tion than did a moderate Fe deficiency which in turn resulted in more CO₂ fixation than did an adequate Fe level may explain the observations that in lime-induced chlorosis, organic acid contents increased and then decreased as the disorder advanced (3, 12).

SUMMARY

Fixation of CO₂ with PEP (carboxylase and carboxykinase systems each included) and R5P as substrates was greater in homogenates of plants grown at a slightly deficient Fe level than in those grown at an adequate Fe level. For severe Fe deficiency the fixation catalyzed by PEP carboxylase was decreased. A chelating agent sometimes increased the amount of CO₂ fixation with the PEP carboxykinase reaction. A chelating agent was necessary in the reaction mixture for maximum activity of the reaction catalyzed by PEP carboxylase as well as for the carboxylation enzyme. Addition of Fe to assay mixtures inhibited CO₂ fixation through PEP. Kinetic studies indicated this to be a competitive inhibition. Fe also inhibited CO₂ fixation when R5P and G6P were used as substrates and activity was increased with both substrates by use of chelating agents.

LITERATURE CITED


LOSS OF PHOSPHORUS-32 BY PLANT ROOTS AFTER FOLIAR APPLICATION 1,2

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The fact that plant roots are capable of losing ions to the ambient medium was recognized as early as the nineteenth century, and has been the subject of intermittent attention since that time. Excellent reviews of the early work in this field were compiled by Merrill (10) and True (14). Definite conclusions in these early experiments were often lacking since the work was limited solely to the use of electrolytic or chemical techniques. Such techniques prevented accurate determination of very low concentrations, as well as detection of ion movement in a direction opposite to that of net flow. Recent development of radioisotope methods has made such measurements possible, and has provided a new approach to the study of ion loss by roots. Evidence exists to indicate that root loss of ions may play an important role in the overall nutrient

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2 This paper is based on work performed under contract no. AT(30-1)-2177, Project 281, with the U.S. Atomic Energy Commission.
economy of the plant. Such loss was of sufficient magnitude to be detected chemically in the plant growth medium (2, 5, 7), and to cause a significant reduction in the nutrient content of the plant top (1). Regulated loss of ions by roots has been envisioned as one means by which the plant is able to maintain electrical neutrality while differentially absorbing companion ions (7, 8). Considerable use has already been made of root loss determinations in the development of "free" and "outer" space concepts, and the volumetric measurement of such space (3). In these and related studies loss of phosphorus, sulfur, selenium, rubidium, and other elements by roots to the external medium was noted. The forces and mechanism involved in such transfers are not clearly understood, however, and invite continued investigation.

The present work was undertaken to study the effect of phosphorus in the root environment and of time on loss of radiophosphorus from roots. Test plants were placed in a graded series of phosphorus solutions and also in water, and the effect of such treatments on root loss of $P^{32}$ was studied over long (6 to 7 day) and short (50 minute) time periods. Experimental techniques differed in important respects from classic excised root methods often employed in studies of this type, and these warrant brief initial mention. Firstly, whole intact plants were used so that any possible effect of plant top on the phenomenon under study would not be lost. Secondly, the radioactive material was introduced to the plant through the foliage in order to prevent contamination of the root surface with isotope. Lastly, complete nutrient solutions instead of single salt solutions were employed in order to minimize root loss of materials other than those under observation.

**Materials and Methods**

Bean seeds of the variety Wade were germinated on damp cheesecloth until the radicles were about 2 to 3 cm long, and then moved for further growth to a device designed to eliminate root injury in later transfers. Seeds were placed on a paper towel which was held taut on a wood frame and which was suspended approximately 75 mm above the surface of a reservoir of aerated water. A hole was provided in the towel for each radicle so that it could grow downward into the water. Plants were removed from this system when they were about 8 cm in height by saturating the towel with water and peeling it away from the plants. In this and subsequent manipulations, extreme care was taken not to injure the roots.

The young plants were next moved to a controlled environment facility and grown in 2 liters of aerated complete nutrient solution for 4 days. Solutions were then discarded, roots rinsed with water, and new treatment solutions added. Radioactive phosphorus was applied to the surface of 1 of the primary leaves of each plant immediately following the solution change. The area of application was approximately 4 cm², and was previously outlined with a black water emulsion paint not toxic to plants. Extent of root loss of foliar applied isotope was estimated 6 to 7 days after application. Ten ml of nutrient solution from each plant container was evaporated to dryness in a stainless steel planchet and assayed for radioactivity. Just prior to the time the solution samples were taken, the test plants were removed from the solution and also prepared for counting. The foliar area upon which the isotope was originally placed was removed as a leaf plug, and the remaining plant top tissue was placed in a crucible, dried, and weighed. The tissue was finally ashed at 450°C until gray, and then assayed for radioactivity. Roots were treated separately in the same manner as the plant tops except that dry weights were not taken.

The controlled environment facility consisted of an enclosure 16 by 20 feet in which light intensity, light duration, day temperature, and humidity were controlled. A day length of 14 hours with a light intensity of 1200 ft-c 1 foot above the plant containers was maintained in all studies. Day temperatures fluctuated 8 degrees around a mean of 20°C. and dropped to between 10 and 15°C at night. Relative humidity was kept at 50%.

The radioactive phosphorus was obtained from the Oak Ridge National Laboratory as $H_3P^4O_4$ in dilute hydrochloric acid. Acid normality of the various shipments ranged between 0.42 and 0.58. Specific activity of all shipments was above $1 \times 10^8$ millicuries per gram phosphorus. Dilutions were made of the original shipments so that 25 microcuries of carrier free isotope were applied to each plant in 0.3 ml solution. Adjustments in pH of the acid isotope solution were not made since greatest foliar uptake of phosphorus occurs at low pH values (13). Treated leaves were held horizontal with a wire arm to prevent running off of isotope.

Two systems of counting were necessitated by the vast differences in activity between the solution and tissue samples. Plant tissues were counted in the crucible in which they were ashed, using a thin end-window Geiger-Müller tube (1.4 mg/cm²). Where activity was beyond the capacity of the instrument, a 5.4 mg/cm² filter was placed between the sample and end-window. Results obtained with the filter are so identified in the text. All solution samples were counted in a gas-flow counter equipped with an ultrathin end-window (150 μg/cm²). Both Geiger-Müller and gas-flow counters were connected to a conventional amplifier-scaler-high voltage system. It is important to emphasize here that it was not possible to calibrate the different instruments used against a common source so that results for the solution and tissue samples are not comparable.

Sixteen phosphorus treatments were studied ranging from 0 to 14 millimicroms phosphorus per liter solution. Treatments are designated in the text according to the millimicroms phosphorus in 1 liter of solution. The fact was mentioned earlier that treatments were not single salt solutions but contained a balanced compliment of essential mineral elements. Content of all elements except phosphorus was held constant.
among treatments (N = 18 milliliters/1; K = 6; Ca = 6; Mg = 1; S = 1). Microelements were added according to Hoagland (6). Changes in H₂PO₄⁻ were accommodated by alterations in the NH₄⁺ to NO₃⁻ ratio. The pH value for fresh solutions ranged between 6.5 and 6.8. Tap water also served as a treatment. Chemical tests for calcium, potassium, and phosphorus in the tap water proved negative. Flame methods were used for the bases, while the colorimetric ammonium molybdate method was used for phosphorus. Electrical resistance of the tap water was 2.2 × 10⁴ ohms, as compared with 7.9 × 10⁴ for demineralized water. The pH of tap water was 6.5 which was similar to that of demineralized water.

A record of solution pH changes was maintained during the treatment periods early in the investigation, but was eventually abandoned because changes were not considered to be of sufficient magnitude to be nutritionally important. In addition, non-metabolic root loss of ions of the type studied here has been shown to be virtually insensitive to pH (3).

The overall set of 14 treatments was divided into 4 groups, each of which was studied at a different time. Each treatment was replicated 8 times, and plants were arranged in randomized block order. Results were analyzed according to conventional analysis of variance methods.

**Results**

**Long Duration Experiment:** Radioactivity of treatment solutions at the conclusion of the experiment is shown in figure 1. Results indicate that root loss of P⁴² did occur to the graded series of solutions and that this loss increased with increasing stable phosphorus in the root environment. Loss in this series appeared as an exponential function of environmental phosphorus. Radiophosphorus was also lost to tap water at a level slightly greater than that to the 0.0 P treatment.

The phosphorus economy of a plant is known to have an influence on foliar uptake and distribution of this element in the plant (13). A check was therefore made to determine whether nutrient treatments affected foliar uptake of P⁴² in the present experiment, and whether this was a factor to consider in interpreting the root loss data. Tops and roots of the test plants were assembled according to treatment at the conclusion of the experiment, and assayed for P⁴² according to procedures outlined. Results for the run in which water was a treatment are given in table I.

No statistically significant differences in activity occurred between the graded phosphorus series of treatments, either for root or for top samples. Loss data for these plants plotted according to tissue activity yielded essentially the same curve as that in figure 1. Tissue activity for the water treated plants was considerably below that of the phosphorus group, however, and indicated a relatively lower foliar uptake of isotope by these plants. A reploting of loss data according to plant top activity elevated the water treated plants to a level of that of the 0.8 P plants, while a recalculation based on root activity elevated the water plants above all other treatments.

Although solution treatments did not substantially affect foliar uptake of P⁴² (except in the water treatment), it is reasonable to expect that they did influence the overall phosphorus economy of the plants and thus caused a shift in the ratio of stable to radioactive atoms (specific activity) in the tissue. The shift would be toward a dilution of radioisotopes (a lowering of specific activity) as phosphorus in the treatment solutions increased, and would in effect tend to minimize the loss values for the high phosphorus treatments. Loss of a greater total number of phosphorus atoms would be required for each ordinate unit in figure 1 for the high phosphorus treatments than for the low phosphorus treatments. Treatment differences in root loss could therefore be expected to be somewhat greater than those given in figure 1, were absolute values available.

Dry weights of the plant tops were taken to determine whether root loss results were conditioned by growth differences among plants. In no case were weight differences among treatments statistically significant.

**Short Duration Experiment:** Methods and results: In the interest of simplicity of presentation, solution activity was regarded up to now as a direct indicator of the extent to which roots lost P⁴². The existence of such a simple relationship may be questioned when critical examination is made of the test conditions of the experiment. Six to seven days elapsed from the time of isotope application to the time root loss determinations were made. During this time, solutions in which the test plants were grown were not changed. Opportunity was therefore ample for recapture by plants of a portion of the P⁴² atoms once lost to the solution. Ion recapture of this type would tend to be greatest for the low phosphorus and least for the high phosphorus treatments. A true picture of ion loss by roots is further complicated in the earlier experiments by possible influences of solution treatment on the specific activity of the plant tissue.

A second study was undertaken under conditions designed to largely eliminate these experimental weaknesses. Root loss was measured over much shorter

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**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Radioactivity as counts per minute *</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>0.0 P</td>
</tr>
<tr>
<td>Roots</td>
<td>284**</td>
</tr>
<tr>
<td>Plant tops</td>
<td>1095**</td>
</tr>
</tbody>
</table>

* Counts made through a metal filter.
** Statistically significant at the 5% level. LSD 0.05 roots = 838; LSD 0.05 tops = 1083.
time periods than those employed earlier, thus avoiding prolonged exposure of the plants to the different phosphorous regimes. In addition, treatment solutions were periodically drained and renewed throughout the test period to reduce the chances of recapture by the root of ions which had previously passed out of the root.

Experimental procedures for starting plants were similar to those reviewed. Bean plants were germinated and then grown in 2 liters of aerated complete nutrient solution. The method and activity of isotope application was also similar. The complete nutrient solutions were not discarded upon \( \text{P}^{32} \) application, however, but plants were allowed to remain in them for 2 more days. Plants were then individually removed from the solutions, and the roots lowered into glass tubes 10.0 cm long and 2.5 cm in diameter. Tubes were sealed at the lower end except for a solution drain and an air inlet. At the time of plant transfer, each tube contained 30 ml of the old complete nutrient solution.

The old solution was next drained from each tube and replaced with 30 ml of treatment solution. Solutions were again drained and renewed at 5, 15, 30, and 50 minute intervals, using the first drainage as the base time. All tubes were vigorously aerated throughout the course of the study. Four ml of each drainage was evaporated to dryness in a stainless steel planchet and counted to determine extent of root loss of \( \text{P}^{32} \) to the solution.

Treatments were 0.0 P, 0.4 P, 1.0 P, 6.0 P, and were again designated according to milliatoms stable phosphorus in a liter of solution. Tap water and demineralized water also served as treatments. Electrolyte content and pH values for the 2 waters were given earlier.

The experimental procedures were carried out twice, each treatment being replicated 4 times in the 1st run and 6 times in the 2nd run. Results for the 2 runs were identical in virtually every respect, and were therefore composited for presentation in figure 2.

Results indicate that \( \text{P}^{32} \) was lost by the test plants throughout the entire course of the study. Greatest loss occurred in the 1st 5 minutes of the study, and decreased exponentially with time. Solution treatments did not affect the exponential nature of the curve, but did influence the level at which it occurred. De-
mineralized water showed the greatest loss, followed in order by the 6.0 P, 1.0 P, 0.4 P, tap water, and 0.0 P treatments. Treatment differences remained consistent throughout the entire study. Results for the phosphorus series of treatments showed loss of \textsuperscript{32}P as a function of stable phosphorus in the root environment and are in agreement with those of the long term experiment given in figure 1.

**Discussion**

The results indicated clearly that radiophosphorus passed from the root to the external medium. These findings are not in themselves new, and have been reported by others (1, 5, 11, 12). The present experiment departed from techniques generally employed in such studies, however, in that introduction of isotope was made through the foliage and not the root of the plant. Surface contamination of roots with radioactive material during the introduction process, and subsequent release of the contaminant during loss studies, was thus avoided. All radiophosphorus which entered into the root loss calculations came originally from the root interior, having at one time or another followed the sequence of foliar absorption and internal distribution. Exact definition of terms as tissue “interior”, “external”, and root “surface” in this instance is difficult in light of conflicting opinions regarding root morphology as it applies to transport of ions. Definitions may vary according to the school of interpretation favored. If, as in the concept of “free” space, the interface barrier to free diffusion of ions in the root is taken as a natural line of division, root “exterior” could be designated on the one hand as any area external to the physical structure of the root itself or again, as certain areas extending to the endodermis (8), or even to within the stele of the root (4).

Root loss of phosphorus was conditioned in this experiment by composition of the root environment. Referring for the present only to the graded phosphorus series of treatments and not to water, loss occurred as a positive function of ambient phosphorus concentration. A similar relationship was shown by Hevesy (5) for wheat plants and suggests that an isotopic exchange reaction is largely involved in the loss process. Such a system of isotopic exchange would indicate that root phosphorus occurs to a considerable extent in the bound rather than the freely diffusible state. These conclusions are in agreement with Overstreet et al (12) who showed a non-metabolic binding of newly absorbed phosphorus in barley roots. The exponential nature of the loss in the present experiment further suggests that differences exist in tenacity with which individual phosphorus ions are maintained in the bound state.

Loss of radiophosphorus to the water treatments cannot, however, be reconciled with the proposed model of isotopic exchange. Loss was often greater to water which was virtually free of exchange materials than to nutrient solutions which were abundantly supplied with such materials. The fact that loss to demineralized water was significantly different than that to tap water adds further to the difficulty of arriving at a reasonable explanation for the water treatments.

The present results and also those by Overstreet et al (12) showed an effect of time on the magnitude of phosphorus loss. Such a time effect is difficult to interpret since it could occur as a product of various and unrelated phenomena. A diminishing root content of total phosphorus during the test period, or at least that fraction which is easily exchangeable, could result in a drop in root loss values with time. A time effect could further be explained as a function of distance the exchange components necessarily traversed into and out of the root tissue to complete the loss process. A gradual recession into the root interior of the reaction responsible for the large scale release of \textsuperscript{32}P as equilibrium between stable and radioions was approached in the exterior regions, could effectively reduce root loss of radioions with time.

The above interpretations should be considered in light of 2 important limitations in experimental procedures worthy of emphasis. Firstly, recapture of \textsuperscript{32}P atoms by roots after they had once passed out of that tissue, was not prevented entirely, even in the short term experiment. Ion recapture was possible during the time intervals between solution drainings, although the frequency with which solutions were changed undoubtedly reduced the incidence of recapture. Recapture of radiophosphorus was also possible between the time of isotope application and actual utilization of the plant in root loss studies. A buildup of \textsuperscript{32}P on the root surface could have occurred in this manner and influenced subsequent loss figures, especially in the early time periods of the study. Influences of ion recapture such as these could not in any respect be compensated for in the present experiment, and root loss figures must therefore be considered apparent and not absolute throughout.

Secondly, while root loss of \textsuperscript{32}P was considered here exclusively as a root phenomenon, such loss may indeed involve forces residing in tissues outside of the root. A differential replenishing of root phosphorus by aerial tissues during the test period could well have occurred, for instance, to influence the root loss results. That aerial tissues do indeed influence loss results has already been demonstrated by Jacobson et al (8).

**Summary**

Root loss of \textsuperscript{32}P to a graded series of phosphorus solutions and to water was studied over a long (7 day) and a short (50 minute) time interval. Intact bean plants were used in which the isotope was introduced through the foliar tissue.

In the long term experiment, loss to the phosphorus solutions occurred as an exponential function of ambient phosphorus concentration. Foliar uptake of radiophosphorus was conditioned by the water treatment so that loss values for this treatment varied according to the mode of calculation used.
Loss to the graded phosphorus solutions also occurred as an exponent of ambient phosphorus in the short term experiment. Loss to demineralized water was considerably greater than that to tap water. Loss to all treatments was greatest in the 1st 5 minute period of the study, and decreased exponentially with time. Solution treatments did not affect the basic nature of the time-loss curve, but did influence the level at which it occurred.

LITERATURE CITED

GERMINATION OF LIGHT SENSITIVE SEED IN CROSSED GRADIENTS OF TEMPERATURE AND LIGHT
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Seed germination is strongly temperature dependent and is also frequently influenced by light. These variables are interrelated: the relationship of germination to temperature is dependent on the light treatment and conversely the relationship of germination to light treatment is dependent on the temperature. (2, 4, 8, 9) Using standard methods, a complete study of the interrelation of these 2 variables, on the rate or attainable percentage of germination of a single strain of seed, requires the correlation of a large number of separate measurements. It seemed likely that the crossed gradients principle might be useful for seed germination experiments.

In this paper we shall describe an attempt to survey, in a single experiment, the effects of combinations of a wide range of temperature and light treatments. This was done with an apparatus developed originally to study algal growth in crossed gradients of temperature and light intensity (6, 7). In the previous work the algae were grown on an 11 × 12 inch agar surface in a shallow chamber with a temperature gradient from left to right and with a light intensity gradient from front to back. Thus a single experiment gave a 2-dimensional picture of the growth response to the 2 variables.

Drs. E. H. Toole and S. B. Hendricks suggested that lettuce seed (Lactuca sativa L. var. Grand Rapids) would be suitable for testing the utility of the crossed gradients principle for seed germination investigations.

The effect of the interaction of temperature and light on germination in this variety of lettuce has been studied in great detail by several workers. This particular seed is very light sensitive, giving a greater germination percentage in light than in darkness. The photo-response varies with the temperature (3) and the type of radiation—red light promotes and far-red radiation inhibits the germination of water-imbibed seeds (5). The action of the red and far-red radiation is repeatedly reversible (1, 8). Inhibition by far-red radiation is particularly temperature sensitive if seeds are first given a saturating dosage of red light and then exposed to a less than saturating far-red dosage (9). We therefore decided to germinate seeds in a temperature gradient crossed with a far-red radiation