FOLIAR ABSORPTION OF MINERAL NUTRIENTS WITH SPECIAL REFERENCE TO THE USE OF RADIOISOTOPES AND THE "LEAF WASHING TECHNIQUE"

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By

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AN ABSTRACT

Submitted to the College of Agriculture, Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Approved [signature]

Foliar absorption rates for radiophosphorus (P^{32}) and calcium (Ca^{45}) in the bean plant were determined by washing from the surface of the leaf, at intervals after treatment, the non-absorbed residue. The nature of the washing solution and the presence of moisture on the leaf surface on P^{32} uptake, and the effects of pH on Ca^{45} absorption were also determined.

Bean seedlings were germinated in quartz sand and then transferred to aerated half strength Hoagland's solution. When the primary leaves were partially expanded, one drop of treating solution was applied to the center of the upper surface of the primary leaf by means of a tuberculin syringe. The phosphate treating solution consisted of 20 millimolar orthophosphoric acid, at pH 3, containing 3.5 to 13 microcurie per millilitre of P^{32}. A 25 millimolar solution of calcium chloride enriched with 16 microcurie per millilitre of Ca^{45} constituted the calcium treating solution. There were seven or more single plant replicates for each treatment. The non-absorbed residue was removed from the leaf surface by washing the site of application with distilled water or other test solutions. The wash solution along with the various plant parts were brought to dryness, and the radioactivity determined by means of an end-window G-M tube and standard scaler circuit. P^{32} or Ca^{45} not recovered in the wash solution was considered absorbed and was expressed as percent of total applied.

The leaf washing technique was evaluated (a) by determining how much distilled water was needed to wash off the non-absorbed residue from one drop of P^{32} labeled solution applied to the upper surface of the primary of the bean plant, (b) by measuring the comparative effectiveness of different solutions including distilled water, H_3PO_4 at 10^{-1}, 10^{-2}, 10^{-3} or 10^{-4}, and $CaCl_2$, KH_2PO_4 and NaOH at 10^{-2}M, on removal of residues by washing, and (c) finally by comparing foliar absorption of P^{32} as measured by the leaf washing technique with the leaf disc removal technique. All washing procedures and measurements were made after a 24-hour absorption period.

The results showed that (a) 10 millilitres of distilled water was sufficient to remove the non-absorbed residue from one drop of foliar applied P^{32} labeled ortho-phosphoric acid solution, (b) distilled water was as effective in washing the residue from leaf surfaces as any other solution prepared, and (c) the leaf washing technique provided greater precision than leaf disc removal for measuring absorption of foliar applied nutrients.

The leaf washing technique was then utilized in studies of the initial absorption rates of foliar applied P^{32} and Ca^{45}, in an evaluation of the effects of the presence of surface moisture on foliar uptake of P^{32}, and in determining the effects of pH on foliar absorption of Ca^{45}. The absorption rate for

Ca^{45} was much higher than that of P^{32}. Fifty percent of the foliar applied Ca^{45} was absorbed in about 3 hours and 99 percent in 96 hours, while only 27 percent of the P^{32} was absorbed in 24 hours and about 40 percent after 96 hours. Translocation of Ca^{45} out of the treated leaf was negligible. The presence of surface moisture significantly increased foliar absorption of P^{32} but did not alter transport out of the treated leaf. Ca^{45} absorption was significantly greater at pH 5 and pH 6 than at pH 4. It was proposed that the presence of moisture on the leaf surface aids foliar absorption of P^{32} by increasing permeability and providing an aqueous pathway through the cuticle, and that changes in the electric charge on the cuticle could explain the difference in Ca^{45} absorption when the pH was altered.

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INTRODUCTION

The agricultural importance of foliar applications of mineral nutrients has progressively achieved greater proportions in recent years. This has developed as a result, primarily of knowledge accumulated by research workers that has raised the veil of growers' doubt concerning the potentialities of foliage feeding. Dr. Sylvan H. Wittwer in Michigan has summarized the limitations and potential benefits from foliage feeding as follows (60):

> "The greatest values of foliage feeding are now realized on crops where certain deficiency disorders can be easily corrected by spray treatment, where spraying for other reasons is already an established practice, on crops where total leaf areas are large, where conditions are not optimum for nutrient uptake by roots (cold soils in early spring and the far north), and when there is a great demand for nutrients such as during flowering and early fruit set."

Thus growers have been intrigued by the possibilities offered through foliage feeding in increasing yields, hastening maturity, improving quality, and the availability of a means of readily correcting nutritional disorders, plus its use as supplemental fertilizing program.

Much fundamental research has been completed and much remains to be done on the penetration of nutrients through leaves and mechanisms of absorption and distribution and redistribution of foliar applied nutrients. For instance, Dr. Sylvan H. Wittwer in Michigan has suggested ratios of

2-1-2, 2-1-1 or 4-1-4 as generally most suitable for foliar fertilization based on plant requirement, and studies of absorption rates and mobility of foliar applied nutrients (60).

A convenient procedure for accurately measuring foliar absorption of mineral nutrients is greatly needed. With this objective the leaf washing technique was evaluated, comparisons made with the leaf disc removal technique, foliar absorption rates accurately determined for P^{32} and Ca^{45}, and some variables affecting absorption of P^{32} and Ca^{45} studied.

LITERATURE REVIEW[1]

Methods of Applying Treating Solutions

One of the first considerations with respect to foliar absorption studies of mineral nutrients must be where and how the treating solutions are to be applied. Although the method of application is often determined by the nature and magnitude of the experiment, as well as the plants to be treated, plant spraying has been commonly used, and has constituted the exclusive method for most field experiments, and for application to crops where nutritional disorders were to be corrected. Either the entire above-ground parts have been sprayed (14, 27, 38, 42, 45, 50, 51, 53, 58), single leaves or selected leaves (9, 19, 22, 23, 24, 31, 32, 40, 48, 52, 65), or only a limited part of the leaf surface (7, 8, 10, 16). Specially designed spray equipment was used in many of these studies. Nutrient solutions have also been painted onto leaf surfaces (4, 28, 43, 46, 51, 59, 66, 67, 68, 69, 70). Dipping has been a common method of treatment (1, 2, 3, 12, 29, 30, 33, 36, 37, 59, 62, 64). The one drop technique has also been used (4, 11, 24, 25, 30, 31, 59). Other methods, although rarely employed, have been the multiple drop technique (11, 48), injection (8, 9, 31), dusting (55), and vacuum infiltration (18, 68). Moreover

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[1] The author is greatly indebted to Dr. S. H. Wittwer and the review article that he prepared in collaboration with Dr. F. G. Teubner entitled "Foliar absorption of mineral nutrients" which appeared in Ann. Rev. Plant Physiol. 10: 13-22, 1959, in preparation of this Literature Review.

it is important to note that the lanolin ring method, a modification of the one drop technique, was used by Gustafson (25) to retain the drop of treating solution at the site of application where such a condition was desired. Another modification of the one drop technique was made by Colwell (18), and may be termed the plasticine well method. A plasticine well was set carefully on the middle of the upper surface of the leaf to be treated, into which the treating solution was poured.

Leaf Washing Technique

The leaf washing technique has been developed as perhaps the most promising in the field of foliar absorption of mineral nutrients. However, this technique was not reported prior to 1952. In that year Cook and Boynton (19) employed a leaf washing technique in their studies on urea nitrogen absorption through McIntosh apple leaves. The sprayed leaf was cut from the tree at the end of absorption period and washed with a low pressure jet of distilled water containing a wetting agent. They wetted the surface of sprayed leaf and flushed it with water four to six times, making a total wash solution volume of 500 millilitres. They also tested the efficiency of the above volume of wash solution by washing with a second 500 and a third 500 millilitres of water. Less than 5 and only 0.05 percent of total applied nitrogen were recovered in the second and in the third 500 millilitres of water, respectively. Thus, their experiments were standardized in that only one washing of 500 millilitres was used. The amount of urea nitrogen not recovered in the wash, as compared with that applied, was considered as the amount absorbed. In further tests with nitrogen absorption by apple leaves, Rodney (42) washed each leaf previously sprayed with different nitrogenous compounds through three changes of distilled water. He standardized with three changes of water, since he found only trace amounts in the third rinse. After drying at 105°C, the samples were analyzed for total nitrogen by the Kjeldahl method.

Volk and McAuliff in 1954 (57) thoroughly washed the sprayed tobacco leaves with distilled water in their foliar absorption studies with N^{15} labeled urea nitrogen. The samples were then dried, ground and analyzed for nitrogen by the micro-Kjeldahl method. They did not, however, describe their washing technique in detail.

Fisher and Walker (23) in their studies on absorption of radiophosphorus (P^{32}) and magnesium through the lower surface of apple leaves, adopted the washing technique described by Cook and Boynton (19). The wash solution used, however, by Fisher and Walker contained not only a wetting agent (0.1 percent Triton X-100), but was acidified with one millilitre of concentrated hydrochloric acid per liter, because they found that this prevented the absorption of P^{32} on the Geiger-Müller immersion tube with which they counted the non-absorbed P^{32} and also this solution resulted in more accurate counts of radioactivity.

Cain in 1956 (16) employed the leaf washing technique in his studies on the absorption and metabolism of urea nitrogen by coffee leaves. Wash water was applied with a dropping pipette to wash off non-absorbed urea, while an especially designed mop was squeezed out into a micro-Kjeldahl flask. Although four washings were considered sufficient, the exact quantity of water that was used to wash the marked area on the leaf surface sprayed with urea was not reported. The combined washings were analyzed for a determination of the non-absorbed nitrogen.

Thorne in 1955 (50) washed the tops of each two-months-old sugar beet plant, previously sprayed with macro nutrients, in 300 millilitres of distilled water to remove the non-absorbed nutrients that remained on the outside of the treated leaves. The leaves were then wiped with filter paper which was, in turn, washed with hot water. The tops were once again rinsed with 100 millilitres of clean water by means of a pipette and then dried. The collected washings including that from the filter paper were used for chemical analysis of nitrogen, phosphorus and potassium.

Wallihan and Heymann-Herschberg in 1956 (59) washed the treated leaf with an acidified detergent solution to remove the residue from one drop of radio-zinc (Zn^{65}) applied to either side of an orange leaf. Absorption of Zn^{65}

was calculated from the counts detected on either side of surfaces of the leaf after washing. It is important to note that the effects of different wash solutions on washing-off the non-absorbed Zn^{65} residue were studied. A solution of Dreft detergent, which was acidified to 0.3 normal hydrochloric acid, was more effective in removing the non-absorbed residue of Zn^{65} than either sodium-ethylene-diaminotetraacetate (NaEDTA) solution or Ivory soap solution. The quantity of wash solution was not, however, indicated.

Oland and Opland in 1956 (40) adopted a somewhat different leaf washing technique. They wiped the apple leaf, previously sprayed with magnesium sulfate, with a dot of cotton wool, repeatedly wetted, and rinsed it in distilled water. After this washing procedure to remove the non-absorbed residue, samples were dried at 75° C, wet-digested, and chemically analyzed for magnesium. The amount of water used was not given.

Walker and Fisher in 1957 (58) also used a washing method. They washed apple leaves, which had been sprayed with either magnesium sulfate or chelated magnesium, to remove the residue that remained outside of the leaf after the absorption period. The leaves were then analyzed chemically for magnesium to determine which one of the compounds was most efficiently absorbed. However, their leaf washing technique was not described.

In 1957 Thorne (51) reported on the results of two different leaf washing techniques. She washed the foliage of three-month-old sugar beets, which had been treated with labeled nitrogen (N^{15}), P^{32}, and potassium, with spray water before she prepared samples for radioactive assay through dry ashing. In a second procedure she washed the top of each sugar beet plant individually, in such a way that the resulting solution was made to one litre and analyzed for non-absorbed nutrients.

Wittwer, Teubner and McCall in 1957 (62) washed tomato fruits with distilled water to remove surface contamination, since they had dipped the top of the plant into a P^{32} labeled phosphorus solution at the initial flowering stage, in their studies on the comparative utilization of P^{32} labeled phosphorus from foliage and soil applications. The fruits were dry-ashed and prepared for chemical and radio-phosphorus assay.

Freiberg and Payne in 1957 (24) used somewhat different leaf washing techniques for banana leaves treated with N^{15} labeled urea nitrogen. One hundred inches taken from a longitudinal half of a treated leaf were washed by wiping with glass wool soaked in distilled water. This glass wool was squeezed out and rinsed several times with clean water until the total volume was brought up to 100 millilitres. In their other experiments, they collected discs, 2.2 centimetres in diameter, by punching them out of the treated leaf. One hundred were then placed in a jar containing 50 millilitres of distilled water, and shaken vigorously for one minute. The solution was then collected in an Erlenmeyer flask. Four millilitres of either of the above solutions thus prepared were analyzed manometrically for the non-absorbed urea.

In 1957 Barrier and Loomis (4) used a leaf washing technique to remove the residue from one drop of P^{32} labeled phosphorus applied to the "unifoliate" leaf of the soybean seedling. The treated leaves were rinsed through a series of four water-Dreft solutions and finally tap water. Results were expressed as counts per minute detected in the dried sample.

Hagler in 1957 (27) washed leaves or canes of the grape, which had been sprayed with several magnesium compounds, with one percent hydrochloric acid solution, and rinsed with distilled water, to remove non-absorbed residues. After drying at 80° C in a forced draft oven, the samples were ground in a Wiley mill, the magnesium contents chemically determined, and the efficiency of absorption of various compounds noted. No details of the leaf washing technique were provided.

In recent experiments (1958) Mederski and Hoff (37) harvested the tops of 5-week-old soybean plants which had been dipped into one or five percent manganese sulfate solutions, and washed them in six successive 400 millilitre lots of distilled water. After washing, the plant samples were oven dried, weighed, ground, and analyzed for total manganese. They used six washings, since no detectable quantity of manganese was found in additional washings. They considered the difference in manganese content between the dipped and non-dipped plants after washing as foliar absorbed manganese.

Burr _et al._ in 1958 (13) divided the leaf of sugar cane, treated with N^{15} labeled urea nitrogen, into several anatomical parts and scrubbed them thoroughly to remove non-absorbed urea residues. No further information on their leaf washing technique was given.

Thorne (52) has recently (1958) described an excellent leaf washing technique. The swede leaf sprayed with radioactive phosphorus solution was washed, after an absorption period, by dipping into a solution of two percent sodium phosphate containing five percent hydrochloric acid and a wetting agent. It was then rinsed in a jet of distilled water. The total washings used for washing each leaf were made to a volume of 100 millilitres. The treated leaf and the remainder of the plant, separated into stems and roots, were wet-digested and the samples prepared for radioactive assay. It is of interest to note how she proved that 100 millilitres of water was sufficient to remove the non-absorbed residue without leaching of nutrients from within the leaf. She washed a leaf of a plant rich in P^{32} labeled phosphorus which had been applied to, and absorbed by, the roots in the usual way. No leaching of radioactivity was observed, and she found negligible radioactivity in washing beyond 100 millilitres in the original washing procedure.

Of late, Bukovac, Teubner and Wittwer in 1959 (11) have developed a leaf washing technique. A bean leaf treated with Mg^{28} labeled magnesium by the one drop method was washed with 25 millilitres of distilled water. The wash solution was applied as a fine jet stream from a polyethylene wash bottle. The oven dried materials were then assayed directly for radioactivity. The amounts of magnesium absorbed and translocated out of the treated leaf were calculated from the counts detected in each part of the plant as compared with total counts detected in all parts of the plant including the washing solution used for removing the non-absorbed residue from the leaf surface.

Leaf Disc Removal Technique

The leaf disc removal technique has been frequently used as one of the means of measuring foliar absorption of mineral nutrients. With this technique, there is no concern as to whether or not the non-absorbed residue of applied nutrients is completely removed by washing, and there is no danger of leaching nutrients out of the leaf from excess washing. But the leaf disc removal technique has two serious sources of error: (a) the absorbed nutrients not transported beyond the area punched out are not included in the absorbed amount, and (b) without particular care, the treated area might not be punched out exactly, although the lanolin ring method could reduce the possibility of such error. However, the lanolin ring method to delineate more precisely the treated area has been used in but few cases (25).

Several investigators, however, have used the leaf disc removal technique. Kaindl in 1956 (30) "plugged-out" treated areas on leaves of Solanum nigra and Galinsoga parviflora which had been treated with P^{32} by the one drop technique, and separated the plant into the remainder of the treated leaf, non-treated leaves, stems, and roots. The samples were then dry-ashed and assayed for radioactivity. Wallihan and Heymann-Herschberg in 1956 (59) "punched-out" treated areas as large as one square centimeter after the designated absorption period. They noted the merits of this technique in their studies on the absorption and translocation of Zn^{65} through the citrus leaf. Because they found that there was strong "fixation" of zinc chloride on the leaf surface, they could hardly expect the complete removal of the non-absorbed residue by the washing technique. In this connection they also suggested the use of the single-drop technique by which consistent results could be obtained if the drop were kept small and did not spread. The amount of Zn^{65} absorbed was calculated from the counts detected in plant parts other than in the leaf disc that was punched out. The total radio-activity was closely estimated by adding the counts detected on the leaf disc that was punched out and those on the remainder of the treated leaf.

Gustafson in 1958 (25) described an excellent "plugging" technique in his studies of foliar absorption and translocation of Co^{60} in bean and cucumber plants. At the end of the absorption period he punched out the treated area with a cork borer and discarded it, and then separated the plant into the treated leaf, the remainder of the top, and the roots. Then these samples were dried and coarsely ground for assay of radioactivity. An excellent modification was the use of a lanolin ring by which he could keep the treating solution from spreading. The lanolin ring was outlined with ink in hot weather when the lanolin would easily melt. Attention was directed to keeping the treating solution within the lanolin boundary. Gustafson (26) used this technique again in his subsequent study on the comparative absorption of Co^{60} by upper and lower epidermis of leaves. Bukovac and Wittwer (12) used the leaf disc removal technique extensively with 14 radioisotopes (Na^{22}, P^{32}, S^{35}, Cl^{36}, K^{42}, Ca^{45}, Mn^{52-54}, Fe^{55-59}, Cu^{64}, Zn^{65}, Rb^{86}, Sr^{89}, Mo^{99}, $BaLa^{140}$), in their studies on the foliar absorption and mobility of these elements in the bean plant. Plants treated during the expansion of the first trifoliate leaf were harvested at different time intervals. After punching out an area one centimeter in diameter which encircled the treatment site, each plant was separated into the remainder of the treated leaf, stem plus non-treated leaves, and roots. The samples were then dried and counted directly using an end-window G-M tube and standard scaler circuit. Absorbed amounts were expressed as percentages of total radioactivity recovered in the sum of all plant parts, other than the leaf disc that was removed, which encompassed the site of application.

Sample Preparation Methods for Assay of the Absorption of Foliar Applied Mineral Nutrients

Foliar absorption of non-radioactive mineral nutrients may be determined in a gross way by standard chemical methods. Tracer techniques, however, have allowed for much greater precision and sensitivity, and, if combined with standard chemical determinations, permit a differentiation within the plant of foliar absorbed nutrients and those previously and concurrently taken up by the roots. Pertinent literature is reviewed here in which radioactive analyses were employed in studies of foliar absorption of mineral nutrients.

Direct counting of oven dried samples has been the most simple method and was used by Bukovac, Teubner and Wittwer (11), Bukovac and Wittwer (12), Teubner, Wittwer, Long and Tukey (48), and Swanson and Whitney (47). Swanson and Whitney (47), however, used a partial digestion method for leaf and root sample. These samples were partially digested in concentrated nitric acid, plated out and dried in one-inch stainless-steel cupped planchets, and then counted.

Dry ashing, prior to radioactive assay, has been used in other studies, and has the advantage of eliminating considerable self-absorption by the plant material where weak beta emitting radioactive isotopes (S^{35}, Ca^{45}) are used. Mayberry (36), Chen (17), Romney (43), and Koperzhinsky and Sheberstov (32) ashed plant samples in a muffle furnace at temperatures ranging from 500 to 600°C, and then counted them directly for radioactivity. Mayberry (36) added 3 millilitres of 10 percent magnesium nitrate solution to each plant sample before placing them in the furnace to prevent volatilization of phosphorus compounds. Asen, Wittwer and Teubner (2), Higashino and Yatazawa (28), and Yatazawa (66) ashed oven dried samples, with or without grinding, in a muffle furnace at the temperatures described above. They dissolved the ash in concentrated hydrochloric acid, evaporated under an infra-red lamp, and assayed for radioactivity. An end-window Geiger Müller counting tube was used in all the above studies. Wallihan and Heymann-Herschberg (57) and Thorne (51), however, employed a glass-walled dipping counter or M6 liquid containing tube Geiger-Müller counter for measuring the radioactivity of the ash dissolved in a dilute hydrochloric acid solution. Kaindl (29) used a somewhat different method, in that he put the ashed or ground sample into a hole on a glass slide as an alcoholic suspension, and covered it with a one millimetre thick layer of paraffin. In most cases 60 milligrams per square centimetre were prepared and then these were counted.

Wet-digestion methods have also been used. In radio-sulfur (S^{35}) migration studies, Biddulph, Cory and Biddulph (8) cut off one inch segments of stem of the bean plant, the primary leaf of which had previously been treated with S^{35}. These segments were then wet digested. No further information on the digestion procedure or counting techniques was found in their report. In radio-phosphorus (P^{32}) studies Thorne (52) separated each plant into (a) the treated leaf sprayed with P^{32}, (b) the remainder of the top, and (c) the roots. (The treated leaf was washed by the washing technique which has already been reviewed). She then digested each part separately with sulfuric and nitric acids, made the resulting solution to 50 millilitres, and determined P^{32} with a Geiger-Müller counter using a 10 millilitre M6 liquid containing tube. Koontz and Biddulph (31) digested each part of the bean plant, a leaflet of which had been sprayed with P^{32}, in nitric acid, and then made to a constant volume. After drying the aliquots on porcelain, radioactivity was determined by using an end-window Geiger-Müller tube. Absorption was expressed as a percentage of the total phosphorus applied. The amount translocated out of the treated leaflet was considered as the absorbed amount.

MATERIALS AND METHODS

Plant Material and Plant Growing

Phaseolus vulgaris, var. black seeded Blue Lake[1], was selected as the experimental plant because of its rapid germination and uniformity (12). Seeds were spaced approximately 2 inches by 3 inches and germinated in coarse No. 8 quartz sand[2]. They were sown about one inch deep, and no nutrient solution, other than tap water, three to five times a day, was supplied. Seedlings were retained in the sand for seven to eight days after sowing until they were transplanted to the solution cultures. At this time the primary leaves were partially expanded, and the first trifoliate leaf was just emerging. Plants of uniform height and leaf size were selected. The aerated solution culture method of Asen, Wittwer and Teubner (2) was emplyed, except that one gallen mason jars were used instead of the 2-gallon glazed crocks.

Environmental Conditions

All experiments were performed in the greenhouse. Experiments I to V were conducted from March 9 through April 12, during which time the temperature within the greenhouse was maintained between 21 and 27°C. Tem-

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[1]Stock No. 42335, Rogers Brothers Seed Company, Twin Falls, Idaho.

[2]American Graded Sand Company, Chicago, Illinois.

perature control was difficult from June 22 through July 12, during which time Experiments VI and VII were conducted. The mean temperature during that period was 27°C, and ranged from 21 to 32°C. Daily mean temperatures were calculated from the means of temperatures recorded at 2:00 to 3:00 p. m. and 3:00 a. m. Other environmental conditions, such as humidity and light, were beyond control.

Preparation of Treating Solutions

Treating solutions for Experiments I to V consisted of a 20-millimolar solution of ortho-phosphoric acid, at pH 3, containing 3.5 to 13 micro-curie per millilitre of P^{32}. The radioactivities of the treating solutions for each experiment will be indicated later. All treating solutions were adjusted to a pH of 3, using ammonium hydroxide and hydrochloric acid, since many earlier reports (41, 47, 48, 57, 63) had indicated peaks in absorption rates at low pH levels. The treating solution for Experiments VI and VII consisted of 25 millimolar calcium chloride containing 16 micro-curie per millilitre of Ca^{45}. The pH was adjusted to 5 by means of ammonium hydroxide and hydrochloric acid for reasons established by the author[1].

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[1] Leaf burning occurred when a 25 millimolar calcium chloride solution at pH 3 was applied. But no leaf burning was found at pH 4, 5 or 6, even though a 25 millimolar calcium chloride solution was used. On the other hand, a calcium chloride solution alone (pH about 5) caused no leaf burning at a concentration as high as 500 millimolar.

Treatment of Leaves with Radioactive Solutions

One day after the bean seedlings were transferred to the solution cultures, one of the primary leaves was treated with one drop (ca. 0.01 millilitre) of treating solution by means of a tuberculin syringe fitted with a No. 27 guage stainless steel needle. The drop was applied to the upper surface on the main vein, midway from the apex to the base, since it had been previously determined that absorption was greatest from this region of the leaf (34, 48, 59). Special care was exercised not to induce leaf injury with the tuberculin syringe needle, since Volk and McAuliffe (57) reported that ten times more absorption occurred with urea nitrogen on tobacco leaves following slight injury from a camel's hair brush. All experimental plants were treated from 8:00 to 8:30 a.m., since absorption has been found to be greatest during the morning (6, 48).

Harvesting, Leaf Washing, and Leaf Disc Removal Techniques

With the leaf washing technique, plants were harvested, at the designated time intervals, and separated into the treated leaf cut at the base of the petiole, and the remainder of the plant. During washing the tip of the treated leaf was directed toward the inside of a 50 millilitre beaker and the treatment site washed with 10 millilitres of distilled water, supplied dropwise by means of a 10-millilitre pipette. After the initial experiments, directed toward the objective of determining how much water was needed, 10 millilitres of distilled water was considered sufficient to remove the non-absorbed residue from one drop of P^{32} labeled ortho-phosphoric acid applied to the upper surface of the primary leaf of the bean plant. An additional one to two millilitres of distilled water were applied to the margin of the leaf after washing to assure no contamination. Time spent in washing a single leaf ranged from 30 to 45 seconds. For Experiments VI and VII, 1 3/4 ounce paper cups were used instead of the 50-millilitre glass beakers, since they could be easily disposed of as expendable equipment and problems of decontamination following the use of Ca^{45} were minimized.

With the leaf disc removal technique, the treated area on the leaf was punched out with a cork borer one centimetre in diameter according to the technique described by Bukovac and Wittwer (12). The harvested plant was separated into (a) the treated area punched out, (b) the remaining portions of the treated leaf, and (c) a composite sample consisting of roots, stems and remaining leaves. Particular care was exercised to keep the applied drop from spreading along the veins. Small drops were helpful in this respect.

Preparation of Samples for Radioactivity Assay

The various plant parts, washings, and punched-out leaf discs were placed in 50-millilitre glass beakers or 1 3/4 ounce paper cups, and dried in the forced draft oven at 70°C for 12 to 24 hours. Before the dried samples were counted directly, using an end-window Geiger-Müller tube and standard scaler circuit, they were pressed firmly against the bottom of the container with a smasher made of a rubber stopper. This corrected variations in the geometrical placement of the samples relative to the window of the counting tube.

Calculations and Estimates of Variability

The amounts of labeled nutrients not recovered in either the leaf washings or leaf discs, that were punched out, were considered as absorbed amounts. These were expressed as percentages of total labeled nutrients applied. Total applied radioactivity was determined by a summation of the counts detected in each part of the plant plus that removed by the washings or the leaf disc. Data were subjected to an analysis of variance where desired, and values for the least differences necessary for significance at five and one percent levels are given in the tables. Replicates within a treatment and the treatments were completely randomized.

RESULTS AND DISCUSSION

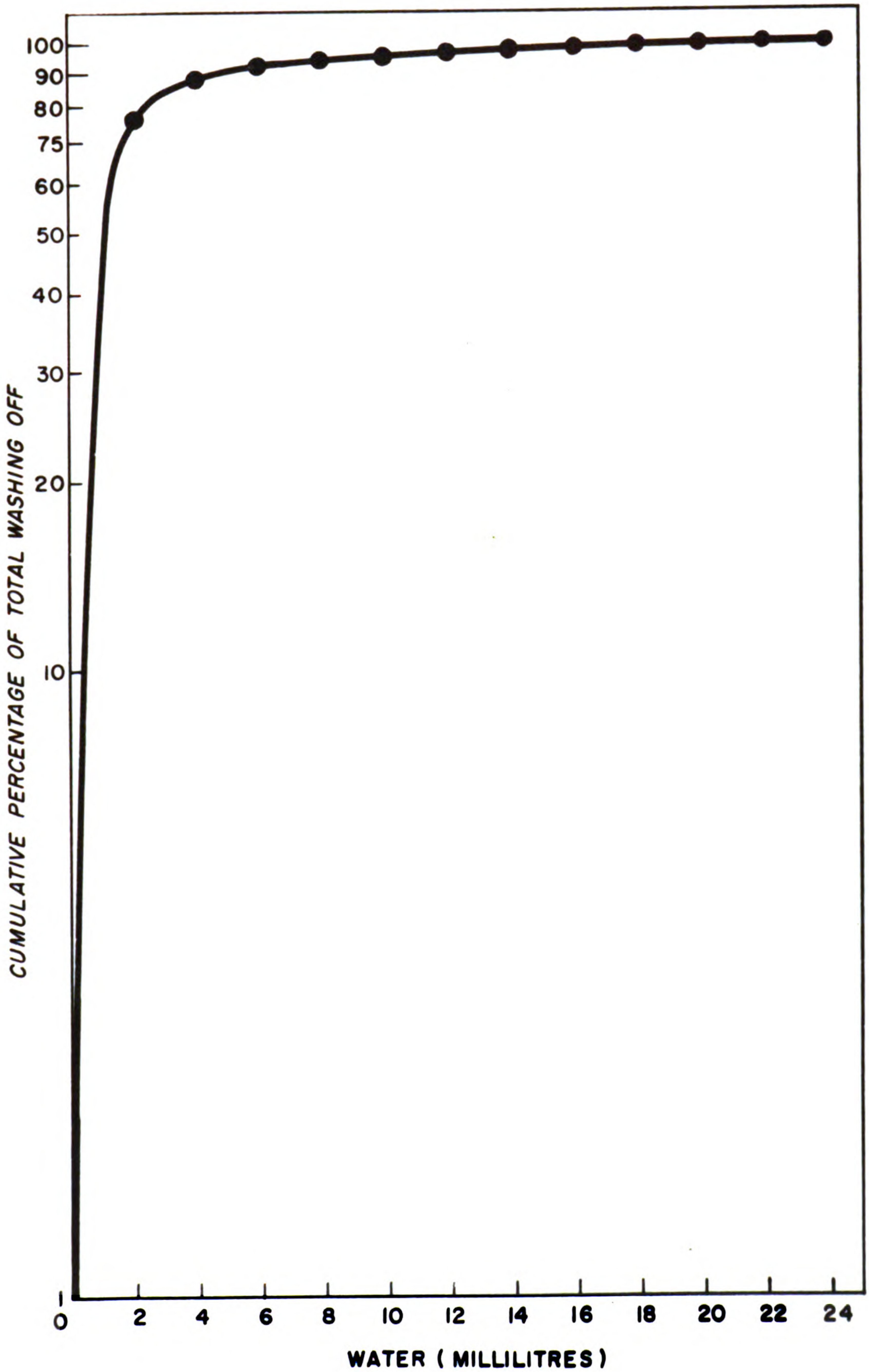
Experiment I

The primary objective of this experiment was to determine how much distilled water was sufficient to remove the non-absorbed residue from one drop of P^{32} labeled phosphorus applied to the upper surface of the primary leaf of the bean plant. The treating solution consisted of 20 millimolar orthophosphoric acid, at pH 3, containing 13 micro-curie per millilitre of P^{32}. After 24 hours absorption, the treated area on the leaf was washed with 12 successive two-millilitre aliquots of distilled water. Each washing was collected separately in a 50-millilitre beaker. After being dried, the wash solutions were counted as described earlier, and the P^{32} recovered in each two-millilitre wash aliquot was expressed as a percent of total P^{32} recovered in the summation of all washings, consisting of 24 millilitres of distilled water. Cumulative percentages are shown in Figure 1.

Since 96.4 percent of the non-absorbed P^{32} was recovered by the first 10 millilitres of wash water, all subsequent experiments were standardized by using this volume. The P^{32} recovered in each additional two-millilitres of wash water was consistently about 0.5 percent of total P^{32} recovered in the washings. Accordingly, it is difficult to say whether or not this might have resulted from leaching of P^{32} from inside the leaf. This could have

Figure 1

The quantity of distilled water needed to remove the residue from one drop of P^{32} labeled ortho-phosphoric acid applied to the upper surface of the primary leaf of the bean plant as determined by washing the site of application with 12 successive 2-millilitre aliquots of distilled water. Recovered P^{32} in each 2-millilitres was expressed as percent of total P^{32} washed off, and was cumulated against the quantity of distilled wash water. Leaf surfaces were washed 24 hours after the application of P^{32}. Each value was a mean of eight replications.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CUMULATIVE PERCENTAGE OF TOTAL WASHING OFF
0
2
4
6
8
10
12
14
16
18
20
22
24
WATER (MILLILITRES)

Reg. No. 21721-3

been proved, as Thorne (52) did, by successively washing the leaf of a plant which had received P^{32} through the roots, but this was not done.

The standardization of the washing procedure with 10 millilitres of distilled water, as has been reported here, should be compared in its simplicity with the method of Barrier and Loomis (4). They washed the non-absorbed residue remaining from one drop of P^{32} applied to the "unifoliate" leaf of the soybean plant with a series of four water-Dreft solutions and with a fifth solution consisting of tap water.

Experiment II

In this experiment the efficiency of different washing solutions in removing the non-absorbed residue from one drop of P^{32} labeled ortho-phosphoric acid applied to the upper surface of the primary leaf of the bean plant was studied. Washing solutions tested, with results from each, are given in Table I. There were no statistically significant differences ($P = 0.05$) among them, however, 10^{-1} molar ortho-phosphoric acid gave the highest recovery. It was not clear whether this might have been the result of a direct effect of pH or that some exchange between the leaf absorbed P^{32} and non-absorbed P^{31} used in washing may have occurred. Washing solutions, other than distilled water have been employed for removal of non-absorbed P^{32} labeled phosphate solutions. Barrier and Loomis (4) used a water-Dreft solution, Fisher

TABLE I

The Effect of Different Washing Solutions as a Means of Removing the Residue from One Drop of P^{32} Labeled Ortho-Phosphoric Acid Applied to the Upper Surface of the Primary Leaf of the Bean Plant*

Washing Solutions: Added Compounds	Washing Solutions: Molarity	Percentages Recovered
None (distilled water only)		77.8
H_3PO_4	10^{-1}	87.3
	10^{-2}	78.7
	10^{-3}	77.7
	10^{-4}	76.9
$CaCl_2$	10^{-2}	74.1
KH_2PO_4	10^{-2}	76.7
NaOH	10^{-2}	71.7
L. S. D.		
5%		11.5
1%		13.6

*Data are expressed as percentages of total phosphorus applied which were recovered in 10 millilitres of each solution after 24 hours absorption. Each value was a mean of eight replications. Treating solution consisted of 20 millimolar ortho-phosphoric acid (pH 3) containing 7 micro-curies of P^{32} per millilitre.

and Walker (23) a 0.1 percent Triton X-100 solution acidified with one millilitre of concentrated (37-38%) hydrochloric acid per litre, and Thorne (52) a 2 percent sodium phosphate solution containing 5 percent hydrochloric acid and a wetting agent. Perhaps Thorne (52) had reasons for using solutions of the same compound as she sprayed the foliage with, highly acidified solutions, and solutions containing a wetting agent. No explanations, however, were given. Fisher and Walker (23) used an acidified solution, simply because it aided in making radioactive measurements with the immersion Geiger-Müller tube. Nevertheless, a trend for increased efficiency in removal of residual P^{32} from leaf surfaces has been noted when the acidity of the washing solution was increased. Phosphate solutions have also been more effective than solutions of other compounds. Further evidence for their greater effectiveness, however, is needed.

Experiment III

The comparative efficiencies of the leaf washing and the leaf disc removal techniques as they apply to studies of foliar absorption of P^{32} labeled ortho-phosphoric acid were compared in Table II. Although data heretofore published indicated no significant differences in percent uptake of foliar applied nutrients, the absorption as measured by the leaf disc removal procedure must always be less than that recorded for the properly employed leaf washing

TABLE II

Absorption of Foliar Applied P^{32} Labeled Ortho-Phosphoric Acid as Measured By the Leaf Washing and Leaf Disc Removal Techniques*

Techniques Employed	Test I	Test II
	Total Absorbed (Percent)	Total Absorbed (Percent)
Leaf washing	23.5	17.8
Leaf disc removal	22.9	16.5
L. S. D.		
5%	7.3	11.5
1%	10.2	16.0

*Treating solutions consisted of 20 millimolar ortho-phosphoric acid (pH 3) containing 12 and 3.5 micro-curie of P^{32} per millilitre in Tests I and II, respectively. One drop of these treating solutions was applied to the upper surface of the primary leaf of the bean plant. All measurements were taken after 12 hours absorption. Each value is a mean of eight replications.

technique, since a definite amount is absorbed directly beneath the site of application but not transported beyond the punched-our area.

Precautions were taken to keep the applied drop from spreading along the veins when the leaf disc removal technique was used. How effectively this was done, however, depended on the skill of the operator and his keenness of vision. Perhaps no difficulty would have been encountered, if the lanolin ring (25) or plasticine well (18) method had been used. Leonard (34), although he worked with 2, 4-D, modified the lanolin ring method which modification is suggested for future studies of foliar absorption. Since the lanolin ring did not necessarily confine the treating solution, he made a ring of a mixture of lanolin and starch, and set a plastic ring in this mixture. Thus, he successfully confined the treating solution to the desired area.

At any rate, as far as the studies of foliar absorption of mineral nutrients are concerned, it appears that the leaf washing technique is a more accurate means of measuring absorption of foliar applied nutrients than is the leaf disc removal procedure.

Experiment IV

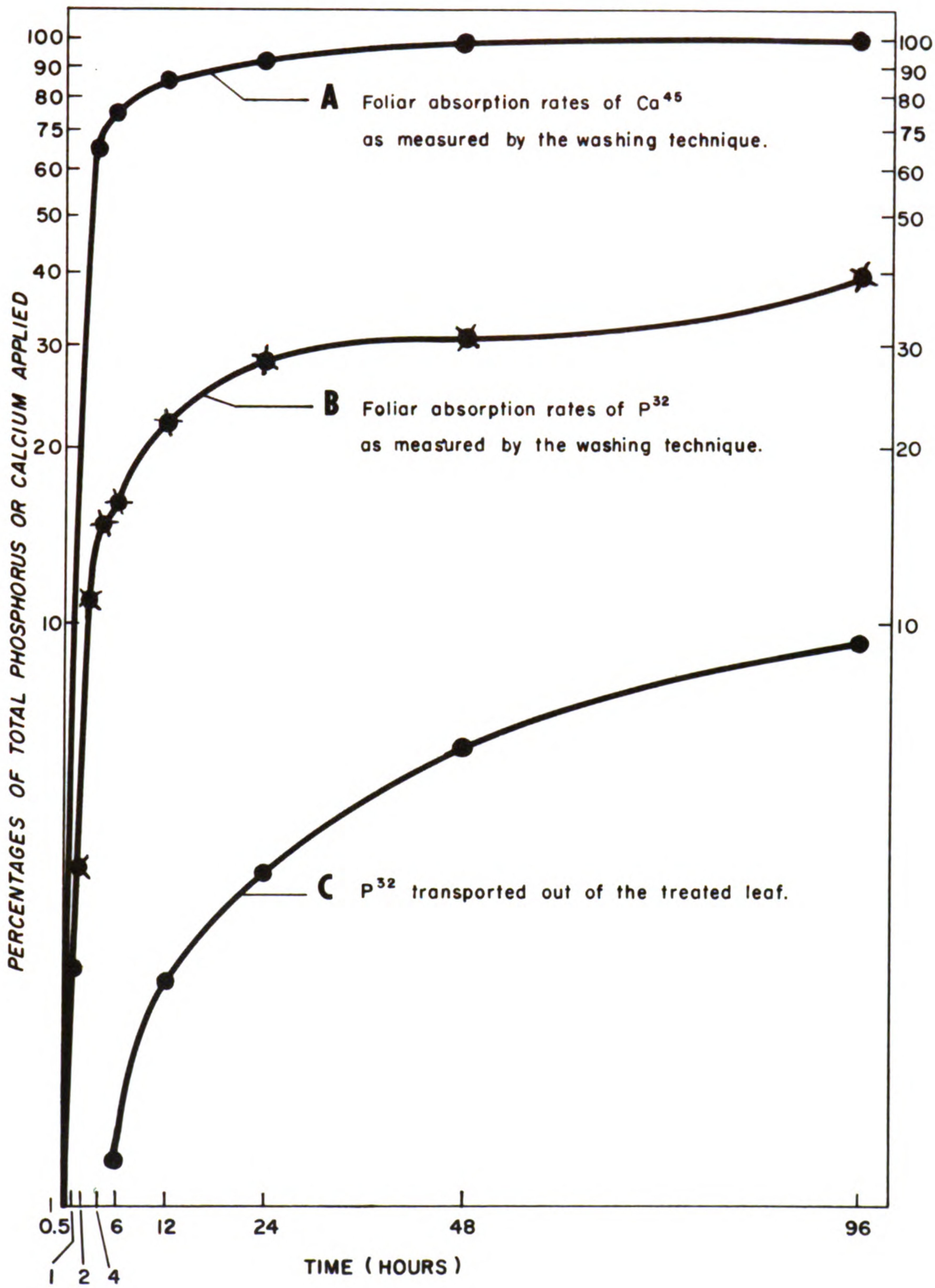
The foliar absorption rate of P^{32} labeled ortho-phosphoric acid by bean leaves as determined by the leaf washing technique was studied in this experiment. The treating solution consisted of 20 millimolar ortho-phosphoric acid, at pH 3, containing 10 micro-curie per millilitre of P^{32}. The results are summarized in Figure 2.

The foliar absorption rate for P^{32} labeled phosphorus herein reported varied somewhat from other published reports (12, 44, 52). In some instances the absorption rates for the bean leaf were lower than in the present experiment--about 23 percent in 48 hours (12), and for sugar cane only 50 percent in 15 days (15). In other cases the absorption rates for the bean leaf were higher than those reported by the present data--50 percent in 30 hours, and 60 percent in 96 hours (31), and 16 percent in 2 hours (4), and for swede leaves 50 percent in 3 days (49). Phosphate absorption rates for apple leaves (23) were comparable to the present values for the bean.

As to the translocation rates out of treated bean leaves, Mayberry (36) observed that 45 percent of total applied P^{32} was transported in 160 hours. According to Koontz and Biddulph (31) 34.5 percent of the applied P^{32} was translocated out of the treated leaf of the bean plant in 96 hours. In both cases the rates of transport were higher than those reported by the present data, since less than 10 percent was removed in 96 hours (Figure 2). It is possible that the specific activity of the treating solutions may have altered the values cited above, but this was not determined in the present study.

Figure 2

Foliar absorption rates of Ca^{45} and P^{32} as measured by the leaf washing technique, and transport rates of P^{32} out of treated bean leaves. Non-absorbed residues were removed by washing the site of treatment with distilled water at the designated intervals after the application of one drop of the treating solution to the upper surface of the primary leaf. Each value was a mean of seven replications for "A", and of eight replications for "B" and "C".



100
90
80
75
60
50
40
30
20
10
1
PERCENTAGES OF TOTAL PHOSPHORUS OR CALCIUM APPLIED
A Foliar absorption rates of Ca^{45} as measured by the washing technique.
B Foliar absorption rates of P^{32} as measured by the washing technique.
C P^{32} transported out of the treated leaf.
0.5
1
2
4
6
12
24
48
96
TIME (HOURS)

Experiment V

The effect of surface moisture on the absorption and transport of foliar applied P^{32} labeled ortho-phosphoric acid was next studied. The experimental moisture conditions imposed at the site of treatment, and the results are given in Table III. Absorption of P^{32} through the upper surface of the bean leaf was almost doubled when the treated area was kept moist as compared to similar treatment areas which were allowed to dry and were not re-wetted. Where moisture was supplied to treated areas, distilled water was added dropwise in the same way as the treating solution was originally applied. In general, absorption was increased with the time during which the treated area was subsequently kept moist.

A possible explanation of the above may be found in van Overbeek's theory (56) that water causes the swelling of cutin, and results in an increase in the permeability of the cuticle. Also, Crowdy's suggestion (21) that "conditions of high moisture may actually increase the permeability of the cuticle in addition to increasing the diffusion of solutes" might be helpful to explain the present data. According to Crafts (20), nutrient salts seem to enter the leaf through an aqueous pathway. This suggestion also could explain the present data.

On the other hand, some reports on the effects of re-wetting on foliar absorption of mineral nutrients are not in agreement with the above, and

TABLE III

Effect of the Presence of Surface Moisture on Foliar Absorption and Transport of P^{32} Labelled Ortho-Phosphoric Acid*

Moisture Conditions at the Site of Treatment	Test I		Test II	
	Total Absorbed (Percent)	Total Transported (Percent)	Total Absorbed (Percent)	Total Transported (Percent)
Kept moist	43.1	2.4	35.0	2.4
Moistened every 1.5 hours	-	-	26.2	2.7
Moistened every 3 hours	27.6	2.7	23.2	2.5
Moistened every 6 hours	22.8	2.2	21.9	2.6
Moistened 5 minutes before harvest	22.8	2.4	16.5	2.2
Kept dry	23.5	2.5	17.8	2.2
L.S.D.				
5%	9.0	N.S.	11.5	N.S.
1%	11.0		13.9	

*One drop of the treating solution (ca. 10 micro-litre) was applied, on the main vein, midway from the apex to the base, on the supper surface of one of the primary leaves of the bean plant. The treating solutions consisted of a 20 millimolar solution of ortho-phosphoric acid (pH 3) containing 12 and 3.5 micro-curie of P^{32} per millilitre in Tests I and II, respectively. All measurements were taken after 12 hour absorption. Each value represents a mean of eight replications.

results have been inconsistent. Thorne (52) reported that re-wetting increased uptake of P^{32} through swede and French bean leaves only when the humidity was high, and in her earlier report (49) she failed to increase uptake of P^{32} through swede leaves by re-wetting. Furthermore, the uptake of magnesium through apple leaves was not increased by re-wetting (40).

As to the effect of re-wetting on translocation, Koontz and Biddulph (31) found that translocation of P^{32} out of the treated bean leaflet was proportional to moisture retention on the treated leaflet, while Single (46) reported that only a slight increase in translocation of manganese occurred out of the painted wheat leaf. Thus, according to the present data, it is evident that the presence of surface moisture significantly increased the uptake of foliar applied phosphorus, but it did not aid in the transport of P^{32} out of the treated leaf during a 12-hour absorption period.

Experiment VI

The leaf washing technique was next used as means of determining the foliar absorption rate for Ca^{45} labeled calcium chloride. The treating solution consisted of 25 millinolar calcium chloride (pH 5) and contained 16 microcurie per millilitre of Ca^{45}. A wash solution volume of 10 millilitres of distilled water was considered, as with P^{32}, adequate for removal of residual of Ca^{45} since the latter was more leachable from leaf surfaces than was P^{32} (54).

Few reports are available that give rates for foliar absorption of Ca^{45} other than that of Bukovac and Wittwer (12). However, the rates reported by them were much lower than those given in the present study (Figure 2). A possible explanation might be that different pH levels were employed for the treating solutions in the two experiments, and that minimum absorption occurred at the pH of 3 used by Bukovac and Wittwer (12). In this connection Orgell (41) has suggested that the cuticle is more negatively charged and expanded at a high (above 6) pH. If this were true, then more attraction between calcium ions and cuticle would result in greater absorption. However, a compensatory affect could be that non-absorbed calcium ions might be bound to the leaf surface so strongly that removal by the leaf washing technique would not occur. It should also be pointed out that Bukovac and Wittwer's data for calcium absorption rates were based on the leaf disc removal technique, which would invariably give lower values than those realized through the properly conducted leaf washing procedure. Meanwhile, the translocation of Ca^{45} out of the treated leaf was negligible. This is in agreement with several other earlier reports (5, 6, 12, 35, 39).

Experiment VII

As has already been indicated, the effect of pH of the treating solution on the absorption of foliar applied Ca^{45} may be an important consideration. The results from variable pH levels on calcium absorption are, accordingly, given in Table IV. It is obvious, within the range tested, that the higher the pH the more rapid was the uptake of Ca^{45} labeled calcium chloride. No explanation for the effect of pH on calcium uptake is offered other than the possibility already mentioned by Orgell (41).

TABLE IV

The Effect of pH on Foliar Absorption of Ca^{45} Labeled Calcium Chloride*

pH	Total Absorbed (Percent)
4	68.0
5	90.7
6	97.0
L.S.D.	
5%	12.1
1%	15.7

*Data are expressed as percentages of total calcium applied in a drop of solution to the upper surface of one of the primary leaves of the bean plant.

All measurements were ascertained by means of the leaf washing technique, and after a 24-hour absorption period. Each figure is a mean of seven replications.

The treating solution consisted of a 25 millimolar solution of calcium chloride containing 16 microcurie of Ca^{45} per millilitre.

SUMMARY

The radioactive isotopes P^{32} and Ca^{45} were utilized, and a special leaf washing technique was developed for removal of non-absorbed residues, in studies designed to resolve the effects of certain factors on absorption of mineral nutrients applied to the primary leaves of the bean plant.

In the leaf washing technique it was determined that 10 millilitres of distilled water was sufficient to remove the non-absorbed residue from one drop of P^{32} applied to the upper surface of the primary leaf, if the water was supplied dropwise. Distilled water was equally as good as H_3PO_4 at 10^{-1}, 10^{-2}, 10^{-3} or 10^{-4}M, or KH_2PO_4, $CaCl_2$, or NaOH at 10^{-2}M. Washing efficiency, however, was improved slightly by the use of 10^{-1}M H_3PO_4. It was determined that the leaf washing technique was a more accurate means for measuring foliar absorption of mineral nutrients than the leaf disc removal procedure.

The leaf washing technique thus developed was utilized in studies of the initial absorption rates of foliar applied P^{32} and Ca^{45} by taking measurements of percentages absorbed at various intervals (30 minutes to 96 hours) after treatment. It was found that the absorption rate for Ca^{45} was much higher than that for P^{32}. Fifty percent of the foliar applied Ca^{45} was absorbed in about three hours with over 99 percent in 96 hours, while only 27 percent

of the foliar applied P^{32} was absorbed in 24 hours and about 40 percent in 96 hours. Translocation of Ca^{45} out of the treated leaf was negligible.

The leaf washing technique was also used as a means of evaluating the effect of the presence of surface moisture on the uptake of labeled ortho-phosphoric acid, and the effect of pH on foliar absorption of Ca^{45} labeled calcium chloride. It was determined that absorption of P^{32} was increased in direct proportion with the time during which the treated area was kept moist. However, the presence of surface moisture at the site of treatment did not aid in the transport of P^{32} out of the treated leaf. There was a pronounced effect of pH on the absorption of foliar applied Ca^{45}, with absorption significantly greater at pH 5 and 6 than at pH 4.

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