LOSS OF NUTRIENTS FROM ABOVE - GROUND PLANT PARTS BY LEACHING AS DETERMINED BY RADIOISOTOPES

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LOSS OF NUTRIENTS FROM ABOVE-GROUND PLANT PARTS BY LEACHING AS DETERMINED BY RADIOISOTOPES

By

HAROLD BRADFORD TUKEY JR.

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
State University of Agriculture and Applied Science
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HAROLD B. TUKEY JR. ABSTRACT

Losses of nutrients from above-ground parts of plants by the action of aqueous solutions were determined, utilizing radioisotope techniques. Several factors affecting these losses were investigated.

Seedlings were grown in aerated nutrient solution cultures containing half-Hoagland solution. Radioisotopes (Ca^{45}, Cl^{36}, Fe^{55-59}, K^{42}, Mg^{28}, Mn^{54}, Na^{22}, P^{32}, S^{35}, Sr^{90}-Y^{90}, and Zn^{65}) were added to the root medium and root-absorbed by the plants. Leaves were leached either by immersing in aqueous solutions, or by applying a water mist spray. The leachates were concentrated and analyzed for radioactivity, as were the leached leaves.

Losses of nutrients from very young leaves were quantitatively small, but increased directly with the maturity of the leaf. Losses were largest as leaves approached senescence.

Susceptibility to leaching varied among crops. Bean, corn, and squash lost greater quantities of nutrients than did tomato and sugar beet.

Losses increased directly with the duration of the leaching period and were measurable after only 30 minutes of leaching.

There is apparently a replenishment mechanism within plants which replaces leached nutrients in the leaf from other plant parts. For example, attached leaves lost larger amounts of nutrients than did detached leaves. Losses were greater from leaves of plants growing in a culture solution in which the Ca^{45}-level was maintained than were losses from leaves of plants growing in a Ca^{45}-level which was allowed to deplete naturally by root absorption. Further, when leaves were being leached, the roots were simultaneously absorbing nutrients from the root medium.

Plant constituents leached from one plant species were reabsorbed by the roots of the same and of different species of plants.

Losses (percentage basis) of a given nutrient were greater from plants deficient in that nutrient than from healthy plants. Leaves which were cut, bruised, or diseased lost greater amounts of nutrients than did healthy leaves.

Losses of most of the radioisotopes studied were apparently not affected by light intensity. However, losses of P^{32} and S^{35} were greater at night than in the day.

The relative leachability of 11 radioisotopes from young leaves was determined. Isotopes which were readily leached were Na^{22} and Mn^{54}. Those moderately leached included Ca^{45}, Mg^{28}, S^{35}, K^{42}, and Sr^{90}-Y^{90}. Those leached with difficulty were Fe^{55-59}, Zn^{65}, P^{32}, and Cl^{36}.

Losses of P^{32} were greatly increased by oil in the leaching spray. However, losses of Ca^{45} were not increased by exposure of the plant to ether or a chelating agent.

Potassium in the leaching solution increased the loss of Ca^{45} and K^{42}. Phosphate increased the loss of P^{32}. However, calcium and sodium depressed the loss of both Ca^{45} and K^{42}.

In addition to mineral nutrients, large amounts of carbohydrates were leached from leaves. These losses were apparently directly related to light intensity, being largest when the light intensity was high.

Losses of materials from fleshy fruits were also determined. Carbohydrate loss varied directly with the maturity of the fruits, being highest from fully ripe fruit. Losses of Sr^{90}-Y^{90} were approximately 75 percent of the isotope in the fruit regardless of the maturity of the fruits.

Leaf analyses showed a lower concentration of leachable nutrients in plants grown out-of-doors as compared to plants either protected from rain by a plastic roof or grown in the greenhouse.

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DEDICATION

To H. B. Tukey Sr., whose enthusiasm and thoughtful criticism has been a constant source of encouragement, and whose abilities and achievements will continue to serve as a reminder for superior accomplishment - to you this thesis is dedicated in grateful appreciation.

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INTRODUCTION

The root has been commonly accepted as the sole organ concerned with the uptake of water and nutrients by plants. From time to time this classical concept has been challenged with the indication that leaves may also participate in absorption of water and nutrients.

It naturally follows that if plants can take up nutrients through the above-ground parts, they may also be able to lose nutrients through these same parts. It is the purpose of this thesis to present additional facts pertaining to leaching of nutrients from above-ground parts of plants by rain and dew, with special reference to the use of radioactive isotopes.

LITERATURE REVIEW

Absorption by above-ground plant parts

Mariotte in 1717 (59) and Hales in 1727 (33) suggested that plants could absorb water through the leaves. Further, Böhm in 1877 (14) showed that bean leaves could take up calcium and water, and L. Hiltner (37, 38), E. Hiltner and Kronberger (36), and Wille (102) demonstrated that plants could be "nourished" by salt solutions applied to the foliage. Not only ions, but also large-molecule-sugars could be leaf absorbed (63, 78). Wetzel (97) suggested that even the uptake of nutrients by leaves might be sufficiently great to be of value in the growth of plants. He also found that absorption was not hindered by a thick cuticle.

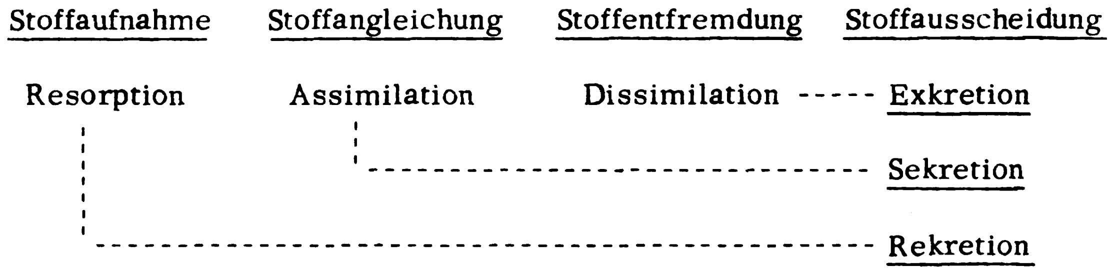
The modern use of radioactive isotopes has clearly and conclusively demonstrated that a number of nutrient elements may be absorbed by the plant through the leaves, the bark, and even the fruit (86, 103). So-called "foliar", "non-root", or "above-ground" feeding has assumed considerable economic importance. It has proved of value in correcting deficiencies of such minor elements as zinc, magnesium, manganese, iron, and boron in horticultural crops. Sprays of such macro-nutrients as nitrogen, phosphorus, and calcium are accepted as a method of applying fertilizers, especially at critical times in plant development.

Losses from above-ground plant parts

Due to confusion in the literature and the difficulty of accurate translation, it is advisable to define the terms used in this thesis.

Prior to 1900, many authors seem to have used the term "washing out", or "elimination", or "Stoffausscheidung" to describe the removal of nutrients from leaves by rain. Haberlandt (32) proposed that the term "Exkretion" be used for the localization and elimination of a substance "within the cell", and that the term "Sekretion" be confined to the elimination of materials from the cell entirely. Arens (7, 8) used the term "kuticuläre exkretion" for the disposal of materials through the cuticle from within the plant, subsequently to be washed away by rain and dew.

Frey-Wyssling (28), proposed the following diagrammatic definition:



Rekretion was the elimination of absorbed but unmetabolized products such as potassium ions; sekretion was the elimination of assimilated or metabolized products, such as sugars; and exkretion was the elimination of products of dissimilation, such as calcium oxalate.

Following the lead of Frey-Wyssling, Mes (61) used the terms "Excretion (recretion)" or the removal of waste products and unmetabolized products, to describe the process by which materials are removed by rain or dew.

For the purpose of this thesis, the term "leaching" will be defined as the removal of constituents from plant parts by the action of aqueous solutions. This definition makes no differentiation between the various sources of the leached plant constituents, only that the materials were derived from the plant in some manner and form. Although various authors have used different terms to describe this phenomenon, it is apparent from their writings that they all referred to the same process which we choose to call "leaching".

As early as 1804, de Saussure (72) in his classic volume Recherches chimiques sur la végétation stated that water could wash materials from plant leaves. He soaked hazelnut leaves (Corylus avellana) in water for eight periods of one-half hour each and then analyzed the tissue. Leaves which were not washed contained 26 percent soluble salts and 23.3 percent phosphates in the ash, while those which were washed contained only 8 percent soluble salts and 19.5 percent phosphates. These findings were substantiated by Gaudichaud in 1841 (31) and Sachs (70) who found that water which had been placed on leaves became alkaline.

In 1883, Buchenau (15) observed that grass grew more vigorously in the early spring under the spread of shade trees, and attributed this growth to the effect of nutrients washed from the foliage of the trees during the preceding season. He quoted von Hartig as having observed water dripping from buds of trees, which apparently carried nutrients with it. He further noted that this was not a revolutionary or unexpected phenomenon inasmuch as the leaching of nutrients from leaves by rainfall was already well recognized in vegetable crops.

Von Homeyer (40) supplemented the observations of Buchenau by writing that the growth of grass under shade trees varied with species of tree involved. He associated this with the varying nature of the leachate from the different tree species. For example, grass under trees of linden (Tilia spp.), beech (Fagus spp.), and maple (Acer spp.) grew vigorously, whereas grass under poplar and willow (Populus spp.) was only slightly stimulated. Further, grass under birch (Betula spp.) showed no vigorous early spring growth.

During the latter half of the 19th and the early part of the 20th centuries, analytical chemists, principally Europeans, examined the mineral composition of many plant species growing under a variety of environmental conditions. One such study by Councler (21) reported that the mineral composition of leaves from plants growing in the greenhouse was considerably higher than from leaves of field-grown plants. For example, ash from leaves of Acer negundo growing under glass contained 12.2 percent phosphate, 27.2 percent calcium, and 45.5 percent potash, as compared with 3.4 percent phosphate, 14.9 percent calcium, and 33.9 percent potash in field-grown plants.

Dulk (24) noted a reduction in potash and phosphorus in 4-year-old pine needles (Pinus spp.) as compared with 3-, 2-, and 1-year-old needles. Contrastingly, calcium, manganese, and silica increased in the older needles.

Von Leiningen (48) found a higher ash and salt content in shade leaves of trees as compared with sun leaves.

It was reported by many investigators that the composition of plants differed not only among species, but also with the season, and even fluctuated during the same growing season (3, 4, 5, 6, 26, 27, 29, 45, 64, 65, 67, 69, 70, 71, 73, 82, 84, 95, 96, 105). Representative of these studies were those of Wilfarth, Römer, and Wimmer (100). Working with wheat (Triticum spp.), barley (Hordeum vulgare), peas (Pisum sativum), and potatoes (Solanum tuberosum), they found that the amounts of nutrients increased steadily throughout the growing season, reaching a peak at the time of flowering and heading. As plants approached maturity, however, the nutrient composition declined steadily; at senescence and leaf fall there was a sharp reduction in dry matter, nitrogen, phosphorus, sodium, and potassium. Forty-seven percent of the sodium, 38 percent of the potassium, and 23percent of the nitrogen were depleted.

This decrease in the amounts of nutrients at leaf senescence had been observed many times previously (29, 67, 70, 84, 96) and was a popular topic of discussion for several years (45, 64, 65, 66, 69, 73, 82). It had been first explained by Sachs in 1863 (71) as due to the downward movement of ash-laden sap from the leaf to the stem, roots, and soil. This explanation was generally accepted. In fact, it was so well accepted that when Wehmer in 1892 (95) suggested that the nutrient depletion of leaves could also be explained by the leaching action of rain and dew, his statements were immediately subjected to severe attacks from all sides.

Wehmer's theory found little support in Europe. With the exception of the works of Swart (82) and André (3, 4, 5, 6), most writers continued to accept the "Rückwanderung". Ramann (64, 65) published several papers illustrating the movement of nutrients back into the stems at the time of leaf senescence. Working with oak (Quercus spp.), beech (Fagus spp.), and birch (Betula spp.), he found that materials did not migrate back from the leaves into the stem as long as the leaves were alive. With aging and death of the leaf, 25 to 71 percent of the protein moved back into the stem. Potash

(5 to 56 percent) and phosphorus (18 to 41 percent) also moved back into the stem, but were influenced in amount by the nutritional status of the stem. However, calcium and silicic acid accumulated in the leaf and did not move out at senescence. He also reported that "Rückwanderungen" took place in a relatively short period of time during yellowing and death of the leaf.

In another report, Ramann (66) killed beech leaves by freezing and noted the movement of nutrients out of the leaves. Proteins did not move out of the leaves, but potash and phosphorus moved back into the stem in the same manner as at natural leaf senescence.

From these experiments, Ramann agreed with Sachs that the reduction in the amounts of nutrients in foliage as plants matured and senesced was due to a movement of the nutrients out of the leaves into the stems. He again discredited the importance of leaching by rain or dew.

Despite the violent criticism of Wehmer's leaching theory, it began to find some favor in other parts of the world. An important contribution in support of leaching was presented by Le Clerc and Breazeale (45). Recognizing the controversy over the decrease in mineral content at senescence and perhaps understanding it better because of their distance from the field of combat, they proposed three hypotheses to account for this decrease:

1. "The backward flow of the salts of the plant juices through the stem and roots to the soil.
2. "The decay and drying and falling off of leaves.
3. "The action of rain, dew, and other climatic agencies, or a combination of all these causes to a limited degree."

They showed that as senescence approached, potash, nitrogen, and phosphorus did migrate out of the leaf into the stem. However, the subsequent movement of nitrogen and phosphorus was in an upward direction toward the seeds, not downward to the roots, as Sachs (71), Wilfarth *et al.* (100), and others had supposed. Also, potash did not move downward to the roots, but remained somewhat stationary in the stem. Thus, the "backward flow" hypothesis was discredited by them.

Further, the losses reported by Wilfarth *et al.* were too great to be explained on the basis of falling leaves. Accordingly, they discredited this hypothesis as well.

In order to determine the importance of dew and rainfall, they performed interesting experiments in soaking and syringing leaves of several plants with water. From this they observed that barley (*Hordeum vulgare*) plants after soaking in water for several minutes showed losses of 1.6 percent of the nitrogen in the plants, 36 percent phosphoric acid, 65 percent potash, 52 percent soda, 45 percent magnesium, and 75 percent chlorine. Green potted rice (Oryza sativa) plants which were syringed with artificial rain, showed considerable loss of mineral nutrients, as did mature potato plants (Solanum tuberosum). Twigs of apple trees (Malus spp.) with leaves still attached were soaked in water for several minutes and then analyzed. The leaves lost 3 percent of their total nitrogen content, 25 percent phosphoric acid, 18 percent potash, 22 percent soda, 6 percent lime, 12 percent magnesium, and 40 percent chlorine. These experiments not only demonstrated leaching, but also showed that fruit crops as well as field crops were subject to this phenomenon.

In another experiment, wheat (Triticum aestivum) plants in three stages of maturity were leached for 5 to 10 minutes in water. The mineral loss was considerable and increased proportionately with the maturity of the plants. Further, air-dried plants lost more nutrients than did living plants.

Mature wheat plants were exposed to four days of natural rain amounting to one inch. The plants lost 27 to 32 percent nitrogen, 20 to 22 percent phosphoric acid, 63 to 66 percent potash, 46 to 65 percent soda, 51 to 59 percent lime, 54 to 67 percent magnesium, and 90 percent chlorine.

Oat plants (Avena sativa) were grown out-of-doors and the natural rainfall, after passing over the plants was collected. Upon analysis, it was found that the rain washed out 2 percent of the total nitrogen in the mature plants, 33 percent of the phosphoric acid, 36 percent of the potash, 23 percent of the soda, 40 percent of the lime, 45 percent of the magnesium, and 40 percent of the chlorine.

LeClerc and Breazeale also calculated that the equivalent of 700 pounds of kainit, 10 pounds of sodium nitrate, and 36 pounds of 16-percent phosphate may be washed by rain from the foliage of one acre of wheat in one season.

They showed, as had Councler (21), that greenhouse-grown crops contained a higher concentration of mineral elements than did plants grown out-of-doors, and that greenhouse soils became exhausted more rapidly than field soils. They suggested that the depletion of greenhouse soils was due to the lack of leaching action of rainfall in the greenhouse which occurred out-of-doors and which returned nutrients to the soil.

Despite the quality of this research and the importance of its findings, this paper became almost forgotten. Mention of leaching phenomena is almost entirely lacking from the literature from that time until soon after World War I.

In 1925, Mann and Wallace (58) reopened interest in leaching with the observation of a diseased spotting of the leaves of the Cox's Orange Pippin variety of apple (Malus domestica) during the very wet year of 1924.

This foliar spotting was not apparent in trees grown in the greenhouse, nor during the drier season of 1923. They concluded that the disease was produced by the natural leaching of nutrients from the leaves by rain; in fact, they produced the disease symptoms by washing Cox's Orange Pippin leaves in water, which removed 26 percent of the dry matter of the leaves. They suggested that the outer leaf surface controlled the leaching and that varieties differed as to this control.

Later, Wallace (92) demonstrated clearly that substantial amounts of materials could be leached from leaves of fruit plants by cold water. He found that leaves of apple (Malus domestica), pear (Pyrus communis), plum (Prunus spp.), and gooseberry (Ribes spp.) lost 40 to 50 percent of the total ash content of the leaves, 94 to 98 percent of the potash, and 20 to 36 percent of the dry matter during four consecutive 24-hour leaching periods. Black currant (Ribes nigrum) leaves were the most resistant to leaching of any species tested, but the Victoria variety of plum (Prunus domestica) was the most resistant single plant of all. The major portion of the leached nutrients was organic matter which amounted to eight times the quantity of the ash constituents leached.

Lees (46) stated that rainfall during one season greatly affected fruiting the next by altering the nutritional status of the tree.

Cholodny in 1932 (19) reported on leaching experiments with cereal crops. He had observed that the yield of wheat was often reduced by a rainy spell occurring during head development. Accordingly, plants of barley (*Hordeum* spp.), wheat (*Triticum* spp.), and rye (*Secale* spp.) were subjected to 3 to 4 days of artificial rainfall during the heading stage; the dry weight was reduced 22 to 31 percent by the treatment. The early milk stage proved to be the most susceptible to leaching of sugars, largely from the endosperm.

Similarly, fruits of raspberry (*Rubus idaeus*), blackberry (*Rubus* spp.), and mulberry (*Morus* spp.) lost large quantities of sugars by soaking in water for 15 to 16 hours; losses were considerably less from cherry (*Prunus* spp.), and currant (*Ribes* spp.) fruits. From this work Cholodny postulated that the poor flavor of soft fleshy fruits during protracted rainy periods might be the result of a loss of sugars.

About this same time (1930 to 1940) considerable interest developed in the function of dew in plant growth. Measurements were made of the amount of dew present in certain geographical areas and seasons. Hiltner (34, 35) using mustard (*Raphanus* spp.), and oats (*Avena* spp.) as experimental plants, suggested that dew supplied salt-free water which tended to reach equilibrium with the salts absorbed through the roots and so reduce the salt concentration within the plant. This natural equilibration, he maintained, was necessary for "healthy" growth. He did not consider leaching as a mechanism for the adjustment of a plant to its environment, but rather as a distinct and unrelated phenomenon.

Zattler (106, 107) agreed with Hiltner and enlarged on the latter's experimental evidence. He showed that the effect of dew was greatest on plants growing at high nutrient levels. Dewed plants grew more vigorously, became reproductive, and produced larger yields than undewed plants, which remained vegetative. He also showed that salt concentrations were lower in the dewed plants. Both Zattler and Hiltner seemed to overlook the possibility of dilution of salt concentration in the leaves through the actual loss of salts by the leaching action of the dew.

In 1934, Arens (7) published a comprehensive review on the subject of leaching or "exkretion" from the leaves. Upon examining droplets of dew on leaf surfaces of some 78 species of plants, he found that the pH of such droplets varied between 7.0 and 9.0, compared with a pH of 4.5 to 7.6 in guttated droplets. From this he concluded that salts were being actively excreted through the epidermal cells of the leaf--salts which were not appearing in the gutation waters. Droplets on upper surfaces were more alkaline than those on lower surfaces. He found that potash, calcium, magnesium, phosphorus, and organic matter were leached from leaves, with potash and calcium being leached most easily. Loss of ash constituents by leaching varied between 15 and 50 percent on a weight basis for many crops. Increases in temperature increased the loss of ash constituents.

Arens calculated the loss of materials from a hectare of sugar beets (Beta vulgaris) containing 80,000 plants during 18 to 24 hours of rainfall to equal 62.2 kg of ash, 38.58 kg of phosphoric acid, and 5.26 kg of CaO. He found that sun leaves were more easily leached than shade leaves, thus providing a possible explanation for the lower mineral content of sun leaves as compared to shade leaves reported by von Leiningen (48). Further, he wrote that it was a well known fact among "practical" men that rain had a more beneficial effect upon plants than did ground irrigation. This agreed with the findings of Hiltner (34, 35) and Zattler (106, 107).

Arens pointed out that the leaching of nutrients from leaves could explain many naturally occurring phenomena, including the higher concentration of salts in greenhouse-grown plants, as compared to field-grown plants, and the apparent "Rückwanderungen" of nutrients from dying leaves.

As long ago as the Fourth Century B. C., Aristotle (9) had suggested that since plants possessed no organ of excretion similar to those of animals, the dropping of fruits and seeds served this essential function. Stahl (79) and his students emphasized the essentiality of an excretory mechanism in plants, such as hydathodes, nectaries, and the precipitation of materials within cells as crystals. Van der Volk (91) proposed that leaf drop in autumn served as an excretory process.

In this connection Arens (7) suggested that leaves might be considered analagous to organs of excretion in the animals. He concluded that in leaching, plants possessed an adaptive mechanism for active excretion of salts. It had already been shown by the work of Wiesner (98, 99) and Merkenschlager and Klinkowski (60) that plants could be divided into two classes, namely, (a) ombrophilic plants, which preferred to be wetted with dew and rain, such as the potato (Solanum tuberosum) and (b) ombrophobic plants, such as lupine (Lupinus spp.) and alfalfa (Medicago sativa), which did not prefer wetting. These observations and those of von Leick (47) recognized dew as an ecological factor in plant behavior.

The importance of the preference of certain plants for wetting of the foliage was also shown by Spiekermann (77). He noted a higher ash content in potato plants infected with leaf roll disease--a disease which occurred only in areas deficient in rain and dew (60). Arens (7) concluded from these results that some plants, such as the potato, were not tolerant of a high salt content within the plant and so preferred a maritime climate where rain and dew could wash out the excess salt accumulation from above-ground parts. In contrast, lupine was adapted to a continental climate with less dew and rain because it was tolerant of a high salt content and did not require the removal of excess salts.

He observed that in desert areas, plants often possessed special adaptative mechanisms for ridding themselves of salts, such as glandular hairs which accumulated salts and then abscissed. This type of adaptative mechanism was seldom observed among natural plant populations in moist climates where rain and dew could serve as salt-removing agents. He regarded the rain forest as a plant association highly adapted to cuticular excretion.

Arens reported that the higher the osmotic concentration in the leaf, the greater was the loss of nutrients by excretion. He found that aquatic plants, which were continually bathed in water, had a low osmotic pressure which greatly reduced the leaching effect. In contrast, land plants had higher osmotic concentrations, and the leaching effect readily occurred when water was present on the leaf surface.

From these arguments and findings, Arens developed the concept that leaching was essential in maintaining the nutritional equilibrium in plants. He felt that the ability of plants to utilize this mechanism was a significant and important ecological factor in plant adaptation and distribution. He thought that the concept of an impermeable leaf surface had been greatly over-emphasized, and agreed with Linsbauer (49) that the epidermis might serve other than for protection and reduction of water loss.

Lausberg (44) supported Arens' concepts of the leaf as an agent of active cuticular excretion. She found that attached leaves of castor bean (*Ricinus* spp.) soaked 1 hour daily for 18 days gave off nearly three times as much potassium and twice as much calcium as was found in the leaves at the start of the experiment. She found that soft rain caused greater loss than did hard rain, and that old leaves lost more nutrients than did young leaves. The upper surfaces of leaves gave off 69 percent more materials than did lower surfaces, corroborating the findings of Arens. She found that potassium was lost in greater quantities than magnesium, with calcium lost in least amounts. Onions (*Allium* *cepa*) excreted large amounts of chlorine, some sulfate, but only traces of phosphorus.

Engel (25), however, in 1939 could not agree with the concepts of Arens. After making detailed studies of the amount of water which collected on plants from rain and dew, and the pH of such solutions, he proposed that only passive excretion occurred in leaves. He favored the idea of diffusion of leaf substances from the leaf to the water, rather than an active process of excretion.

The papers of Arens (7) and Lausberg (44) had great influence on acceptance of leaching as a normal physiological process in plants. Achromeiko (1) showed that leaching could take place from roots as well as from leaves. Roots of grain crops excreted minerals only when leached with water;

whereas with legumes, root excretion, especially of phosphorus, was a frequently occurring process, even before flowering. Loehwing (51, 52) also demonstrated root leaching.

By 1950 the practical importance of leaching had been recognized and scientists intensified their investigation of this phenomenon (30). Tamm (83) in Sweden found that a considerable amount of alkaline salts and organic matter, and a lesser amount of nitrogen and phosphorus were leached from conifer and hardwood forest trees. During a 40-day period in the autumn, 2 to 3 pounds per acre of potassium, sodium, and calcium were carried to the ground from conifers by rain. Will (101) found similar losses in his work in New Zealand.

Ingham (41) compared open field rain and rain as it dripped from trees and found the latter contained six times as much P_2O_5 and three to four times as much CaO and ammonium nitrogen. Linskens (50) noted that leaves were not wetted by water to the same extent in different stages of ontogeny, being more hydrophobic in the younger than the older stages.

Dalbro (22) reported the results of leaching experiments with fruit trees, in which he collected samples of rain water in vessels suspended in apple trees. From this he calculated a loss from apple trees of more than 1, 000 pounds per acre, including 25 to 30 pounds of potassium, 10. 5 pounds of calcium, and 9 pounds of sodium. The greatest part of the leachate residue was organic, principally carbohydrates. Losses reached seasonal peaks at times of flowering and leaf senescence and were steady even throughout the dormant season. Dalbro also noted the obvious implications of leaching on soil analysis, leaf analysis, appearance of deficiency symptoms, soil texture, and soil microorganisms. He quoted the work of Bloomfield (13) and Lutwick *et al.* (55, 56) in which tree leachates had an important effect on soil texture.

With the coming of the Atomic Age and the availability of radioactive isotopes, several investigators enlisted the aid of tracers in their studies of leaching. Mes (61), working in South Africa, investigated the loss of P^{32} from several vegetable and field crops. Tomatoes (*Lycopersicon esculentum*), potatoes (*Solanum tuberosum*), corn (*Zea mays*), and beans (*Phaseolus vulgaris*) lost between 2.2 and 5.0 percent of the root-applied P^{32} during a 30-minute exposure to artificial rain. It was determined that the nutrients lost from the leaves by leaching were replaced by nutrients translocated from other plant parts. There seemed to be no direct relation between the amount of the tracer absorbed by a plant and the amount lost. Instead, the stage of plant development and the prevailing climatic conditions were of greater importance. The ash content of all plants tested was higher when the plants were protected from rainfall than when plants were not protected.

Long, Sweet and Tukey (53) also using radioisotopes, found little or no loss of P^{32} from young seedlings of bean (Phaseolus vulgaris), sweet potato (Ipomaea batatas), and poinsettia (Euphorbia pulcherrima). However, stem cuttings of bean and rose (Rosa spp.) lost 1.5 and 12.8 percent, respectively, of the P^{32} previously absorbed through the cut surfaces. Bean plants which root-absorbed radiopotassium in the dark lost 42 to 71 percent of the same K^{42} during a 4-hour leaching period. Losses from plants in the light were only 5 to 12 percent. In addition, a 10 percent reduction in the content of calcium, magnesium, nitrogen, and phosphorus was found in the leached plants. Chromatographic separations suggested the presence of a polypeptide and large amounts of a galactan in the leachate.

While making leaf analyses to determine foliar uptake of nutrients, Corin et al. (20) noticed that washing the leaves prior to analysis resulted in a loss of nutrients. For example, detached apple leaves which were soaked in water for 5 hours lost 12.5 percent of the P^{32}, 20 percent of the Ca^{45}, and 27.8 percent of the S^{35} in the leaves. None of the Fe^{59} was lost.

MATERIALS AND METHODS

Laboratory and Greenhouse Studies

Preliminary studies were begun in the fall of 1955 in the greenhouse to determine suitable plant materials, methods, and procedures for use in the investigation of factors influencing the loss of nutrients from foliage by leaching.

Bean (*Phaseolus vulgaris* L., variety Contender) and squash (*Cucurbita pepo* L., variety Early Prolific Straightneck) were chosen as the principal experimental plants. They are both economically important, a supply of seed is readily available, germination and growth are rapid and uniform, and the leaves are large and erect and well adapted to leaching experiments. Both crops have been found useful in research of this type, and results obtained have been found applicable to other crops.

In addition to bean and squash, several other plants were used at various times during the course of the studies, including cabbage (*Brassica oleracea* L.), corn (*Zea mays* L.), lettuce (*Lactuca sativa* L.), sugar beet (*Beta vulgaris* L.), and tomato (*Lycopersicon esculentum* Mill.).

The following radioisotopes were included in the experiments: Ca^{45}, Cl^{36}, Fe^{55-59}, K^{42}, Mg^{28}, Mn^{54}, Na^{22}, P^{32}, S^{35}, Sr^{90}-Y^{90}, and Zn^{65}. The commercial sources of these isotopes, their carriers, and radiological half-lives are given in Table I.

TABLE I

Commercial Source, Carrier, and Radiological Half-Life of Isotopes Studied.

Isotope	Commercial Source	Carrier	Half-Life
Ca^{45}	Oak Ridge National[1] Laboratory	$CaCl_2$ in HCl solution	152 days
Cl^{36}	Oak Ridge National Laboratory	HCl solution	4.4×10^5 years
Fe^{55-59}	Oak Ridge National Laboratory	$FeCl_3$ in HCl	45.1 days
K^{42}	Oak Ridge National Laboratory	K_2CO_3 solid	12.44 hours
Mg^{28}	Brookhaven National[2] Laboratory	$MgCl_2$ in conc. HCl	21.4 hours
Mn^{54}	Nuclear Science and[3] Engineering Corp.	$MnCl_2$ in HCl	310 days
Na^{22}	Oak Ridge National Laboratory	NaCl isotonic solution	2.6 years
P^{32}	Oak Ridge National Laboratory	PO_4 in HCl solution	14.3 days
S^{35}	Oak Ridge National Laboratory	SO_4 in HCl solution	87.1 days
Sr^{90}-Y^{90}	Oak Ridge National Laboratory	$SrCl_2$ in HCl solution	Sr- 28 years Y - 61 hours
Zn^{65}	Oak Ridge National Laboratory	$ZnCl_2$ in HCl solution	250 days

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[1] Oak Ridge National Laboratory, Oak Ridge, Tennessee.

[2] Brookhaven National Laboratory, Upton, Long Island, New York.

[3] Nuclear Science and Engineering Corporation, Pittsburgh, Pennsylvania.

Treating solutions were prepared from the original isotope shipments by adding a predetermined amount of sample to 50 ml of distilled water. This diluted the radioactivity of the sample for safety in handling. With K^{42}, which was shipped as a crystalling solid (K_2CO_3), the entire sample was dissolved in 1 liter of distilled water. All treating solutions were stored in a lead-lined safe to reduce the hazard of radiation.

Seeds of the plants to be used were germinated either in white quartz sand (No. 8 size, American Graded Sand Company, Chicago, Illinois) or in moistened rolls of paper toweling. The young seedlings were then transferred to aerated solution cultures (10) in 3.5-liter glass jars (5 plants to the jar) containing half the nutrient intensity of Hoagland and Arnon's standard solution (39), as seen in Figure 1. The seedlings were supported by styrofoam plugs (expanded polystyrene, Dow Chemical Company, Midland, Michigan) inserted in covers of tempered masonite. Air was supplied to the culture solutions from a compressor through Tygon plastic tubing (U. S. Stoneware Company, Akron, Ohio) and porous air stones (Ward's Natural Science Establishment, Rochester, New York).

To avoid contamination from radioactive materials, the solution culture jars were placed inside 7-liter glazed earthenware crocks. Eight of the crocks were placed in a large metal tray 48 by 27 by 4 inches, which was lined with absorbent paper, as seen in Figure 2.

Figure 1

Aerated nutrient culture (3.5-liter jar) used in the experiments. Note plants supported by styrofoam plugs inserted through holes cut in tempered masonite covers, and air line and porous air stone in liquid.

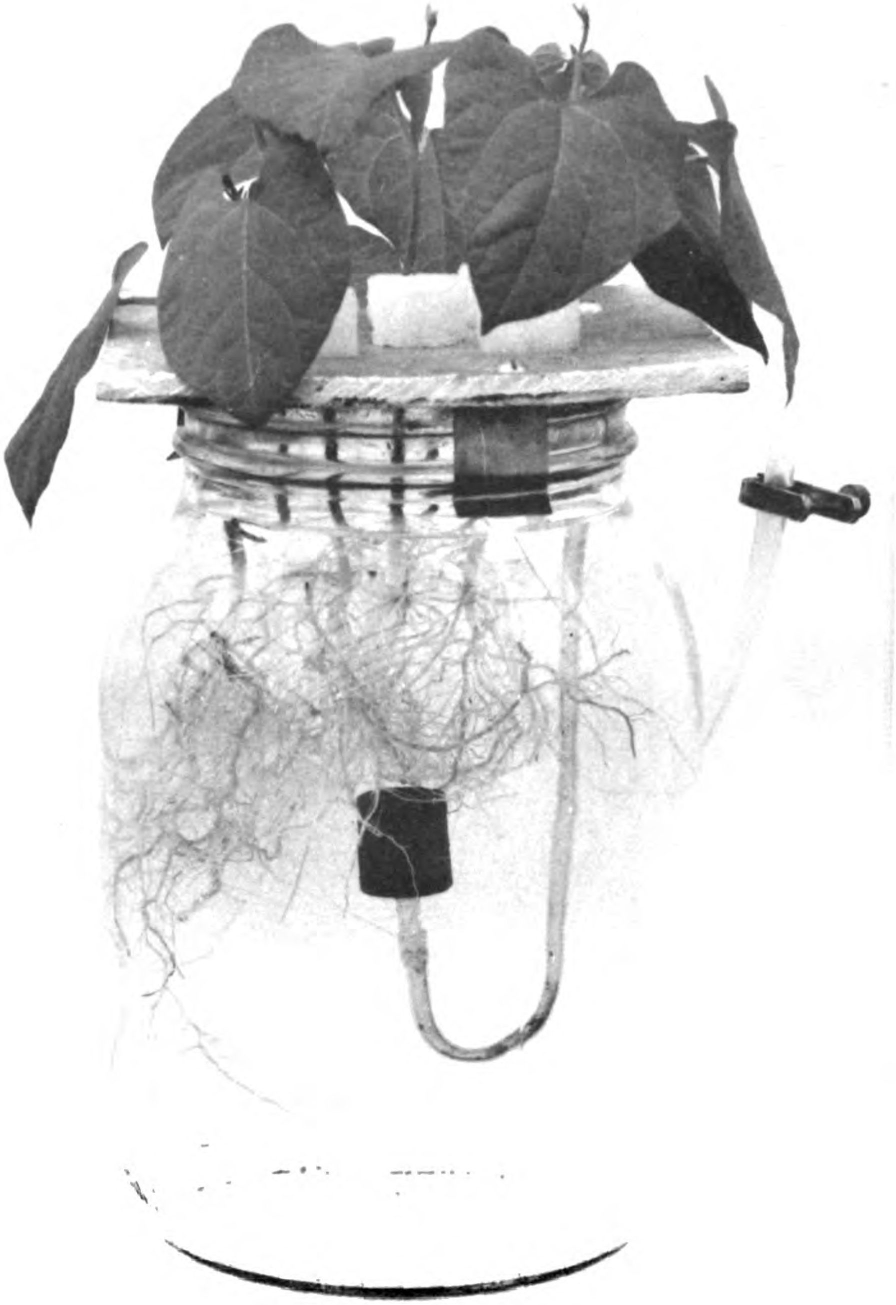
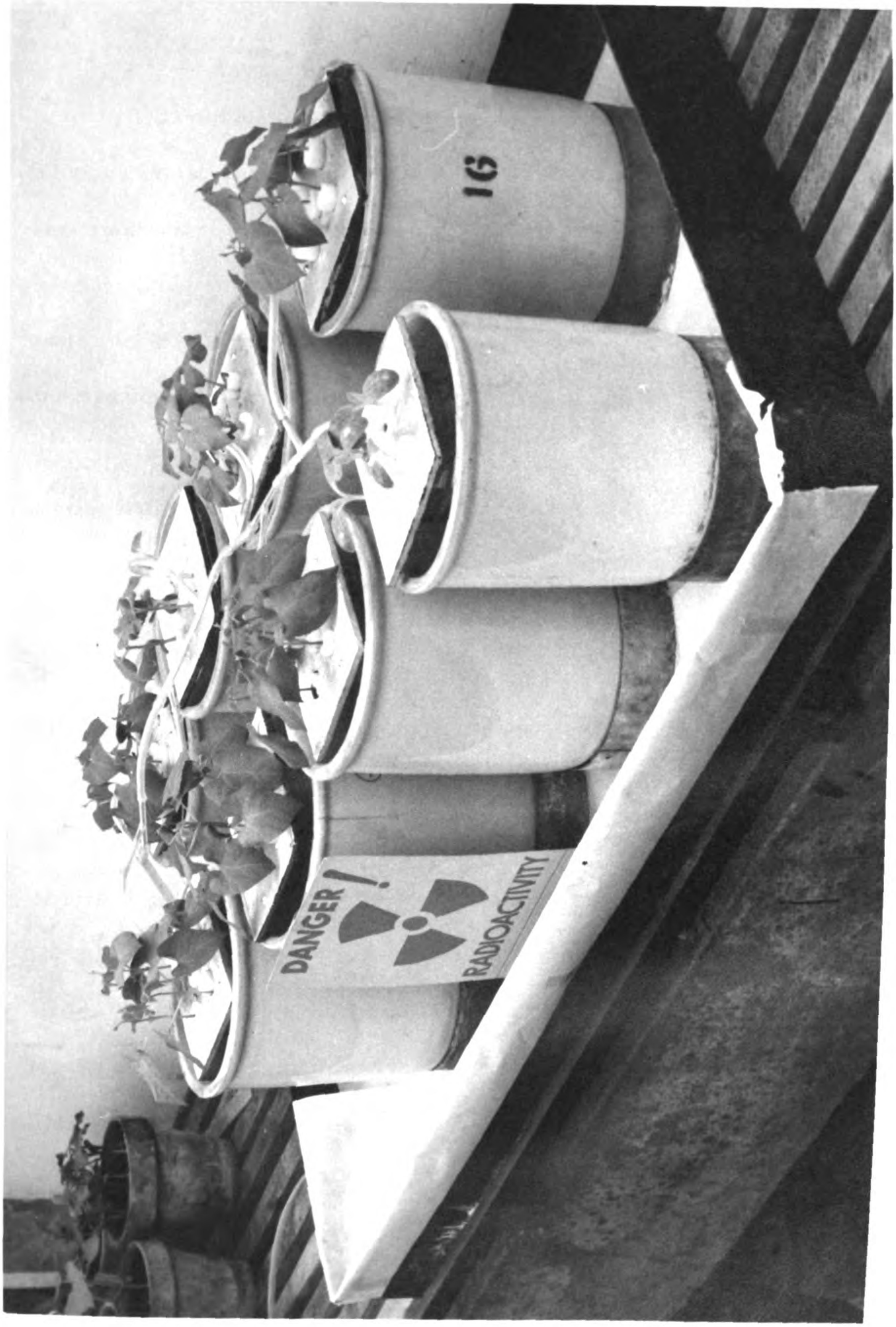


Figure 2

Battery of aerated nutrient solution cultures placed in 7-liter crocks in a paper-lined, water tight tray.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DANGER !
RADIOACTIVITY

Radioisotopes of the various nutrient elements were added to the solution cultures (50 uc/jar) so that as the plants grew, the radioisotopes were root-absorbed and distributed within the plants. High activity isotope solutions (greater than 20 microcuries per ml) were used in all cases to avoid altering the concentration of nutrients in the nutrient cultures as much as possible.

After 10 to 15 days in the culture solutions, a primary leaf of bean or the second initiated leaf of squash was leached by one of three methods: (a) continuous immersing of the leaf in distilled water; (b) immersing of the leaf in distilled water, but having the solution replaced with fresh water at hourly intervals; or (c) misting the leaf with a water spray.

With the short lived isotopes K^{42} and Mg^{28}, a modified treatment procedure was used. Seedlings were allowed to grow in non-labeled solution cultures for 10 to 15 days; K^{42} or Mg^{28} was then added to the cultures. After 24 hours, during which the plants root-absorbed and distributed the isotopes, the leaves were leached by one of the three methods mentioned above. An initial activity of 10 millicuries of K^{42} per jar was necessary to have sufficient activity to analyze at the completion of the experiment. This was not necessary with Mg^{28}.

In the first two methods of leaching (Figure 3), the leaves were immersed in 130 ml of distilled water in a flat vessel covered with a watch

Figure 3

Method of leaching root-absorbed nutrients from young leaves by immersing in an aqueous solution in a flat vessel.

glass to keep the leaf submerged. The solutions in the vessels were either replaced at hourly intervals or poured off at the end of the experiment and evaporated to dryness in 50-ml beakers on a steam bath. The dried samples were then analyzed for radioactivity directly in the beakers by a mica end-window Geiger-Müller tube and a Tracerlab Sc-1M Autoscaler (Tracerlab, Inc., Waltham, Massachusetts) equipped with a shielded counting chamber.

Recognizing the possible variables involved in soaking leaves, such as altered respiration and gas exchange, a third method of leaching was devised as seen in Figure 4. The leaves were subjected to a mild mist spray of distilled water for 24 hours. Satisfactory results were secured by employing a high-volume air compressor which atomized 0.5 liters of water per hour through each nozzle. The water was gravity fed to the nozzles from four stainless steel tanks holding 6 gallons each. The mist spray, after passing over the leaves was collected in funnels and channeled through Dowex 1-Dowex 50 anion-cation exchange resins (Dow Chemical Company, Midland, Michigan) which adsorbed the leached plant constituents. The resins were held in glass columns 8 inches long and of 3/4 inch diameter by glass wool plugs and corks. At the completion of the leaching period, the resin columns were eluted by soaking for 6 hours in 25 ml of 10 percent hydrochloric acid solution. The eluants were decanted off and evaporated to dryness on a steam bath in 50-ml beakers, and the samples

Figure 4

Method of leaching root-absorbed nutrients from young leaves by misting with distilled water.

(Top) Atomizing nozzles with water lines and air lines;

(Center) Plants in nutrient cultures, and funnels;

(Bottom) Columns of anion-cation exchange resins.


DANGER
RADIATION HAZARD

assayed for radioactivity as in the first two methods.

For the determination of S^{35}, which emitted only weak beta rays, the eluants from the resin columns were passed through saturated $Ba(OH)_2$ solution which precipitated the S^{35} as $BaS^{35}O_4$. The $Ba(OH)_2$ solution was analyzed after the removal of the precipitated $BaS^{35}O_4$ and found to be free of radioactivity. The precipitate was washed, dried, and analyzed for radioactivity in metal planchets utilizing a Nuclear 172 Ultra Scaler with D47 gas-flow attachment (Nuclear Instrument and Chemical Corporation, Chicago, Illinois).

In all procedures, the leached leaves were harvested at the termination of the experiment, the oven-dry weights recorded, and the radioactivity determined directly in 50-ml beakers. The dried leaf samples containing S^{35} were counted directly in planchets in the gas-flow counting apparatus mentioned before.

Most samples were counted for at least 20 minutes, unless the radioactivity was above 500 counts per minute, when a shorter period of time was adequate. Ten counts per minute above existing background for shielded chambers and 5 counts per minute for the gas-flow apparatus were deemed sufficient for significance. No corrections for self-absorption were made inasmuch as each leachate was counted as an infinitely thin sample and self-absorption was negligible with dried plant materials.

Direct counting of dried plant materials has been successfully utilized in this laboratory with no differences being noted on a comparative basis with ashed samples.

Calculations of radioactive decay for use in adjusting counting data and determining treating amounts were made from the tables in the Radiological Health Handbook (42) which are based on the following equation:

$$A_t = A_o e^{-\lambda t}$$

where

A_t = activity at time interval t.

A_o = activity at initial time.

e = base of natural logarithm (2. 3026).

λ = decay constant for the isotope used.

t = time elapsed.

Losses from leaves were expressed as percentages of the total radioactivity (counts per minute) in the leaves as compared with the total radioactivity (counts per minute) in the leachates.

Losses of carbohydrates from plant leaves were determined by the method of Lunt and Sutcliffe (54), involving a colorimetric evaluation of the products of sulfuric acid hydrolysis by resorcinol-4:6-disulfonic acid[1/]. Five-ml samples of the carbohydrate solution to be tested plus

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[1/] The assistance of Dr. T. L. Rebstock, Department of Agricultural Chemistry, Michigan State University, in preparation of the sulfonic acid reagent is gratefully acknowledged.

one ml of 0.5 percent solution of the calcium salt of resorcinol-4:6-disulfonic acid were hydrolysed by 10 ml of concentrated sulfuric acid for 1 hour in darkness. Optical densities of the mixtures as compared to blank solutions were read on a Bausch and Lomb Spectronic 20 colorimeter (Bausch and Lomb Optical Company, Rochester, New York) at 490 mu wave length in 1-cm cells. Comparison of the optical densities with those of a previously established standard curve gave quantitative carbohydrate losses. Losses of carbohydrates were expressed as a percentage of the dry weight of the leaves as compared with the total carbohydrates in the leachates.

Strawberry plants (Fragaria spp., variety Catskill) were transplanted from the field, to pots in the greenhouse[1/]. Fruits in four stages of maturity ranging from immature (green) to very mature (fully ripe) were selected for treatment, and 0.02 ml of Sr^{90}-Y^{90} (10 uc/ml) were placed on the peduncles of the fruits utilizing a tuberculin syringe with a No. 27 needle. After 2 days, during which the isotope was absorbed and translocated into the fruits, the fruits were detached from the plants at a point acropetal to the site of application and fresh weights determined. The fruits were then immersed in distilled water for 24 hours. The cut peduncles were not in contact with the water. At hourly intervals, the solutions were

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[1/] Strawberry plants courtesy of Mr. Jerome Hull, Department of Horticulture, Michigan State University.

replaced with fresh water, dried on a steam bath, and analyzed for radioactivity in 50-ml beakers as before. At the completion of the experiment, the strawberry fruits were oven-dried, weights recorded, and the radioactivity analyzed in the beakers. Losses were expressed as the percentage of the total radioactivity in the fruit as compared to the total radioactivity in the leachates.

Losses of carbohydrates from strawberry fruits were determined colorimetrically (54), and were expressed as the percentage of the oven-dry weights of the fruits.

Autoradiograms were prepared by a procedure developed by Wittwer and Lundahl (104) to show the uptake and distribution of root-absorbed isotopes. Following treatment plant materials were immediately pressed and dried between layers of 8 by 10 inch botanical blotting paper and steel plates in an oven at 85° C. After 1 to 2 hours, the materials were taken from the oven, and the pressed, dried specimens were carefully moved from the original paper to new sheets and covered with Saran wrap (Dow Chemical Company, Midland, Michigan) to act as a protectant and to hold the plants in place. The mounted specimens were then placed in contact with 8 by 10 inch Kodak blue-brand X-ray film (Eastman Kodak Company, Rochester, New York) and enclosed in a fitted light-proof box. After an exposure of 3 weeks, the films were removed, and processed with Kodak developer and fixer.

Dark or shaded spots on the film indicated presence of radioactivity in the plants.

The radioisotope tracer technique was found to be very useful. First, the source of radioactivity in the leachate was clearly identifiable as that originally applied to the root medium, absorbed by the roots, transported to the leaf, and leached from the leaf. Second, the analysis was much simpler, and more rapid than by standard chemical procedures. Third, a single element could be determined at one time without interference from other elements. Fourth, quantitative measurements could be made easily at a very low level. Fifth, autoradiograms gave a clear picture of uptake and distribution of radioactive nutrients as no other method of analysis could.

Field Studies

Eight 5-year-old apple trees (*Malus domestica* Bork., varieties Golden Delicious and Jonathan) growing in the orchard were selected for study. Two trees of each variety were covered with a roof of clear plastic as a protection from rain, and two other trees of each variety were left uncovered, as seen in Figure 5[1/]. Leaf samples were collected from each tree midway down the new shoot growth on June 11, and September 18, 1957. The

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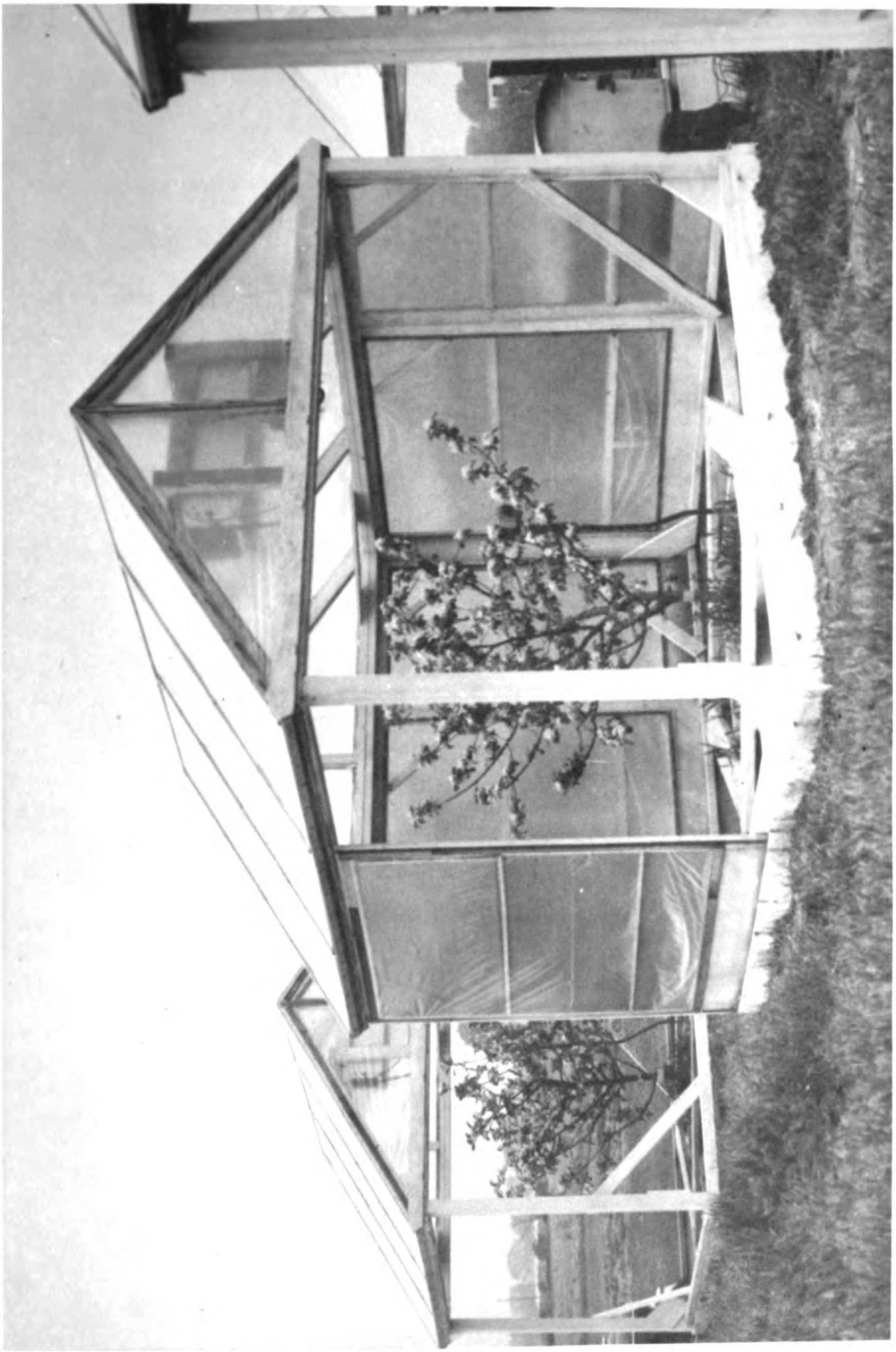
[1/] Trees and protective covers courtesy of Dr. A. E. Mitchell and Mr. Dale Kretchman, Department of Horticulture, Michigan State University.

Figure 5

Apple trees (*Malus domestica*) covered with a clear plastic roof as a protection from rain. Sides were open during the experiment.



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samples were wiped off with a damp rag to remove any residues, oven-dried, and ground to a 20-mesh size. They were analyzed quantitatively for ten nutrient elements[1]. Nitrogen was determined by the Kjeldahl method, potassium by a flame photometer, and boron, calcium, copper, iron, magnesium, manganese, phosphorus, and zinc by spectrographic analysis. Nutrient concentrations were expressed as a percentage of the oven-dry sample weight from averages of replicated determinations.

Samples of mature leaves of cherry (Prunus cerasus L.), and blueberry (Vaccinium corymbosun L.), and mature petioles of grape (Vitis labrusca L.) were collected from plants growing either in nutrient gravel or sand cultures both out-of-doors and in the greenhouse (2, 11, 23, 62). The samples were handled in the same manner as described above, and were analyzed for the same ten elements.

Significant differences between means were determined by analysis of variance ("F" test) (76), and were labeled "significant at the 5 percent level".

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[1] Analyses by Dr. E. J. Benne and Mr. S. T. Bass, Department of Agricultural Chemistry, Michigan State University.

RESULTS

Demonstration of Leaching

A preliminary experiment was conducted to determine the possible loss of P^{32} and the extent of this loss from bean leaves. Bean seeds were germinated in coarse sand and the seedlings transferred to the aerated nutrient solution cultures. The plants were separated into three groups of four plants each. To the first group, 20 uc of P^{32} (5 uc/ml) were added to the root medium and root-absorbed by the plants as they grew. After 12 days a primary leaf was leached by immersion in 130 ml of distilled water in a flat vessel for 5 consecutive hours. At the end of the 5-hour leaching period, the leaf was removed from the water, which was analyzed for radioactivity as described in the section on Materials and Methods.

The second group of four plants was allowed to grow for 9 days before treatment. Five drops (0.5 ml) of P^{32} (5 uc/ml) from a tuberculin syringe and No. 27 needle were placed on one primary leaf of each plant. The drops were positioned in a semi-circle on five principal veins at the base of the leaf and about 2 cm from the leaf petiole. After 3 days, the opposite primary leaf from that treated was leached for 5 hours and the leachate analyzed as in the first group. The third group of four plants was also allowed to grow untreated for 9 days. Five drops of P^{32} (5 uc/ml)

were placed on a primary leaf as described above. After 3 days the plants were removed from the solution cultures and the roots were rinsed with distilled water and immersed in 130 ml of distilled water in a flat vessel for 5 hours. The leachate was handled in the same manner as in the first two groups.

The results given in Table II show clearly that root-absorbed P^{32} can be leached from leaves and that leaf-absorbed P^{32} can be leached from roots and non-treated leaves of the same plant. The uniformity of loss was not due to the comparative leachability of the various plant parts, but rather to the different quantities of P^{32} applied to the plant. Based on this experiment and similar studies with Ca^{45}, a suitable technique was developed for further investigation.

Age of Leaf

It has been suggested in the literature that as the leaf matures, it becomes more susceptible to leaching by rain and dew (7, 19, 44, 45). To investigate this suggestion the second initiated leaf from 2-week-old squash plants (four plants) which had previously absorbed Ca^{45} through the roots was leached by immersing in 130 ml of distilled water in a flat vessel for 24 hours. At hourly intervals, the solutions in the vessels were poured off and replaced with fresh water, and the radioactivity of each leachate

TABLE II

Demonstration of the Ability of Young Bean Roots and Leaves (*Phaseolus vulgaris*) to Lose Root- and Foliar-Absorbed P^{32} by Continuous Soaking in Distilled Water for 5 Hours

Treated Plant Part	Leached Plant Part	Total Radioactivity Leached (cpm per plant part)				
		1	2	3	4	Mean
Primary leaf	Opposite primary leaf	23	21	24	20	22
Primary leaf	Roots	26	23	22	23	23.5
Roots	Primary leaf	21	22	21	20	21

determined as described in the section on Materials and Methods. To compare the effect of age, the same procedure was followed with the second initiated leaf from 4-week-old plants (four plants).

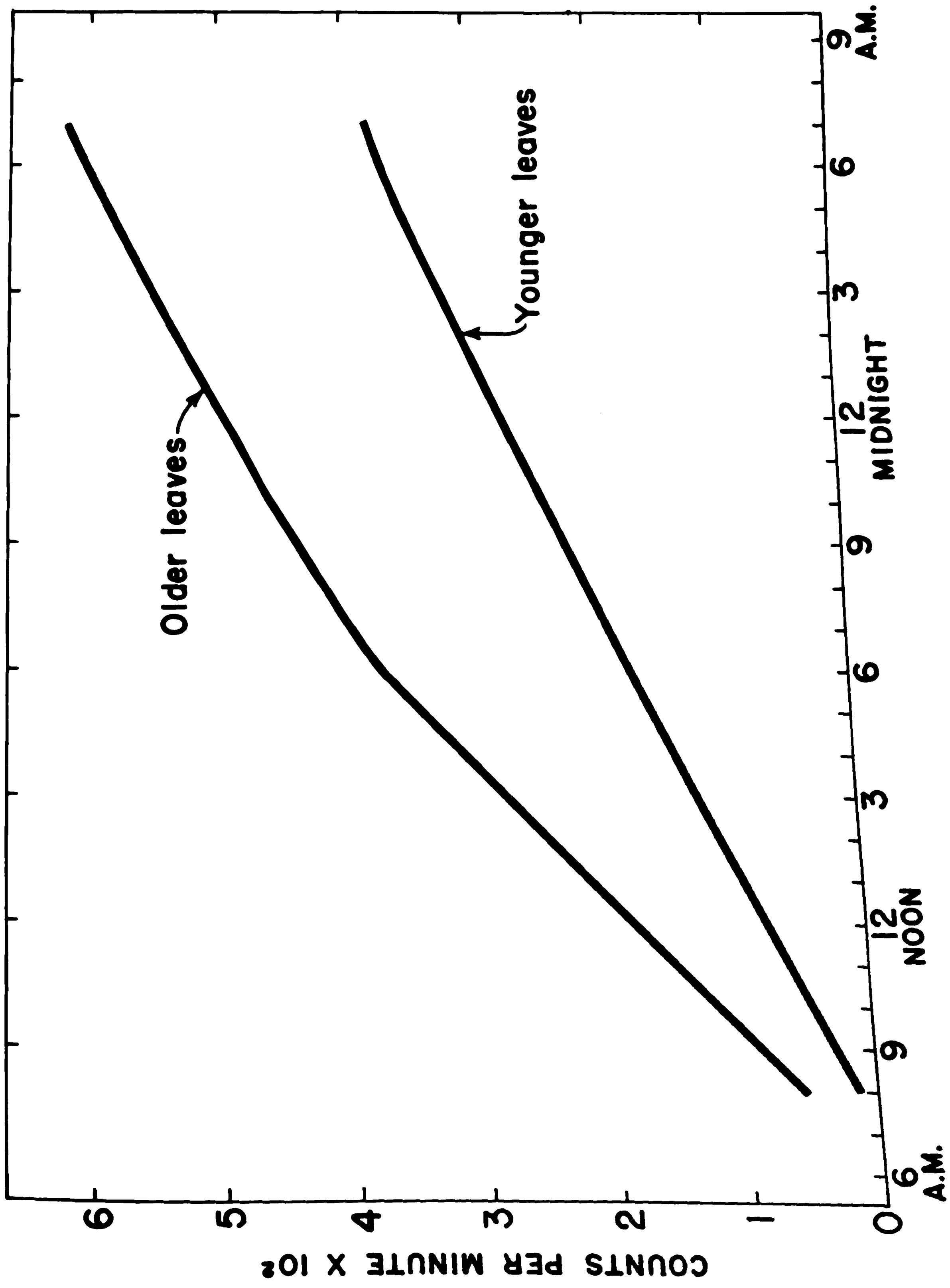
At the completion of the experiment the leached leaves were harvested, oven-dried, and analyzed for radioactivity. Average hourly loss of Ca^{45} during the 24-hour period was expressed as a percentage of the total radioactivity in the leaves.

The results of the experiment are given in Figure 6, in which the curves of the cumulative hourly losses of root-absorbed Ca^{45} from both the 2-week-old (younger) and the 4-week-old (older) leaves are plotted. The losses from the older leaves were largest during the first ten hours of the experiment and were consistently larger than the losses from the younger leaves. The average loss from older leaves during the 24 hours was more than 600 cpm. This was 3.4 percent of the root-absorbed Ca^{45} in the leaves. The average loss for younger leaves was less than 400 cpm, which was only 2.3 percent of the Ca^{45} in the leaves. It should be pointed out that all leaves used in this experiment were relatively young, including those from the 4-week-old plants.

These findings support the work of Cholodny (19) who theorized that although very young leaves appear delicate and fragile, they are less susceptible to leaching loss than are older leaves. Linskens (50) showed

Figure 6

Cumulative loss of root-absorbed Ca^{45} from squash leaves (*Cucurbita pepo*) by leaching with distilled water as influenced by age of leaf.



COUNTS PER MINUTE X 10²
0
1
2
3
4
5
6
6 A.M.
9
12 NOON
3
6
9
12 MIDNIGHT
3
6
9 A.M.
Older leaves
Younger leaves

that young leaves were hydrophobic and were wetted with difficulty. Hydrophoby decreased with the maturity of the leaves. Thus, the susceptibility of a leaf to leaching may be dependent on the wetting properties of the leaf.

Arens (7) proposed that this ability of young leaves to resist a nutrient loss by leaching was an adaptive mechanism which had been developed through the processes of evolution. Without such a protective mechanism, plants could quickly become deficient in certain nutrients. For example, early in ontogeny, the leaf is growing rapidly and is in need of a large amount of nutrients; yet it is during the spring season that moderately heavy rainfall and heavy dew occur. Lausberg (44) in recognizing the degree of leaching from young and mature leaves, agreed with Arens' conclusions.

At the same time it is well recognized that nutrients are readily leached from mature leaves and from leaves approaching senescence. LeClerc and Breazeale (45) reported losses of 52 percent of the calcium from mature barley leaves (Hordeum vulgare) during a "few minutes" soaking in water. Similarly, losses of calcium from apple leaves (Malus domestica) amounted to more than 6 percent of the calcium in the leaves.

Type and Nature of the Plant

There is considerable evidence in the literature that the amount of leaching depends to a great degree on the species and type of plant (7, 45, 58, 92). Cholodny (19) showed greater leaching losses from summer wheat (*Triticum* spp.) than from barley (*Hordeum* spp.) or rye (*Secale* spp.). Buchenau (15) and von Homeyer (40) suggested that leaching occurred more readily from leaves of beech (*Fagus* spp.), maple (*Acer* spp.), and linden (*Tilia* spp.) than from birch (*Betula* spp.), poplar and willow (*Populus* spp.).

To study the possible effect of crop on leaching losses, seeds of squash, corn, bean, tomato, and sugar beet were germinated in moistened paper toweling and the seedlings transferred to the aerated solution cultures in 3.5-liter jars. Three jars each (five plants of one crop per jar) of squash, bean, and tomato, and two jars each of corn and sugar beet were included in the study.

Fifty microcuries per jar of P^{32} were added to the root medium of one jar each of the bean, corn, squash, tomato, and sugar beet plants. Similarly, 50 uc per jar of Ca^{45} were added to the root medium of one jar each of the bean, corn, squash, and tomato plants. After 14 days, during which time the isotopes were root-absorbed and distributed in the plants, a second initiated leaf of squash, corn, tomato, and sugar beet, and a primary leaf of bean were removed from the parent plant by severing at the base of the petiole. The detached leaf blades were immersed in 130 ml of distilled water in a flat vessel in such a manner that the cut petioles were not in contact with the water. After 2 hours of leaching, the solutions in the vessels were poured off and the radioactivity in the leachates and the leached leaves determined.

The four remaining jars (one each of bean, squash, tomato and sugar beet) were allowed to grow in nutrient cultures for 13 days before treatment. Ten millicuries of K^{42} were then added to the root medium in each jar. After 24 hours of root-absorption, the leaves were leached in the same manner as described above. Losses were expressed as the percentage of the total radioactivity in the leaves.

The results of the experiment are shown in Table III. In general, losses of root-absorbed nutrients were greater from bean, corn, and squash leaves than from tomato and sugar beet leaves. For example, losses of root-absorbed Ca^{45} during the 2-hour soaking period from leaves of bean, corn, and squash were 3.6 to 4.1 percent of the isotope in the leaf as compared with only 1.1 percent loss from tomato leaves. Similarly, bean and squash leaves lost 1.6 and 2.4 percent respectively of the root-absorbed K^{42} in the leaf, whereas tomato lost 1.0 percent and sugar beet, with its waxy leaf surface, lost only 0.3 percent of the K^{42}. Similar results were obtained with P^{32}.

TABLE III

Percentage Loss of Root-Absorbed Ca^{45}, P^{32}, and K^{42} from Detached Young Leaves Soaked in Distilled Water for 2 Hours

Isotope	Crop (Percent Leached)				
	Bean	Corn	Squash	Tomato	Sugar Beet
Ca^{45}	3.6	3.9	4.1	1.1	--
P^{32}	0.4	0.1	0.5	0.1	trace
K^{42}	2.4	--	1.6	1.0	0.3

This experiment clearly demonstrated leaching differences among plant species, and suggested the possible importance of the cuticle in controlling these losses. Arens (7) showed that by removing the cuticle of cabbage leaves (Brassica oleracea) the loss of ash by leaching could be increased greatly, as compared with loss from intact leaves. Waxing the leaf surface reduced leaching losses. Mann and Wallace (58) suggested that the outer leaf surfaces controlled the amount of leaching loss. It would appear from this that plants with a thick waxy leaf cuticle, such as young sugar beet, would be less susceptible to leaching than plants without such a waxy cuticle, as young corn, bean, and squash. Arens also showed that considerable losses occur from mature sugar beet leaves.

Duration of the Leaching Period

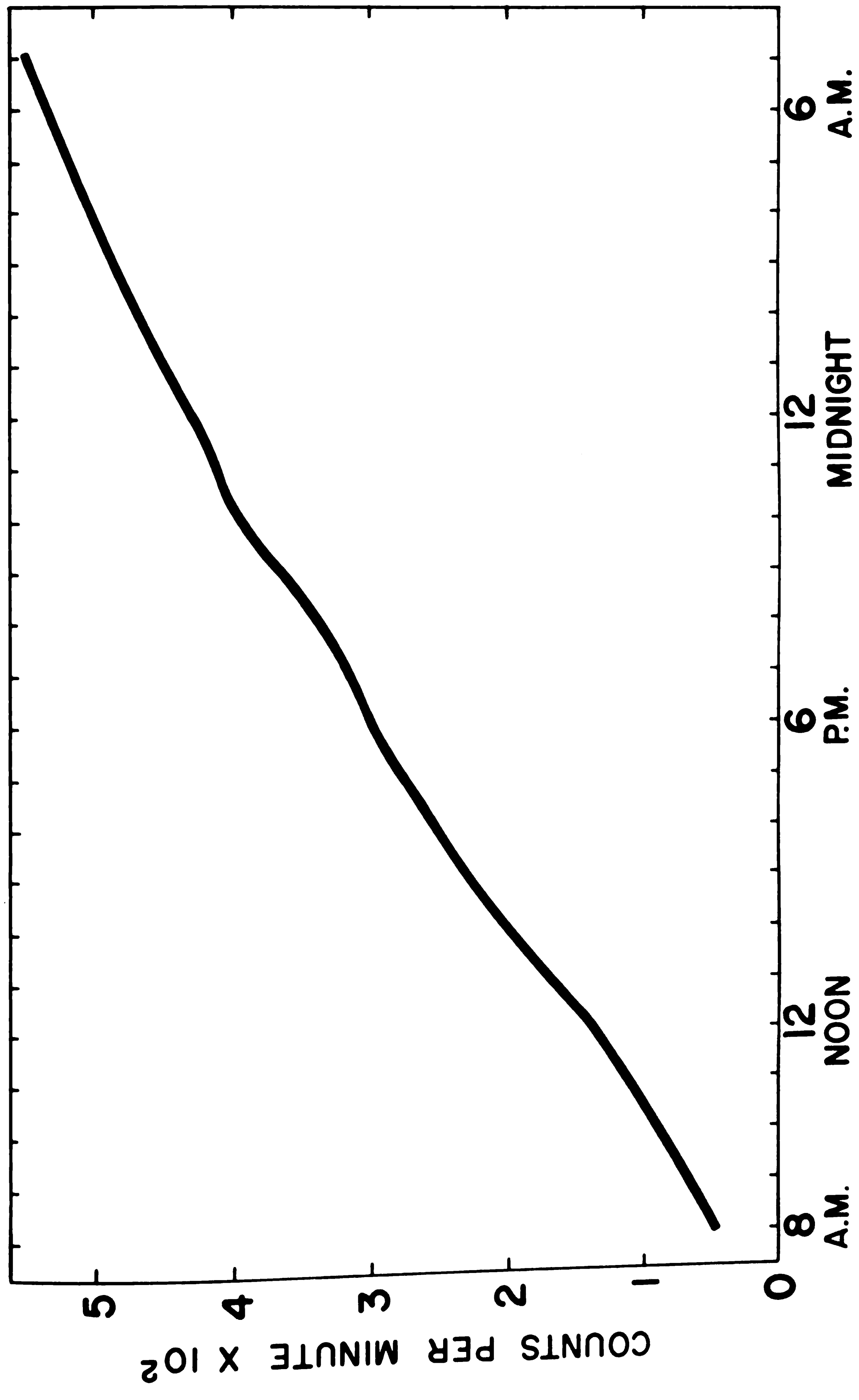
The hourly leaching losses of root-absorbed Ca^{45} over a 24-hour period were determined for intact young squash leaves immersed in distilled water. The cumulative curve of average hourly losses is plotted in Figure 7. Losses of Ca^{45} were almost a direct function of time except for the last few hours of the experiment, when they declined slightly. The average total loss of Ca^{45} per leaf was approximately 550 counts per minute, which was 3 percent of the total root-absorbed Ca^{45} taken up by the leaf. If leaching had been continued, as shown by Lausberg (44), losses would have increased until they eventually totaled several times the amount of the nutrient contained originally in the leaf.

The curve in Figure 7 also shows that measurable amounts of nutrients may be leached from leaves in a very short period of time; in fact, the curve rises most rapidly during the first few hours of the experiment. Similarly, Mes (61) found that losses of P^{32} from tomato plants were greatest during the first 30 minutes of leaching.

In another study of the effect of time on loss of nutrients by leaching, young squash plants were permitted to root-absorb Ca^{45} and P^{32} for 14 days, and K^{42} for 24 hours. Leaves were then removed from the parent plant and leached by continuous soaking in distilled water for three different intervals of time, namely, 1/2, 2 and 24 hours. Analyses were made of the radio-

Figure 7

Cumulative loss of root-absorbed Ca^{45} from squash leaves (*Cucurbita pepo*) leached with distilled water during a 24-hour period.


COUNTS PER MINUTE X 10^2
0
1
2
3
4
5
8 A.M.
12 NOON
6 P.M.
12 MIDNIGHT
6 A.M.

activity in the leachates and in the leaves, as shown in Table IV. The losses of root-absorbed nutrients are expressed as a percentage of the total radioactivity in the leaves. Noticeable losses of all three isotopes were detected after only 30 minutes of leaching. After 2 hours, these losses, at least those for Ca^{45} and K^{42} increased markedly. After 24 hours of continuous soaking, 18 percent of the Ca^{45} and 3 percent of the K^{42} in the leaves were leached.

Other workers have also reported results which agree with these findings (7, 19, 22, 44, 72). It should be remembered that the losses reported in the literature were usually from mature leaves, while in the experiments reported in this thesis, significant losses at short-time intervals were shown from young immature leaves, which are leached with greater difficulty than are older leaves.

The results show clearly that significant losses may occur after even a few minutes of leaf exposure to water, and that leaching may be a factor to consider in crop production in connection with rainstorms, night dew, or foggy, misty periods.

TABLE IV

Percentate Loss of Root-Absorbed Ca^{45}, K^{42}, and P^{32} from Young Detached Leaves of Squash (Cucurbita pepo) Immersed in Distilled Water for 1/2, 2 and 24 Hours

Isotope	Leaching Period		
	1/2 Hour (percent)	2 Hours (percent)	24 Hours (percent)
Ca^{45}	0.5	4.1	18.0
K^{42}	0.8	1.56	3.0
P^{32}	0.2	0.5	1.5

Replenishment of Leached Nutrients

The findings of Lausberg (44) that continued leaching resulted in nutrient losses several times the amount found in the leaf at any one time, indicated that leached nutrients were being replaced in the leaf from other plant parts. Similar results were reported by Mes (61), in which nutrients leached from tomato leaves were replaced by nutrients from other plant parts.

To investigate this suggested replacement mechanism, young squash plants were permitted to root-absorb either Ca^{45} or P^{32} for 13 days, or K^{42} for 24 hours. The second initiated leaves of these plants were then leached by immersing for 2 hours in distilled water while either (a) still attached to the parent plant; or (b) detached from the parent plant. A greater potential supply of radioactive nutrients was thus available to leaves which remained attached to the parent plant. The cut petioles of the detached leaves were not in contact with the water. At the completion of the 2-hour leaching period, the leaves were harvested and analyzed for radioactivity along with the leachates.

The results are shown in Table V. Considerably more Ca^{45} was leached from attached leaves (9. 4 percent of the Ca^{45} in the leaves) than from detached leaves (4. 8 percent). Similarly, 3. 7 percent of the K^{42} was lost from attached leaves and only 2. 3 percent from detached leaves. Inasmuch as the uptake of both Ca^{45} and K^{42} in both attached and detached leaves was somewhat alike, it is suggested that a significant replacement of leaf calcium and potassium may have occurred in intact leaves during the experiment.

In contrast, however, the loss of P^{32} from detached leaves was more than three times as great as the loss from attached leaves. A possible explanation of this phenomenon is that phosphorus is active in the metabolic processes of the living plant, perhaps to a greater degree than potassium or calcium, and is in a form which is not available or cannot be leached from the intact plant. If these metabolic processes within the plant are interrupted, as when the leaf is detached, phosphorus may be freed or changed to a form more readily leached. If this is the case, then

TABLE V

Comparative Loss of Root-Absorbed Isotopes from Attached and Detached Leaves of Young Squash Plants (*Cucurbita pepo*) Immersed in Distilled Water for 2 Hours

Isotope	Total Radioactivity of Leaf*		Radioactivity in Leachate		Loss	
	Detached (cpm)	Attached (cpm)	Detached (cpm)	Attached (cpm)	Detached (percent)	Attached (percent)
Ca^{45}	1735	3080	87	318	4.8	9.4
K^{42}	32, 768	29, 430	755	1089	2.3	3.7
P^{32}	10, 900	11, 047	190	50	1.7	0.5

*Radioactivity in the leaf after leaching plus radioactivity in the leachate.

additional supplies of phosphorus would not increase the leaching of phosphorus from intact leaves.

On the other hand, calcium and potassium may exist in the leaf in forms which may be more readily leached from both intact and detached leaves. Thus, when there is a ready supply of replaceable nutrients to the leaf, more can be leached.

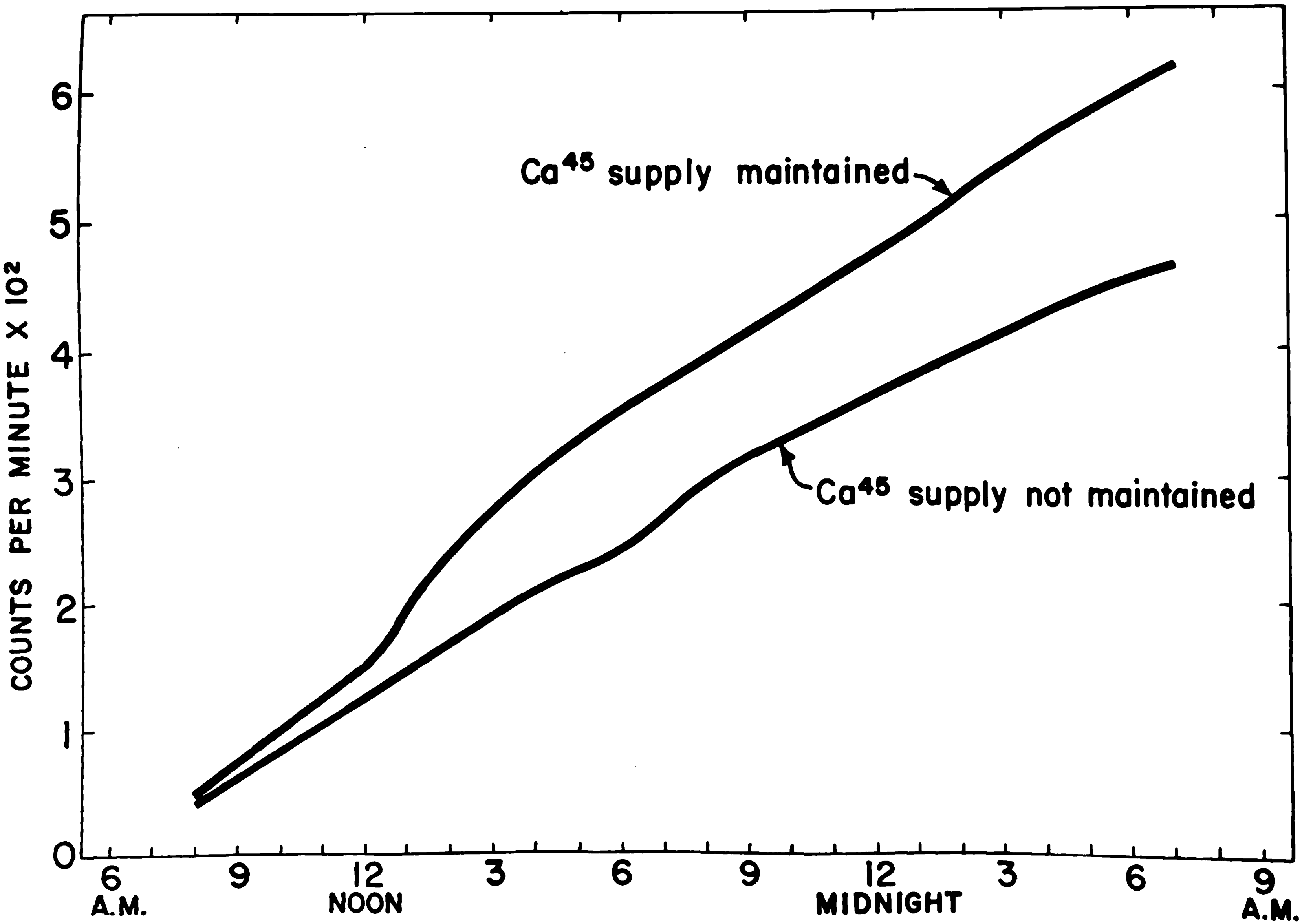
To further investigate this indicated replenishment mechanism, intact leaves of squash plants, which had previously root-absorbed Ca^{45} for 13 days were leached by immersing in distilled water for 24 hours. Two levels of Ca^{45} were maintained in the root media during the leaching process. In the one medium, the Ca^{45} was maintained at a relatively constant level. In the other medium, the Ca^{45} was allowed to deplete naturally during the course of the experiment through root absorption. Hourly measurements were made of the removal of Ca^{45} from the leaves of both sets of plants during the 24-hour period.

The cumulative average hourly losses of Ca^{45} are shown in Figure 8. More Ca^{45} was lost from the leaves by leaching when there was a constant supply (upper curve) of that nutrient in the root medium than when the supply was gradually depleted (lower curve). A replacement of the leached nutrients from other parts of the plant is also suggested.

Determinations were made of Ca^{45} uptake by the plants from the two

Figure 8

Cumulative loss of root-absorbed Ca^{45} from squash leaves (*Cucurbita pepo*) by leaching with distilled water as influenced by the supply of Ca^{45} to the root medium.


COUNTS PER MINUTE X 10²
0
1
2
3
4
5
6
6 A.M.
9
12 NOON
3
6
9
12 MIDNIGHT
3
6
9 A.M.
Ca⁴⁵ supply maintained
Ca⁴⁵ supply not maintained

root media. Five 1-ml aliquots were taken from the root medium of the plant at the start of the experiment and at 8-hour intervals. The aliquots were placed in metal planchets, dried under a heat lamp, and analyzed for radioactivity.

The results of these analyses are given in Table VI. It can readily be seen that the root medium in which the Ca^{45} level was maintained varied only slightly in radioactivity throughout the experiment. In contrast, the root medium in which Ca^{45} was not maintained shows a steady decrease in radioactivity indicative of nutrient uptake by the plant.

The results shown in Figure 8 and Table VI demonstrate that as nutrients are leached from leaves, the roots are absorbing more nutrients to replace those lost. Thus, leaching may not only affect the single leaf in direct contact with the leaching solution, but also may alter the nutrient content of the entire plant and of the medium in which it is growing.

Reabsorption of Leached Nutrients

Since plant constituents which are leached by rain and dew are soluble in water, they may perhaps enter the soil solution and be reabsorbed by roots of the same or of different plants.

To study these possibilities, root-absorbed Ca^{45} was leached from young squash leaves by immersing in distilled water in a flat vessel (Figure 3).

TABLE VI

Radioactivity (cpm) of the Root Media of Young Squash Plants (Cucurbita pepo) During a 24-Hour Period When the Leaves were Being Leached as Influenced by the Supply of Ca^{45} to the Root Media

Ca^{45} Level in Root Media	Time in Hours			
	0 (cpm)	8 (cpm)	16 (cpm)	24 (cpm)
Maintained	227	219	215	223
Not maintained	231	202	174	160

Young seedlings of cucumber, lettuce, corn, tomato, and cabbage were placed so that their roots were in the distilled water which at the same time was leaching Ca^{45} from the squash leaves. After 24 hours, the squash leaves were removed, but the seedlings were left in the leaching solution for an additional 24 hours. Autoradiograms of the seedlings were then prepared as described in the section on Materials and Methods.

Figure 9 is an autoradiogram of cucumber (left) and lettuce (right) seedlings showing the uptake and distribution of Ca^{45} which had been leached from squash leaves. The dark or shaded portions indicate zones of isotope accumulation. Likewise, Figure 10 is an autoradiogram of corn (left) and tomato (right) seedlings, showing the uptake and distribution of leached Ca^{45}. Similar results were obtained with cabbage seedlings.

Figure 9

Autoradiogram of (left) cucumber (*Cucumis sativus*) and (right) lettuce (*Lactuca sativa*) seedlings, resulting from root absorption and distribution of Ca^{45} which had been leached from squash leaves (*Cucurbita pepo*).

Figure 10

Autoradiogram of (left) corn (*Zea mays*) and (right) tomato (*Lycopersicon esculentum*) seedlings, resulting from root absorption and distribution of Ca^{45} which had been leached from squash leaves (*Cucurbita pepo*).

This experiment conclusively demonstrates that the nutrients leached from the above-ground parts of one plant may be root-absorbed by the roots of the same or another species of plant. It further demonstrates the last link in the complete cycle of nutrients through the plant and the soil. Nutrients may be absorbed by either foliage or roots, translocated within the plant, leached from the leaves by the action of dew and rain, deposited on the soil beneath, and then reabsorbed by the roots of the same or of different plants.

Light and Darkness

The leaching of most of the radioisotopes studied was apparently not affected by changes in light intensity. For example, in Figure 11 the hourly loss of Ca^{45} from young squash leaves by immersing in distilled water for 24 consecutive hours is compared to the natural variation of sunlight. The upper curve is the fluctuations of solar radiation in foot candles, and the lower curve shows the hourly loss of Ca^{45} in counts per minute.

No apparent relation can be noted between the two curves. The loss of Ca^{45} seems to be a function of time rather than of light intensity. Similarly, no relation between losses and temperature (which in the greenhouse is often a function of light intensity) could be demonstrated.

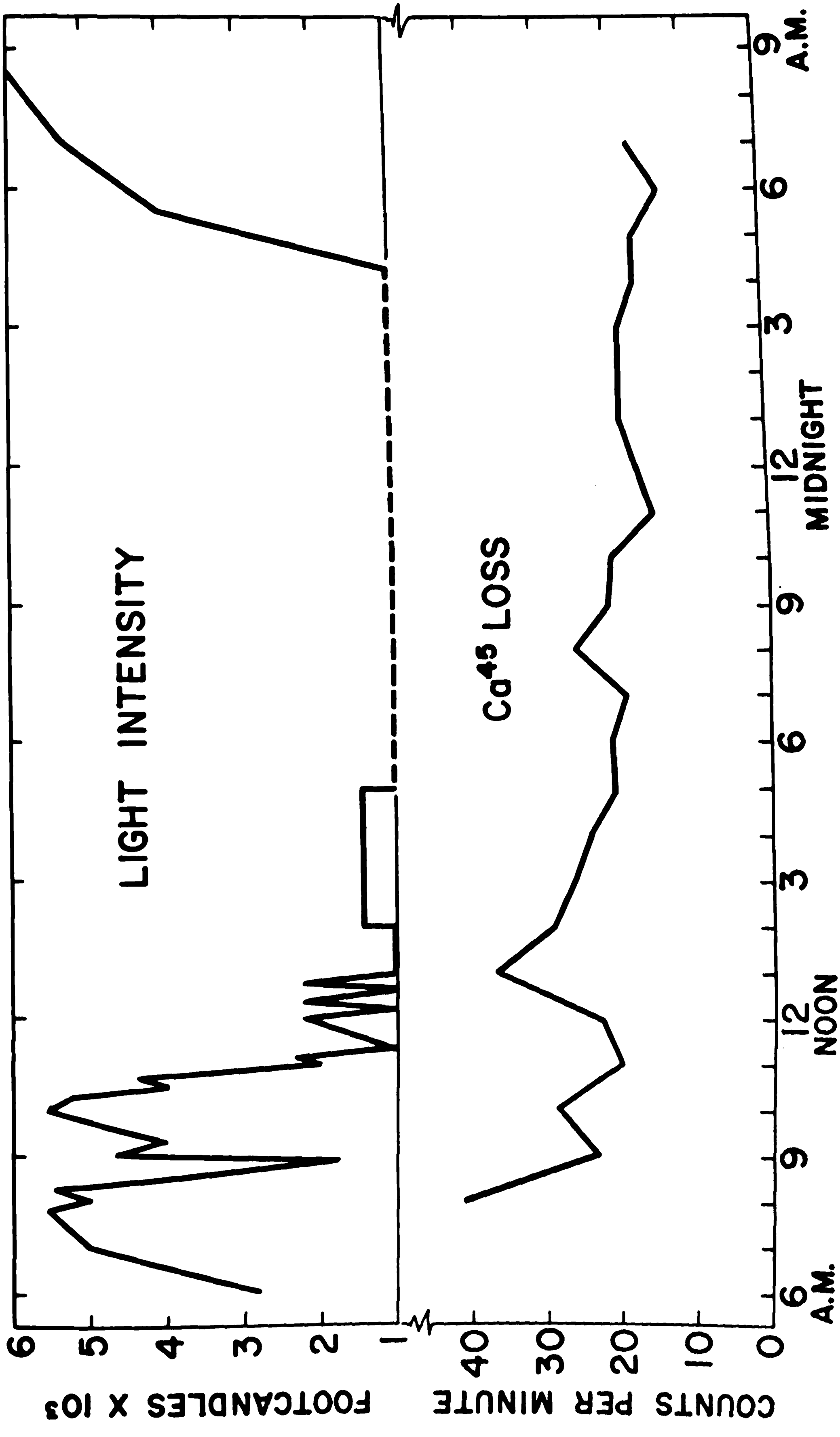
All of the other isotopes studied (Cl^{36}, Fe^{55-59}, K^{42}, Mg^{28}, Mn^{54}, Na^{22}, P^{32}, S^{35}, Sr^{90}-Y^{90}, and Zn^{65}) followed the same pattern as for Ca^{45}

Figure 11

Loss of root-absorbed Ca^{45} from squash leaves (*Cucurbita pepo*) leached with distilled water as related to light intensity.

(Top) Variation of light intensity in foot candles.

(Bottom) Hourly Ca^{45} loss in counts per minute per leaf.


LIGHT INTENSITY
Ca45 LOSS
FOOTCANDLES X 10³
6
5
4
3
2
1
COUNTS PER MINUTE
40
30
20
10
0
6
A.M.
9
12
NOON
3
6
9
12
MIDNIGHT
3
6
9
A.M.

with two major exceptions. Losses of P^{32} from young squash and bean leaves, and losses of S^{35} from young squash leaves were consistently greater in the darkness than in the light, as seen in Table VII. Hourly losses of P^{32} from squash and bean leaves were almost twice as great in the darkness as in the light, and losses of S^{35} from squash leaves were three times as great in darkness as in light. However, losses of S^{35} from bean leaves were slightly greater in the light than in the darkness.

Nutrient Level

In all of the previous experiments, optimum levels of all factors necessary for growth were provided, including light, temperature, moisture, and nutrients. Even in the experiment utilizing two levels of Ca^{45}, the supply of nutrients was adequate.

The question naturally arises as to the degree of foliar leaching from nutritionally deficient plants. Accordingly, squash seeds were germinated in quartz sand and the seedlings transferred to aerated solution cultures at two nutritional levels. One culture contained half the nutrient intensity of Hoagland's standard solution (39)--the solution generally used throughout these investigations. In the second solution, phosphorus was withheld, and the osmotic concentration of the resulting solution was adjusted with the other nutrient components to approximate the osmotic concentration of the

TABLE VII

Loss of Root-Absorbed Isotopes from Plant Foliage by Misting with a Water Spray for 24 Hours as Influenced by Light and Darkness

Plant	Isotope	Total Loss		Loss per Hour*	
		Darkness (cpm)	Light (cpm)	Darkness (cpm)	Light (cpm)
Squash	P^{32}	95	29	6.0	3.2
Bean	P^{32}	123	49	8.4	5.2
Squash	S^{35}	276	105	25.2	8.4
Bean	S^{35}	198	215	15.2	19.6

*Calculated from Total Loss.

half-Hoagland solution. The first solution (half-Hoagland) was designated the "control-phosphorus" solution, and the second solution was called the "low-phosphorus" solution.

Equal amounts of P^{32} were added to each culture vessel regardless of nutrient concentration, and the plants were allowed to root-absorb the P^{32} for 14 days. At that time, the second initiated leaf from plants grown at both phosphorus levels was leached by a water mist spray for 24 hours and the radioactivity in both the leachate (as adsorbed on resin columns) and the leached leaves was determined. Before leaching, the low-phosphorus plants were somewhat dwarfed and showed a slight yellowing of the leaves.

The results are given in Table VIII. Loss of P^{32} from the low-phosphorus plants amounted to 1.5 percent of the total radioactivity in the leaf, as compared with a loss of only 0.09 percent from the control plants. This is a better than 15-fold increase in the percent of phosphate leached from phosphorus-deficient plants as compared with those grown at the half-Hoagland phosphate level.

Uptake of P^{32} (cpm/g dry weight) was 50 percent higher in the low-phosphorus plants than in the control-phosphorus plants. This would be expected, as the only source of phosphorus to the low-phosphorus plants was from the small amount of labeled phosphorus (less than 1 ppm) added to the root medium, as the original phosphorus in the seed had been already

utilized in germination and growth. The control-phosphorus plants, on the other hand, had two sources of phosphorus, namely, the P^{32} added and the non-radioactive P^{31} phosphorus from the nutrient solution. The latter source contributed most heavily to the total phosphorus uptake by the plants. The dwarfed appearance of the low-phosphorus plants is shown by the lower dry weight of leaves from these plants, as compared with the dry weights of leaves of the control-phosphorus plants.

A second experiment utilizing Ca^{45} and squash plants employed the same procedures as with P^{32}. Two levels of calcium in the root media were used, namely, half-Hoagland nutrient solution (control-calcium) and osmotically adjusted half-Hoagland solution minus calcium (low-calcium). However, since the seedlings failed to grow in the low-calcium solution, 3 ppm of calcium were added. Equal amounts of Ca^{45} were added to each culture vessel, and after 14 days the second initiated leaves of the plants were leached by a distilled water spray and the losses determined as with P^{32}.

The results are given in Table VIII and are similar to those from the P^{32} studies. Loss of Ca^{45} from the low-calcium plants was 2.1 percent of the isotope in the leached leaves, as compared with 1.3 percent from the control-calcium leaves. Uptake of Ca^{45} (cpm/g dry weight) was 75 percent higher in the low-calcium leaves than in the control-calcium

TABLE VIII

Leaching Loss of Root-Absorbed Ca^{45} and P^{32} from Leaves of Young Squash Plants (*Cucurbita pepo*) by Spraying with Distilled Water for 24 Hours as Influenced by Two Levels of Calcium and Phosphorus in the Root Media

Isotope	Root Medium	Loss (percent)	Dry Weight of Leached Leaf (g)	Total Radioactivity* of Leaf (cpm/g dry weight)
P^{32}	Low-phosphorus	1.5	0.1052	106,455
	Control-phosphorus	0.09	0.1923	70,410
Ca^{45}	Low-calcium	2.1	0.1652	42,643
	Control-calcium	1.3	0.2201	24,836

*Radioactivity in the leaf after leaching plus radioactivity in the leachate.

leaves. The low-calcium leaves were lower in dry weights.

These findings suggest two important points. First, leaching of mineral nutrients from leaves is not just an overflow mechanism by which plants lose nutrients only when the supply is in excess of requirements. The results show that plants which were deficient in a given nutrient, lost more of that nutrient (percentage basis) through leaching than did plants which were not deficient. Second, plants deficient in mineral nutrients or otherwise unhealthy, may be more susceptible to leaching than are healthy plants. In further support of this second statement, it has been observed in our studies that leaves which were cut or bruised or showed necrotic areas, lost greater amounts of nutrients than did apparently intact and healthy leaves.

Two explanations of this increased loss from unhealthy leaves may be given. First, the maturity of diseased or nutritionally deficient plants may be accelerated, and it has been shown in this thesis that older leaves lose more nutrients than do younger leaves. Second, cuts or lesions may provide a direct pathway for cell contents to wash from the leaf.

Nature of the Leaching Solution

Rain water contains varying amounts of dissolved substances (22). As it drips downward from leaf to leaf through a plant, the content of dissolved substances may increase, so that the lower leaves of the plant may be subjected to leaching from water with a higher salt concentration than are the upper leaves.

Accordingly, the possible effect of various salts in the leaching solution on the loss of nutrients from plant leaves was studied. Solutions of 0.1 M KH_2PO_4, 0.01 M KH_2PO_4, 0.25 M NaOH, 0.25 M $CaCl_2$, and tap water containing small amounts of Ca and Mg salts were utilized. Distilled water was used as the control. Leaves of young bean, corn, squash, and tomato plants which had previously root-absorbed Ca^{45} or P^{32} for 14 days, or K^{42} for 24 hours, were removed from the parent plant and immersed for 2 hours in 130 ml of the above-named solutions. Care was taken to prevent the cut end of the petiole from coming into contact with the solution.

Table IX shows the percent of the root-absorbed nutrients in the leachate as compared with the leached leaf. Losses of Ca^{45} from bean, corn, and squash leaves varied between 3.6 and 4.1 percent, but tomato leaves lost only 1.1 percent of the Ca^{45}. Losses of P^{32} varied between 0.1 and 0.5 percent for all four crops. Losses of K^{42} from squash and

TABLE IX

Percentage Loss of Root-Absorbed Ca^{45}, P^{32}, and K^{42} from Detached Young Leaves Soaked in Various Aqueous Solutions for 2 Hours

Aqueous Solution	Bean		Corn		Squash			Tomato		
	Ca^{45}	P^{32}	Ca^{45}	P^{32}	Ca^{45}	P^{32}	K^{42}	Ca^{45}	P^{32}	K^{42}
Distilled water (control)	3.6	0.4	3.9	0.1	4.1	0.5	1.6	1.1	0.1	1.0
0.1 M KH_2PO_4	--	--	5.0	0.4	4.8	1.7	--	3.0	0.5	--
0.01 M KH_2PO_4	4.7	0.9	4.6	0.3	3.9	1.1	2.3	--	--	3.7
0.25 M NaOH	1.3	0.1	1.1	1.1*	0.7	11.5*	--	1.1	0.4	--
0.25 M $CaCl_2$	0.5	trace	0.8	trace	0.6	0.3	0.6	0.6	0.1	0.7
Tap water (Ca, Mg)	4.4	trace	4.5	0.1	4.4	0.3	1.3	1.3	0.1	0.4

*Severe leaf injury.

tomato leaves varied between 1.0 and 1.6 percent of the total isotopes in the leaves.

With phosphate in the leaching solution, the loss of P^{32} was increased. With potassium in the leaching solution, the loss of K^{42} and Ca^{45} was increased in proportion to the concentration of potassium in the solution. Similarly, in other experiments, 0.1 M KCl solution increased the loss of Ca^{45} and K^{42} as compared to losses in distilled water.

In contrast, with sodium and calcium in the leaching solution, there was no increase in the loss of Ca^{45} or K^{42}. In fact, the presence of these salts seemed to depress the loss of all three isotopes from all four crops, except where severe leaf injury occurred. In another experiment, dextrose in the leaching solution also depressed losses of Ca^{45} and P^{32} as compared to losses in distilled water. Tap water, however, which contained small amounts of calcium and magnesium salts increased slightly the loss of Ca^{45}, but had no effect on loss of K^{42} or P^{32} as compared to distilled water.

It should be pointed out that there were differences among the three leaching methods (described in the section on Materials and Methods), as regards their ability to remove nutrients from the leaves. These differences may be explained on the basis of the salt concentration in the leaching solution. Greatest losses of nutrients occurred when the leaves were soaked continuously in the same solution. The next greatest losses occurred when leaves were soaked for the same length of time, but with the leaching solutions replaced with fresh water at hourly intervals. The smallest losses occurred with the water mist which continued to spray water onto the leaves.

The greater leaching from continuous soaking can, in part, be attributed to the accumulation of salts in the leaching solution. The other two methods, respectively, provide less opportunity for salt accumulation and result in a decrease in the amount of leaching. However, other factors, such as variations in the wetting properties of the leaf, aeration, and gas exchange, must also be considered.

Effect of Di-ethyl Ether and Chelating Agents

Bukovac (16, 18) has reported that exposure of bean leaves to the vapors of di-ethyl ether allowed basipetal transport of foliar-applied Ca^{45} from the leaf into the stem and the roots.

A similar technique was used in an attempt to increase the loss of Ca^{45} by leaching. Young squash plants which had previously root-absorbed Ca^{45} were exposed to the vapors of 50 ml of di-ethyl ether for 30 minutes in a closed belljar. At the end of the exposure time, the second initiated leaf, either still attached to the parent plant or detached from the plant, was soaked in distilled water for 2 hours. Leaves from control plants non-exposed to the ether vapors were also leached by the same method.

Losses of Ca^{45} from either attached or detached leaves of ether-

exposed plants were approximately the same as losses from comparable leaves non-exposed to ether vapors.

In a second experiment, the same procedure was followed except that half of the ether-exposed and non-exposed leaves were soaked in a 5-percent solution of di-sodium ethylenediamine tetra-acetate (Alrose Chemical Company, Providence, Rhode Island). This compound has been used as a chelating agent for calcium.

Once again, losses of Ca^{45} from either attached or detached leaves exposed or non-exposed to ether vapors, leached by distilled water or a 5-percent solution of a calcium chelating agent, did not vary significantly. Vapors of di-ethyl ether, or use of a chelating agent in the leaching solution, had no apparent effect on the leaching of Ca^{45} under the conditions provided.

Relative Leachability of Isotopes

A survey was made of the relative leachability of the isotopes studied (Ca^{45}, Cl^{36}, Fe^{55-59}, K^{42}, Mg^{28}, Mn^{54}, Na^{22}, P^{32}, S^{35}, Sr^{90}-Y^{90}, and Zn^{65}). Young bean and squash seedlings were grown in nutrient solution cultures containing 50 microcuries of the various isotopes (initially 10 millicuries of K^{42}) as described in Materials and Methods. After 13 days of root-absorption (24 hours for K^{42} and Mg^{28}) an intact primary leaf of each bean plant and the intact second initiated leaf of each squash plant were leached by a water mist spray for 24 hours and the leached isotopes adsorbed on resin columns. The resin was later eluted and the radioactivity of the leachate and the leached leaves was determined. Losses were expressed as a percentage of the radioactivity in the leached leaf as compared with the radioactivity in the leachate. The relative ease of leaching of these isotopes from young leaves is given in Table X.

The most readily leached nutrients were Na^{22} and Mn^{54}. Forty-five percent of the Na^{22} in the leaves was leached during the 24-hour period. Radiosodium was lost in equal amounts whether it was absorbed through the roots or through the foliage. Losses of Mn^{54} amounted to 25 to 30 percent of the isotope in the leaves.

Losses of Ca^{45}, Mg^{28}, S^{35}, K^{42}, and Sr^{90}-Y^{90} were moderately great in decreasing order as named, and varied between 1 and 10 percent of the isotope in the leaves. The heavy metals, Fe^{55-59} and Zn^{65} were leached with difficulty (less than 1 percent) as was also true with P^{32}. Losses of Cl^{36} were extremely small. The low rate of radiochlorine removed may help to explain the occurrence of chlorine toxicity in plants. It is interesting to note that chlorine accumulated in the vascular tissue of the leaf, and thus may not be easily removed by the leaching action of water.

TABLE X

Relative Order of Leachability of Eleven Radioisotopes from Young Squash (Cucurbita pepo) and Bean (Phaseolus vulgaris) Leaves by Spraying with a Water Mist for 24 Hours

Relative Ease of Leaching (in Order Named)		
Easily (25 to 45 percent)*	Moderately (1 to 10 percent)*	With Difficulty ($<$ 1 percent)*
Na^{22}	Ca^{45}	Fe^{55-59}
Mn^{54}	Mg^{28}	Zn^{65}
	S^{35}	P^{32}
	K^{42}	Cl^{36}
	Sr^{90}-Y^{90}	

*Percentage of total radioactivity in leaves.

It should be re-emphasized that young leaves were used in this experiment. If mature leaves had been used, the relative leachability of nutrients would have been much the same, but the quantitative losses would have been greatly increased (see literature review). One exception was potassium, which was leached at a moderate rate from young leaves, but was the most readily leached nutrient from mature leaves. Relative losses of chlorine are also increased from mature leaves (44, 45).

Effect of Oil on Loss of Phosphorus

During the determination of the relative leachability of P^{32}, a small amount of lubricating oil from the air compressor escaped the oil trap and entered the mist spray lines and was sprayed on the leaves along with the water. The loss of root-absorbed P^{32} increased considerably during the time the oil was sprayed on the leaves, although the leaves themselves were apparently not injured.

In an attempt to verify this accidental finding, P^{32}-labeled squash leaves were leached with the water mist for 24 hours. Half of the leaves were leached by distilled water as usual, and the other half of the leaves were leached by distilled water plus a trace of lubricating oil from the compressor.

The results of this experiment are shown in Table XI. Removal of P^{32} from the leaves sprayed with the oil-water mixture averaged 823 cpm per leaf during the 24-hour period. This was almost 3 percent of the P^{32} in the leaf. In contrast, the loss of P^{32} from the control leaves (sprayed with water) was 21 cpm which was only 0.07 percent of the P^{32} in the leaf. Until oil was introduced into the spray lines, losses of P^{32} were low as in the control leaves; but when oil was introduced into the water lines, the losses of P^{32} increased markedly within 30 minutes.

These observations suggest consideration of the possible effect of oils, spray chemicals, and wetting agents on the leaching of nutrients from leaves.

TABLE XI

Loss of Root-Absorbed P^{32} from Young Squash Leaves (Cucurbita pepo) by Spraying for 24 Hours with a Water Mist which Contained a Trace of Lubricating Oil

Treatment	P^{32} Leached (cpm)	Percentage Leached
	(means of four replications)	
Controls (no oil present)	21	0.07
Oil present in mist spray	823	2.95

Loss of Carbohydrates

In addition to mineral nutrients, large amounts of organic materials, principally carbohydrates, can be leached from leaves. Dalbro (22) reported that more than 800 kilograms of organic materials per hectare can be lost from apple foliage during one growing season. Similarly, many other workers have noted that the dry matter constituents of leaves decrease when the leaves are soaked in water (7, 19, 45, 58, 92).

To study the losses of carbohydrates by leaching, bean leaves from plants previously grown in solution cultures for 14 days were immersed in distilled water in a flat vessel for 24 consecutive hours. At hourly intervals the solutions in the vessels were replaced with fresh water and analyzed

colorimetrically for carbohydrates by the method developed by Lunt and Sutcliffe (54). Dry weights of the leached leaves were also recorded.

The curve of the cumulative hourly losses of carbohydrates in milligrams from young bean leaves is shown in Figure 12. It can be seen that in general, the curve increases consistently with time, but at a decreasing rate toward the last few hours of the experiment. The average total loss of carbohydrates during the 24-hour period was 7.5 milligrams per leaf, which was 4.8 percent of the dry weight equivalent of the leached leaves.

The variations in the curve of Figure 12 indicated that some factor other than the duration of the leaching period was affecting the loss of carbohydrates. Accordingly, the influence of several environmental factors was investigated and an interesting relationship between carbohydrate loss and light intensity was noted. This relationship is presented in Figure 13. The upper part of the graph shows the variations of light intensity in foot candles during the 24-hour period, and the lower half shows the hourly losses of carbohydrates in micrograms per leaf. The two curves are remarkably similar, showing an apparent direct relationship between light intensity and leaching of carbohydrates. It will be observed that greatest removal of carbohydrates occurred during the periods of highest light intensity. Only small losses occurred during darkness.

Figure 12

Cumulative loss of carbohydrates from bean leaves (*Phaseolus vulgaris*) by leaching with distilled water during a 24-hour period.

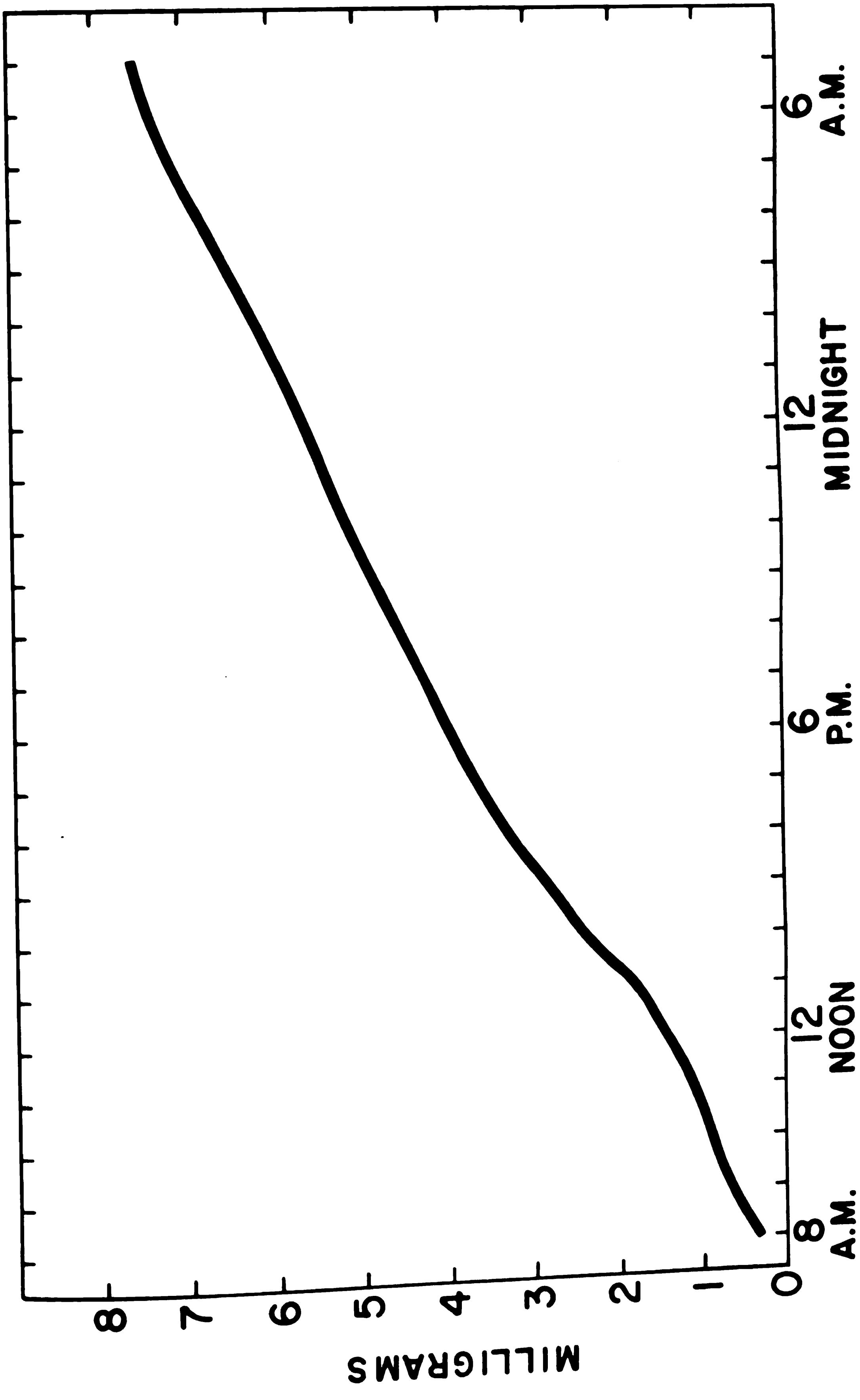
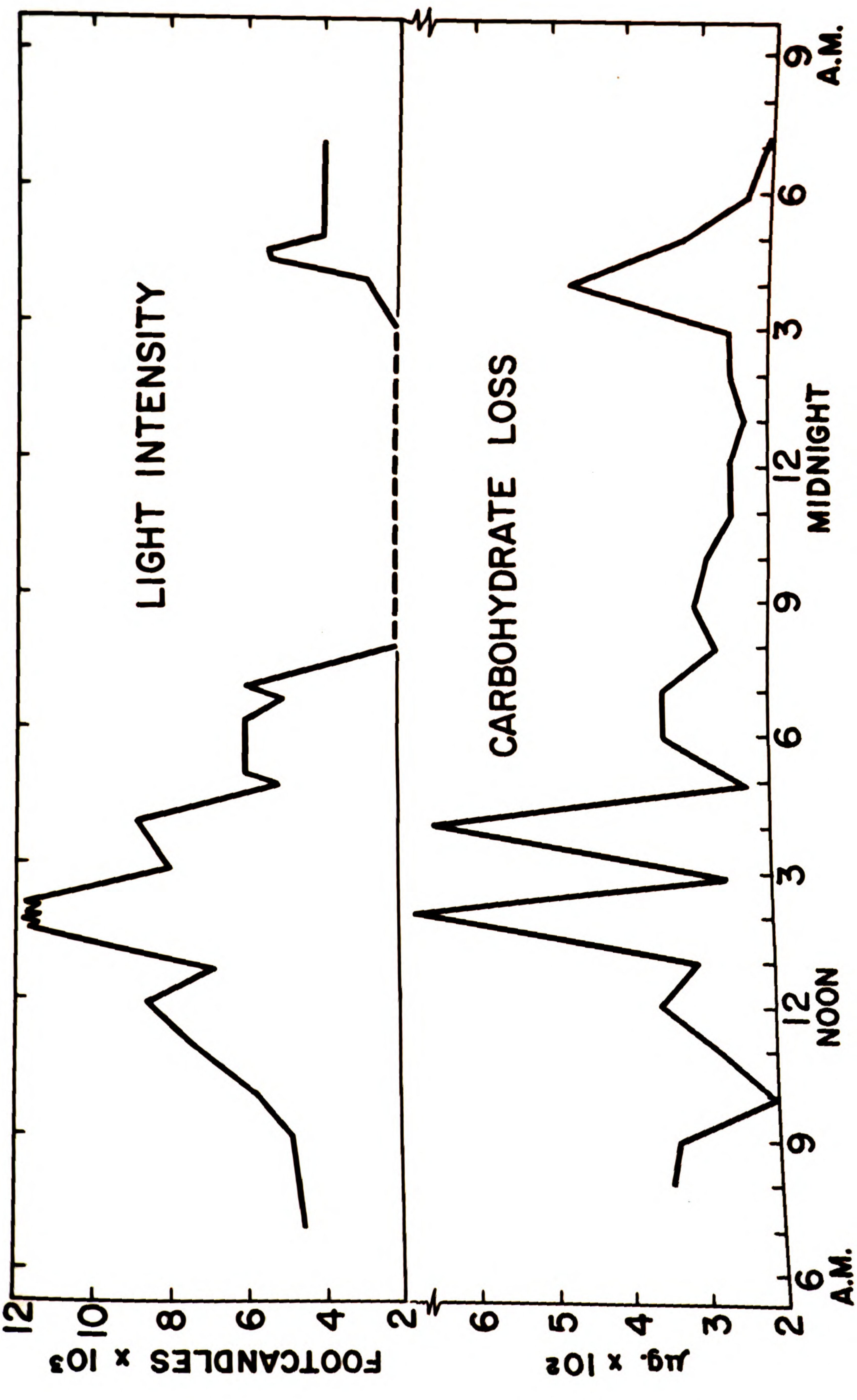

MILLIGRAMS
0
1
2
3
4
5
6
7
8
8 A.M.
12 NOON
6 P.M.
12 MIDNIGHT
6 A.M.

Figure 13

Loss of carbohydrates from bean leaves (*Phaseolus vulgaris*) by leaching with distilled water as related to light intensity.

(Top) Variation of light intensity in foot candles.

(Bottom) Hourly carbohydrate loss in micrograms per leaf.


LIGHT INTENSITY
CARBOHYDRATE LOSS
FOOTCANDLES x 10³
12
10
8
6
4
2
µg. x 10²
6
5
4
3
2
6
A.M.
9
12
NOON
3
6
9
12
MIDNIGHT
3
6
9
A.M.

To further substantiate the relationship between light intensity and carbohydrate loss, two variations were introduced into the experimental procedure; (a) the leaf being leached was left exposed to light and the remainder of the plant was covered with a black cloth; and (b) the leaf being leached was covered with a black cloth and the remainder of the plant exposed to light. Once again, hourly losses of carbohydrates were measured.

In general, the results were similar to those reported earlier. When the leaf was left exposed to light (a) loss of carbohydrates paralleled the intensity of light; when the leaf was covered with the cloth (b), carbohydrate losses were consistently low and did not fluctuate with the changes of light intensity. Although the temperature of the leaching solution and the covered leaf (b) rose somewhat during the hourly intervals, no relation between these rises and the carbohydrate loss could be determined.

Two hypotheses may be suggested to explain the above phenomena. First, increased solar radiation stimulates the photosynthetic activity of the leaf. Since newly elaborated carbohydrates are readily water soluble, they may be in a condition to be easily leached immediately after manufacture. The loss of carbohydrates by leaching may merely be a function of the carbohydrate content of the leaf.

Second, the mechanism of carbohydrate removal may be affected. There is evidence that the number of plasmodesmal connections from the cells to the leaf surface increases in the light (43). The plasmodesmata may aid in establishing a pathway for carbohydrates to be leached from the leaf. Arens (7) has also shown that the sun leaves of trees are more susceptible to leaching than are the shade leaves.

Loss of Nutrients from Fleshy Fruits

The fact that materials can be leached from leaves suggests that perhaps they can also be leached from fruit, especially from fleshy fruits, such as the strawberry (Fragaria spp.).

Strawberry fruits of the Catskill variety were selected in four stages of maturity, ranging from very mature (fully ripe) to immature (green). Utilizing a tuberculin syringe and a No. 27 needle, 0.02 ml of Sr^{90}-Y^{90} (10 uc/ml) were placed on the peduncles of the fruits. Since strontium moves almost exclusively in an acropetal direction (17), the absorbed Sr^{90}-Y^{90} accumulated in the fruit. Two days after treatment, the fruits were removed from the plant by cutting the peduncles at a point distal to the site of application. Fresh weights were recorded. The fruits were immersed in distilled water for 24 consecutive hours, but with the solutions replaced by fresh water at hourly intervals. Care was taken to prevent the cut ends of the peduncles from coming into contact with the water.

The leachates were dried on a steam bath and analyzed for radioactivity in 50-ml beakers. The fruits were oven-dried, weights recorded, and the radioactivity analyzed. Losses were expressed as a percent of the total radioactivity in the fruit.

The results are summarized in Table XII. Losses of Sr^{90}-Y^{90} were approximately 75 percent of the isotope in the fruit, regardless of the stage of maturity of the fruit. This was the largest percentage loss observed from any of the plant tissues studied.

Losses of carbohydrates from strawberry fruits in the same four stages of maturity by the same leaching procedure were determined colorimetrically (54). Dry and fresh weights of the fruits were also recorded. Losses were expressed as a percent of the dry weight of the fruit as compared with the total carbohydrate content of the leachate.

Table XIII shows the results from this experiment. Losses of carbohydrates were directly correlated with fruit maturity. From very mature (fully ripe) fruit, the loss during the 24-hour period was 84 milligrams or 6.02 percent of the dry weight of the fruit. Loss from green, immature fruits was only 4 milligrams, or 1.65 percent of the total dry weight.

During the first 7 hours of leaching, losses of carbohydrates from fully ripe fruit were relatively low but steady at the rate of 1 milligram per hour. For the next 17 hours, losses increased progressively until they reached 7 milligrams per hour during the last 2 hours of the experiment.

TABLE XII

Leaching Losses of Sr^{90}-Y^{90} from Strawberry Fruits (Fragaria spp.) by Immersing in Distilled Water for 24 Hours as Influenced by the Maturity of the Fruits

Fruit Maturity	Total Radioactivity* of Fruit (cpm)	Radioactivity of Leachate (cpm)	Loss (percent)
Mature - fully ripe	807	607	75.2
Mature - firm ripe	742	561	75.6
Immature - coloring	829	637	76.8
Immature - green	761	568	74.7

*Radioactivity in the fruit after leaching plus radioactivity in the leachate.

TABLE XIII

Leaching Loss of Carbohydrates from Strawberry Fruits (Fragaria spp.) by Immersing in Distilled Water for 24 Hours as Influenced by the Maturity of the Fruits

Fruit Maturity	Fresh Weight (g)	Dry Weight (g)	Carbohydrate Loss (mg)	Percent Dry Weight Leached
Mature - fully ripe	18.62	1.39	83.94	6.02
Mature - firm ripe	25.64	1.63	66.96	4.10
Immature - coloring	21.91	1.81	65.02	3.60
Immature - green	1.97	0.25	4.03	1.65

After 24 hours of leaching, the fruits were insipid, lacking in sweetness, poor in color, and of decidedly poor quality. The water in which the fruits had been soaked carried the characteristic aroma of ripe strawberry fruits, but the strawberries themselves had little of this aroma. This substantiates the hypothesis of Cholodny (19) that during wet seasons strawberry fruits may have poor flavor due to leaching of sugars from the fruit by rainfall.

From these observations, it would also appear that a limited exposure to water would not greatly affect the quality of the marketable strawberry fruits, which is of interest to packers and shippers who wish to use hydrocooling as a method of preserving and prolonging fruit quality. However, continued exposure of the fruit to water would certainly entail a sharp reduction in sugar content and general appearance to the point that the product would be unsuitable for sale.

Leaf Analyses of Plants Protected and Non-protected from Rainfall

In order to relate the laboratory and greenhouse findings to field conditions, the degree of leaching by natural rainfall on orchard-grown fruit trees was studied. Eight 5-year-old apple trees (*Malus domestica*) of Jonathan and Golden Delicious varieties growing in the orchard were utilized. Four of the trees, two of each variety, were covered with a roof of clear plastic, as a protection from rain (see Figure 5). The sides were left open, but the roof projected over the edges of the trees to give ample protection except from wind-driven rain. Four other trees, two of each variety, were left unprotected from natural rainfall.

Early in the season (June 11, 1957), after 5.84 inches of rainfall during the preceding 31 days, leaf samples were taken from each tree midway down the new shoot growth and analyzed quantitatively for boron, calcium, copper, iron, potassium, magnesium, manganese, nitrogen, phosphorus, and zinc. Concentrations of nutrients in the leaf samples were expressed as a percentage of the oven-dry weight.

The results of the leaf analyses are shown in Table XIV. The percent calcium was higher in covered trees, as was true with manganese and nitrogen, indicating that these three elements may be leached from young apple foliage. Levels of boron, copper, iron, potassium, magnesium, phosphorus, and zinc were approximately equal from both sets of trees, indicating that these elements were not readily leached from young apple leaves. These results, with the exception of magnesium, agree with the relative leachability of nutrients determined by the radioisotope technique.

A second sampling of leaves from the same eight apple trees was made on September 18, 1957 when the leaves were approaching maturity. Rainfall during the preceding 57 days totalled only 2.10 inches. The samples were handled in the same manner and analyzed for the same ten

TABLE XIV

Comparative Mineral Content of Young Leaves of Orchard-Grown Jonathan and Golden Delicious Apple Trees (Malus domestica) Protected and Non-protected from Natural Rainfall

Nutrient	Non-protected (percent of dry weight)	Protected (percent of dry weight)
B	0.0023	0.0023
Ca	0.85*	0.95
Cu	0.0026	0.0028
Fe	0.0181	0.0180
K	2.06	2.03
Mg	0.31	0.32
Mn	0.0066*	0.0076
N	2.68*	2.90
P	0.20	0.21
Zn	0.0020	0.0021

*Significantly lower than values for protected trees at the 5 percent level.

elements. The results of the analysis are given in Table XV. Levels of calcium, copper, potassium, and manganese were significantly lower in the non-protected trees than in the protected trees, indicating that these nutrients were leached from mature leaves. Levels of iron, magnesium, and nitrogen were lower, but not significantly so, in the trees in the open as compared with the protected trees. Levels of boron, phosphorus, and zinc were approximately the same in both sets of trees.

These results also agree closely with the laboratory and greenhouse findings. A comparison of Tables XIV and XV shows that potassium is more readily leached from mature leaves than from young leaves. The lack of rainfall in the month immediately preceding the leaf analysis undoubtedly reduced the margin of significance between the levels of nutrients in the two sets of trees.

A comparison was made of the leaf composition of plants grown in the greenhouse and plants grown in the field. Cherry trees (*Prunus cerasus*) and grape vines (*Vitis labrusca*) were grown in nutrient gravel cultures both out-of-doors and in the greenhouse. The respective crops received the same treatments in the two environments with the exception that the plants grown out-of-doors were exposed to natural rainfall and dew, and the greenhouse plants were not. Samples of mature leaves from the cherry trees and mature petioles from the grape vines were collected at the end

TABLE XV

Comparative Mineral Content of Mature Leaves of Orchard-Grown Jonathan and Golden Delicious Apple Trees (Malus domestica) Protected and Non-protected from Natural Rainfall

Nutrient	Non-Protected (percent of dry weight)	Protected (percent of dry weight)
B	0.0026	0.0028
Ca	1.44*	1.54
Cu	0.0030*	0.0037
Fe	0.0353	0.0375
K	1.29*	1.50
Mg	0.34	0.37
Mn	0.0074*	0.0102
N	2.24	2.29
P	0.18	0.18
Zn	0.0019	0.0020

*Significantly lower than values for protected trees at the 5 percent level.

of the season. The samples were ground and analyzed quantitatively for calcium, iron, potassium, magnesium, manganese, nitrogen, and phosphorus.

The results of the analyses are presented in Table XVI. In general, levels of all nutrients except iron were lower in plants grown out-of-doors than in plants in the greenhouse. Greatest differences occurred with potassium, calcium, magnesium, and nitrogen in the order named.

In a second experiment, young blueberry plants (Vaccinium corymbosum) were grown in nutrient sand cultures out-of-doors. In July, half of the young plants were brought into the greenhouse; the other half of the young plants remained out-of-doors. Samples of mature leaves were taken from both sets of plants and analyzed as described above. The results of the analyses are shown in Table XVII. Levels of all nutrients except copper and iron were lower in the field-grown plants as compared with the plants in the greenhouse. Greatest differences occurred with manganese, calcium, and magnesium in the order named.

These results agree with the relative leachability of isotopes as given in Table X. A comparison of this table with Tables XVI and XVII shows that, in general, the nutrients which were more readily leached were present at lower levels in the field-grown crops than in the green-

TABLE XVI

Comparative Nutrient Composition of Mature Cherry Leaves (*Prunus cerasus*) and Grape Petioles (*Vitis labrusca*) Grown Out-of-Doors and in the Greenhouse

Nutrient	Cherry Leaves		Grape Petioles	
	Out-of-Doors[1] (percent)	Greenhouse[2] (percent)	Out-of-Doors[2] (percent)	Greenhouse[3] (percent)
Ca	1.35	2.54	0.44	0.99
Fe	0.020	0.0179	---	0.0035
K	1.42	3.24	3.03	5.72
Mg	0.72	0.90	0.32	0.45
Mn	0.010	0.0161	---	0.0063
N	2.81	3.63	0.88	1.06
P	0.186	0.180	0.22	0.37

[1] After E. L. Proebsting, Jr. (62).

[2] After D. R. Dilley (23).

[3] After E. L. Bergman (11).

TABLE XVII

Comparative Nutrient Composition of Mature Blueberry Leaves (*Vaccinium corymbosum*) Grown Out-of-Doors and in the Greenhouse[1]

Nutrient	Out-of-Doors (percent of dry weight)	Greenhouse (percent of dry weight)
B	0.0231	0.0266
Ca	0.47	0.63
Cu	0.0042	0.0033
Fe	0.0287	0.0203
K	0.736	0.920
Mg	0.34	0.45
Mn	0.0066	0.0216
N	2.25	2.39
P	0.22	0.28
Zn	0.0010	0.0011

[1] After H. J. Amling (2).

house-grown crops. These data may suggest one reason why greenhouse crops often have a higher content of certain nutrients than do the same crops grown out-of-doors where rain and dew can leach nutrients from the foliage.

Mann (57) and Councler (21) also noted the higher nutrient content of greenhouse plants as compared to field plants, and Arens (7) was among the first to attribute these differences to leaching by rain and dew.

GENERAL DISCUSSION

The data reported herein and the references cited from other workers prove conclusively that significant losses do occur from above-ground parts of plants, including leaves, fruit, and branches. These findings suggest several areas of practical application.

Foliar Analysis

The use of foliage analysis for the determination of the nutritional status of plants has become an important research tool. It has been shown earlier in these studies that measurable amounts of nutrients are lost from leaves after only a very brief exposure to water (30 minutes), and that after a 24-hour exposure, the losses may be quantitatively large. This should be recognized when taking foliage samples, and the time of sampling in relation to previous precipitation should be noted. It would seem reasonable to recommend that comparative leaf samples should not be taken after prolonged rainy periods, or even directly after a short rain storm, as suggested by Arens (8) and Dalbro (22).

Leaching becomes greater as the season progresses, inasmuch as mature leaves are much more susceptible to leaching than are young leaves. Many of the leaf sampling techniques for grower service throughout the country involve taking samples from trees in the late summer or early fall when leaves are susceptible to leaching. Washing the samples prior to analysis will also remove nutrients as Corin *et al.* (20) noted. In addition, some of the variations in composition during a 24-hour period may be caused by the leaching action of dew during the night hours.

Effects on Soil

Leaching not only affects the nutrition of the foliage and plant, but it also may greatly affect the soil or media in which the plant is growing. Bloomfield (13) has shown the effect of leachates on the deflocculation of clay minerals, which would greatly affect the soil texture, aeration, permeability, and exchange capacity beneath the spread of plants. Similarly, Lutwick *et al.* (55, 56) have shown the effect of leachates from decomposing leaves on soil-forming materials, especially as concerned with iron-containing materials. From these reports it would appear that the leaching of nutrients from leaves by rain could markedly alter the soil texture and soil-forming processes, which, in turn, greatly affect the fertility of the soil.

As Dalbro (22) has suggested, the large amounts of carbohydrates in the leachates must undoubtedly have a beneficial effect on the microorganism population in the soil, which, in turn, would hasten the decomposition of organic matter and the release of nutrients for use by the plant. This process could also mean a more rapid depletion of the soil beneath plants and the need for soil building practices.

The use of soil analysis techniques to determine the fertilizer needs of a crop should be based on a knowledge of leaching phenomena. Just as with foliar analyses, soil samples should not be taken from beneath plants immediately after a prolonged rainy period, as these samples may not be representative of the true condition of the soil, but only of its temporary nutrient level.

Application of Water to Leaves

Many modern agricultural practices involve the application of aqueous solutions to above-ground parts of plants. The normal spraying procedures for control of insects and diseases is just one example. It has been shown in this paper that oil in the leaching spray greatly increased the loss of P^{32}. Similarly, the presence of various salts in the leaching solution greatly increased the losses of Ca^{45}, K^{42}, and P^{32}. Commercial spray preparations contain various amounts and combinations of chemicals, including oils, which may influence leaching losses.

Linskens (50) has shown that young leaves are wetted with difficulty (hydrophobic) as compared with more mature leaves. He also noted that surface active agents (wetting agents) influenced the wetting of all ages of leaves. Many pesticides contain wetting agents to increase their effectiveness. Since the major portion of the spray program is applied in the spring and early summer, the wetting agents may increase the leaching action of sprays by reducing the naturally hydrophobic properties of young leaves. Further, during the early part of the growing season there is heavy demand for nutrients by the plant. To lose appreciable amounts at this time by leaching might be critical.

Mist propagation is a second agricultural practice which involves the application of water to above-ground plant parts. The usual procedure is to mist cuttings continuously, or at frequent intervals, for several weeks or until rooting is accomplished. During this period the cuttings and attached leaves may be almost continually bathed in water. Long *et al.* (53) and Sharpe (74) have noted considerable losses of nutrients under mist propagation. Arens (7) suggests that a light rain of this type over a long period of time will leach more nutrients than will a heavy downpour of a few minutes, although the same amount of water falls on the foliage in both cases.

Many growers of floricultural crops grown in the greenhouse syringe plant foliage with water to control certain pests as well as to improve the quality of the crop. The suggestion is made that the syringing, in addition to its pest-control benefits, may also leach organic materials and nutrients from the plants, thus providing a nutrient balance more favorable to high quality.

Overhead irrigation is another agricultural practice which applies to foliage. The application may continue for 6 to 12 or more hours. The irrigation water often contains large quantities of dissolved salts, which will increase the loss by leaching as shown earlier. The increasing use of sprinkler-type irrigation equipment, especially in the eastern United States, suggests that a further investigation of this phase of leaching be undertaken. It is interesting in this connection to observe the comment of Arens (7) that among "practical" men it is well known that rain produces more beneficial effects to plants than does the same amount of water applied as ground-irrigation.

Benefits of Mulch

Wander and Gourley (93, 94), among others, have reported that available potassium increases to a greater degree under a heavy mulch in an orchard than under sod or clean cultivation. Similarly, calcium, magnesium, phosphorus, and boron are found in larger amounts directly beneath a heavy mulch. The authors have attributed these findings to the fact that mulch contains rain-leachable nutrients and decomposable organic matter which are washed from the mulch into the soil. However, the higher concentration of nutrients in the soil under mulch can also be explained in part by the possible effect of the foliar leachate on the mulch. Rain and dew, after passing over the leaves and leaching the nutrients, fall onto the mulch, which acts to prevent run-off; the salt concentration of the water dripping from the leaves is higher than that of rain water so that the leaching efficiency on the decomposing mulch is increased.

Winter Injury

It is well known among fruit growers that a rainy fall season often is followed by winter injury to fruit trees. This has generally been attributed to delayed maturation and hardening-off of the trees prior to severe winter cold.

It is suggested that winter injury after a rainy fall might be caused in part by the leaching of nutrients from the foliage, so that the trees enter the winter season in a nutrient-depleted or deficient state. Dalbro (22) has shown that losses occur even from dormant trees. Therefore, leaching could occur throughout the winter season, and further deplete the already low nutrient supply.

Nutrient Levels of Greenhouse Crops Versus Field Crops

The results included herein give added support to the theory that the natural leaching action of rain and dew is responsible for the low concentration of nutrients in field-grown crops as compared with greenhouse-grown crops.

Councler (21), Mann (57), and others (7, 45) have noted such differences. Arens (7) was among the first to suggest leaching as a causal factor.

As reported herein, the nutrients which were at a lower level in field-grown crops (exposed to rain) as compared to greenhouse-grown crops (protected from rain) were those nutrients which were readily or moderately leached. Not only is there a difference in the nutrient level of crops in the two environments, but also the differences occur only with those nutrients which are readily leached. This is a strong supporting argument for Arens' suggestion.

One may argue that many other factors influence the accumulation of nutrients, such as sunlight, temperature, and humidity. An answer to this question is supplied from the results reported for orchard trees in close proximity to one another, except that some trees were protected from rain on the foliage. Foliar analyses of these trees again showed a lower concentration of leachable nutrients in trees in the open as compared with the protected trees.

Rückwanderung

The apparent Rückwanderung of nutrients from senescing leaves back into the stem has always been a topic for discussion. After reviewing the literature and the experimental results of this investigation, it seems that no single explanation will suffice, but rather that the phenomenon is the result of at least three separate functions. First, leaching of nutrients from leaves by rain and dew occurs throughout the season, becoming especially pronounced as leaves approach maturity. Many nutrients, principally calcium, potassium, sodium, magnesium, manganese, and sulfur may be removed by this method. Second, large amounts of nutrients are removed from the plant in leaf fall during the season, especially in the autumn. Third, some nutrients migrate out of the leaves back into the stem as Sachs (71) suggested, but by no means is this process the sole explanation for *Rückwanderung*.

Leaching and the Tropics

Varying amounts and intensities of rainfall occur in different parts of the world and at different seasons of the year. Presumably, plants in tropical areas are especially subject to the leaching action of rain. Bhan *et al.* (12) have mentioned that banana trees produce poor fruit yields during the rainy season, intimating that this might be due to the leaching of essential nutrients by the rain.

The profuse vegetative growth of the tropical rain forest can be explained in part by leaching. The heavy rainfall is capable of leaching large amounts of carbohydrates, so that the carbohydrate-nitrogen relationship may be appreciably altered and result in conditions favorable to vigorous growth.

Arens (7) has suggested that the rain forest is a natural plant population particularly well adapted to cuticular excretion, which employs rain for excess salt removal.

Mechanism of Leaching

Arens (7) has suggested that excess salts are actually excreted through the cuticle and onto the leaf surface. The salt residue is subsequently washed off the leaf. This view of active excretion was also accepted by Lausberg (44) and Mes (61) as evidenced by their use of the term "excretion". Engel (25) felt that a passive diffusion of substances from the underlying cells to the leaf surface might occur, but he did not agree with Arens' active excretion concept.

Mann and Wallace (58) proposed that the outer leaf surfaces controlled leaching in some way. Varieties and species differed as to the degree of control. Arens also showed the effect of the cuticle on leaching. When the cuticle was removed from cabbage leaves, the loss of nutrients increased. Waxing the leaf surface decreased the losses. Arens theorized that the epidermis was not impermeable as had been previously accepted, and that pores actually existed in the cuticle.

Evidently the stomata are not the primary agent in the leaching mechanism. Lausberg showed that upper leaf surfaces lost up to 70 percent more nutrients than did lower leaf surfaces. Arens noted the same situation with leaves from many plants. Seemingly, if stomata were active in excretion, losses should have been greater from the lower surfaces where stomatal openings were found in greater abundance in the plants studied.

Strugger (80, 81) used dyes to trace the movement of water and dissolved material from cells. He found that substances did not pass directly between cells, but rather moved along the cell walls. He also noted that the materials followed the cell walls to the cuticle and passed through pores or openings in the cuticle to the leaf surface and outside atmosphere. The rate of movement was apparently related to the intensity of transpiration, being most rapid during periods of increased transpiration.

Since that time, it has been shown that the cuticle is not impermeable, nor is it a continuous layer over the surface of the leaf (75). Investigators of foliar absorption of nutrients (75, 104) have noted that the cuticle is broken in many places, and in fact is not present in certain leaf areas. Absorption of nutrient sprays is in part apparently by diffusion and osmosis through the cell walls of the epidermis.

Other workers have found that pectinaceous materials (68) and plasmodesmata (43) project through the cuticle from the epidermal cells which provide a direct link with the leaf surface. Similarly, pits have been found in the outer cell walls of the epidermis of many plants, including conifers (49). Thus, the leaf has many direct connections between the inner cells of the mesophyll and the leaf surface.

It would appear from these reports that leaching can take place by a variety of methods. First, in areas where the cuticle is absent or discontinuous, materials can escape to the leaf surfaces by diffusion or osmosis through the epidermal cells. Second, where the cuticle is present, materials can follow the transpiration stream along the cell walls and upward to the leaf surface by means of the plasmodesmata or pectinaceous projections. Third, materials may pass through the pits in the epidermal cell walls and be washed to the ground by rain or dew.

Thus apparently, the opposing views of Arens and Engels may be reconciled in that leaching may consist of both an active excretion and a passive diffusion.

Conclusions

The suggestion is made that more attention should be given to leaves, fruit, and branches as organs capable of both uptake and loss of organic and inorganic constituents through surface contact with aqueous solutions. This ability of above-ground plant parts to absorb as well as lose constituents may have pronounced effects on yield and quality, susceptibility to disease and nutritional disorders, and plant adaptation and distribution.

SUMMARY

Losses of plant constituents from above-ground parts of plants by leaching with aqueous solutions were determined by radioisotope techniques. Several factors which were investigated affect these losses.

Greater losses occurred from leaves of 4-week-old squash plants than from leaves of 2-week-old plants. The amounts from very young leaves were small, but increased directly with the maturity of the leaves. Losses were largest as leaves approached senescence.

Susceptibility to leaching varied among crops. Bean, corn, and squash lost greater quantities of Ca^{45}, K^{42}, and P^{32} than did tomato and sugar beet.

Leaching of Ca^{45} from young squash leaves increased directly with the duration of the leaching period. Measurable amounts of loss were detected after only 30 minutes of leaching.

Attached leaves lost greater amounts than did detached leaves suggesting a replacement of leached nutrients within the leaves. Losses were greater from leaves of plants growing in culture solutions in which the Ca^{45}-level was maintained than were losses from leaves of plants growing in a Ca^{45}-level which was allowed to deplete naturally by root-absorption. When the leaves were leached, the roots were simultaneously absorbing nutrients from the root medium. These findings indicate that as

nutrients were leached from leaves, they were replaced by nutrients from other plant parts including the roots.

Calcium^{45} leached from squash leaves was reabsorbed by the roots of cabbage, corn, cucumber, lettuce, and tomato seedlings, indicating that materials leached from one plant may be root-absorbed and utilized by plants of the same or of different species.

Phosphorus-déficient squash plants lost fifteen times as much P^{32} (percentage basis) as did control plants. Calcium-deficient squash plants lost 2.1 percent of the Ca^{45} present in the leaves as compared to only 1.3 percent from control plants. Leaves which were cut, bruised, or diseased lost greater amounts of nutrients than did healthy leaves.

The leaching of Ca^{45} was apparently not affected by changes in light intensity. This was true of most of the other isotopes studied (Cl^{36}, Fe^{55-59}, K^{42}, Mg^{28}, Mn^{54}, Na^{22}, Sr^{90}-Y^{90}, and Zn^{65}). However, losses of P^{32} and S^{35} were greater at night than in the day.

The relative leachability of eleven radioisotopes from young leaves of squash and bean was determined. Isotopes which were readily leached were Na^{22} and Mn^{54}, whose losses varied between 25 and 45 percent of the isotope in the leaves. Isotopes moderately leached included Ca^{45}, Mg^{28}, S^{35}, K^{42}, and Sr^{90}-Y^{90}. One to 10 percent of these isotopes were removed. Losses of Fe^{55-59}, Zn^{65}, P^{32}, and Cl^{36} were less than 1 percent of the isotope in the leaves.

Removal of P^{32} was greatly increased by the presence of oil in the leaching spray. Losses of Ca^{45} were not increased by exposure of the plant to vapors of di-ethyl ether, and to a solution of di-sodium ethylenediamine tetraacetate, a calcium chelating agent.

Potassium in the leaching solution increased the loss of Ca^{45} and K^{42} as compared to losses in distilled water. Phosphate increased the loss of P^{32}. Calcium and sodium depressed the loss of both Ca^{45} and K^{42}. Dextrose in the leaching solution depressed the loss of all three isotopes.

In addition to mineral nutrients, large amounts of organic materials, principally carbohydrates, were leached from leaves. Losses were apparently directly related to light intensity, being largest when the light intensity was high, and being low during hours of darkness. Losses from young bean leaves amounted to almost 5 percent of the dry weight equivalent of the leaves.

Materials were also leached from a fleshy fruit (strawberry). Carbohydrate loss varied directly with the maturity of the fruits, being 6.0 percent of the dry weight from fully ripe fruits, and only 1.7 percent of the dry weight of immature green fruits. Losses of Sr^{90}-Y^{90} were approximately 75 percent of the isotope in the fruits regardless of the maturity of the fruits.

Leaf analyses of apple, blueberry, cherry, and grape showed a lower concentration of leachable nutrients in plants grown out-of-doors as compared to plants protected from rain either by a plastic roof or in the greenhouse.

The suggestion is made that more attention might well be given to above-ground plant parts as organs of both uptake and loss of nutrients.

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