

THE ENTRY OF NUTRIENTS THROUGH
THE BARK AND LEAVES OF
DECIDUOUS FRUIT TREES AS INDICATED BY
RADIOACTIVE ISOTOPES

Thesis for the Degree of Ph. D.

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DECIDUOUS FRUIT TREES AS INDICATED
BY RADIOACTIVE ISOTOPES

By

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A THESIS

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I. INTRODUCTION

Although the usual path of entry into plants of mineral nutrients is through the young roots and root hairs, the above-ground portions are also capable of nutrient absorption. Interest in the application of nutrients to above-ground parts has been stimulated by the observations that several physiological diseases, induced by deficiencies of specific elements, can be corrected by sprays of the deficient elements, and that nitrogen can be supplied to McIntosh apple trees through foliar sprays of urea.

However, the foliar application of urea nitrogen has raised the question, whether twigs and branches may not also play a part in absorption and utilization. Further, dormant applications of minor elements have shown nutritional value. Accordingly, this investigation deals specifically with the nature and extent of uptake and utilization of nutrients labeled with radioactive isotopes applied to the bark and leaves of certain horticultural plants during various seasons of the year.

II. REVIEW OF LITERATURE

Bark Application of Nutrients

Forsyth (22) in the third edition of his book, "A Treatise on the Culture and Management of Fruit Trees" published in 1803, gave a formula for a dressing to be applied to pruning wounds and to cavities of trees after removal of decayed wood, as follows: 1 bushel of fresh cow dung, 1/2 bushel of lime rubbish of old buildings or hydrated lime, 1/2 bushel of wood ashes, and 1/16 of a bushel of pit or river sand. An improvement of the original formula was the addition of urine and soap suds to make a thick paint so that it could be brushed on to the cleaned wounds of a tree. Before the compound dried, it was sprinkled with a powder composed of 2/3 wood ashes and 1/3 ashes of burnt bone. Trees made rapid and vigorous growth after the compound was applied according to Forsyth, who was a gardener for King George III of England. For disclosing this information to the public, he was presented a medal by the King.

Gris (24), working in France in 1843, found that chlorosis in plants could be corrected by supplying iron sulfate either through the roots or directly to the leaves.

In America, Downing (14) recommended a wash of 2 pounds of potash in 2 gallons of water to be used for insect control. This solution, painted on the tree during the dormant period, also promoted the growth of the tree and improved the color of the bark.

Ballard and Volck (2) in 1914 reported increased growth following both foliar and dormant season sprays of sodium nitrate made to fruit trees in California. When caustic potash was added to the sodium nitrate, the trees bloomed earlier than when sprayed with sodium nitrate alone. A five-fold increase in yield of Yellow Belleflower apple was found when the trees were sprayed when the soil was dry. Apples and pears responded to the treatment but stone fruits did not.

Work in Oregon on tree applications of sodium nitrate was reported by Lewis (35, 36, 37) in 1914. He used a spray of 135 pounds of sodium nitrate, 19 pounds of sodium hydroxide, and 135 gallons of water. This spray was applied March 17, 1913, when the plants were in the green-tip stage. Dry sodium nitrate was applied to the soil around the trees April 25, and a solution of sodium nitrate was sprinkled on the ground around the trees May 7. The trees were greener, made increased growth, and gave a higher yield when the fertilizer was applied to the tree as compared with either type of ground application and no fertilizer application. However, no rain fell after the soil treatments were applied. The following year all treatments were made in March and no differences were noted in response to method of fertilizer application.

Interest was renewed in dormant tree applications after several workers (21, 34, 54) showed that some physiological diseases or disorders which were caused by deficiencies of mineral nutrients could be corrected by such treatments. Roach (49), by using dyes, followed the movement of solution injected into a plant. To correct chlorosis,

he suggested inserting tablets containing the missing element into holes drilled into the tree. Bennett (3) working in California also found that injections of iron salts would correct lime induced chlorosis.

Many workers in Australia and New Zealand have reported that dormant applications are effective in curing zinc and manganese deficiencies. Kilpatrick (34) in 1941 recommended the use of 1 pound of manganese sulfate in 2 gallons of water to cure manganese deficiency in peaches. Ward (61) writing in Australia in 1944 reported the most rapid and most enduring treatment for the little-leaf disease was found to be a 2 1/2- or 5-percent spray of zinc sulfate during the dormant season. For complete recovery it is necessary to spray 2 years in a row and in alternate years thereafter. A statement was made by Skepper (54) in an article in 1950 on fertilizers for fruit trees that "Applications of zinc compounds to the soil do not have any beneficial effects but fortunately trees are able to absorb zinc through their leaves and bark". He recommended 10 pounds of zinc sulfate and 5 pounds of hydrated lime in 100 gallons of water for citrus fruits. For deciduous trees he suggested the same rates for foliar applications as for citrus trees, during the dormant season 40 or 50 pounds of zinc sulfate in 100 gallons of water may be used on deciduous trees. With apples in New Zealand, Forester (21) has cured little leaf with 25 pounds of zinc sulfate in 100 gallons of water.

Thompson (57) in a paper written in 1944 discussed methods, advantages, and disadvantages of both solid injections and dormant sprays

made to fruit trees. He suggested that sprays are easier to apply and also easier to use to test for a deficiency by spraying a small limb. Disadvantages are that results are often variable and that the effect may last only one season. Also, ferrous sulfate, used for iron deficiency, may cause injury when used as a spray. Solid injections have the advantage that they are reliably effective in the cure of severe iron chlorosis, although considerable injury to the trunk may follow. Also injections are not well adapted to use with young trees. Thompson further suggests a dormant spray for iron deficiency of 10 pounds of ferrous sulfate in 100 gallons of water in a severe case and 5 pounds in other cases.

Manganese deficiency in cherries has been cured with dormant sprays at concentrations of either 4 or 16 percent manganese sulfate according to Thompson and Roberts (58). The 16-percent manganese sulfate solution was more effective than the 4-percent.

Movement of radioactive phosphorus from a nutrient solution into the base of dormant stems of excised red maple and McIntosh apple trees was studied by Eggert (16) in New Hampshire. He found the red maple would take up P^{32} from the nutrient solution but that the McIntosh did not take up P^{32} rapidly until the blossom buds began to swell. Apple buds when pink contained 10 times as much P^{32} as the nutrient solution on a unit weight basis. The primary leaves contained 7 times as much while spurs contained only $1/5$ as much as the nutrient solution. The small branches contained even less P^{32} .

Harley (30) was not able to find movement of radioactive phosphorus through the bark during the dormant period. However, at the time of bud swell there seemed to be some intake through uninjured bark, and when the bark was injured there was a decided increase in the absorption of phosphorus.

Foliar Application of Nutrients

Hamilton, Palmiter and Anderson (29) tried foliar application of various nitrogen carriers in the early 1940's. At that time, they found that Uramon (urea) induced darker leaf color with less damage to the plants than did the inorganic forms of nitrogen which they used.

A basis for the absorption of urea or any other substance through the leaves was found by Roberts, Southwick, and Palmiter (50) who studied McIntosh apple leaves to determine the relationship of cell wall constituents to the penetration of spray solutions. Their observations indicated that the cuticle is not a continuous layer but "exists in lamellae parallel to the outer epidermal cell walls. The pectinaceous substances form a continuous path from the outside of the leaf and extending to the walls of the vein extensions, which also contain a large amount of pectinaceous substances...The amount and location of the pectinaceous substances present in the leaves account for the entrance of water soluble materials such as minor elements, nitrogen, hormones, and organic fungicides sprayed upon apple leaves."

Fisher (20) gives three principles that underlie foliage applications of nitrogen fertilizer. The first is that yields obtained following foliar sprays are as high as from comparable soil applications.

The second is that a greater nitrogen effect is obtained the later the spray is applied, up to the time of the "second cover" spray. The third is that a foliar spray produces a "nitrogen effect" of equal magnitude more rapidly than does a comparable soil application but that the effect of the spray application is less lasting.

Some factors affecting the absorption of urea by McIntosh apple leaves have been reported by Cook and Boynton (13). Lower leaf surfaces absorbed urea more readily than the upper surfaces, and young leaves more readily than old leaves. A leaf which was originally high in nitrogen had a greater absorbing capacity than a leaf low in nitrogen. Low temperature, low vapor pressure, and the addition of a wetting agent increased urea utilization. A marked effect on absorption in some cases was caused by changing the pH of the solution. No effect on absorption was noted in trees kept in the dark to reduce synthesis of carbohydrates. When sucrose was added to the spray solution, the amount of injury was reduced but also the rate of uptake was depressed. The uptake was most rapid the first few hours after spraying but continued at least 48 hours at a measureable rate.

Rodney (51) at Ohio State worked with calcium nitrate, ammonium sulfate, and urea as foliar sprays on Richared apple trees. The urea caused less leaf burning than did the other two compounds. He also found that the amount of entry through the upper surface of the leaves was nearly equal to the amount which enters through the lower leaf surface. This indicates that nitrogen is capable of entering the leaf directly through the cuticle as there are no stomates on the upper surface of the apple leaf.

When hydrated lime and a suitable spreader were added to urea sprays, Norton (45) was able to obtain both a color and growth response in peach trees. Starch, molasses, ammonium carbonate, ammonium citrate, and sodium bisulfite did not increase the response from a urea spray. He found that urea at 10 pounds per 100 gallons of water plus Kolofog (a bentonite-sulfur) at 6 pounds per 100 gallons, as sticking agent, was the most effective material.

In Washington State, Bullock and others (9) concluded that Elberta peach foliage was able to absorb urea but that the greatest nitrogen response occurred when part of the nitrogen application was made to the soil and part to the foliage. They found that when the leaf nitrogen was increased, the maturity of the fruit was retarded.

The cure of manganese deficiency in peaches and apples by foliar applications has been reported by Woodbridge and McLarty (63). Effective spray solutions were 2 pounds of manganese sulfate in 100 gallons of water or 1 pound each of manganese sulfate, boric acid, ferrous sulfate, and zinc oxide in the same amount of water.

Epstein and Lilleland (19) found that manganese deficiency in deciduous fruit trees could be cured by foliar sprays, by liquid injections into branches, and by placing dry manganese salts in holes drilled into the trunk of the tree.

Mottle leaf on orange has been alleviated so that trees produce larger leaves, longer internodes and more xylem tissue when sprayed with 10 pounds zinc sulfate and 5 pounds hydrated lime in 100 gallons of water, according to Reed and Parker (47).

It has been estimated by Woodhams (64) that in the citrus region of southern California there have been 402,000 trees sprayed with zinc salts, 35,000 with copper salts and 17,000 with magnesium salts.

Tracer Techniques

In the present problem radioactive isotopes were utilized to follow the movement of applied nutrients. Accordingly, a review of some of the methods utilized in isotopic research is included. Measurement depends on the emission of radiation which either affects a photographic plate or actuates a Geiger-Müller tube.

Solution counting using the immersion type of Geiger-Müller tube has been favored by several investigators (40, 42) on the grounds that the solutions are easier to handle and show less variation due to position. The Geiger-Müller tube is placed in a solution cup which has a funnel side arm and a bottom drain. The cup holds 10 milliliters of solution plus the counting tube. After a sample is counted, the solution cup is drained. The cup need not be rinsed unless the difference in activity of the following solution is greater than 50 percent, since less than 1 percent of the solution remains in the cup. Martin and Russell (40) report that if the pH of the counting solution is below 3 the phosphorus is not absorbed by the glass walls.

The end window Geiger-Müller tube, which is more efficient (recording 25 percent of the emissions compared to 10 percent by the immersion Geiger-Müller tube) is used for counting dry samples. Hall and MacKenzie (28) state that solid samples should be used because when phosphorus is in a low concentration (1-2 ppm) there is a high

percentage absorption by glassware. Their method involves the precipitation of the phosphorus first as ammonium phosphomolybdate and a re-precipitation as magnesium ammonium phosphate. The second precipitate, which is counted after drying, is collected on filter paper supported by a metal ring.

MacKenzie and Dean (38) have described a method of making briquettes of ground plant material so that the plant material has uniform sample geometry. This procedure is adapted particularly to isotopes of high energy radiation such as phosphorus and where large amounts of plant material are available.

Another technique using dried ground plant material has been described by Mitchell and Linder (43). A weighed amount of ground plant material is placed on a disc of Scotch Tape supported by a metal ring. After the ring is tapped to obtain uniform distribution, the excess plant material is removed for weighing.

The preceding techniques using electronic counting are all of a quantitative nature but the other important method, that of exposing films by means of radiation, is primarily a qualitative method.

Wittwer and Lundahl (62) have described a technique by which the gross absorption and distribution of radioisotopes in young whole plants can be evaluated. The young plants are dried rapidly under heat and pressure before being placed in a special exposure box with 8 x 10-inch Kodak No-Screen X-ray film. After an exposure of 3 to 10 days for a phosphorus treatment, the film is developed and the distribution of the nutrient can be seen.

More detailed studies of the distribution of isotopes can be made by using Eastman nuclear track plates such as types NTB and NTB2. Duggar and Moreland (15) used this type of emulsion in working with the distribution of C^{14} from $C^{14}O_2$ in sections of hydrangea leaves 15 microns thick. Localization of the radioactive carbon nearly to the exact cellular location is possible by this technique.

Tracer Experiments

Some of the earliest tracer experiments using an element which occurs naturally in plants were done by Gustafson (26) in 1937 using cyclotron produced P^{32} . Stem cuttings with the xylem removed were found to translocate as much phosphorus as intact cuttings. In a further experiment Gustafson and Darkin (27) removed the xylem from one cutting, the phloem from another, and left one other intact, and found that either portion of the vascular system could transport the phosphorus equally well.

In a later experiment, Gustafson (25) found that Bryophyllum cuttings which were intact were able to transport more phosphorus than a cutting with either the xylem or phloem removed. However much less phosphorus reached the top of the plant when the xylem was removed than when the phloem was removed.

Stout and Hoagland (55) using isotopes of potassium, sodium, phosphorus, and bromine concluded that as long as the bark and wood of the willow cuttings were in contact, the concentration of isotope was the same in both tissues regardless of the species of plant or of the ion used. Radiation was detected in the upper leaves of a rapidly transpiring plant one hour after P^{32} was applied to the soil.

The movement of phosphorus in the bean plant (Phaseolus vulgaris) has been extensively investigated by Biddulph (4). In his first work he found that phosphorus moved rapidly throughout the plant, following the transpiration stream, with the greatest concentration of phosphorus occurring in the young leaves. In an experiment in 1941 he (5) found that there was a difference in rate of migration and in distribution at different hours of the day. The greatest downward movement took place at or near 10 A.M. and the least near 10 P.M.. The most pronounced upward migration occurred near noon, but was comparatively small.

Colwell (11) studied translocation from a pool of P^{32} solution held on the leaf by a plasticine ring. The movement of the phosphorus was toward the nearest points of utilization. By girdling the stem he found that leaf-applied phosphorus was carried by the phloem of cotton plants (Gossypium spp.). This was verified when the phloem of the petiole was destroyed by scalding. The movement of the phosphorus was then much slower than when the leaf was undamaged.

The cotton plant was also used by Biddulph and Markle (6) in studies of migration of P^{32} solution injected into the leaf. A concentration gradient was found both above and below the leaf with downward movement in excess of 21 centimeters an hour. The upward movement varied from 0 to 40 percent of the mobile phosphate, while downward movement accounted for 60 to 100 percent of the mobile phosphate.

Very rapid upward movement of bromine, 15 feet in 5 minutes, was found in a cucurbit plant from KBr^{82} introduced into the nutrient solution by Stout and others (56). In other experiments they found zinc

Zn⁶⁵ accumulated in seeds and conducting tissues of the tomato. It was determined that less than 1 percent of a soil application of phosphorus was recovered in 43 days.

A study of the metabolism of elemental sulfur applied to lemons was made by Turrell and Chervenak (59) using S³⁵. Metabolic products of the sulfur were H₂S, SO₂ and SO₄ with a very high proportion of the sulfur in the hydrogen sulfide and SO₄ being derived from the elemental sulfur. The SO₂ formed was derived largely from a source within the fruit.

Mitchell and Linder (44) studied the effects of co-solvents and surface agents on the absorption and translocation of radioactive 2,4-diiodo (I¹³¹) phenoxyacetic acid when the same amount of radioactive material was applied to an equal area. A very decided increase in absorption over the distilled water solution was noted with several additives ranging up to 350 percent with Tween-20.

Simultaneous movement of P³² and C¹⁴ in opposite directions in phloem tissues was detected by Chen (10). He also found that the xylem carried P³² when applied to the roots, whereas the phloem was the channel for foliar application.

Several interesting facts have come from a study of chlorosis using radioiron, by Wann and others (60). Chemical analysis of green foliage varied only slightly from that of chlorotic foliage. Resistant plants contained four times as much iron on lime-free soils as on high lime soils while susceptible plants contain eight times as much iron. Iron from injections remains in an active state indicating that the inactivation of iron must occur in the soil or in the roots.

Biddulph and Woodbridge (7) have worked on the relationship of phosphorus and iron in bean plants. They found that as the amount of phosphorus in the nutrient solution was varied from low to high levels, the concentration in the tissues increased but at different rates. As the phosphorus level was raised above that necessary for growth, there was a precipitation of iron and phosphorus in the roots interfering with the movement of both ions.

Silberstein and Wittwer (53) tested various organic and inorganic phosphorus compounds for foliar application on vegetable crops. Best growth response was obtained with ortho-phosphoric acid. In a field trial, there was increased early but not total yield of tomatoes as compared to a broadcast soil application. Autoradiograms showed that P^{32} had moved from the foliage of tomatoes, corn, and beans to the roots within 6 hours.

In Sweden, Ehrenberg and Granhall (18) have made injections of radioactive substances into fruit trees in an effort to secure mutations. The advantage of this method is that the radiophosphorus and radiosulfur tend to concentrate in the buds where mutations are induced, without damage to the rest of the tree.

The effect of exposure of the meristematic regions of barley subjected to a constant, relatively high level of radiation from P^{32} has been studied by Mackie, Blume, and Hagen (39). They found cell division ceased, cells enlarged, cytoplasm became less dense, and cell walls thickened. Damage to shoot meristems was more pronounced than to root meristems when solutions of comparatively low specific activity were used.

Two British workers, Russell and Martin (52) have reported damage to plant tissue at levels much lower than reported by American workers. They found reduced root growth at a level of 10 microcuries per liter of culture solution. The solution gave 0.4 "equivalent roentgen"* per day but due to selective accumulation in the meristematic regions, the root tip received 300 "equivalent roentgen"* per day.

Blume (8) studied the effect of different ratios of P^{32} to P^{31} on the growth of plants in soil. The only reduction in growth of barley seedlings occurred at the highest concentration of 12,500 microcuries of P^{32} per gram of P^{31} .

The movement of calcium into tomato, alfalfa, red clover and wheat from a soil application has been studied by Ririe and Toth (48). They found greater amounts of Ca^{45} in the lower leaves than in the upper leaves. Split root studies with tomatoes indicate little movement of Ca^{45} through the roots in a solution containing Ca^{45} to other roots in solutions of varying levels of calcium.

The relative absorption of phosphorus by apple trees was studied by Eggert et al (17) working in New Hampshire. They have shown conclusively that phosphorus in water solutions sprayed on the foliage and small branches of apple trees can be absorbed and translocated to other parts of the tree. Over half the phosphorus sprayed on the plants was found to have been translocated to the roots while 2 to 3 percent of the total phosphorus in the plant came from the foliar spray.

* A "equivalent roentgen" (er) is essentially the same as a "Roentgen-equivalent-physical" (rep) which is defined as "That dose of any ionizing radiation which produces energy absorption of 93 ergs per gram of tissue".

A device for the measurement of the rate of urea hydrolysis has been reported by Hinsvark, Tukey, and Wittwer (31). The relative rates of $C^{14}O_2$ production were studied for several vegetable and woody plants and it was ascertained that the rate of urea hydrolysis as indicated by $C^{14}O_2$ evolution was closely correlated with the amount of injury produced by the spray. The greater the rate of hydrolysis the less tolerant a plant was found to be to a given concentration of urea.

Fraser and Mawson (23) found a uniform distribution in a narrow spiral band to a height of 15 feet following an injection during the growing season of Rb^{86} below the surface of a trough holding potassium chloride solution which was attached to the trunk of the tree. The rate of movement into the plant from the solution decreased after the initial rapid intake. Upward movement of Rb^{86} ceased in October when leaves became senescent but downward movement continued. In healthy trees, the movement was confined to a narrow path, while in diseased trees, the movement was over a fan-shaped area.

III. MATERIALS AND METHODS

All radioactive materials, which were used in these experiments, were obtained from the Atomic Energy Commission at Oak Ridge, Tennessee. Radioactive calcium (Ca^{45}), radioactive phosphorus (P^{32}) and radioactive Potassium (K^{42}) were used to study the rate and extent of absorption and subsequent translocation following foliar and bark application of nutrient elements.

Radioactive calcium (Ca^{45}), which was received as a solution of calcium chloride, was added to a 2 percent solution of stable calcium chloride.

Radioactive phosphorus (P^{32}), which was received as a solution of phosphoric acid, was prepared in most experiments as a 0.3 percent solution of stable phosphoric acid to which radioactive phosphorus (P^{32}) was added to make concentrations ranging from 1 to 10 microcuries per milliliter. In certain experiments, concentrations of stable phosphoric acid ranging from 0.25 to 8.0 percent were used, while in these same experiments the number of microcuries per milliliter was varied from 0.01 to 53.3.

Radioactive potassium (K^{42}), which was received as irradiated units of potassium carbonate, was prepared as a 0.3 percent solution of potassium carbonate.

Various procedures were used for applying the radioactive materials depending upon the nature of the plant material under study. For leaf application, the leaves were dipped into a 600-milliliter beaker

containing the desired solution, the leaves being forced into the solution by means of a glass stirring rod.

When the solution was applied to limbs or branches, paint brushes were used, a 3-inch brush being used on the old limbs and a 1/8-inch brush for water sprouts and small limbs. For some of the dormant applications, a measured amount of solution was pipetted onto cotton gauze wrapped around a limb. Spraying with a Shur-Shot sprayer at 60 to 100 pounds pressure was another method used for young dormant trees. This work was done in the middle of an open field, mask and protective plastic cover being worn by the operator.

Samples that were collected from experimental treatments were placed in a drying oven at 70°C. Because of the large volume of some of these samples, principally those in the out-of-doors experiments with phosphorus and calcium sprays, the samples were dried at 120°F. in a large circulating air dryer such as used for food dehydration.

To reduce the shielding effect of the tissue and to prepare the samples for chemical analysis, the tissue was reduced to ash in 50 milliliter porcelain crucibles in the muffle furnace at 600°C. Phosphorus samples were handled as described by the A.O.A.C. (1), adding first .5N magnesium nitrate, then concentrated hydrochloric acid. Magnesium nitrate will form magnesium pyrophosphate with the phosphorus compounds present in the plant tissue, a compound which is thermally stable at 600°C. The hydrochloric acid partially digests the plant material which permits close contact of the magnesium nitrate and the phosphorus compounds.

Before ashing, leaves and current-season's shoots were ground to 20 mesh in a Wiley mill. Watersprouts and other older tissues were reduced to small pieces, then excess hydrochloric acid added to insure penetration of the magnesium nitrate.

Ash from foliage treatments was dissolved in 2N hydrochloric acid then transferred to a 25-milliliter volumetric flask and made to volume. An aliquot of 5 milliliters was removed for counting. Ash from woody samples was dissolved in acid at the rate of 5 milliliters of acid to 1 gram of dry plant material. Five milliliters of the acid solution of the woody material was evaporated to dryness on an electric hot plate and used as a sample for counting. A Tracerlab autoscaler equipped with a shielded chamber was used for counting the samples.

Autoradiograms were used to evaluate the gross intake and distribution of foliar and bark applied nutrients. Autoradiograms were prepared by the method outlined by Wittwer and Lundahl (62) with one modification. The treated leaves were removed from the shoot to prevent blurring of the autoradiogram rather than washing the leaves to remove the excess phosphorus or potassium which causes the blurring.

The steps in the preparation of autoradiograms are as follows:

1. Place plant part between two sheets of botanical specimen paper and dry under pressure with infra-red heat lamps.
2. Remove botanical specimen paper from both sides of plant part and replace with a sheet of pliofilm on one side and a new sheet of absorbent paper on the other.
3. Press plio-film side against 8 x 10 inch, No-screen Kodak X-ray film with steel plates and leave in the dark (photo-

graphic) room 5 to 7 days for exposure.

4. Process films, using X-ray developer and fixative.

Information is given on weather conditions when they were unusual and had a probable affect on the results of an experiment.

More detailed information on materials and methods will be described in each experiment.

IV. RESULTS

The research undertaken in this project may be divided into preliminary experiments not using radioactive materials and experiments using radioactive materials which are grouped according to season (a) applications to the bark during the dormant season, (b) applications to both bark and leaves during the early spring season, and (c) applications to the leaves during the mid-summer and late summer season.

Preliminary Experiments

Experiment I

Object: To determine the total bark area of twigs, branches, and trunk of a dormant apple tree, so as to gain some appreciation of the surface exposed to nutrient applications.

Part 1

Materials and Methods: Two 3-year-old McIntosh apple trees were used in preliminary measurements to determine a practicable means of determining the surface area of a dormant tree. Two methods were compared (a) computation from measured diameters, and (b) computation from volume as determined by actual water displacement.

Both methods depended on finding the surface area and weights of stem sections 80 millimeters in length. These stem sections were cut into eight diameter classes, namely 3, 4, 5, 6, 8, 11, 13, and 15 millimeters which represented all the diameter sizes found on the 3-year-old McIntosh apple trees.

In the first method, it was assumed that the stem section was a perfect cylinder. Since the diameter and the length of each section was known, the surface area of the cylinder could be calculated by using the formula for the surface area of a cylinder, πDL . D was the diameter of the section, and L was the length of the section.

In the second method, the 80-millimeter sections were immersed in graduated cylinders and measurements made of the volume of water displaced. It was then necessary to determine the radius of a stem 80 millimeters in length which would have this measured volume. This was done by substituting the value obtained for the measured volume into the formula for the volume of a cylinder, $\text{Volume} = \pi R^2 L$ or $\text{Radius}^2 = \frac{\text{Volume}}{\pi \text{ Length}}$. This calculated value for the radius was used to determine the surface area of the 80-millimeter stem sections.

A proportion was then used to find the total surface area of stems of a given diameter class, as follows:

$$\frac{A_1}{A_2} = \frac{W_1}{W_2} \quad \text{or} \quad A_2 = \frac{A_1 W_2}{W_1}$$

A_1 = calculated surface area for a 80 millimeter section of diameter D (calculated by either of the 2 methods)

A_2 = total surface area of wood of diameter D

W_1 = weight of a 80 millimeter section of diameter D

W_2 = total weight of wood of diameter D

Results: The bark areas of the two 3-year-old McIntosh apple trees are given in Tables I and II. These data show that the surface area obtained by the two methods are similar, with the value computed by the measured diameter method being the more conservative estimate.

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TABLE I

BARK AREA IN SQUARE CENTIMETERS OF A 3-YEAR-OLD McINTOSH

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APPLE TREE (TREE A) AS DETERMINED BY TWO METHODS

Age	Aver. Diam. of Branches (Mm.)	Area of 80-mm. Section by Diameter Method (Sq. Cm.)	Wt. of 80-mm. Section (Gms.)	Vol. of 80-mm. Section (Ml.)	Total Weight (Gms.)	Total Area by Diameter Method (sq. cm.)	Area of 80-mm. Section Volume Method (Sq. Cm.)	Total of Area by Volume Method (Sq. Cm.)
1-yr.	3	7.55	1.81	.8	9.97	41.6	8.97	47.8
	3	7.55	1.81	.7			8.42	
Wood	4	10.06	2.72	1.2	11.78	47.6	11.00	49.7
	4	10.06	2.26	1.0			10.03	
2-yr.	4	10.06	2.72	1.4	5.44	21.5	11.87	23.8
	6	15.10	3.62	3.0	8.15	31.9	17.36	39.1
Wood	8	21.00	5.44	4.5	16.77	64.7	21.30	62.6
3-yr.	11	27.63	9.54	9.0	42.20	122.4	30.50	135.0
	13	32.61	13.13	11.9	44.80	111.4	34.62	118.2
Wood	15	37.71	16.77	15.0	32.60	73.4	38.90	75.7
Total						514.3		551.9

TABLE II

BARK AREA IN SQUARE CENTIMETERS OF A 3-YEAR-OLD McINTOSH

APPLE TREE (TREE B) AS DETERMINED BY TWO METHODS

Age	Aver. Diam. of Branches (Mm.)	Area of 80-mm. Section by Diameter Method (Sq. Cm.)	Wt. of 80-mm. Section (Gms.)	Vol. of 80-mm. Section (Ml.)	Total Weight (Gms.)	Total Area by Diameter Method (Sq. Cm.)	Area of 80-mm. Section by Volume Method (Sq. Cm.)	Total Area by Volume Method (Sq. Cm.)
1-yr.	3	7.55	1.81	.7	9.54	39.6	8.42	46.4
	3	7.55	1.81	.85			9.26	
Wood	4	10.06	2.72	1.29	8.15	30.2	11.39	35.4
	4	10.06	2.72	1.50			12.23	
	5	12.57	3.18	2.2	16.32	64.7	14.91	78.5
	5	12.57	3.18	2.4			15.56	
2-yr.	5	12.57	3.18	1.8	4.08	16.2	13.47	17.3
	6	15.10	3.53	2.3	8.60	35.9	15.22	36.0
Wood	8	21.00	5.44	4.6	12.68	49.0	21.30	49.5
3-yr.	11	27.63	9.08	8.7	23.55	72.5	29.55	76.9
	13	33.61	12.68	13.5	39.40	104.5	36.10	109.0
Wood	15	37.71	17.63	17.0	40.75	89.3	41.80	99.2
Total						501.9		

Part 2

Materials and Methods: During January 1952, the total surface area was determined of a 25-year-old McIntosh apple tree with a limb spread of 26 feet 8 inches and a height of 20 feet 10 inches by the measured diameter method.

The tree was cut down and the wood grouped into 19 diameter classes, ranging from 5 millimeters to 305 millimeters, as shown in Table III. Branches up to 8.5 centimeters in diameter were cut into 80-millimeter lengths and the average weight of 10 sections was determined. This average weight and calculated surface area were substituted in the proportion used in part 1 of this experiment. For limbs with a diameter greater than 8.5 centimeters, the area was calculated directly assuming the limb to be cylindrical.

It will be seen from the accompanying table (Table III) that the surface area of the 25-year-old McIntosh apple tree was 85.99 square meters. Interestingly enough, wood of 6 millimeters or less in diameter constituted slightly over one-third (35.7 percent) of the entire surface area, and over one-half (53.7 percent) was 10 millimeters or less in diameter. Thus a large portion of the surface area of the tree occurred in wood most adapted for the intake of mineral nutrients because of the relatively thin layer of periderm.

TABLE III
 BARK AREA IN SQUARE METERS AND SQUARE FEET
 OF A 25-YEAR-OLD McINTOSH APPLE TREE

Aver. Diam. of Branches (Mm.)	Area of 80-mm. Section (Sq. Cm.)	Total Area (Sq. M.)	Total Area (Sq. F.)	Portion of Tree %	Aver. Wt. of 80-mm. Section (Gms.)	Total Weight (Gms.)
5	12.57	30.74	330.76	35.7	1.84	44,990.68
7	17.59	8.78	94.47	10.2	3.39	16,925.71
9	22.62	6.73	72.41	7.8	5.60	16,669.89
11	27.64	5.01	53.91	5.8	8.24	14,940.35
14	35.19	7.70	82.85	9.0	13.73	30,050.79
18	45.24	5.38	57.89	6.3	21.99	26,167.61
25	62.83	6.57	70.69	7.6	42.79	44,735.31
35	87.96	3.87	41.64	4.5	85.27	37,535.93
45	113.10	2.92	31.42	3.4	139.68	36,088.98
55	138.23	1.29	13.88	1.5	208.49	19,448.58
65	163.36	1.57	16.89	1.8	291.20	28,066.79
75	188.50	.80	8.61	.9	387.70	16,499.78
85	213.63	.58	6.24	.7	497.97	13,438.06
95	238.76	.38	4.09	.4	622.04	---
105	263.89	1.21	13.02	1.4	759.88	---
115	---	.84	9.04	1.0	---	---
135	---	.13	1.40	.1	---	---
205	---	.43	4.63	.5	---	---
305	---	1.06	11.41	1.2	---	---
Grand Total		85.99	925.25	99.8	---	---

Experiment II

Object: To test the effectiveness of various sticking agents when used with nutrients applied to the bark of fruit trees.

Part 1

Materials and Methods: A 5 percent solution of phosphoric acid containing 1/100 microcurie of P^{32} per milliliter was used as the basic solution to which were added the following sticking agents at a concentration of 2 ounces to 100 gallons of water: Triton B-1956, Ortho, Dupont Sticker-Spreader, Atlas G-772, Atlas Tween-20, and Methocel 4000 c.p.s. Other sticking agents used were Plant Spray Spreader at 1 ounce to 8 gallons of water, Kolofog at 6 pounds to 100 gallons of water, and Good-rite PE-PS at 6 pounds to 100 gallons of water.

To test the effectiveness of the sticking agents, uniform sections of water sprouts from McIntosh apple trees 6 millimeters in diameter and 8 centimeters in length were immersed in solutions containing the sticking agents. Five stem sections were used with each agent.

A hook-shaped piece of wire was inserted into the pith of each section, in order that the sections could be immersed in the solutions and later hung up until dry.

Standard procedure for phosphorus analysis according to the A.O.A.C. (1) was followed, except that instead of being ground the sections were cut into small pieces before being treated with the reagents in preparation for ashing.

The ash was dissolved in 5 milliliters of 2N hydrochloric and then transferred to metal sample boxes for counting the radiation.

TABLE IV
 RETENTION OF P³² PHOSPHORIC ACID BY McINTOSH APPLE STEM
 SECTIONS AS AFFECTED BY SEVERAL STICKING AND WETTING AGENTS
 AS INDICATED BY COUNTS PER MINUTE FROM P³²

Sticking and/or Wetting agent	Retention Count/min. per sq. cm. of surface	Grams deposited for entire mature apple tree*
Atlas G-772	6.93	179.5
Carbowax 1500	6.34	164.2
Kelofog	5.43	140.7
Dupont S.S.	7.58	196.3
Methocel 4000	8.03	208.1
Ortho	6.35	164.5
PE-PS	5.5	142.5
Plant Spray	6.29	162.9
Triton 1956	6.33	164.0
Tween-20	7.16	185.5

*Computed on the basis of the 5 percent solutions used for these tests and the surface area of a 25-year-old McIntosh apple tree as determined in experiment I.

Results: The results of this experiment given in Table IV are the averages of the amount of radioactivity found on the five sections used for each solution.

Both Kolofog and Goodrite PE-PS are known to be good sticking agents but in this test they showed the lowest figures for retention. A possible explanation is that during the ashing process a compound may be formed with phosphorus which is volatile below 600°C. Phosphorus di-, tri-, penta-, and hepta-sulfides, having boiling points ranging from 337°C. for the disulfide to 523°C. for the heptasulfide, could have been the compounds formed.

Part 2

Materials and Methods: A second series of tests was conducted using Dupont Sticker-Spreader and Methocel 4000 c.p.s. at the same rates as were used in the first tests, plus solutions of Methocel 4000 c.p.s. and Methocel 1500 c.p.s. at the rate of 4 pounds of sticker to 100 gallons of solution.

The ends of the stem sections were dipped in paraffin so as to prevent absorption of solution through the cut ends. After the stem sections were dry, the ends were cut and discarded, leaving a piece of shoot 6 centimeters in length. The stem sections were prepared for analysis in the same manner as described in Part 1.

Results: The results of the second series of tests are shown in Table V.

The values obtained confirmed the results obtained in the first test. Methocel 4000 c.p.s. was again found to be the most effective

TABLE V
 RETENTION OF P³² PHOSPHORIC ACID BY McINTOSH APPLE STEM
 SECTIONS AS AFFECTED BY SEVERAL STICKING AGENTS AS
 INDICATED BY COUNTS PER MINUTE FROM P³²

Sticking and/or Wetting agents	Retention Count/min. per Sq. Cm. of surface	Grams deposited for entire mature apple tree*
Dupont S.S. 2 oz./100 gal.	1.68	90.26
Methocel 4000 2 oz./100 gal.	4.33	232.65
Methocel 4000 4 lbs./100 gal.	3.99	214.38
Methocel 1500 4 lbs./100 gal.	3.18	170.86

*Computed on the basis of the 5 percent solutions used for these tests and the surface area of a 25-year old McIntosh apple tree as determined in experiment I.

sticking agent, but increasing the amount of sticking agent did not result in a corresponding increase in the amount of phosphoric acid retained on the stem section.

Experiment III

Object: To determine the tolerance of McIntosh apple and Montmorency cherry trees when dormant and when in the green tip stage to sprays of mineral nutrients at different concentrations.

Materials and Methods: One year-old Montmorency cherry trees (Knight-Dowd strain) and 2-year-old McIntosh apple trees were obtained from the Greening Nurseries of Monroe, Michigan, in February, 1952, for this experiment. The trees were planted in lard cans which held approximately one cubic foot of coarse quartz sand. Tap water alone, without any added nutrient material, was supplied to the plants.

Solutions or slurries of calcium chloride, phosphoric acid, potassium nitrate, NuGreen (urea), and a 20-20-20 fertilizer were prepared at concentrations of 2, 4, 8, 16, and 32 percent by weight and sprayed onto the plants with a small hand sprayer until the plants were thoroughly wet with the solutions. Potassium nitrate was not used in Part 1 of his experiment but it was used in Parts 2, 3, and 4 because a peculiar marginal chlorosis developed on the trees sprayed with NuGreen and the 20-20-20 fertilizer which both contain urea nitrogen. Two trees were sprayed at each concentration of the five materials and two trees, which were not sprayed, served as check trees for each part of the experiment.

This experiment was divided into four parts: Part 1- Dormant sprays on cherries, Part 2- Greentip sprays on cherries, Part 3- Dormant sprays on apples, and Part 4- Greentip sprays on apples. Cherry trees, which were sprayed in the dormant state, were planted, and were sprayed February 28 in the greenhouse. Cherry trees, which were sprayed in the greentip stage, were planted March 28. These trees remained outside the greenhouse until April 11th, when they were sprayed and then were taken inside the greenhouse. Apple trees, which were sprayed in the dormant state, were planted and were sprayed March 26th. On March 27th these trees were moved to an unheated building for 10 days. Apple trees, which were sprayed in the greentip stage, were planted March 29th and then were left 10 days out-of-doors. The greentip state of bud growth was not reached till after the trees had been in the greenhouse seven days.

Results: The effects of the different materials on the development of the trees in each of the four parts of this experiment will be presented separately.

Part 1 Dormant Spray on Cherry

Inhibition of the rate of development of the buds was shown by trees sprayed with 2-, 4-, and 8-percent solutions of calcium chloride. The inhibition had been overcome by the time final observations were made four weeks after planting and these plants were average in development. The two highest concentrations of calcium chloride killed some buds and caused inhibition of others. Approximately 20 percent of the buds sprayed with the 32-percent solution were dead and another

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35 percent were not growing but were still green inside when cut open. Approximately 5 percent of the buds, most of which were terminal, were killed by the 16-percent solution while another 10 percent of the buds appeared to be inhibited.

No killing of the buds was noted with any of the concentrations of NuGreen used. The 16- and 32-percent solutions did cause a white or yellow marginal chlorosis of the leaves of the developing shoots.

No effect on the development of the buds was noted when concentrations of phosphoric acid 8-percent or less were sprayed on the trees. Only a few lateral buds were destroyed by the 16-percent solution. The 32-percent solution of phosphoric acid proved to be the most toxic solution used in part 1 of this experiment as 75 percent of the buds sprayed with it were killed. The lateral buds were destroyed by this solution in contrast to the terminal buds which were destroyed by the calcium chloride solutions.

Sprays of 20-20-20 fertilizer produced the same results as those described for the NuGreen.

Part 2 Green Tip Spray on Cherry

Calcium chloride caused no inhibition of the rate of development of the buds when used at a concentration of 8 percent or less. At first all the buds appeared to have been destroyed by the 32-percent solution and only one bud on each tree sprayed with the 16-percent solution was growing. Later three buds developed on each tree sprayed with the 32-percent solution while only one bud developed on one of the trees sprayed with 16 percent calcium chloride.

NuGreen had no effect on the trees which were sprayed with a solution of 8 percent or less. About 50 percent of the buds were destroyed by the 32-percent solution. Marginal chlorosis started to appear 18 days after spraying with the 16- and 32-percent solutions.

Phosphoric acid at concentrations up to 8 percent did appear to be toxic. At 16 percent about 50 percent of the buds were killed and at 32 percent all buds were killed although one shoot was produced from an adventitious or latent bud of one of the trees before the final measurements were taken.

Potassium nitrate did not cause injury at any of the concentrations used in this experiment.

No injury in the form of bud killing was found but marginal chlorosis of the leaves occurred following the application of the 16- and 32-percent concentrations of the 20-20-20 fertilizer.

Leaves of shoots, affected by chlorosis, were painted with 0.3-percent solutions of manganese chloride, magnesium sulfate, magnesium nitrate, potassium sulfate, and potassium chloride 21 days after they had been sprayed to find whether a deficiency of magnesium, manganese, or potassium was responsible for the chlorosis. Each solution was painted on all leaves of a single shoot but no recovery was noted on subsequent observations.

Part 3 Dormant Spray on Apple

While the apples, which had been sprayed in the dormant condition, were being held in the unheated storage building, it was observed that certain of the spray materials were sufficiently hygroscopic to accumulate water which ran down the stems. Calcium chloride,

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phosphoric acid, NuGreen, and to a certain extent 20-20-20 showed this property while potassium nitrate apparently was not hygroscopic.

The effects of the materials during the growing season are described as follows:

Calcium chloride had no inhibiting effect at concentrations up to 8 percent. However the terminal buds of the trees sprayed with the 16- and 32-percent solutions did not develop.

NuGreen had no effect when applied at a concentration of 8 percent or less. Some buds were killed by the 16-percent concentration and most buds by the 32-percent concentration. No chlorosis was noted following application of any concentration of NuGreen to the apple trees.

Some lateral buds were destroyed by the 8-percent solution of phosphoric acid but none were injured by the 2- or 4-percent solutions. At first only one bud developed on one tree and two on the other sprayed with 16-percent phosphoric acid. Later two and four shoots respectively were produced by the trees. These shoots and those produced by the trees sprayed with 32-percent solution, which had appeared to be dead, were of adventitious origin.

No effect on the development of the shoots was noted following the use of potassium nitrate at any concentration.

Sprays of 20-20-20 fertilizer of a concentration of 8 percent or less had no effect on the growth of the apple trees. At 16 percent some of the lateral buds failed to develop and most of the growth was confined to terminal regions of the shoots. Most of the lateral branch-

es of one plant were killed by the 32-percent solution while most of the buds were killed on the other plant but in a more scattered manner.

Part 4 Green Tip Spray on Apple

The effects of the materials during the growing season are described as follows:

Calcium chloride at 8 percent killed some of the buds but no effect was noted at the lower concentrations. Only a comparatively few buds developed on trees sprayed with the 16-percent solution. The trees sprayed with the 32-percent calcium chloride at first appeared to be dead but later several buds on each tree developed.

NuGreen and 20-20-20 sprays were without effect.

Phosphoric acid solutions of 8 percent or less concentration had no effect on the growth of the plants. About 50 percent of the buds were killed by the 16-percent solution; all were lateral buds. Both trees sprayed with 32-percent phosphoric acid at first appeared to be dead but later three adventitious buds produced shoots.

Potassium nitrate sprays had no effect on the development of the plants at any concentration.

Entry of Mineral Nutrients Applied to Bark of Limbs
and Branches during the Dormant Season

Experiment IV

Object: To determine whether potassium from K^{42} potassium carbonate enters the plant when applied to the bark of limbs and branches of an apple tree during the late winter.

Materials and Methods: A 0.3 percent solution of K^{42} potassium carbonate to which had been added Triton B-1956 at a concentration of 0.1 percent was used. The activity of the K^{42} was approximately 2 microcuries per milliliter at the time of treatment on February 6, 1951.

Potted 2-year-old McIntosh apple trees growing in the greenhouse and mature dormant Rhode Island Greening apple trees in the orchard were treated with this solution. The application was made at selected positions on the trees by wrapping cotton gauze around the limb and then saturating the gauze. A 4-inch band of gauze was wrapped around a lateral limb of one of the young McIntosh trees and around the trunk of the other 6 inches above the soil line. After 28 hours, samples were taken from the trees.

On the dormant Rhode Island Greening trees in the orchard, the solution was painted directly on 1- and 2-year bark as well as onto a 6-inch cotton gauze band which had been wrapped around a 10-year-old limb. As a precaution, a dam of cheese cloth was placed at the base of the 2-year-old shoot growth to prevent any contamination by the solution beyond this point.

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Sampling of the dormant wood in the field was done after 24 and 48 hours, at each harvest one limb was sawed off and sections removed both at 6 and 18 inches above and below the gauze. Sections were removed 6 and 18 inches basipetal to the treated 2-year-old shoots at 24 hours and again 48 hours after treatment.

The samples at the various distances were divided into bark (phloem and periderm) and wood (xylem) as nearly as was possible. These were placed in crucibles and ashed in the muffle furnace at 600°C.

The sample was counted directly in the crucible with a Tracerlab Autoscaler.

Results: The amount of radioactivity found in the untreated portions of the young trees in the greenhouse was small but in general it was greater than twice the radioactive count of background. However, samples taken 6 inches below the treated area on the lateral limb and also root samples from this same tree did not contain this amount of radioactive material. All samples from the tree, where the solution was applied 6 inches above the soil line, were above twice background in radioactive count. Greater radioactivity was found in the bark samples than in the wood samples, except in the roots where the wood contained more radioactivity than the bark.

The amount of radioactivity found in the tree in the orchard was small as it was not large enough in most samples to produce a radioactive count twice that of background, in general greater radioactivity was found in the bark tissue than in the wood portions. The only counts recorded in the 10-year-old limbs, which were twice that of

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background after 24 hours, were samples of the bark and wood in the treated area. The sample of bark 6 inches above the treated area as well as the bark and wood in the treated area of the 48-hour treatment of the 10-year-old limb showed radioactive counts over twice that of background. The amount of radioactivity found in the 3-year-old portion of the limbs 6 and 18 inches below the treated 2-year-old limbs was not large enough to produce a radioactive count of twice background. While the radioactive count levels were too low to be considered positive, a definite trend towards a higher count value was noticed for the bark sample than for the corresponding wood sample.

Experiment V

Object: To determine the value of a lime slurry as a means of increasing entry of K^{42} potassium carbonate through the bark of apple and peach trees.

Materials and Methods: A stock solution of K^{42} potassium carbonate was made up by adding 11 grams of K^{42} potassium carbonate to 3 liters of water. The stock solution was divided into two portions: agricultural lime was added to one portion to make a slurry and Drefit was added to the other portion to improve the wetting properties of the solution. These solutions were applied to the limbs with a paint brush.

Six uniform 8- to 10-year-old limbs of South Haven peach and four similar limbs of Rhode Island Greening apple tree were used, half the number being used with each material applied.

The treatments were made to the limbs at 4 P.M. on April 3, 1951. The limbs were cut from the trees after 23 hours and thin longitudinal

sections were sawed through area which had both treated and untreated bark. After these sections were dried, autoradiograms were made from them. Trimmings from the pieces of the limbs from which the thin longitudinal sections were cut were ashed and the radioactive count was measured.

Results: No K^{42} potassium was found either in the portions of the wood which were ashed and counted or which were used for autoradiograms. A clear line of radioactive material was shown by the autoradiograms to be present on the epidermis but none was evident in the area of the phloem. However, these results may be questionable because the radioactivity of the sample may have dropped below a detectable level due to the short half life of K^{42} .

Experiment VI

Object: To determine the relation between concentration of phosphoric acid solution and the rate of entry into 1-year-old peach shoots in early winter.

Materials and Methods: Three concentrations of phosphoric acid, 3, 20 and 80 milligrams per milliliter with the same relative specific activity of 0.21 microcuries per milligram of phosphorus were applied to 1-year-old Halehaven peach limbs on December 16, 1952. The amounts of P^{32} per milliliter of solution were 2.0, 13.3, and 53.5 microcuries per milliliter, respectively.

Vertical limbs about 2 feet long were selected for this treatment. In order that translocation within the limb could be studied without

TABLE VI

THE INFLUENCE OF CONCENTRATION UPON ENTRY OF P³² PHOSPHORIC ACID INTO 1-YEAR-OLD PEACH LIMBS DURING DECEMBER AS SHOWN BY RADIOACTIVITY FOUND IN THE TREATED AND UNTREATED PORTIONS OF THE LIMBS

Sample	8 Hours		14 Days	
	Dry Wt. (Gms.)	Radioactivity per 1 gram Dry Wt. (Count/min.)	Dry Wt. (Gms.)	Radioactivity per 1 gram Dry Wt. (Count/min.)
0.3% A Autoradiogram				
Shavings	1.4425	20.4	1.1025	3.8
Treated Portion	4.4623	14,094.0	9.9589	2,172.9
0.3% B Autoradiogram				
Shavings	1.0362	20.8	1.1686	16.3
Treated Portion	3.7726	20,652.0	4.6201	7,019.7
2.0% A Autoradiogram				
Shavings	1.0006	5.9	1.0472	4.7
Treated Portion	3.1939	925.8	3.7779	82.9
2.0% B Autoradiogram				
Shavings	1.1691	7.0	1.0141	8.4
Treated Portion	3.9278	649.2	3.3627	268.9
8.0% A Autoradiogram				
Shavings	1.4602	9.2	0.8532	130.9
Treated Portion	5.4730	35,574.0	5.4438	14,260.3
8.0% B Autoradiogram				
Shavings	1.0661	58.4*	1.0167	33.2
Treated Portion	1.3784	39,282.0	5.1456	13,293.0
Check A Untreated Limbs	1.4123	3.5		
Check B Untreated Limbs	1.1865	9.6		
1/100 ml. of the 3 mg/ml treating solution				723.0
1/100 ml. of the 20 mg/ml treating solution				4,900.0
1/100 ml. of the 80 mg/ml treating solution				17,652.0

*Contaminated

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interfering contamination, only the basal 10 inches of the limbs were painted with the P^{32} solutions.

Four limbs were used for each concentration, two being cut after 8 hours and the other two after 14 days. The weather during the 8-hour period was clear with a low temperature of 42°F . Rain did not fall during the 14 day period until the evening of the fourth day after which some precipitation fell for the following 6 days. This was followed by clear weather until the limbs were cut.

After removing the shoots from the tree, a $1/16$ -inch section was removed from the terminal 9 inches to make autoradiograms. The pieces that were trimmed off the shoots were dried, ashed, and counted, and were designated "autoradiogram shavings".

Results: Radioactive count in treated and untreated portions of peach shoots shown in Table VI indicated that very little entry occurs in the late fall or early winter regardless of concentration of acid. A and B in Table VI refer to replicate limbs for each concentration.

The autographs made of the 8-hour treatment showed no evidence of exposure to radioactive material except for the base of the 8-hour B treatment. However, a faint image was made on all the films exposed to the 14-day treatment with the strongest image being found with the 3 percent treatments.

Experiment VII

Object: To determine the accumulation of P^{32} at 6-, 24-, and 48-hour intervals following a bark application of P^{32} phosphoric acid to Hale-haven peach trees.

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Materials and Methods: Nine horizontal limbs, from which vertical branches arose, were selected on nine 4-year-old Halehaven peach trees. A 0.3 percent solution of P^{32} phosphoric acid, with Dreft as a wetting agent, was applied to the horizontal branches only. The solution froze to the limbs as it was applied with a paint brush at 10 A.M. on March 21, 1951.

The vertical limbs were cut at 6, 24, and 48 hours after treatment and examined to determine whether any radioactive phosphorus had been translocated into them from the application to the horizontal limbs. The vertical branches were separated into untreated buds, xylem, and wood, and the horizontal limbs into treated buds, xylem, and wood. Intact stem sections were designated "wood" while alternate sections which were scraped to remove the phloem tissues were designated "xylem".

Results: The results are given in Table VII. To calculate the figure for the total solution applied, the radioactive count from each horizontal limb and attached vertical branch were totaled and were converted to milliliters of solution. To find the radioactivity of the discarded phloem, it was assumed that this tissue had the same activity as the corresponding wood sections minus the activity of the xylem sections. The percent of material translocated was determined by dividing the total activity found in the plant into the activity found in the untreated limb.

Table VII shows that six hours after application of P^{32} phosphoric acid to horizontal limbs, radioactivity of 169.5 to 222 counts per

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TABLE VII

MIGRATION OF RADIOPHOSPHORUS APPLIED TO DORMANT HORIZONTAL BRANCHES INTO
ATTACHED VERTICAL LIMBS OF THE PEACH AT 6, 24, AND 48 HOURS AFTER APPLICATION

	<u>Time of Harvest</u>								
	Tree 1	6 Hours Tree 2	Tree 3	Tree 1	24 Hours Tree 2	Tree 3	Tree 1	48 Hours Tree 2	Tree 3
Ml. of Radio- phosphorus Solution Applied	1.50	1.78	1.01	3.63	1.10	0.60	1.76	2.05	1.63
Radio- activity of Solution Applied Count/min.	13,987.3	16,534.2	9,385.7	29,613.3	8,996.8	4,887.1	14,791.5	17,311.3	13,698.8
Radio- activity Trans- located Count/min.	187.1	222.0	169.5	138.9	254.5	77.7	117.1	146.9	155.4
Percent of Radio- activity Trans- located	1.34	1.34	1.81	0.47	2.83	1.59	0.79	0.85	1.14

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minute was detected in the 12- to 24-inch vertical branches which arose from the treated horizontal limbs. Only one limb of the three cut 24 hours after treatment and no limbs cut 48 hours after treatment showed a radioactive count higher than the minimum count found 6 hours after treatment. Possibly injury in the treated area, which permitted greater entry, had occurred in the one limb cut 24 hours after treatment which showed a radioactive count of 254.5 counts per minute.

A possible explanation for the lower radioactive counts found in the untreated vertical limbs at 24 and 48 hours after treatment than were found 6 hours after treatment, may be that equilibrium had not been reached 6 hours after treatment between the count of phosphorus which was entering the vertical limb from the treated horizontal limb and the amount which was being translocated to other parts of the plants.

Experiment VIII

Object: To determine the accumulation of phosphorus within 8- to 10-year-old peach limbs after 6, 16, 24, 44, and 72 hours following application of P^{32} phosphoric acid to cotton gauze wrapped around the limbs in early spring.

Materials and Methods: A solution of phosphoric acid contained 0.04 microcuries per milligram of phosphorus was applied April 19 to limbs of South Haven peach trees 1 1/2 to 2 inches in diameter. A continuous supply of phosphorus was provided on the surface of the limbs by using several thicknesses of cotton gauze, which were wrapped around the limbs in a band 4 to 5 inches wide. The gauze was held in place by waterproof tape which also prevented movement of the solution beyond the gauze.

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Longitudinal sections 1/16- to 1/8-inch thick were cut from two limbs at each of the following time intervals of 6, 16, 24, 44, and 72 hours, using an electric table saw. These sections were placed on botanical drying paper and dried between hot steel plates. The outer bark was removed on all but one of the sections at each harvest so that an indication of the amount of P³² which had been applied to the bark and also the amount of P³² which had entered the limbs could be seen in the autoradiograms made from the limbs.

The solution with the exception of the 6-hour samples, which were treated at 9 A.M. of April 20th, was applied at 4:30 P.M. on April 19th. The weather at the time of treatment showed broken clouds and a temperature of 55°F. Limbs for the 16-hour samples were cut at 8:30 A.M. on April 20th. The 24-hour samples were cut at 4:30 P.M. on April 20th. During the day, the weather was clear and the temperature was 60°F. Two limbs were cut at 44 hours or at 12:30 P.M. on April 21st. Final samples were sectioned at 5:00 P.M. on April 22nd after a night of heavy rain.

Results: Activity in the phloem area was seen in the autoradiograms, with the heaviest line at 6 and 16 hours. The density of the line decreased with time so that at 72.5 hours no activity was visible in the phloem tissue. From this information, it would seem that after the initial entry, the phosphorus is moved to other parts of the plant.

Experiment IX

Object: To determine whether P³² phosphoric acid from a bark application would enter dormant 3-year-old apple limbs in February.

Materials and Methods: Fifteen vertical limbs of a Winesap apple tree, which were 3 years old and 8 feet tall, were used in this experiment, which was started February 24, 1953. They were cut from the tree, all the side limbs removed, and 5 layers of gauze 3 inches wide wrapped around the limb 18 inches from the base. The limbs were laid horizontally over saw horses and 5 milliliters of a solution, which contained 0.10 microcurie per milligram of phosphorus, were pipetted onto the gauze. A piece of plastic was fastened over the gauze with masking tape to prevent drying. After all the limbs had been treated, the base of each was slashed and placed in a bucket of water. The treatments were held inside an unheated storage building on the college farm where no freezing occurred until the time that the 96-hour samples were taken.

At time intervals of 12, 24, 48, 96, and 192 hours, three limbs were removed from the bucket and samples 1/4-inch long were removed at 3, 6, and 12 inches below and 3 and 12 inches above the treated area. Because of the smaller diameter of the wood, a 1-inch section was used at 48 inches above the treated area.

Results: Rather high values of radioactive count were found in the samples immediately above and below the treated area, however the variability between samples was great within the limb replications. For the 12-hour samples, this statement is not true as no treatment gave a radioactive count exceeding twice background.

The most interesting fact is that the average minimum count values, although low, showed an increasing value at each successive

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harvest. However the only time at which the minimum amount of radioactivity, usually the sample 48 inches above the treated area, exceeded twice the radioactive count of background was at 192 hours after treatment.

Intake and movement of a large amount of phosphorus over 14 inches in a basal direction can be discounted in this experiment. Only a small amount of radioactivity was found in the water in which the shoots were placed during the experiment.

Experiment X

Object: To determine whether pruning wounds in the treated area affect the rate of entry of phosphorus from P^{32} phosphoric acid as well as to observe the effect of sucrose on the rate of entry of phosphorus from P^{32} phosphoric acid.

Materials and Methods: This experiment, which was started April 23, 1953, using a phosphoric acid solution containing 0.06 microcurie per milligram of phosphorus, followed the same experimental procedure as Experiment IX with some modifications. Black Twig apple limbs 3 to 5 years old were used. Since the distribution of phosphorus within the limb was the main consideration, only one period of sampling, 48 hours, was used. This made it possible to use an increased number of replications. A side shoot or spur was removed from the area which was to be treated of half the limbs. Sucrose was added to part of the original solution to make a 5 percent solution. Each solution was pipetted onto cotton gauze wrapped around the limbs on four of the eight limbs. Each limb was placed upright in a bucket of water immediately after treatment, so as to prevent contamination by horizontal spread of the solution.

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Results: The levels of radioactivity in this experiment were much lower than those encountered in the previous experiment, but the distribution appeared to follow the same trend. The greatest amount of radioactivity was found 3 inches above the treated area where 6 of the 8 limbs had an amount of radioactivity greater than twice the background count level. On the other hand, at 3 inches below, only 2 of the 8 limbs had an amount of radioactivity greater than twice that of background and in one of the limbs this radioactivity was due to surface contamination. The other larger amount of radioactivity was on one of the injured limbs which in general gave evidence of greater entry.

Sucrose did not seem either to increase or decrease entry of phosphoric acid. However, the values recorded are much too close to background to be regarded as conclusive.

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Spring Growing Season Treatments

Experiment XI

Object: To determine whether movement of phosphorus from bark applications of phosphoric acid will take place at the time of bud swell.

Materials and Methods: The basal foot, of eight intact 2-year-old Halehaven peach shoots approximately 2 foot long, was painted with phosphoric acid solutions which contained 0.04 microcurie per milligram of phosphorus on April 3, 1953. Four limbs were painted with a solution containing phosphoric acid only and four with a solution containing phosphoric acid plus 5 percent sucrose.

The temperature was about 50°F. during the 24-hour period allowed for absorption. Rain fell after the solution had been in place for 7 hours.

When the limbs were cut, the basal or treated portion of the limb was separated from the apical or untreated portion. The two portions of each limb were ashed then counted separately.

Results: An amount of radioactivity greater than twice the background level was found in the untreated portion of only two of the eight limbs. Both these limbs had been treated with the solution containing no sucrose. All the radioactive count values were lower on the treated portions of the limbs when sucrose was included in the solution.

Experiment XII

Object: To determine whether increased intake of phosphorus from P^{32} phosphoric acid occurs when the bark of an apple shoot is scraped.

Materials and Methods: The bark of 1-year-old water sprouts of Red Astrachan apple was scraped lightly with a knife, so as to roughen the bark but not remove much of it. The basal 8 inches of four of the shoots were so scraped, but four others were left untouched. On April 23, 1953, all were painted with a solution of phosphoric acid containing 0.10 microcurie per milligram of phosphorus. The solution dried rapidly and twenty-four hours after application, rain fell for 4 hours.

Forty-eight hours after application samples were collected, the shoots being cut 2 inches above the treated area in order to avoid possible contamination. The swelling buds were removed from the untreated portion and were considered as a separate sample for counting purposes.

Results: Only two of the eight samples from the untreated portion gave counts twice that of background and they were buds from scraped limbs. Approximately five times as much phosphorus remained on the scraped shoots as on the unscraped, suggesting that the greater entry into scraped shoots may have been due in part to the greater amount of solution retained by the scraped bark.

Experiment XIII

Object: To determine whether P^{32} phosphoric acid will enter the tomato plant from applications made to the sides of the stem.

Part 1

Materials and Methods: Seeds of Michigan State Forcing variety of tomato were planted September 23, 1952, and the seedlings transplanted to 3-inch pots on October 30, and to 12-inch pots on November 28.

In a preliminary experiment, intake from a continuously moist source of phosphoric acid was compared to a single application by brush. This treatment, which ran from December 4 to 8, utilized a phosphoric acid solution which on November 12, 1952, had an activity of 2 microcuries per milliliter of P^{32} . A 1 1/2-inch band of solution was painted onto one plant, while a 1-inch gauze which was soaked in solution was wrapped around the stem of another. A piece of plastic film prevented the gauze from drying during the experiment.

The two plants were cut into sections consisting of a piece of the stem and an attached leaf. These sections were dried and the dry weight determined for each. Each section was ground to a fine powder with a mortar and pestle before the radioactivity in each section was determined on December 17.

Results: The entry of radiophosphorus as indicated by counts per minute from P^{32} in different parts of the plants is given in Table VIII. The greatest concentration of phosphorus appeared to be in the youngest leaf tissue, as might be expected from previous experiments with tree fruits. The amount that entered the plant was much greater from the gauze treatment than from the single brush application. However, the percent of the total phosphorus that was translocated was less from the gauze treatment than from the single brush application. Only 24.1 percent was translocated during the 4 day period when applied in gauze as compared to 31.4 percent translocated from a single brush application.

TABLE VIII
 DISTRIBUTION OF RADIOPHOSPHORUS IN TOMATO PLANTS FOLLOWING
 THE APPLICATION OF PHOSPHORIC ACID SOLUTION TO THE STEM BY
 BRUSH AND IN COTTON GAUZE AS INDICATED BY COUNTS PER MINUTE

Location of Plant Part	Brush Application		Gauze Application	
	Dry Wt. (Gms.)	Radioactivity 1 Gm. Dry Wt. (Counts/min.)	Dry Wt. (Gms.)	Radioactivity 1 Gm. Dry Wt. (Counts/min.)
Tip	.1549	739.2	.1665	9,493.7
Leaf 5 above	.2257	361.1	.2552	4,367.9
Leaf 4 above	.2870	304.7	.3900	2,637.7
Leaf 3 above	.3071	135.5	.5030	1,031.2
Leaf 2 above	.3321	150.9	.5333	993.2
Leaf 1 above	.3859	132.9	.4185	933.6
Treated Stem	.1019	11,185.5	.2228	83,337.9
Treated Gauze			.3609	45,269.3
Leaf 1 below	.3658	117.6	.2755	689.7
Leaf 2 below	.3353	110.6	.2467	971.2
Roots	.3450	100.9	.4303	1,479.7

The number of radioactive counts per minute measured in 1/200
 of a milliliter of the treating solution was 246.8.

Part 2

Materials and Methods: After December 20, only distilled water was supplied to the tomato plants so that flowering would be induced. On January 14, 1953, flowers of six plants were sufficiently developed to set fruit artificially when the flowers were dipped into a solution of 50 ppm of alpha (p-chlorophenoxy)-propionic acid. On January 18, two plants were moved into each of three greenhouse rooms, which were held at 50°, 65°, and 80°F. constant temperatures.

One day was allowed for the plants to become acclimated to the new environment before the plants were treated by the gauze method with a phosphoric acid solution which contained 0.21 microcurie per milligram of phosphorus. A 6-inch piece of gauze, 1-inch wide, was wrapped around the stem of each plant 17 inches below the developing fruit. Fifteen drops, which was approximately 3/4 of a milliliter, of the solution were applied to each gauze. Plastic film covered the gauze to prevent drying of the solution. An additional piece of cotton was placed at the basal end of the plastic to absorb any excess solution.

Entry of phosphorus into the plant was determined by the activity from P³² which was found in the developing fruit. To obtain a record of the rate of entry of phosphorus into the fruit, a Gieger-Müller tube was placed next to the fruit. The radiation pulses received by the Gieger-Müller tube were recorded by an Esterline Angus chart recorder which was attached to a Tracerlab count rate meter according to the method described by Hinsvark, Tukey, and Wittwer (31). A

record of the temperatures in each room was obtained by means of thermographs.

At each temperature, the largest fruit was placed next to the Gieger-Müller tube to obtain a continuous record of the amount of radioactivity in the fruit. After four days, all fruit were picked and then dried. The dried fruits were pulverized with a mortar and pestle, then a record of the actual counts per minute per gram of dried fruit tissue was obtained with a Tracerlab Autoscaler.

Results: Intake of P^{32} into the developing tomato fruit following a stem application of P^{32} phosphoric acid in gauze was found to be dependant on temperature. The results expressed on the basis of 1 gram of dried plant material in Table IX show that 176 and 300 counts per minute were found in fruit grown at 50° , 572 and 663 counts per minute when grown at 65° , finally 657 and 738 counts per minute in fruit grown at 80° . Similar results were found by Hinsvark and Wittwer (32) who were studying absorption of phosphorus from foliage applications at these same temperatures.

The size of fruit was influenced by the temperature. Large fruits 5.9 and 4.1 grams were produced at the high temperature, one large 4.1 grams and one small fruit 0.5 grams were produced at the intermediate temperatures, and small fruit 1.2 and 0.3 grams were produced at the low temperature.

Experiment XIV

Object: To determine whether a spray application of mineral nutrients applied to the bark can supply a portion of the mineral requirements of developing shoots.

TABLE IX

THE EFFECT OF TEMPERATURE ON THE MOVEMENT OF RADIOPHOSPHORUS INTO THE DEVELOPING TOMATO FRUIT FROM PHOSPHORIC ACID APPLIED IN COTTON GAUZE TO THE STEM OF THE PLANTS AS INDICATED BY COUNTS PER MINUTE

Temperature	Green Wt. (Gms.)	Dry Wt. (Gms.)	Total Radioactivity (Counts/min.)	Radioactivity 1 Gm. Dry Wt. (Counts/min.)
50°	1.222	0.1028	30.8	300.0
	0.276	0.0290	5.1	175.8
65°	4.137	0.3250	158.9	572.0
	0.502	0.0498	32.3	663.0
80°	5.869	0.4127	304.5	738.0
	4.105	0.3029	197.8	657.0

Materials and Methods: Dormant 1-year-old Montmorency cherry trees and 2-year-old McIntosh apple trees from cold storage were planted in 12-inch pots filled with quartz sand on June 11, 1952. These trees were sprayed June 14th with a 2 percent solution of phosphoric acid which contained 0.22 microcurie per gram of phosphorus and which utilized Methocel 4000 c.p.s. as a sticking agent at the rate of 1/4 pound per 100 gallons of water. The spray was applied at a pressure of 100 pounds per square inch with a Sure-Shot sprayer. Twenty trees of each variety were sprayed with the solution and four non-sprayed trees served as controls.

Dormant 1-year-old Montmorency cherry trees and 2-year-old McIntosh apple trees from cold storage were planted in 12-inch pots filled with quartz sand on June 18th. These trees were sprayed June 19th with a 2 percent solution of calcium chloride which contained 0.20 microcurie per gram of calcium and which utilized Methocel 4000 c.p.s. as a sticking agent at the rate of 1/4 pound per 100 gallons of water. The spray was applied at a pressure of 60 pounds per square inch with a Sure-Shot sprayer. Sixteen trees of each variety were sprayed with the solution and four non-sprayed trees served as controls.

As the trees were grown out-of-doors it was necessary to fasten plastic sheet 18 by 42 inches around the tree and around the pot to prevent the entry of rain water, which might carry some of the spray material into the root medium. A piece of cotton, which was placed under the plastic, absorbed any water which seeped between the plastic and the stem. A budding rubber held the plastic and cotton tightly to

the stem. The plastic was fastened to the pot with twine, leaving a covered opening to permit watering of the plant.

The basic nutrient solution was 1/2 Hoagland's (33). Half of the trees of the phosphorus bark spray treatment received complete Hoagland's solution while the other half received a minus phosphorus Hoagland's solution. The same pattern was followed with the calcium treatments, half receiving complete solution and the other half minus calcium.

On August 7, 1952, all the new shoot growth was removed from each tree. After drying, these shoots were ground to 20 mesh in a Wiley mill. One gram of the dried material from each tree was ashed following the procedure described in the A.O.A.C. (1) for calcium or phosphorus analysis depending upon which spray the tree had received. Results: Numerical data from this experiment are shown in Tables X, XI, and XII.

More new growth was produced by the trees receiving the complete nutrient solution than by those receiving the deficient solutions.

No valid count data were obtained from the phosphorus spray because the phosphorus passed through too many half-lives before analysis was made.

Root initiation did not occur in many of the cherry trees, possibly because of the high temperatures at the time of planting. Very little new growth was made by the tops of these plants. The count data obtained were very variable.

TABLE X
 SHOOT GROWTH PRODUCED BY APPLE AND CHERRY TREES UNDER
 DIFFERENT NUTRIENT CONDITIONS FOLLOWING A DORMANT SPRAY
 OF RADIOPHOSPHORUS OR RADIOCALCIUM

Treatment	(Dry weight in grams)*	
	<u>Apples</u>	<u>Cherries</u>
Phosphorus Spray		
-P Nutrient Solution	10.63	2.38
+P Nutrient Solution	14.66	3.03
No Spray		
-P Nutrient Solution	8.09	1.52
+P Nutrient Solution	7.57	1.82
Calcium Spray		
-Ca Nutrient Solution	6.84	1.51
+Ca Nutrient Solution	9.01	2.35
No Spray		
-Ca Nutrient Solution	6.56	1.87
+Ca Nutrient Solution	8.48	2.17

* Mean values for the treatments

TABLE XI

RADIOCALCIUM CONTENT OF APPLE SHOOTS GROWING ON TWO LEVELS
OF CALCIUM NUTRITION PRODUCED FOLLOWING A DORMANT SPRAY OF
CALCIUM CHLORIDE AS INDICATED BY COUNTS PER MINUTE

	Nutrient Level	
	-Ca Radioactivity 1 Gram Dry Wt. (Counts/min.)	‡Ca Radioactivity 1 Gram Dry Wt. (Counts/min.)
1	672	348
2	838	284
3	472	267
4	602	241
5	561	230
6	445	318
7	275*	390
8	473	753*
Total	4063	2078
Mean	580.4	296.9

Differences necessary for significance:

5 percent level 181.7 1 percent level 275.1

*These values were not used in the statistical analysis

TABLE XII
ANALYSIS OF VARIANCE OF RADIOACTIVE COUNTS FROM RADIOCALCIUM
IN APPLE SHOOTS PRODUCED BY TREES GROWING ON COMPLETE AND
MINUS CALCIUM NUTRIENT SOLUTIONS FOLLOWING A DORMANT SPRAY
OF CALCIUM CHLORIDE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	14	419,039		
Treatment	1	281,434	281,434	25.6**
Replication	6	60,631	10,105	0.919
Error	7	76,974	10,996	

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A highly significant difference was found in the radioactive calcium content of the current season shoots of apple trees which received complete nutrient solution and those which received minus calcium nutrient solution, more radioactive calcium being found in the plants which had received the minus calcium solution.

Experiment XV

Object: To determine whether mineral nutrients applied to semi-dormant sour cherry tree would induce a response in growth.

Materials and Methods: Two sprays, NuGreen and 15-30-15 fertilizer, were supplied by spraying the solution at the tree and by spraying the solution at the ground. An equal amount of nitrogen, .07 pounds, was applied to each tree. This amount, which is equivalent to 1/2 pound of sodium nitrate, was contained in 1/2 gallon of spray, which the sprayer delivered in 2 seconds.

The NuGreen spray contained 17 pounds of NuGreen in 50 gallons of water. The complete fertilizer spray was composed of 20 pounds of Bonro 10-52-17 and 25 pounds of Rapid-Gro 23-21-17 in 50 gallons of water. Methocel 4000 c.p.s. was used as a sticking agent for both sprays at a rate of 1/4 pound per 100 gallons of water.

Eighty Montmorency cherry trees, 5 years old, were used for this experiment which was started April 18, 1952. At that time, the circumferences of the trunks 1 foot above the ground were measured and recorded. The sprays were applied to the trees, which were in the green tip stage, on April 20th.

TABLE XIII

TOTAL INCREASE IN CIRCUMFERENCE IN CENTIMETERS OF FOUR TREE REPLICATIONS OF 5-YEAR-OLD MONTMORENCY CHERRY TREES FOLLOWING GROUND AND TREE APPLICATION OF NUTRIENTS

Replication	Check	N-G	N-S	NPK-G	NPK-S
1	15.5	16.4	21.0	18.1	15.9
2	18.0	18.7	17.1	19.5	20.9
3	18.6	18.3	19.2	19.7	20.3
Total	52.1	53.4	57.3	57.3	57.1
Average per tree	4.425	4.117	4.775	4.775	4.758

Differences necessary for significance 5 percent level = .275

Footnote

N-G - NuGreen sprayed on ground

N-S - NuGreen sprayed on tree

NPK-G - 15-30-15 sprayed on ground

NPK-S - 15-30-15 sprayed on tree

Four spray treatments containing four trees each were replicated four times. The check treatment of four replications of four trees received no fertilizer.

Results: The increase in circumference during a 1-year period was used for evaluation of this experiment.

As one replication each in the NuGreen application to the ground and complete spray to the tree were very low as compared to the other replications, it was decided to run the analysis of variance on the three highest replications.

As would be expected on trees that had not been fertilized previously, a significant response to fertilizer application, which is shown in Table XIII, was noted. However, the NuGreen ground treatment was significantly lower than the check, partly because two replications made less than average growth. The important point is that no differences in response were noted from the two methods of application. Response was about the same whether the solution was sprayed at the tree or on the ground around the tree.

Experiment XVI

Object: To determine whether greater nutrient intake from an application of phosphoric acid to the bark of twigs and branches occurs during the period of rapid growth than during the dormant period.

Materials and Methods: Water sprouts and 10-year-old limbs of a single Jefferis apple tree were used in this experiment. With the water sprouts, four treatments were used, with two water sprouts per treatment. In the first treatment, the solution was painted directly

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on the basal 8 inches of an undamaged water sprout. In the second treatment, the solution was painted on the basal 8 inches of a water after the bark had been scraped lightly with a knife. In the third treatment, the solution was applied to a 6-inch gauze wrapped around the basal 8 inches of an undamaged water sprout. In the fourth treatment, the solution was applied to a 6-inch gauze which had been wrapped around a shoot after the water sprout had been scraped. Plastic film was used to cover the gauze to prevent rapid drying.

To study the movement of phosphorus into water sprouts following application to the bark of 1-year-old limbs from which the water sprouts arose, three treatments were used. In the first treatment, the solution was painted on the undamaged bark of a limb from which two water sprouts arose. In the second treatment, the solution was painted onto scraped bark from which three water sprouts arose. In the third treatment, the solution was applied to a piece of gauze 6 x 36 inches which had been wrapped around an area of the limb which had been scraped. The treated area carried two water sprouts. A sheet of plastic film was used to cover the gauze to prevent rapid drying.

A 0.3 percent solution of phosphoric acid containing 0.53 microcurie per milligram of phosphorus was applied to the limbs at noon on May 23rd. The water sprouts were removed for sampling 48 hours later. No rain fell during the period.

Current seasons shoots (leaves and stem) and the untreated portion of the previous seasons shoot (stem) were collected from each water sprout for sampling. Water sprouts that had been treated directly

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were cut two inches above the treated area while shoots that had been growing in a treated area of older bark were cut one inch above the treated area.

After drying, the samples were ashed according to the procedure outlined for phosphorus analysis in the A.O.A.C. (1). Radioactive counts were measured on the total ash of each sample on June 11th. The count recorded on June 11th for several of the samples was higher than the mechanical ability of the scaler to record efficiently. These samples were counted again on July 6, 1953, after being diluted 1 to 20.

Total phosphorus in each sample was determined according to the micro method given in A.O.A.C. (1) on June 30th.

Results: The numerical data obtained in this experiment is presented in Tables XIV, XV, XVI, and XVII.

A greater amount of radiophosphorus entered limbs when the bark was scraped lightly, when cotton gauze saturated with P^{32} phosphoric acid solution was wrapped around the limbs, and when cotton gauze saturated with P^{32} phosphoric acid solution was wrapped around lightly scraped limbs than when the solution was applied to a limb with intact bark.

When the radioactive counts found in current seasons growth of the various treatments were compared to those found in the water sprouts with intact bark (195-339 counts/min.), a 10-fold increase was noted with scraped bark (2,086-5,437 counts/min.), a 30-fold increase following absorption from gauze over intact bark (7,804-10,961

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TABLE XIV

PHOSPHORUS AND RADIOPHOSPHORUS CONTENT OF CURRENT SEASONS GROWTH (LEAVES AND STEMS) OF WATER SPROUTS OF APPLE FOLLOWING APPLICATION OF PHOSPHORIC ACID TO THE BASAL 8 INCHES OF BARK OF THE WATER SPROUTS

Replication	Total Dry Wt. of Leaves and Stems (Gms.)	Radioactivity Per 1 gram Dry Wt. of Leaves and Stems (Counts/min.)	Total Phosphorus per 1 gram dry wt. of Leaves and Stems (Mgs.)
Solution Applied to Intact Bark			
1	1.6005	339.0	4.327
2	1.6875	194.6	4.222
Solution Applied to Cotton Gauze over Intact Bark			
1	1.1787	7,804.2	4.666
2	1.0113	10,961.2	6.180
Solution Applied to Lightly Scraped Bark			
1	1.3868	5,437.8	4.507
2	1.4811	2,086.2	4.692
Solution Applied to Cotton Gauze over Lightly Scraped Bark			
1	0.9322	423,815.0	14.321
2	0.9142	368,177.0	15.314

The phosphorus content of 1/100 milliliter of the treating solution was 0.508 milligrams and the radioactive count of this amount was 37,880.0 counts per minute.

TABLE XV

PHOSPHORUS AND RADIOPHOSPHORUS CONTENT OF CURRENT SEASONS GROWTH (LEAVES AND STEMS) OF WATER SPROUTS OF APPLE FOLLOWING APPLICATION OF PHOSPHORIC ACID TO BARK OF ADJOINING 10-YEAR-OLD LIMBS

Replication	Total Dry Wt. of Leaves and Stems (Gms.)	Radioactivity per 1 gram Dry Wt. of Leaves and Stems (Counts/min.)	Total Phosphorus per 1 gram dry Wt. of Leaves and Stems (Mgs.)
Solution Applied to Intact Bark			
1	1.3300	21.4	3.308
2	0.9030	17.2	3.267
Solution Applied to Heavily Scraped Bark			
1	0.8955	145.5	3.238
2	1.4789	754.4	3.043
3	1.1400	917.9	3.491
Solution Applied to Cotton Gauze Over Heavily Scraped Bark			
1	1.6834	80,511.0	8.094
2	1.3532	62,716.0	6.836

The phosphorus content of 1/100 milliliter of the treating solution was 0.508 milligrams and the radioactive count of this amount was 37,880.0 counts per minute.

TABLE XVI

PHOSPHORUS AND RADIOPHOSPHORUS CONTENT OF PREVIOUS SEASONS
GROWTH (WOODY STEM) OF WATER SPROUTS OF APPLE FOLLOWING
APPLICATION OF PHOSPHORIC ACID TO THE BASAL 8 INCHES OF
BARK OF THE WATER SPROUTS

Replication	Total Dry Wt. of Woody Stem (Gms.)	Radioactivity Per 1 gram Dry Wt. of Woody Stem (Counts/min.)	Total Phosphorus per 1 gram dry wt. of Woody Stem (Mgs.)
Solution Applied to Intact Bark			
1	2.6717	83.7	1.192
2	3.5557	50.2	1.159
Solution Applied to Cotton Gauze over Intact Bark			
1	1.7747	2,196.1	1.155
2	4.2045	1,326.9	1.146
Solution Applied to Lightly Scraped Bark			
1	2.9745	881.7	1.150
2	4.1016	328.2	0.959
Solution Applied to Cotton Gauze over Lightly Scraped Bark			
1	1.1096	154,482.0	8.562
2	2.1403	80,718.0	14.601

The phosphorus content of the 1/100 milliliter of the treating solution was 0.508 milligrams and the radioactive count of the amount was 37,880.0 counts per minute.

TABLE XVII
PHOSPHORUS AND RADIOPHOSPHORUS CONTENT OF PREVIOUS SEASONS
GROWTH (WOODY STEM) OF WATER SPROUTS OF APPLE FOLLOWING
APPLICATION OF PHOSPHORIC ACID TO BARK OF ADJOINING 10-
YEAR-OLD LIMBS

Replication	Total Dry Wt. of Woody Stem (Gms.)	Radioactivity per 1 gram Dry Wt. of Woody Stem (Counts/min.)	Total Phosphorus per 1 gram Dry Wt. of Woody Stem (Mgs.)
Solution Applied to Intact Bark			
1	3.2744	3.2	0.803
2	2.2455	8.5	---
Solution Applied to Heavily Scraped Bark			
1	2.4200	28.1	0.791
2	4.4141	183.1	0.768
3	4.6828	202.1	0.722
Solution Applied to Cotton Gauze Over Heavily Scraped Bark			
1	5.0736	8,336.0	1.375
2	4.1297	5,881.0	1.170

The phosphorus content of the 1/100 milliliter of the treating solution was 0.508 milligrams and the radioactive count of this amount was 37,880.0 counts per minute.

counts/min.), and a 1000-fold increase following absorption from gauze over scraped bark (368,177-423,815 counts/min.). It thus appears that one of the limiting factors in bark application is the small amount of material that is retained by the bark if an aqueous solution of material is applied. Also, exposure of living cells within and beneath the bark aids entry. It is interesting in this connection to observe that reports in old horticultural literature of beneficial results from nutrient applications to the woody portions of trees, usually stress both scraping of the area where the application is to be made, and application of the material in a thick slurry or paste.

Drying of the leaves at the edges, shriveling of the bark, and pink discoloration of the phloem were observed in the shoots which were scraped and then covered with gauze. This was possibly due to phosphorus toxicity as shriveling and pink discoloration of the phloem were observed in experiment III when 2-year-old McIntosh apple trees were sprayed with phosphoric acid solutions at concentrations ranging from 8 to 32 percent.

More radioactive phosphorus was found in the expanding shoots of the current season than in the woody shoots of the previous season. For example in Tables XIV and XVI, the amount of P^{32} absorbed through the intact bark of water sprouts gave 195 and 339 counts per minute in the current seasons growth (leaves and stem) and 50 and 84 counts per minute in the previous seasons growth (stem) whereas the amount of P^{32} absorbed through lightly scraped bark of water sprouts gave 2,086 and 5,438 counts per minute in the current seasons growth and 328 and 882

counts per minute in the previous seasons growth. As the current season shoots were still in active growth, more phosphorus metabolism could be expected there than in the previous season shoots where only the cambial area was in active growth.

Greater entry of radioactive phosphorus occurred following application of the solution to the bark of water sprouts than occurred following application of the solution to the bark of 10-year-old limbs. Thus in Tables XV and XVI counts per minute found in current seasons growth were 195 and 339 when the solution was brushed on the intact bark of watersprouts and 16 and 28 when the solution was brushed on the intact bark of a 10-year-old limb in an area on which two water sprouts arose.

Experiment XVII

Object: To compare the entry and subsequent movement of radiophosphorus applied to leaves alone, bark alone, and leaves and bark together of young peach trees.

Materials and Methods: Three 1-year-old Elberta peach trees growing in pots in the greenhouse, and two 4-year-old Halehaven peach trees growing in the orchard were used. At the time the experiment was begun, February 17, 1951, the leaves were starting to unfold on the trees in the greenhouse. An equal amount of a phosphoric acid solution, which contained 0.07 microcurie per milligram of phosphorus, was used on each tree. In the greenhouse, the solution was applied to the expanding leaves of one tree, to the bark of another, and to both the leaves and bark of a third. The night temperature of the greenhouse was 40°F.

In the orchard, most of the solution was applied to the buds on the basal portion of 1-year-old twigs. The temperature remained above freezing during the night and was in the 40's F. during the day.

Results: No intake of radioactive phosphorus was observed from the application to the dormant buds of peach twigs out-of-doors. However, entry and movement occurred in some cases from applications to the trees growing in the greenhouse. Following an application to either the leaves and bark or to the bark alone, movement to other parts was detected. However, when the application was made solely to the expanding leaves, no movement occurred away from this area, probably because phosphorus was being metabolized in the region of application.

Experiment XVIII

Object: To compare the retention of radiophosphorus solution by leaves of apple, peach, pear, sour cherry, and sweet cherry.

Materials and Methods: One tree each of McIntosh apple, Elberta peach, Bartlett pear, Montmorency sour cherry, and Windsor sweet cherry were used. The trees were actively growing in pots in the greenhouse where they had been in active growth for approximately 3 weeks before the treatments were made.

A solution of 0.3 percent phosphoric acid containing 0.11 microcurie per milligram of phosphorus and containing Dreft as a wetting agent was applied to one of the lower growing shoots of each plant on March 3, 1951. The entire shoot was submerged in a beaker of the radioactive solution after each potted tree had been laid horizontal on the greenhouse bench. The trees were left horizontal until the

applied solution had dried on the shoots so that contamination of the surrounding limbs by lateral movement of the radioactive solution would be eliminated; because any excess solution on the horizontal trees dripped onto paper spread on the greenhouse bench. After the solution dried, the trees were placed upright.

Samples of treated and non-treated shoots, and new roots were collected 6 days after treatment and the radiophosphorus in each part was counted utilizing a Tracerlab Autoscaler. Thus, the total amount of phosphorus solution applied and the amount translocated to the shoots and to the roots were determined.

Results: Data obtained are shown in Tables XVIII and XIX. A relation was observed between adherence and retention of material and the physical characters of the leaves. Thus, apple leaves, which have the most pubescence, showed the greatest retention of the solution, whereas peach leaves, which are glabrous, retained the least.

Translocation of phosphorus was not correlated with the amount which had adhered to the leaves of the different fruits. The data show that the pear and sour cherry were more efficient in absorption as indicated by the percent of the solution translocated of that which was applied. The data also indicate that there may be a difference in direction of movement of the absorbed phosphorus in the different plants. On a unit weight basis, the radioactive count found in the tops of pear and sour cherry was greater than the amount found in the root tissue. In the apple, peach, and sweet cherry on a unit weight basis, the greatest amount of radioactive count from P^{32} was found in

TABLE XVIII

RETENTION OF PHOSPHORIC ACID BY SHOOTS OF APPLE, PEACH, PEAR, SOUR CHERRY, AND SWEET CHERRY FOLLOWING DIPPING IN A SOLUTION CONTAINING RADIOPHOSPHORUS AS INDICATED BY COUNTS PER MINUTE

	Fresh Wt. of Shoot Growth (Gms.)	Total Activity (Counts/min.)	Amount of Solution (ml.)
Apple	3.655	131,532.9	2.91
Peach	3.593	28,594.2	0.633
Pear	1.389	39,332.0	0.87
Sour Cherry	3.761	44,076.8	0.976
Sweet Cherry	7.573	63,661.8	1.41

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TABLE XIX

TRANSLOCATION OF RADIOPHOSPHORUS INTO UNTREATED SHOOTS AND
 ROOTS OF APPLE, PEACH, PEAR, SOUR CHERRY, AND SWEET CHERRY
 FOLLOWING DIPPING OF ONE LOWER SHOOT IN PHOSPHORIC ACID

	Radioactivity in Counts per Minute per 1 gram Fresh Tissue		Total Percent P ³² Translocated to Shoots and Roots
	Shoot	Root	
Apple	88.7	162.0	0.52
Peach	12.7	46.9	0.39
Pear	156.3	104.0	2.65
Sour Cherry	206.8	31.0	3.37
Sweet Cherry	12.4	19.3	0.36

the roots. Thus the movement, after entry of P^{32} into the more efficiently absorbing plants, was apparently different than in the less efficiently absorbing plants.

Summer Growing Season Treatments

Experiment XIX

Object: To determine whether greater entry of potassium occurred when the amount of K^{42} potassium carbonate on the leaf was increased by the use of a lime slurry.

Materials and Methods: The potassium solution was prepared by adding 11 grams of potassium carbonate containing K^{42} to 3 liters of water. To half the solution, Dreft was added as a wetting agent and to the rest of the solution hydrated lime was added to form a slurry.

Median leaves of McIntosh apple, Elberta peach, and Montmorency sour cherry shoots on trees growing in pots in the greenhouse were dipped into the solution using one tree of each variety. The procedure was repeated with the lime slurry using one tree of each variety.

The dipping of the median leaves was done at 9 A.M. on April 4, 1951, and the shoots were cut after 7 hours. The shoots were dried under heat lamps for 16 hours after harvest. Autoradiograms were made by exposing X-ray film with these dried shoots.

Results: Intake of K^{42} by the actively growing apple shoots occurred with both carriers. The intake appeared to be slightly greater in the shoot dipped into the solution. Perhaps this occurred because the wetting agent in the solution wet the pubescent apple leaf more easily, whereas the slurry was prevented by the epidermal hairs from coming in contact with the epidermis of the leaf. Some injury to the leaves was noted where the slurry and leaf epidermis were in contact. Potassium

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was found to be concentrated in the stem of the apple with a diffusion gradient extending in both directions from the treated leaves.

Greater intake of K^{42} by the actively growing peach and sour cherry shoots occurred when the leaves were dipped into the slurry. The peach and sour cherry leaves were not wetted by the solution but the slurry was in contact with most of the epidermis of the leaf. Some burning of the leaves was caused by the slurry. Peach leaves, other than the treated leaves, as well as the stem were visible in the autoradiogram made from the slurry treatment while only the stem was visible in the autoradiogram made from the solution treatment. Petioles and midribs of sour cherry leaves, other than the treated leaves, as well as the stem were visible in the autoradiograms where both treatments were used. The autoradiogram made from the slurry treatment was darker indicating a greater accumulation of K^{42} .

Contact of the material with the epidermis of the leaf was apparently necessary for entry. The nature of the leaf surface was the factor which controlled contact. Thus different responses were obtained with the pubescent apple leaves and the glabrous peach and sour cherry leaves to the solution and the slurry methods of application of K^{42} potassium carbonate.

Experiment XX

Object: To determine whether the distribution of potassium and phosphorus within limbs differed following foliage applications of K^{42} potassium carbonate and P^{32} phosphoric acid.

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Methods and Materials: Four potted 2-year-old McIntosh trees, which were in active growth, were used in a greenhouse experiment. The tips of shoots of two trees were dipped, and the median leaves of shoots of the other two trees were dipped in the solutions at 9:30 A.M. on May 9, 1951. The shoots were cut after 8 hours.

The potassium solution was prepared by adding 11 grams of potassium carbonate containing K^{42} to 3 liters of water. The phosphorus solution was a 0.3 percent solution of phosphoric acid which contained 0.03 microcurie per milligram of phosphorus. Autoradiograms were made from the shoots after they were dried.

Results: Very little movement of either phosphorus or potassium was seen in the autoradiograms of shoots in which the tip leaves were dipped. A faint indication of movement of potassium was seen in the stem immediately below the treated leaves, and the movement of phosphorus was indicated by a faint outline of the leaves immediately below the treated leaves.

More movement occurred when the median leaves of the shoots were dipped. Both elements moved acropetally and basipetally in the stem from the treated leaves. Concentrations of phosphorus appeared in the stem immediately above and below the treated leaves while distribution of potassium appeared to have been more uniform along the stem. Mayberry (41) working with beans and squash found potassium to be uniformly distributed in the plant while phosphorus accumulated in the actively growing regions.

80Experiment XXI

Object: To determine the movement of phosphorus within shoots for a 24-hour period following application of P^{32} phosphoric acid to two leaves of each shoot.

Materials and Methods: Two median leaves of one shoot on each of five 2-year-old McIntosh apple trees were dipped into 0.3 percent solution of phosphoric acid, which contained 0.11 microcurie per milligram of phosphorus on May 18, 1951, at 10:15 A.M. in the greenhouse. All shoots were not in the same stage of growth, as some had matured while others were still in active growth. One shoot was cut after the elapse of each of the following time intervals after treatment: 2, 6, 12, 18, and 24 hours.

Results: Results of this experiment are explained on the basis of the amount of exposure which was seen in the autoradiogram made from each shoot. Increased exposure results from a higher concentration of radioactive phosphorus in the plant material.

At 2 hours after treatment, the terminal leaves of this shoot, which was still growing, were visible in the autoradiogram. More of the phosphorus had apparently moved acropetally in the shoot than had moved basipetally.

At 6 hours after treatment, the terminal leaves were faintly visible although the shoot was more mature than the shoot used for the 2-hour treatment. The stem of this shoot was also visible in the autoradiogram although it was not as dark as the stem of the 2-hour treatment.

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At 12 hours after treatment, the terminal leaves of this shoot, which was still growing, were more clearly outlined in the autoradiogram than were those from the previous two treatments. Also visible were the leaf petioles and a faint outline of some of the leaves above the treated leaves.

At 18 hours after treatment, the terminal leaves of this shoot were clearly visible in the autoradiogram although the shoot was more mature than those used in both the 2-hour and 12-hour treatments. The stem was clearly visible as were some of the leaves above those treated.

At 24 hours after treatment, the terminal leaves of this shoot were clearly visible in the autoradiogram although this was the most mature one in the experiment. The stem and some of the leaf petioles were clearly visible in this experiment even though they were not as dark as the 18-hour treatment.

Continued accumulation of phosphorus in the shoots after entry through the leaves occurred during the 24 hours covered by this experiment. The amount of accumulation of radiophosphorus in the terminal leaves was apparently dependant upon the maturity of these leaves and upon the amount of time allowed for entry. The radiophosphorus content of the shoots appeared to be greater at each successive harvest. However the less mature shoots at 2 hours and 18 hours showed increased accumulation of phosphorus over the 6-hour and 24-hour-treated shoots, respectively.

Experiment XXII

Object: To determine the accumulation in the shoots of radiophosphorus at 2, 4, 6, 8, 10, and 12 hour intervals, following a leaf application of P^{32} phosphoric acid to McIntosh apple trees.

Materials and Methods: The sixth and seventh expanded leaves below the growing point of 30 shoots were dipped June 14, 1951, at 11:00 A.M. into a 0.3 percent solution of phosphoric acid which contained 0.16 microcurie per milligram of phosphorus. At 2, 4, 6, 8, 10, and 12 hours after dipping, five of the shoots were cut. Two shoots at each sampling were vertical shoots and three were horizontal shoots.

After the shoots were cut, two were used to make autoradiograms and three were ashed then counted to record the total activity in the shoots. In the shoots that were counted, an effort was made to avoid contamination of the untreated portion by removing the section of the stem containing leaves numbers 5 to 8 before ashing. The portion of the shoot above leaf number 5 was designated the tip, while that portion of the shoot below leaf number 8 was designated the base.

Temperature at the time of treatment was 70°F. and a maximum temperature of 75°F. was reached during the day.

Results: The autoradiograms showed a progressive accumulation of phosphorus during the period from 2 to 12 hours after treatment, as indicated by increasingly darker images of the untreated areas of the shoots on the X-ray film negatives. However, the negatives were not suitable for reproduction because surface contamination was not removed from the treated leaves and considerable blurring resulted.

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TABLE XX

ACCUMULATION OF RADIOPHOSPHORUS IN McINTOSH APPLE SHOOTS
2, 4, 6, 8, 10, and 12 HOURS AFTER THE APPLICATION OF
PHOSPHORIC ACID TO TWO MEDIAN LEAVES
(Average Values for Three Shoots)

Time of Harvest (Hours)	Radioactivity per 1 Gram Dry Wt. (Counts/min.)	
	Tip	Base
Two	325.9	68.2
Four	920.3	123.7
Six	792.1	188.0
Eight	1,181.9	225.5
Ten	2,862.8	371.4
Twelve	2,625.2	1,517.0

Radioactive counts in one milliliter of treating solution were 64,978.1 counts per minute.

Data obtained from the shoots that were ashed and then counted are shown in Table XX. Considerable variability is shown between the replications, but a trend of increasing accumulation at each successive 2-hour interval of sampling is evident. Also it appears in most cases that the tip area of the shoot contains more radioactive phosphorus than does the base area of the shoot.

Experiment XXIII

Object: To determine the effect of girdling on the distribution of phosphorus within a shoot following leaf application of phosphoric acid.

Materials and Methods: Five sets of three limbs each, on the south side of mature Delicious apple trees, were used in this experiment. Median leaves of the shoots were dipped on June 5, 1951, at 4 P.M. into a 0.3 percent solution of phosphoric acid containing approximately 0.11 microcurie per milligram of phosphorus.

Each set of limbs was divided into three sections: A, used for autoradiographs; B, ashed and counted; and G, girdled, then either ashed or use for an autoradiogram.

The limbs were located on the trees as follows: set 1, terminal shoots of primary limbs; sets 2, 3, and 4, terminal shoots of secondary limbs; and set 5, water sprouts from the interior of the tree.

Girdling was done by drawing a knife completely around the shoot at the base, so as to cut through to the secondary xylem, thus severing the phloem tissue.

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Eighteen hours after the leaves of the shoots were dipped, all shoots were harvested. The shoots upon which the radioactive count was to be determined were ashed immediately after the fresh weight of the untreated portions of the shoots had been determined. Autoradiograms were made from the shoots to be used for that purpose after the shoots were dried.

Results: The stem as well as the terminal leaves were visible in the autoradiogram made from the water sprout. Only a faint outline of the stem was seen in the autoradiograms made from the four non-girdled shoots. The autoradiograms made from the girdled shoots, one of which was the terminal shoot of a primary limb and the other, the terminal shoot of a secondary limb, showed leaf petioles as well as the stem but the terminal leaves were not visible.

The amount of radiophosphorus in the untreated portion of the shoots as indicated by counts per minute is shown in Table XXI. Two non-girdled shoots had a level of radioactivity considerably above the rest of the limbs. One was the terminal shoot of a primary limb while the other was the terminal shoot of a secondary limb.

In the autoradiograms, it appeared that girdling might have induced accumulation of phosphorus in the shoots but this was not shown in the count data. Because the effect of girdling on accumulation was questionable it was not used in further experiments.

No distinction between terminal shoots of primary limbs and of secondary limbs was made in further experiments because there is a comparatively small number of primary limbs on the tree and because

TABLE XXI

THE INFLUENCE OF GIRDLING AND LOCATION OF SHOOTS ON THE
ACCUMULATION OF RADIOPHOSPHORUS FROM AN APPLICATION OF
PHOSPHORIC ACID TO TWO MEDIAN LEAVES

Limb	Fresh Weight (Gms.)	Radioactivity 5 grams Fresh Wt. (Counts/min.)
1 B Primary terminal shoot	4.341	471.2
2 B Secondary terminal Shoot	4.041	56.2
2 G Secondary terminal shoot, girdled	3.475	80.3
3 B Secondary terminal shoot	5.880	228.3
4 B Secondary terminal shoot	6.036	49.4
4 G Secondary terminal shoot, girdled	5.715	40.2
5 B Water sprout	2.786	121.1

Radioactive counts per minute in one milliliter of treating
solution were 64,978.1 counts per minute.

no conclusive evidence was found to show that there was a difference between the two types.

No consistent difference was noted in the amount of absorption of radiophosphorus or in the subsequent distribution between the terminal shoots of primary limbs and of secondary limbs. Therefore no distinction was made between the two types in further experiments.

Experiment XXIV

Object: To determine the distribution of phosphorus in the apple fruit following leaf application of P^{32} phosphoric acid.

Materials and Methods: Two median leaves of two secondary shoots on a spur containing two apples were dipped June 27, 1951, into a beaker containing a solution of phosphoric acid whose radioactive strength was 0.08 microcurie per milligram of phosphorus. These fruits and shoots were harvested July 1, 1951. One fruit was cut into longitudinal sections and the other into transverse sections. Autoradiograms of the fruit were made. See figure 1.

Results: Phosphorus was found to move into adjacent fruit from spur leaves to which it was applied. The greatest concentrations occurred in the seed and vascular system with a second high concentration at the periphery of the fruit. Fruit on spurs both above and below the treated spur showed no radioactivity. These facts agree with other findings that phosphorus moves to the nearest point of mobilization.

Experiment XXV

Object: To determine whether different varieties of apples absorbed P^{32} from P^{32} phosphoric acid at different rates following foliar application.

Figure 1. Autoradiogram of an apple fruit showing the distribution of radiophosphorus in the fruit subsequent to foliage application.



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Materials and Methods: The sixth and seventh expanded leaves below the growing point of five shoots each of Delicious, Jonathan, McIntosh, and Northern Spy apple trees were dipped June 27, 1951, at 12:00 P.M. into a 0.3 percent solution of phosphoric acid which contained 0.08 microcurie per milligram of phosphorus. Terminal buds of the Delicious and Jonathan shoots were still growing at the time of treatment while the terminal buds of McIntosh and Northern Spy shoots had ceased growth.

The temperature was about 80°F. when the median leaves were dipped. A maximum of 85°F. was reached at 4:00 P.M. before the onset of a rain storm which lasted 3 hours.

Because the rain occurred soon after treatment, only two of five shoots of each variety were harvested and were used to make autoradiograms to see whether the rain had caused contamination of the untreated leaves with radioactive material.

Results: The autoradiograms made from the shoots gave no evidence that radioactive phosphorus had been washed from treated to untreated leaves.

Differences between the amount of absorption of phosphorus between the different varieties were noted but they appeared to be related to maturity of the shoots rather than variety. Both of the Delicious shoots and one of the Jonathan shoots showed a concentration of phosphorus in the expanding terminal leaves. Neither of the matured Northern Spy shoots showed evidence of phosphorus accumulation in the autoradiograms. One of the autoradiograms of the matured McIntosh shoots showed phosphorus concentrations in the stem and leaf petioles.

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These results indicated the need for using comparable plant material for an experiment because the age of the plant material apparently affects the amount and manner of accumulation.

Experiment XXVI

Object: To determine whether leaves of different varieties of apples and peaches absorbed radiophosphorus at different rates.

Materials and Methods: The seventh and eighth expanded leaves of five shoots each of Delicious, Jonathan, and McIntosh apple trees and Elberta, Halehaven, and South Haven peach trees were dipped July 7, 1951, at 10:00 A.M. in a 0.3 percent solution of phosphoric acid containing 0.21 microcurie per milligram of phosphorus and no wetting agent. The fourth and fifth leaves of five shoots of Northern Spy apple trees were dipped in the same solution. The shoots were cut after 26 hours.

The temperature was 80°F. at the time the leaves were dipped. A maximum temperature of 85°F. was reached before rain started falling at 3:30 P.M. The rain continued till 5:30 P.M. and intermittent showers fell during the night.

Two shoots of each variety were used to make autoradiograms and three shoots were ashed then counted to obtain the true amount of radioactive phosphorus which had been absorbed. Before the shoots were ashed, leaves six through nine and the corresponding section of the stem were removed. The section of the shoot above the sixth leaf was designated the tip and the section below the ninth leaf was

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designated the base. After the shoots were dried, they were ground in a Wiley mill. A 0.85 gram sample was ashed according to the procedure given for phosphorus analysis in the A.O.A.C. (1).

Results: Results shown by the autoradiograms made from the shoots are described for each variety of fruit.

Maturation of the terminal buds of the Delicious shoots had occurred but the terminal leaves were not expanded. The stem, leaves, and aphids on the young leaves were visible in the autoradiograms made from both shoots. Phosphorus was more concentrated in the bodies of the aphids than in the shoots.

The terminal buds of the Jonathan shoots had matured and the terminal leaves were fully expanded. Autoradiograms of both shoots showed the stem, and the leaf petioles. A faint outline of all the leaves above the treated leaves was visible in one of the autoradiograms but only a faint outline of the youngest leaf was visible in the other autoradiogram.

The terminal buds of the McIntosh shoots had matured and the terminal leaves were expanded. Only a portion of the stem near the treated leaves appeared in the autoradiogram of one shoot while the outline of the stem and some of the leaves near the treated ones appeared to have been contaminated.

Northern Spy shoots were completely mature at the time of treatment. The stem and the leaf petioles were visible in autoradiograms made from both shoots but leaves above the treated leaves were visible in only one autoradiogram.

Figure 2. Autoradiogram of a Halehaven peach shoot showing distribution of radiophosphorus in the shoot subsequent to application of phosphoric acid solution to the seventh and eighth leaves of the shoot.



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A large accumulation of radioactive phosphorus occurred in the young expanding leaves of the growing Elberta shoots and a lesser concentration occurred in the older leaves and stems according to the autoradiograms.

More phosphorus had accumulated in the expanding shoot tips of Halehaven and in the stem than had accumulated in the same areas of the Elberta stems. An outline of the older leaves was also visible in the autoradiogram. See figure 2.

South Haven shoots were still growing at the time of treatment but the rate of growth was not as vigorous as the preceding two varieties of peaches. The autoradiograms showed the terminal leaves and stem but not as clearly as did the autoradiograms of Elberta and Halehaven.

From the autoradiograms it can be concluded that a growing peach shoot absorbs more phosphorus than does a mature apple shoot. However, it is possible that the direction of translocation after absorption may be different in a mature shoot than in a growing shoot.

Count data from the shoots that were ashed are presented in Table XXII. These results also showed that the growing peach shoots in general absorbed more phosphorus than did the mature apple shoots. The phosphorus content from the foliage application was found to be higher in the younger tip area than in the older base area.

Experiment XXVII

Object: To determine whether the accumulation of P^{32} from P^{32} phosphoric acid by different fruit varieties varied with the stage of maturity of the shoots.

TABLE XXII

COMPARISON OF ABSORPTION OF RADIOPHOSPHORUS APPLIED TO THE SEVENTH AND EIGHTH LEAVES OF APPLE AND PEACH SHOOTS DURING

JULY

(Average Value of Three Shoots)

Variety	Radioactivity in Counts per Minute per 1 gram Dry Wt. Tip	Base
Delicious	450.9	107.7
Jonathan	123.9	64.4
McIntosh	332.4	324.7
Northern Spy	516.7	217.3
Elberta	840.5	280.5
Halehaven	549.9	465.2
South Haven	854.2	240.3

Radioactive counts per minute in one milliliter of treating solution were 15,484.6 counts per minute.

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Materials and Methods: The seventh and eighth expanded leaves of five shoots each of delicious, Jonathan, and McIntosh apple trees and Elberta, Halehaven, and South Haven peach trees were dipped August 3, 1951, at 12:00 P.M. in a 0.3 percent solution of phosphoric acid containing 0.10 microcuries per milligram of phosphorus and no wetting agent. The fifth and sixth leaves of Northern Spy were dipped in the same solution. A high temperature of 80°F. was reached during the day. A strong wind was blowing while the limbs were being dipped. The shoots were cut after 12 hours.

Two shoots of each variety were dried under heat lamps, and then used to make autoradiograms. Three shoots of each variety were ashed and then counted to obtain the true amount of radioactive phosphorus which had been absorbed. Before the shoots were ashed, they were cut above the sixth leaf and below the ninth leaf to remove the treated leaves and other possible contamination. The portion of the shoot above the sixth leaf was designated the tip while the portion of the shoot below the ninth leaf was considered the base. The tip and base pieces were dried in a drying oven, and then ground in a Wiley mill. A 0.50-gram sample was ashed according to the procedure given for phosphorus analysis in the A.O.A.C. (1).

Results: Maturation of the terminal buds of the apple shoots of all varieties had occurred when this experiment was started. All leaves of the shoots were fully expanded at this time.

Similar results were visible in all the autoradiograms produced from the apple shoots. A faint outline of the stems was visible in

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all the autoradiograms while an outline of the leaves was visible in some autoradiograms. Black spots on the leaves, indicating contamination may have been caused by the wind whipping the shoots after they were dipped.

Maturation of the terminal buds of the peach shoots varied with variety at this time. No maturation had occurred in the Elberta peach and the shoots were still growing. The young leaves and stems were clearly outlined in the autoradiograms.

Terminal buds of the Halehaven peach trees had matured but elongation of the stem was occurring in most shoots. All of the younger leaves, midribs of older leaves, and all of the stem were visible in the autoradiogram made from one shoot. The other shoot had matured and the younger leaves were only faintly visible in the autoradiogram. Neither the stem nor the older leaves were visible except near spots indicating contamination.

Shoots of South Haven peach were completely matured. One shoot was visible in the autoradiogram near a dark spot indicating contamination. The youngest leaves of the other shoot were visible in the autoradiogram.

These results suggest that the entry of radiophosphorus into peach shoots was influenced by the maturity of the shoot, as was found with shoots of different apple varieties in previous experiments. Thus the greatest accumulation of radiophosphorus occurred in the Elberta shoots which were still in active growth at the time of treatment.

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Radioactive count data for the shoots that were ashed are presented in Table XXIII. Considerable variability existed among the replicates. Translocated phosphorus from a foliar application in August into apple and peach shoots appeared to be similar in amount in the terminal region. The amount of phosphorus, which had been translocated into the base of the apple shoots, appeared to be greater.

TABLE XXIII

COMPARISON OF ABSORPTION OF RADIOPHOSPHORUS APPLIED TO THE SEVENTH AND EIGHTH LEAVES OF APPLE AND PEACH SHOOTS DURING AUGUST
(Average Value of Three Shoots)

Variety	Radioactivity in Counts per Minute per 1 gram Dry Wt. Tip	Base
Delicious	946.6	1,346.4
Jonathan	1,275.0	1,409.8
McIntosh	1,213.8	771.6
Northern Spy	699.0	*
Elberta	1,263.4	419.2
Halehaven	610.1	350.1
South Haven	920.6	430.0

*Not enough plant material of base to weigh and count

Radioactive counts per minute in one milliliter of treating solution were 231,180. counts per minute.

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V. DISCUSSION

During the course of this research, it was found that calcium, phosphorus and potassium would enter the above-ground portions of fruit trees. Some of the factors which may influence the entry of mineral nutrients into the leaves, twigs, and branches of fruit trees were studied. These factors were: season of the year, length of absorption period, concentration of nutrients, differences between varieties, and method of application.

Some entry of mineral nutrients occurred following an application of P^{32} phosphoric acid or K^{42} potassium carbonate to bark of twigs or branches of fruit trees during the dormant period. The amount of entry, during this dormant period as indicated by radioactive counts in the untreated portions of the plants, was small as these counts were very seldom twice background. Entry of radiophosphorus during May after new growth occurred, was very large ranging up to several thousand times background count in the untreated portions of plants when the solution was applied to injured bark, when the solution was applied to cotton gauze wrapped around the limb or when the solution was applied to cotton gauze over injured bark. Negligible entry occurred at this time following application of P^{32} phosphoric acid solution to uninjured 10-year-old bark but sufficient entry to produce counts 10 to 15 times background occurred when the solution was painted on uninjured 1-year-old bark.

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Others who have worked with entry of mineral nutrients into dormant and growing trees have obtained similar results. Harley and Jefferson (30) found that almost no entry occurred during the dormant period but entry did occur after active growth resumed in the spring. Eggert (16) reported that entry of a solution containing radioactive phosphorus into the excised stem of a McIntosh apple tree occurred only after the buds of the stem swelled. Movement of rubidium Rb^{86} injected into the trunk of a yellow birch tree was toward the leaves during the growing season and toward the roots during the dormant season according to Fraser and Mawson (23). This directional movement with the seasons may be the reason activity was not found in some dormant season experiments as in most instances sampling was done above the treated area rather than toward the roots.

The distribution of phosphorus following foliar application and possibly the amount of entry were influenced by the season of the year. Differential mobilization of phosphorus during active growth occurred; thus a concentration of phosphorus was found in the shoot tips. Mobilization of phosphorus after maturation of the shoots was apparently not selective within the shoots; thus concentrations of phosphorus in a particular region of the shoot did not occur. However, at the time of shoot maturation the developing fruit is rapidly metabolizing phosphorus and movement of phosphorus which enters the plant is towards the fruit. But it is possible that less entry of phosphorus rather than direction of movement after entry explains why there was less phosphorus in a mature shoot tip than in young shoot

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tip. This idea is supported by the fact that several workers (13, 51) have found that young leaves absorb urea more readily than do mature leaves.

A difference in distribution of phosphorus occurred when applied to different tissues of plants. Phosphorus applied to expanding peach leaves did not move out of the leaves while phosphorus applied to the bark at this time did move to the leaves and to the roots.

Continued absorption of foliar applied phosphorus was found up to a period of 24 hours, the longest interval used in the experiments in this project. However phosphorus absorption continues over a longer period. Mayberry (41) found that absorption was still occurring after 160 hours following a rapid initial intake. Absorption continuing over a 30-day period following a foliage application of radioactive phosphorus was reported by Eggert (16).

Results obtained concerning entry following bark application of P^{32} phosphoric acid were of a conflicting nature. Decreased entry, no effect, and increased entry with increasing time intervals occurred in different experiments. Phosphorus applied to 10-year-old limbs of South Haven peach trees showed in autoradiograms the highest radioactivity in the phloem tissue after 6 and 16 hours following treatment. The amount of radioactivity decreased as indicated by the amount of exposure of the film between each successive sampling at 24, 44, and 72 hours. The level of radioactivity found in 1-year-old portions of Halehaven peach limbs was nearly the same when samples were taken at 6, 24, and 53 hours following an application of solution to the 2-year-

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old portions of the limbs. Increased entry from 12 to 192 hours after treatment occurred in apple limbs.

Rapid initial intake followed by movement of the phosphorus to areas of mobilization may be the explanation of these results. Rapid intake was shown by the high levels of radiophosphorus which occurred 6 hours after treatment of 2-year-old Halehaven limbs and of 10-year-old South Haven limbs. Translocation following this entry would explain why the radioactivity level in samples which were adjoining the treated area dropped in the 10-year-old peach limbs. However, the radioactivity in the 1-year-old limbs of Halehaven peach came from translocation of P^{32} phosphorus following entry and not from entry of P^{32} phosphorus alone thus this radioactivity might be less subject to further translocation than would be radioactivity from radiophosphorus which had entered the plant but which had not been translocated from the site of entry. The radioactivity level in the parts of the apple limbs most distant from the point of application increased because of translocation to these regions.

In order to determine whether the concentration of the phosphoric acid would influence the rate of entry, 0.3, 2.0, and 8.0 percent solutions of phosphoric acid, which had the same relative specific activity of radioactive phosphorus, were applied to 1-year-old limbs of Halehaven peach trees. Very little entry occurred with any concentration. This response probably occurred because this experiment was done during early December. However, no increased entry occurred with the higher concentrations so they were not used in further experiments.

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Several experiments were conducted to determine whether there was a difference in response to foliar application of phosphoric acid by different fruit varieties. Varieties used were Delicious, Jonathan, McIntosh, and Northern Spy apples and Elberta, Halehave, and South Haven peaches. In experiment XVIII, it was found that the retention of phosphoric acid by the leaves of apple, peach, pear, sour cherry, and sweet cherry, differed. Differences observed in absorption of phosphorus by the different varieties appeared to be more closely correlated with maturity of the shoot than with variety. This result was more clearly illustrated in the autoradiograms than it was in count data. Autoradiograms made from shoots which were still growing produced a much darker image on the X-ray negative than did mature shoots.

Effective means for increasing the amount of nutrients which entered the plant were (a) injuring the plant, (b) using a cotton gauze to hold the solution, and (c) using hydrated lime to obtain an increased deposit. Harley and Jefferson (30) reported entry into stems and branches which were mechanically injured. Results obtained in some of the experiments of this project confirm those obtained by Harley and Jefferson but the influence of the season of the year and the activity of the plant were found to be greater. During the dormant season, increased entry was not noted following mechanical injury although more material adhered to the branches. Increased entry following mechanical injury was noted in the spring after active growth of the tree had occurred. Variations in results obtained between replications in some

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bark absorption studies may have occurred because of growth cracks or insect and disease damage.

A very effective method for increasing the amount of phosphorus that entered a plant was to saturate a strip of cotton gauze which had been wrapped around the stem. This procedure proved to be more effective than scraping or injuring the shoots of apple trees to stimulate entry. Increased entry of phosphoric acid to the extent that toxicity was produced, occurred when a gauze was wrapped around an area of a shoot which had been previously scraped. A pink discoloration of the phloem tissues of the shoots occurred, which was a symptom of toxic concentrations of phosphoric acid.

While the procedure of scraping and providing a reservoir for continued absorption was only of an experimental nature, it may explain why Forsyth's compound was effective. One of the first points stressed by Forsyth for the use of his compound was the need to scrape down to living tissue before making an application. Another reason why the compound may have been successful was that in the moist climate of England the manure and wood ash mixture might stay almost continuously wet. Thus the two conditions which promoted the greatest entry in Experiment XVI were fulfilled by Forsyth's method.

Entry of radiopotassium into leaves appeared to be controlled by the amount of contact between the applied K^{42} potassium carbonate and the leaf epidermis. Thus increased absorption of potassium by peach and sour cherry leaves occurred when hydrated lime was added to a solution alone. Greater absorption of potassium by apple leaves occurred

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when the solution rather than the lime slurry was used. The pubescence of the apple leaves prevented contact of the lime slurry with the epidermis. Conversely, the pubescence of apple leaf was easily wetted by the solution which contained Dreft as a wetting agent while the peach and sour cherry leaves were not easily wetted.

Concentrations of chemicals higher than the 0.3 percent solutions used for foliar application would be necessary if the mineral nutrient requirements of a fruit tree were to be supplied by a dormant spray. Therefore a series of experiments were conducted to determine the tolerance of McIntosh apple and Montmorency sour cherry to different levels of nutrient sprays while the trees were dormant and while the buds were in the green tip stage.

The chemicals used ranged widely in their phytotoxicity with concentration, plant material, and growth stage. No injury was found with the 2 or 4 percent concentrations and only rarely with the 8 percent concentration of any of the materials. However, the 16 and 32 percent concentrations of the chemicals other than potassium nitrate caused varying degrees of injury.

It was determined that the injury produced by 32 percent NuGreen was related to the time of application to the apple. Buds and some small limbs of 2-year-old McIntosh trees were killed when the trees were sprayed while dormant. This same solution when applied to the trees when the buds were in the green tip stage was not injurious. The same effect was produced by a spray of a 20-20-20 fertilizer at the 32 percent concentration but to a lesser degree. This injury was probably

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caused by urea toxicity since NuGreen is a commercial preparation of urea. Hinsvark, Wittwer, and Tukey (31) have determined that apple twigs are capable of rapid hydrolysis of urea. Rapid hydrolysis of urea during the dormant period when utilization of nutrients was low may have resulted in toxic levels of nitrogen compounds accumulating within the plant. During the growing season, these materials would be utilized and so not accumulate to toxic levels.

Urea injury of the sour cherry showed as a form of chlorosis of the leaves in which the margins turned yellow. This injury occurred where 16 and 32 percent solutions of NuGreen or 20-20-20 fertilizer were used.

The 16 and 32 percent solutions of calcium chloride and phosphoric acid were the most phytotoxic. The injury produced by the two higher concentrations of calcium chloride appeared to be a form of desiccation. When the buds of the sour cherry were examined, multiple layers of what appeared to be bud scales were found to be dehydrated immature leaves. Terminal buds were destroyed or inhibited by the high concentrations of calcium chloride. Phosphoric acid destroyed the buds and the tissues near the buds when applied as a 16 or 32 percent solution. Shrunken areas around the buds and leaf scars were the typical injury produced in the McIntosh trees. Buds on the sour cherry trees were also destroyed. In both apple and sour cherry, lateral buds were destroyed, while buds in the terminal position were not as readily destroyed.

After entry, distribution of phosphorus and potassium was found to differ. Phosphorus tended to accumulate in the rapidly growing

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regions while potassium tended to have even distribution throughout the shoots. Similar results were found by Mayberry (41) working with beans and squash.

From the results obtained in this project, it would seem that sprays of urea or other mineral nutrients would be most successful if they were applied as a delayed dormant spray. At this time, the danger from urea hydrolysis without utilization, has passed. Also, before this time it was found that very little absorption of mineral nutrients occurred.

If the nutrient spray is combined with the regular pest control sprays, the cost of application is negligible. It would save time and money by eliminating a special trip through the orchard to distribute fertilizer. Even though the percent of nutrients absorbed directly by the top of the tree may be small, the material not absorbed would be washed to the ground by rain and thus become available to the plants through the roots.

One of the major reasons for dormant sprays might be to promote recovery of winter injured trees. Partridge (46) observed that peach trees which had been damaged by cold often exhibited symptoms of potassium deficiency. This injury results from an impaired conductive system rather than lack of potassium in the soil. Here an application of potassium to the above-ground parts of the tree, by relieving the potassium deficiency, may promote faster recovery of the tree. The greatest effect on the recovery probably would occur following a delayed dormant spray.

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VI. SUMMARY

1. In a series of experiments, the leaves and the bark of shoots and branches of several types of fruit trees were treated with solutions containing radioactive calcium (Ca^{45}), radioactive phosphorus (P^{32}), and radioactive potassium (K^{42}) and with solutions of other materials. Most of the experiments were undertaken to study the rate and extent of absorption and subsequent translocation.
2. Methods of applying the solutions varied with the material under study. Foliar treatments were made by dipping the leaves into a beaker containing the solution. Bark treatments were made by (a) painting the solution on the limb with a brush, (b) soaking a piece of cotton gauze wrapped around the limb with the solution, or (c) spraying the solution onto the plant.
3. Leaf and growing shoot samples were dried, ground, and prepared for analysis by the methods outlined in the A.O.A.C. (1). Woody samples were cut into small pieces and then dried. To insure the penetration of the magnesium nitrate used in phosphorus analysis, extra hydrochloric acid was added to the woody sample.
4. Radioactivity in the samples was measured directly by the use of a Tracerlab Autoscaler, or it was measured indirectly by the use of Autoradiograms.
5. The bark surface area of a 25-year-old McIntosh apple tree was found to be 86 square meters. Of this total area, 35.7 percent

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occurred on limbs of 6 millimeters or less in diameter and 53.7 percent occurred on limbs of 10 millimeters or less in diameter.

6. Methocel 4000 c.p.s. was the most effective of ten sticking or wetting agents which were tested by the amount of a 5 percent solution of P^{32} phosphoric acid which adhered to sections of McIntosh water sprouts.

7. Calcium chloride, phosphoric acid, potassium nitrate, NuGreen, and a 20-20-20 fertilizer as 2, 4, 8, 16, and 32 percent solutions were sprayed on 1-year-old Montmorency cherry trees and 2-year-old McIntosh apple trees. Spraying was done either when the trees were dormant or when the buds were in the green tip stage. Injury to the trees was found to depend upon concentration of solution, stage of growth, and type of tree but injury rarely resulted from any material used at a concentration of 8 percent or less.

8. Some evidence of entry of radiopotassium through 8- to 10-year-old bark of apple and peach limbs during the dormant period. On the other hand, much greater entry of radiopotassium occurred through bark of actively growing 2-year-old apple trees in the greenhouse.

9. Radiophosphorus entered 2-year-old peach limbs following a dormant application of phosphoric acid. Approximately the same amount of radiophosphorus was found in untreated portions of the limbs whether cut 6, 24, or 48 hours after treatment. No appreciable difference was found in the amount of entry from 0.3, 2.0, or 8.0 percent phosphoric acid applied to the bark.

10. Radiophosphorus entered 8- to 10-year-old South Haven peach limbs when a solution of phosphoric acid was applied in cotton gauze to the

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bark during the dormant season. Autoradiograms showed that the greatest amount of phosphorus was in the phloem tissues near the point of application 6 and 16 hours after the solution was applied.

11. Radiophosphorus was found to have entered 3-year-old apple limbs following application of phosphoric acid to gauze wrapped around the limbs during the dormant season. Injury in the treated area did not seem to stimulate entry during the dormant period.

12. Radiophosphorus entered through the sides of a tomato stem in a larger amount when the solution was applied to gauze wrapped around the stem rather than when the solution was brushed on the stem. At the three temperatures used in this experiment, 50°, 65°, and 85°F., greater entry occurred with each increase in temperature.

13. Radiocalcium entered 2-year-old McIntosh apple trees when calcium chloride was sprayed onto the bark while the trees were dormant. Greater entry occurred into trees grown on minus calcium nutrient solution than into trees grown on complete nutrient solution.

14. NuGreen and a 15-30-15 fertilizer were sprayed onto Montmorency trees which were in the green tip stage. A greater increase in circumference was made by the trees receiving fertilizer than was made by the non-fertilized check trees.

15. Radiophosphorus entered 1-year-old water sprouts and 10-year-old limbs of Jefferis apple trees when a solution of phosphoric acid was applied to the bark during the growing season, in May 1953. The amount of entry as indicated by radioactive counts per minute in untreated new growth was 195-339 when the solution was applied to undamaged shoots;

9,199-11,085 when the solution was applied to cotton gauze over intact bark; 3,090-7,541 when the solution was applied to scraped bark; and 368,177-423,815 when the solution was applied to cotton gauze over scraped bark.

16. Radiophosphorus did not move from the expanding Elberta peach leaves that had been treated with phosphoric acid. Movement was found when the solution was applied to both the expanding leaves and the bark or to the bark alone.

17. The amount of radiophosphoric acid, which was retained by the leaves of apple, peach, pear, sour cherry, and sweet cherries, was found to differ with the greatest amount being retained by apple leaves and the smallest amount by peach leaves. Efficiency of translocation also varied with the greatest percentage of translocation occurring in the sour cherry and the smallest percentage in the sweet cherry.

18. Radiopotassium entered peach and sour cherry shoots from a foliage application in a greater amount when the potassium carbonate was applied to the leaves in a lime slurry rather than as a 0.3 percent solution. The reverse was true of apple shoots as the greater entry occurred when the leaves were dipped in solution rather than in the slurry.

19. Distribution following entry of foliar applied radiophosphorus and radiopotassium was found to differ. Phosphorus accumulated in the rapidly growing areas of the shoot while the distribution of potassium was characteristically more uniform in the shoots.

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20. Radiophosphorus entered McIntosh apple shoots for at least 24 hours after a solution of phosphoric acid was applied to the leaves. This was the longest period of time for foliar entry in these experiments.

21. Radiophosphorus entry and subsequent distribution was found to be influenced very little by whether the shoot was girdled or not girdled, whether the shoot was terminal on a primary or a secondary branch, or whether the shoot was in a horizontal or vertical position.

22. Radiophosphorus entered McIntosh apple fruit after the application of phosphoric acid to the leaves of the spur on which the fruit was growing. Concentrations of radiophosphorus were visible in the autoradiogram in the seeds, in the vascular system, and at the periphery of the fruit.

23. To find whether different plants absorbed radiophosphorus at different rates, solutions of phosphoric acid were applied to the leaves of shoots of Delicious, Jonathan, McIntosh, and Northern Spy apples and Elberta, Halehaven, and South Haven peaches. Maturity of the shoots rather than variety of the shoots appeared to be the principle cause of variation.

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