and clover (66). Generally, P absorption was maximum at soil temperatures near or slightly above optimum for plant growth, and was generally increased by P fertilization.

Simpson (69) grew oats in the greenhouse at two soil temperatures on a soil high in organic matter, and found that temperature had a greater influence upon absorption of soil P than on absorption of fertilizer P. However, Power et al. (58) showed that fertilizer P absorption was more dependent upon soil temperature than soil P absorption. These differences, however, may have resulted from differences in techniques or soils.

Variations in soil temperature may also affect nutrient uptake through changes in amount of root extension. When temperature is reduced below optimum, root growth and extension are also reduced. This would probably be due to reduced translocation of carbohydrates from the tops, or to reduced nutrient uptake from soil or both (3). The relative contributions of these effects are not known. Dul'Bunskaya and Yuzenasov (16) demonstrated that low temperature (5° to 7° C) decreased P<sup>32</sup> uptake by wheat seedlings, particularly

the movement of  $P^{32}$  from the roots to the shoots. Low temperature also decreased the rate of inclusion of  $P^{32}$  into nucleic acids in the roots and shoots to the same extent as it decreased the rate of  $P^{32}$  uptake. The reduced growth at low temperatures have been attributed to reduced translocation of growth substances within the plant (15, 72). Stankovic and Bukovac (72) were able to show that translocation of foliar applied  $P^{32}$  is greatly reduced by lowering soil temperature.

In a pot experiment with barley, Ivanova found that cooling for two weeks decreased the total yield and grain yield per pot with the N-K treatment but not with the N-K-P treatment. Possibly, P fertilization is able to eliminate the harmful effect of low temperature (28). Ketchenson (31) reported that P applications to corn at a soil temperature of 13° C counteracted the harmful effect of low temperature to some extent. In general, applications of P fertilizers at low temperature conditions sometimes affect growth beneficially, but growth of plants never reached the same level as when they were grown at a higher soil temperature (31, 64).

Band placement of the fertilizer compared to mixed placement at 13° C resulted in a relatively greater increase in dry matter than at 20° C (31). Robinson et al. (66) found that the band application of P in clover was more effective at low soil temperature (10° C) than at a high soil temperature (27° C). P, mixed with the soil, was utilized less efficiently at lower temperatures. The increase in yield due to banding was about 260% at 10° C and about 35% at 27° C.

### Mineralization of organic P

Mineralization of organic P is known to take place naturally, presumably by microbial action, when soils are allowed to stand in a warm, moist condition (76).

The evidence of mineralization of P is not so clear-cut as that of mineralization of N in which the release of ammonium or nitrate is easily determined over short periods of time.

According to Tisdale and Nelson (76), evidence of the mineralization of organic phosphate can be visualized in two ways: (a) the organic P level in soils is lowered as a result of long-continued cultivation. Further, it has been observed that when virgin soils are brought under cultivation, the content of organic matter decreases. With this decrease in organic matter, there is an initial increase in the citric acid soluble phosphorus, a measure of the level of inorganic phosphate in soils, and (b) the results of short laboratory experiments in which decreases in organic phosphorus content of soils are related to increases in the dilute acid extractable inorganic phosphate.

Increased soil temperature may raise the concentration of soluble soil and fertilizer P by increasing the rate of mineralization of organic P or the chemical decomposition of insoluble inorganic forms of P (3, 4, 17, 77). However, higher soil temperatures may reduce P solubility by increasing the rate of immobilization and chemical fixation of P in the soils (7, 25, 42). Thus, the net result of the increased soil temperature on P solubility will depend on the relative rates at which these processes change with temperature.

Eid et al. (17) showed that at low soil temperature (20° C), the availability of soil P to plants was dependent almost entirely upon the inorganic fraction because of the low rate of release of organic P. At high temperature (30° C), however, both inorganic and organic fractions were significantly related to the amount of plant-available P. From the results, they concluded that the availability of the inorganic P fraction was not significantly influenced by differences in temperature but that availability of the organic P fraction was significantly influenced by differences in temperature. Therefore, at the beginning of a season, P present in organic form in soils may contribute substantially to the P nutrition of plant growth during the season (77).

Unlike the mineralization of organic carbon and nitrogen, which is not increased by increases in soil pH, mineralization of organic P has been shown to increase with increasing soil pH (1, 76).

Working on the Bladen organic, acid soils (pH 4.6), Army and Miller (4) indicated that increased soil temperature affected P uptake only at high liming level. This suggests that organic P was the main P component being rapidly released due to organic matter breakdown.

Mack and Barrer (43) found that soil incubated at low temperature (-20.5° C) released more P when leached with water than soil incubated at 2.7° C. They suggested that the incubation at low temperature may have caused the formation of new P compounds from both organic and inorganic sources or simply may have changed the surface area of the compounds present.

The effect of freezing and thawing on the physical properties of soils and mineralization of organic nitrogen

A. Physical properties of soils

It has been known for some time that seasonal variations, through the actions of freezing and thawing, and wetting and drying, affect soil aggregates.

According to Baver (5) and Chepil (11), freezing and thawing cause a granulating action on clods. The work of Slater and Hopp (68), however, shows that freezing and thawing decreases the water stability of soil, the most detrimental effects being found for soils of high moisture concentration. Chepil (11) reported that frost action tends to break down large aggregates but tends to aggregate fine particles to an intermediate size aggregate. Soulides and Allison (70) demonstrated that drying and freezing per se had an adverse effect on the stability of soil aggregates.

According to Willis (80), soils which had undergone the effect of freezing and thawing generally had a lower percentage of aggregates above 0.25 mm than those which had not been subjected to the process. Leo (37) reported that, after freezing and thawing at saturated moisture, the total porosity of eight Canadian soils was decreased and their coefficient of permeabilities was increased. However, the maximum water-holding capacity and noncapillary porosity appeared no different between frozen and unfrozen soils.

Jung (30) has made an extensive study of the nature of frost action on soils and has shown that freezing may cause either aggregation or dispersion. The nature of the crystallization of ice is the determining factor.

Crystallization is influenced by the rapidity of cooling. With slow cooling, the ice crystals form in the tension-free pore spaces. These crystals serve as growth centers to which water is drawn from around the particles. This process causes a dehydration of the particles, which makes possible more intimate contact between the soil grains. Moreover, there is a possibility of a certain amount of flocculation, due to any electrolytes that might be released during dehydration. The mechanical force developed from the pressure of ice crystals probably plays an important role in aggregation. If cooling is rapid, large numbers of small crystals are formed which cause a breaking up of aggregates by the expansion of freezing water.

Penner has made an extensive investigation on frost action. He states that destruction due to frost action can occur when water freezes and expands in the porous structure of solid materials. Most cases of severe frost action in soils can be attributed to the formation of ice lenses which initially cause heaving and subsequently result in the loss of bearing strength (57). He postulated the mechanism of frost heaving which involved the interaction of the three frost action factors, namely, a water supply, a frost susceptible soil, and below freezing temperatures (56).

Frese and Czeratzky (19) showed that the effect of frost on soil structure depends on the duration, intensity and temperature trend of the frost. In addition, effects of frost on soil structure were influenced by the mechanical composition of the soils, density and bedding of soil materials, water content of the layer affected by frost and others. In soils with fine particles, the formation of the typical frost structure resulted with the

crystallization of ice in layers between soil material. In soils with coarse particles, water and soil froze to a homogeneous mass.

Cycles of freezing and thawing have been shown to cause a significant breakdown of aggregates from sods at all moisture levels employed except the one equivalent to 1.0 atm. pressure. The destructive effect of alternate freezing and thawing increased with increasing moisture content in the well-aggregated soil, but the effect of increasing moisture content on a poorly aggregated soil was not significant (40).

#### B. Mineralization of Nitrogen

Rapid freezing caused marked changes in the biological activity and appreciably increased the mineralization of nitrogen, as reflected in greater uptake of nitrogen by millet (41).

Ivarson and Sowden (27) noted that freezing caused a marked increase in the total amount of free amino acids extracted, and attributed the effect of soil freezing on the acceleration of microbial activity to the release of available energy sources. They also found that the increase of microbial activity was not related to organic matter or soil nitrogen content. In contrast to their findings, Paul and Tu (55) concluded no change in the total free amino acids of soil frozen at 0°-16° C for one and 24 weeks. The difference might have been due to the different extracting solutions used by them.

Gasser (20) showed that when fresh soil samples were frozen at -10° C, and alternated with thawing at 2° C, there was more mineralization of soil organic nitrogen than when the samples were kept at 2° C. On the other hand,

Soulides and Allison (70) found that freezing resulted in only a slight release of  $\text{NH}_4\text{-N}$ , but no release of  $\text{NO}_3\text{-N}$ .

#### Factors affecting P availability

Wild (79) and Hemwall (23) have made a thorough review of literature on the problems of phosphate fixation in soils. The author, therefore, will review only some of the contributing factors which pertain to the present research.

##### A. Effects of neutral salts

It is known that salts of different cations affect the solubility of soil phosphate in different ways. The results of experiments with phosphate compounds of low solubility have suggested that solubility is increased by certain cations and reduced by others. This problem is important in view of the high rate of fixation of phosphate applied to cultivated soils and the consequent low recovery in the crop.

The effects of salts on the solubility of phosphate in calcareous soils have been explained as (a) a common-ion effect (8, 38, 62), (b) a salt effect (36, 71), and (c) changes brought about in the pH of the medium by hydrolysis of the salt (73).

Liebig (39) showed that sodium chloride, sodium nitrate, and ammonium salts increased the water solubility of calcium phosphate. He urged that this increased solubility would still be exerted in the presence of soil, and hence these salts would increase the diffusion of phosphate fertilizers through the soil. Later, McGeorge and Breazeals (44) found that all the neutral salts that they tried decreased the solubility of rock phosphate and

soil phosphate. Greaves (22) concluded that potassium chloride, sodium chloride, calcium nitrate, and calcium sulfate decreased the solubility of the phosphate of these two forms, but sodium nitrate, potassium sulfate and ammonium sulfate increased the solubility.

Hibbard (24) found that calcium sulfate and calcium and magnesium carbonates considerably depressed the water solubility of the phosphate that have been applied to four soils, but sodium sulfate and ammonium sulfate had practically no effect and potassium and magnesium sulfates had only a slight depressive action. In contrast to his results, Srivastava and Agrawa (71) indicated that magnesium sulfate enhanced the solubility of dicalcium phosphate. Also, when the  $\text{Ca}^{+2}/\text{Mg}^{+2}$  ratio was decreased, the solubility of phosphate was correspondingly increased. After making a comparison with sodium sulfate, they strongly emphasized the specific role of  $\text{Mg}^{+2}$  in increased phosphate concentration.  $\text{Mg}^{+2}$  helped to stabilize the system in such a way that more dicalcium phosphate could be formed, which resulted in a greater concentration of phosphate for plant uptake.

Lehr and Wesemaele (36) made an investigation on the effect of neutral salts on P solubility in pure dicalcium phosphate solution and in soil. They concluded that all neutral salts decreased the solubility of the soil phosphate. They explained this depressing effect as the replacement of Ca ion at the absorptive complex by other cations. The effect was found to be more pronounced with higher salt concentration. In the case of salts with the same anions, the depressing effect increased in the order of the lyotropic series:  $\text{Na} < \text{K} < \text{Mg} < \text{Ca}$ . When pure dicalcium phosphate solution was used, all

neutral salts increased the phosphate concentration, except for calcium salt which was shown to decrease phosphate solubility owing to the common-ion effect.

The depressive effect of neutral salts on the solubility of phosphate in soils has also been interpreted by Schofield (67) and Mattson (45) by assuming that the phosphate ion, along with the changeable cations, is absorbed on the clay particles. Clark and Peech (13) postulated the existence in soil of a crystalline phosphate compound having a definite solubility product.

#### B. Effect of temperature on the solubility of P fertilizers

Temperature during the reaction of water-soluble P fertilizers with soil has been found to influence subsequent uptake of P by crops as shown by Bouldin and DeMent 1/ and others (7, 65).

Beaton et al. (6) prepared pellets of P fertilizers from different sources and measured the rate of dissolution at different incubation temperatures and periods. They found that rate of dissolution of water-soluble P fertilizers markedly increased with increasing temperature, incompletely dissolved at 5° C at the end of 14 days, completely dissolved at 35° C in 4 days. For each 15° C increase of temperature, there was about 33% decrease in concentration of water soluble P and the proportion of dissolved fertilizer P, the decrease being rapid during the first 14 days and slower afterwards.

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1/ Personal communication, TVA, Wilson Dam, Alabama. 1969.

Regarding the solubility of phosphate fertilizer, Bouldin and DeMent and Beaton and Read (7) in a short-term absorption study observed that availability of monoammonium phosphate and diammonium phosphate was greater when these fertilizers were added to cold soils than to warm soils. On the other hand, the availability of monocalcium phosphate was relatively unaffected by temperature. On acid soils, Robinson (65) and Bouldin and DeMent found that the uptake of P from monocalcium phosphate increased as the temperature during the reaction period decreased.

Nakayama and Yamashita (47) incubated volcanic-ash soils with three sources of P fertilizer at 4° C and 30° C for three weeks and then planted tobacco in the greenhouse. They observed that with super, ignited and diammonium phosphates, dry matter production and P uptake by the plants were greater with the 4° C than with 30° C treatment. With fused phosphate yield and P uptake were much greater with the 30° treatment. P concentration in the plants was much greater with the 4° C than with the 30° C treatment, irrespective of the form of P used.

More water-soluble P was extracted from a calcareous Saskatchewan soil when monocalcium phosphate was allowed to react with it at 5° C than at either 16° or 27° C, according to Beaton and Read (7). Following the addition of  $K_2HPO_4$  solution to Texas soils, Fraper (18) found that the amount of P fixed was less when the soils were kept in ice bath for five hours than when they were incubated at either 31° or 40° C. Dadykin (14), who used diammonium phosphate and monopotassium phosphate as sources of P, concluded that low temperature reduced fixation and increased both the amount of available P and the mobility of added P. Incubation temperatures above 15° C

reduced water- and bicarbonate-soluble P added as concentrated superphosphate (58).

B. Effects of  $\text{CaCO}_3$ , Ca ion and pH

Working on three acid soils and two alkaline soils, Terman et al. (74) showed that dicalcium phosphate dihydrate was appreciably more available than anhydrous dicalcium phosphate in all soils. Monocalcium phosphate was slightly more available than the dicalcium phosphates on the alkaline soils, but was less available on the acid soils.  $\alpha$ -Tricalcium phosphate was nearly as available as anhydrous dicalcium phosphate on the acid soils, but was of very low availability on the alkaline soils. Hydroxyapatite was of very low availability of all soils. Availability of octocalcium phosphate was less than that of the dicalcium phosphates and decreased with increasing liming rates on low P, acid soils.

Benne et al. (8) studied the effect of Ca ion and reactions upon the solubility of P, by treating phosphoric acid solutions at varying pH values with CaO,  $\text{CaCO}_3$  and  $\text{CaCl}_2$ . Large excesses of  $\text{CaCO}_3$  failed to precipitate the phosphate from solution apparently because of the slight solubility of  $\text{CaCO}_3$ . Slight additions of CaO reduced phosphate concentration in solution to a minimum at pH 7.36 and maintained the low phosphate concentration at high pH's. Large excesses of  $\text{CaCl}_2$  precipitated no phosphate from solution until the pH was raised to 7.36. In all cases, they found that the Ca ion did not precipitate phosphate from solution below a pH of approximately 5.6. Clark and Peech (12) showed that calcium phosphate could account for the P content found in extracts from many neutral and calcareous soils but not

from acid soils. Teakle (73) determined the solubility of phosphates in aqueous solutions and soils at various reactions. He concluded that calcium phosphate is insoluble under alkaline conditions, and that the main effect in the depression of phosphate solubility in alkaline soils is the presence of Ca ions. He also precipitated Ca in one of the solutions with ammonium oxalate, and caused a twenty-fold increase in phosphate concentration. He was able to reprecipitate this phosphate by adding Ca ions and thereby reduce the phosphate concentration in the soil to its original value. Lewis et al. (38) found that salts of Ca having a common ion with fertilizer phosphate caused fixation of phosphate. Burd (9) noted that with calcareous soils containing  $\text{CaSO}_4$ , the pH had to be lowered almost one unit before the effect of the common ion, Ca, was overcome and an appreciable increase in the phosphate concentration of the soil occurred. He stated that in soils containing gypsum, it is difficult for the plant to shift the equilibrium in favor of dissolution of phosphate. Pratt and Thorne (62) measured the concentration of phosphate between pH values of 4 and 10 in Na-clay and Ca-clay suspensions. The concentration of phosphate was greater over this pH range in the Na-clay system than in the Ca-clay system, again showing the effect of the common ion, Ca, on phosphate solubility.

McGeorge and Breazale (44) attributed the depressing effect of solid  $\text{CaCO}_3$  on the solubility of rock phosphate to a reaction which occurs between the  $\text{CaCO}_3$  and the rock phosphate, resulting in the formation of a new compound containing more  $\text{CaCO}_3$  and having a very low solubility. Olsen (53) suggested that possibly the absorption of carbonate and Ca ions onto the surface of the rock phosphate crystals would modify the nature of the outer

layer of ions by enriching the surface layer with Ca ions, and thus decrease the activity of the phosphate ions. The solid phase  $\text{CaCO}_3$  would also absorb phosphate ions from solution onto its surface, and the relatively higher activity of Ca ions from the  $\text{CaCO}_3$  compared to the Ca ions from rock phosphate would lower the activity of the phosphate ions. The nature of the  $\text{CaCO}_3$  surface may be such that the phosphate ions fit well onto the surface of the crystals, thus causing an additional lowering of the phosphate activity.

Clark and Peech (12) stated that monocalcium phosphate cannot persist in soils; if added as a fertilizer, it will be converted to some other form. Dicalcium phosphate can exist only in soils in which the phosphate concentration is unusually high. It may occur as a transition product. Hydroxyapatite is probably the predominant solid phase in neutral and alkaline soils.

## MATERIALS AND METHODS

### Materials

Soil was collected from the surface six inches at each site, brought to the laboratory, air dried, crushed finely and passed through a 2-mm. sieve. The organic debris and gravel were separated from the soils. Preliminary soil tests, including tests for mechanical and chemical properties, were made before initiating the greenhouse experiment. The locations and various characteristics of soils are given in Tables 1 and 2.

Measurements of particle size distribution show the textures to be loam, silt loam, and sandy loam for the Avalanche, Amsterdam, and Richlie soils, respectively. These three soils are of mixed mineralogy, and the cation exchange capacities are medium.

The pH's of Avalanche loam and Amsterdam silt loam are considered to be in the satisfactory range for plant growth, whereas the pH of Richlie sandy loam indicates a sodic condition. All three soils are calcareous; the  $\text{CaCO}_3$  equivalents are not too high. The measurements of exchangeable  $\text{Na}^+$  and conductivity show that the soils have a slight excess of exchangeable  $\text{Na}^+$  but are unlikely to produce saline effects detrimental to most plants. The total nitrogen and nitrate-nitrogen contents are relatively low, and the organic matter percentages are medium.

The results of the determination of  $\text{NaHCO}_3$  soluble P of the soils, according to Olsen (54), suggests an "unlikely response". However, using the modified Bray procedure adopted by the Soil Testing Laboratory of Montana State University ( $\text{NH}_4\text{F-HCl}$  extraction), the available P contents of the soils were 112, 59, and 186 pounds per acre. Thus, the available P status is

Table 1. Locations and subgroup names of the three soils studied a/

Series	Subgroup	Location
Avalanche	Borollic Calciorthid	Dillon, Montana
Amsterdam	Pachic Cryoboroll	Belgrade, Montana
Richlie	Borollic Calciorthid	Dillon, Montana

a/ Information obtained from Dr. G. A. Nielsen, Plant and Soil Science Department, Montana State University.

Table 2. Measurements of various characteristics for the three soils studied.

Characteristic	Avalanche	Amsterdam	Richlie
pH	7.8	7.9	8.5
Conductivity, mmhos/cm	1.78	0.97	1.56
Organic matter, %	3.82	3.41	2.30
Bulk Density, gm/cm <sup>3</sup>	1.38	1.46	2.30
Total nitrogen, %	.062	.091	.056
NO <sub>3</sub> -N, lb/A	1.5	1.5	0.8
NaHCO <sub>3</sub> soluble P, ppm	16.4	14.0	22.0
Exchangeable cations			
meg/100 gm			
Sodium	7.8	3.92	9.2
Potassium	1.7	0.8	1.9
Calcium	15.4	7.0	12.3
Magnesium	6.7	5.2	7.9
Cation exchange capacity meg/100 gm	37.5	24.5	34.3
Particle size distribution			
Sand, %	35.4	23.4	67.4
Silt, %	46.0	50.0	22.0
Clay, %	18.6	26.6	10.6
CaCO <sub>3</sub> equivalent, %			
Neutralization method	8.35	4.1	6.94
Gravimetric loss of CO <sub>2</sub>	6.96	2.15	5.56

considered to be medium, very low, and high for the Avalanche loam, Amsterdam silt loam and Richlie sandy loam soils, respectively.

## Methods

### A. Potting procedure and fertilization

Each soils was thoroughly mixed, then 2.5 kilogram masses were weighed into plastic pots. Treble superphosphate (0-45-0) was mixed with the soils at rates of 0, 150 pounds per acre (0.995 g/pot), and 300 pounds per acre (1.980 g/pot). Suitable amounts of water were added to bring the soils to field capacity.

Half of the total pots were taken into the cold room where the temperature was kept at  $-26.5^{\circ}$  C. The other half remained in the laboratory at room temperature which ranged from  $25^{\circ}$  to  $29^{\circ}$  C, depending upon the variation of the outdoor temperature. Several pieces of cardboard were used to cover the pots in the cold room to reduce moisture loss. Pots in the laboratory were watered twice daily with estimated amounts necessary to maintain field capacity.

At the end of every six weeks, 27 pots from the cold room and the laboratory, respectively, were moved to the greenhouse where the temperature was about  $26.7^{\circ}$  C. The frozen soils were allowed to thaw for two days. Then, measured amounts of nitrogen and potassium nutrient solutions were pipetted into each pot. The amounts applied depended on the original contents of nitrogen and potassium in each soil. Solutions of minor elements were also applied to ensure against any effect arising from minor element deficiencies. Sources and amounts of nutrients applied are reported in Table 3.

Table 3. Amounts and sources of mineral nutrients applied to 2.5 kilograms of soils b/

Soil	Nitrogen, mg/pot	Potassium, mg/pot
Avalanche	312.5 ( $\text{NH}_4\text{NO}_3$ )	125 ( $\text{K}_2\text{SO}_4$ )
Amsterdam	300.0	250
Richlie	350.0	125

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In addition to the above nutrients, to each soil was added:

Mg, 12.5 mg/pot ( $\text{MgSO}_4$ )	Zn, 12.5 mg/pot ( $\text{ZnSO}_4$ )
B, 2.25 mg/pot ( $\text{H}_3\text{BO}_3$ )	Mn, 2.28 mg/pot ( $\text{MnCl}_2$ )
Cu, 0.196 mg/pot ( $\text{CuSO}_4$ )	Mo, 0.0089 mg/pot ( $\text{H}_2\text{MoO}_4$ )
Fe, 6.27 mg/pot (Fe-citrate)	

b/ Modified from Power et al. (60). To convert to lb/A multiply 0.8.

After fertilization, each pot was seeded with 15 kernels of winter wheat (Triticum aestivum L.), Cheyenne variety. The pots were thinned to 10 plants per pot ten days after germination. In the winter time, incandescent light was turned on to supply a daylength of 8 hours. Measured amounts of water were applied daily. The amounts added depended on the texture of each soil and the vigor of plant growth on it. Plant height measurements were made every three weeks.

B. Dry matter production and P uptake determinations

After the wheat had grown for ten weeks, the above-ground portions were harvested, washed with distilled water, and dried 24 hours at 70° C. The dry plant materials were recorded for the yield index.

The dry plant material was then ground, dry ashed, and the P content of plant tops was determined (32).

C. Physical and chemical methods

The particle size distribution of soils was determined by the hydrometer method.

Exchangeable sodium, potassium, calcium and magnesium were determined using 1 N ammonium acetate as an extractant (29). The conductivity was measured from an extract of the saturated paste (78). Soil pH was measured in a 1:2 soil:water mixture by means of a pH meter.

The CaCO<sub>3</sub> equivalent in percent was determined by both neutralization and gravimetric CO<sub>2</sub> loss methods (78).

The determination of soil organic matter was made by the Walkley-Black method (29). Total nitrogen was measured by the microkjeldal method (29).

$\text{NO}_3\text{-N}$  in soils was determined before P fertilization and after incubation by nitrophenolidsulfonic-yellow color procedure (29).

The  $\text{NaHCO}_3$  soluble P was determined, following Olsen's method (54), at four different times, namely, original or prior to the application of P fertilizer, after application, after incubation and finally after harvest.

D. Experimental design and statistical analyses

A factorial experiment with completely randomized design having three replications, three soils types, three rates of P application, three periods of incubation and two temperatures (frozen and unfrozen) of incubation was adopted.

The analyses of variance for yield, percentage of P content and P uptake in plant tops, as well as linear correlation and regression study, were computerized.

Duncan's multiple range test of significance was applied to means for separation of main effects and interaction of factors detected by the F-test in the ANOVA tables.

## EXPERIMENTAL RESULTS

### Plant growth and dry weight of plant tops

It was observed that the germination of seeds in the soils subjected to the processes of freezing and thawing was generally two to four days later and their emergence was less uniform than in soils kept at room temperature. The delayed germination and poorer initial growth were more apparent at the six-week period than at the later periods. In the first three weeks of growth, the height of plants was similar on all unfrozen pots, regardless of the amounts of P applied, but slight differences appeared among the three soils. These pots had strong plants with healthy and vigorous growth. Heights of plants measured at different ages of growth are reported in Table 4. It is noted that, in some cases at six-week and others at nine-week stages, there were no differences in height in the two treatments. However, the strength, the vigor and leaf sizes were less satisfactory for plants on the frozen soils.

The soils fertilized with P produced taller plants. In most cases, there were not obvious differences in heights between 150 and 300 pounds per acre of P application. This is particularly noted on the frozen pots of Avalanche loam and Richlie sandy loam. The check plants were generally shorter, especially on Amsterdam silt loam.

In general, plant growth and height were not noticeably different among soils and periods of incubation. Plants grown on frozen soils showed poorer initial and less uniform growth throughout the duration of experiment.

Table 4. Plant heights as a function of age and treatment.

a. on Avalanche loam

Plant age weeks	Treatment	Incubation period		
		6-week	12-week	18-week
Inches*				
3	Room	8.26**	9.27	8.48
		8.95	9.16	8.]0
		9.12	9.54	8.52
	Freezing	6.35	7.34	7.23
		6.83	7.15	7.21
		6.54	8.02	7.15
6	Room	16.93	17.26	17.25
		17.20	18.32	18.28
		18.04	19.43	18.67
	Freezing	16.23	16.51	16.27
		16.74	17.21	17.11
		17.25	17.26	18.52
9	Room	22.36	23.22	21.63
		23.63	23.87	24.95
		23.25	24.15	24.00
	Freezing	22.02	21.54	21.02
		22.53	22.48	22.86
		23.87	25.36	23.47

\* Average of eight of the taller plants

\*\* Downward order, on soil received 0, 150, 300 lb/A P, respectively

Table 4, cont.

b. on Amsterdam silt loam

Plant age weeks	Treatment	Incubation period		
		6-week	12-week inches*	18-week
3	Room	9.35**	9.38	9.26
		9.82	9.67	9.03
		8.84	9.21	9.32
	Freezing	8.51	8.23	8.31
		8.48	8.13	8.58
		8.03	8.32	8.56
6	Room	17.52	17.83	17.93
		17.60	17.26	18.05
		17.74	18.27	18.36
	Freezing	17.69	17.72	17.20
		17.75	18.18	18.08
		17.13	18.00	18.73
9	Room	23.26	23.32	23.53
		23.87	24.00	24.69
		22.94	23.48	25.00
	Freezing	23.13	23.16	22.98
		24.46	24.86	24.87
		24.05	24.67	25.86

\* Average of eight of the taller plants.

\*\* Downward order, on soil received 0, 150, 300 lb/A P, respectively.

Table 4, cont.

c. on Richlie sandy loam

Plant age weeks.	Treatment	Incubation period		
		6-week	12-week	18-week
		Inches*		
3	Room	8.52**	8.05	8.96
		9.36	9.46	9.24
		9.42	9.52	9.03
	Freezing	7.23	7.26	7.32
		7.04	7.32	7.97
		7.27	7.30	7.36
6	Room	18.65	18.67	18.23
		18.12	19.25	18.56
		18.17	19.37	19.21
	Freezing	17.76	17.22	17.32
		18.84	17.63	18.43
		19.01	18.26	19.66
9	Room	22.84	22.03	23.63
		24.65	23.89	24.22
		24.54	23.51	25.74
	Freezing	21.03	21.98	21.98
		22.05	21.87	24.36
		22.13	23.56	25.89

\* Average of eight of the taller plants.

\*\* Downward order, on soil received 0, 150, 300 lb/A P, respectively































































































































