HISTOLOGICAL CHANGES AND NUTRIENT LOSSES FROM PLANT FOLIAGE UNDER MIST PROPAGATION

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Dale V. Sweet

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Charles L. Hamner
Major professor

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A THESIS

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ABSTRACT

The effect of a mist treatment on the leaching of root and non-root absorbed radioactive phosphorus^{32}, potassium^{42}, and rubidium^{86} from plant foliage was investigated to determine the nutrient losses from cuttings of certain horticultural plants which were placed under the mist method of propagation. Studies of misted and non-misted cross sections of leaves and stems of *Phaseolus vulgaris* (variety Cranberry), *Malus sylvestris* (Malling IX), and *Prunus mahaleb* (clone 3-16) were included to observe any histological changes which may have occurred during the water-mist treatment.

Rosa odorata (variety Better Times), and *Phaseolus vulgaris* (variety Cranberry) absorbed radioactive phosphorus^{32} for forty-eight hours through the cut surfaces of their stems prior to being placed under a water-mist spray for a similar period of time. The leachate was collected and analyzed for radioactive phosphorus^{32} which was expressed in per cent of total uptake of the isotope in the misted cuttings.

Phaseolus vulgaris (variety Cranberry), and *Ipomea batatas* (variety Gold Coast) which had absorbed phosphorus^{32} through their root systems were not leached of the tracer under the water-mist treatment.

Root and non-root absorbed radioactive potassium^{42} was recovered in greater quantities from the leachate of *Phaseolus vulgaris* (variety Cranberry), *Euphorbia pulcherima* (variety Albert Ecke), and *Ipomea batatas*

(variety Gold Coast) when absorption had occurred in the dark prior to the water-mist treatment. The greatest amount of leaching of radioactive potassium^{42} occurred when the isotope was absorbed in the dark through the cut stem surfaces of plant materials.

Comparative percentages of the leaching of root-absorbed rubidium^{86} from softwood cuttings of *Phaseolus vulgaris* (variety Cranberry) which had received different environmental conditions prior to a forty-eight hour mist treatment indicated that the greatest loss of the isotope occurred after absorption of the rubidium^{86} at full nutrient levels in the dark as compared to low nutrient cultures. Those plants which had absorbed rubidium^{86} in the light under either high or low nutrient levels were not significantly different in per cent of leaching of the tracer during the water-mist treatment.

Histological changes were observed between misted and non-misted cross sections of leaves and stems of plant materials. An absence of reserve foods in the palisade layers of the leaf sections was accompanied with early maturation and accumulation of carbohydrate products in the stem tissues of misted plants as compared to non-misted plants of the same physiological age.

C.L.H

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INTRODUCTION

Fluctuations of nutrient levels within the plant has been under careful investigation since the time of Leibig. The causes of nutrient imbalance have occupied a large part of the scientific literature.

During the last one hundred years, suspicion of nutrient losses from plants due to rain, fog, or dew has been suggested from time to time. These investigators have attempted to gather the leachates from trees, or separated leaves exposed to excessive moisture conditions, to determine the extent of this loss.

Evidence of foliar absorption of nutrients practiced in recent times may further support these theories. The fact that plants can absorb minerals through their leaf surfaces, suggests the possibility of certain mechanisms, which would allow the leaves to lose nutrients under periods of precipitation.

Gradual adjustment of plants to these environmental conditions is shown by observations of leaf morphology of rain forest foliage compared to the structural arrangement of cell types in the leaves of plants native to regions having normal amounts of rainfall.

Recently, new attention to the loss of nutrients from plant foliage by leaching has developed with the use of continuous or intermittent sprays

in the vegetative propagation of plants. This new method for the rooting of cuttings accelerates root growth and increases the number of roots in certain species to a greater extent than that which is possible with similar cuttings rooted under high humidity conditions.

However, nutrient deficiency symptoms on mature leaves growing under mist treatment and chlorosis appearing in the young leaves of newly rooted plants are commonly observed when cuttings are rooted under mist.

The purpose of this investigation was to determine, by the use of radioactive tracers, the leaching of certain nutrients from plant foliage during periods of mist. Histological studies of leaf and stem tissues of certain plants were used to observe any morphological changes which took place in the tissues, and which function to increase or decrease the leaching of nutrients during periods of mist.

REVIEW OF LITERATURE

During the latter part of the nineteenth century, the ash content of plants was actively investigated in an effort to determine the exact fertilizer needs of important field crops.

German chemists noted apparent differences in total ash content of plants in various stages of growth. Immature plants increased in mineral content until the onset of flowering. Following the vegetative condition in which highest activity of nutrient absorption decreased was a period of loss in ash content representing up to fifty per cent of the total amount of some elements at the time of harvest.

Investigators attempted to explain this phenomenon in various ways. Outstanding among these concepts, as reviewed by Mes (17), was the theory of Wilfarth, Roemer and Wimmer (26), who suggested that as the plant matured, the surplus minerals not needed in metabolism including certain nitrogenous materials used by the plant during active growth, were translocated through the stems and roots and "excreted" into the soil. Other workers considered that the loss of leaves due to diseases, insects or defoliation were responsible for a decreased mineral content at maturity.

Kent and Patrick (11) grew corn from the green mature to the mature stage, analyzing samples at four different times. Ash, fat, fiber

and compounds containing nitrogen decreased in the leaves, husks, and stalks. The kernels increased in all these constituents. Although no rain fell during the entire period, considerable dew was evident. There was a certain amount of mechanical loss due to defoliation in harvesting the crop.

LeClerc and Breazeale (14) were the first to conclude that nutrients were washed from plants by the action of rain or dew. In a series of controlled greenhouse experiments with potted plants of wheat, barley, rice, oats and potatoes, they attempted to show through elaborate sampling methods that the nutrients moved upward, not downward, or were translocated to reproductive structures or growing points during the growth processes. The analyses seemed to demonstrate that as the part of an organ dies, the nitrogen and phosphoric acid tend to move from the dying organ into the living tissues and toward seed producing areas, whereas potassium moved only into the living stems and leaves. The upper nodes were found to be richer in ash constituents than the lower nodes. It was believed that if a downward movement of nutrients had occurred in the plant, the lower portion should increase in mineral content. There was no evidence of increased nutrients in the root area from flowering to harvest.

Convinced that the loss of ash constituents was not caused by

the downward translocation of materials, that is, a physiological process, LeClerc and Breazeale carried out experiments on cereals and potatoes to observe the effect of natural and artificial rains, and short time soakings on plant parts. Results of these experiments indicated losses of plant constituents, which usually increased with increasing maturity, from full bloom to the time of maturity. The exposure of plants to natural and artificial rains had no appreciable effect upon the loss of nitrogen during the early stages of plant growth, but as the plants approached maturity or at the dead ripe stage, as much as seven per cent of nitrogen was lost. When plants were in the flowering stage, phosphoric acid was not always leached; however, when the plants were in the mature stage, losses up to thirty-six per cent of phosphoric acid occurred following leaching. Approximately fifty per cent of the potassium, soda, magnesia, and chlorine which was present in plants at the time of heading, was washed from the plants by the action of rain. In the case of continued rains, administered intermittently, most of the leaching usually occurred during the first rainfall.

The investigators finally concluded that a portion of the nutrients used by the plants are returned to the soil, but no complicated physiological process is involved. Nutrient losses occurred by the simple "dissolving" action of rain water on the salts which have been "exuded" upon the surface

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of leaves. They calculated that the analysis of mineral constituents of harvested plants are not a true estimate of the amount of nutrients taken up by the plants from the soil, because some leaching may have taken place at earlier periods or in larger amounts than others.

This evidence appeared to end the active research on leaching for nearly two decades. Mann and Wallace (16) reported on some deficiency symptoms appearing on the leaves of Coxes Orange Pippin apple trees. Purple bloches eventually became necrotic and defoliation followed in severe cases. These indications of leaching were presumed to have been caused by an extremely wet summer of 1924. They were able to duplicate the results artificially the following summer by immersing the apple leaves in cold water for short periods of time. Analyses of the leaves indicated losses in dry matter of 30 per cent, ash content of 56 to 60 per cent, and 80 to 90 per cent of the potassium. By limiting the amount of soaking, the leaves would return to a clear green color.

Lees (15) observed that the previous season's rainfall may have affected apple yields. The leaching of nutrients from the leaves may have had some influence upon the subsequent formation and development of floral primordia and this affecting fruiting.

Arens (1), working with the apple, found that the leaves which were soaked for twenty-four hour periods would lose the equivalent of fifty

per cent of their total ash content, depending upon the environmental factors and the species of the plant. The action was decreased by the use of wax coatings over the leaves. He concluded that excretion was controlled by the epidermal cells, explaining that the phenomenon was an ecological-geographical function.

Lausberg (13), by arranging daily soakings of living material over a period of eighteen days, found that the total excretion in the case of potassium and calcium amounted to two and three times that quantity left in the leaves at the conclusion of the experiment. Soft rains removed more than hard rains. The adaxial surface excreted larger amounts than the abaxial epidermis. Mature plants lost more nutrients than those in active growth.

The composition of rainfall collected underneath the crowns of forest trees was investigated by Tamm (22). He reported that large amounts of organic matter, calcium, potassium and lesser quantities of phosphorus and nitrogen were found in the leachate as compared to rainfall collected in an open field.

Mes (17) reported extensive experiments on potted plants of tobacco, tomato, potato, bean, pea, and maize plants which were subjected to natural and artificial rain and soaking of leaves for very short periods. The plants were grown under several conditions of controlled

light and temperature. Radioactive phosphorus^{32} was absorbed through the root systems of certain plants.

She was unable to show any connection between the proportion of ash contained in plants and their size or weight. Those plants protected from rain contained more ash than exposed plants. However, natural rainfall, supplemented by artificial rains did not further increase the amounts of nutrients lost from plants. Leaves of plants growing in water culture which were soaked for ten minutes, released about ten per cent of the phosphorus^{32} which had previously been absorbed by the plant. No correlation existed between the amount of the isotope taken up by the plants and the quantities released by rain or by the soakings. The amount of "recretion" showed a closer relationship to the time of year than to the total uptake.

Mes used the term "recretion" as defined by Frey-Wyssling (7), stating that it refers to the elimination of substances which have not been assimilated or changed in metabolism of plants, namely mineral nutrients that are excreted by tuttation and glands, insoluble calcium compounds in cells, silica and other deposits capable of being washed out of cell walls. On the other hand, "secretion" should be only organic substances and enzymes which are direct products of assimilation, and "excretion" is named for dissimilation products of metabolism.

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The theory developed by Mes of recreted substances was based on the work of Strugger (21), who found that fluorescence dyes such as the sodium salt of oxypyrenetrisulphonic acid and berberine sulphate were not transported from cell to cell, but were observed to be rapidly transported upward through the cell walls while they moved into the cells at a slower rate. The velocity of transport depended upon the intensity of the transpiration. The dye accumulated in the epidermal cell walls or hairs at the surface. As the water moved out of the plant leaf by evaporation, the dye particle was left behind on the surface.

These spots could be located under ultra violet light with the use of a fluorescence microscope. She claimed that there was no reason to doubt why mineral salts, traveling upward in the intermicellular spaces, could not move in a similar fashion to the dye particle, eventually accumulating in the epidermis and cuticle. Thus, the minerals may be recreted by the action of rain or dew. No mention was made of the method by which the organic substances reported in the literature to be leached from plants were lost.

Will (27) studied the loss of mineral nutrients from tree crowns by rain. Considerable amounts of sodium, potassium and magnesium with lesser quantities of phosphorus and calcium were found to be present in collected water.

Dalbro (5) recently has calculated the loss of nutrients from mature apple trees during one season of rainfall to be approximately 800 to 900 pounds to the acre. Approximately ten pounds is calcium, ten pounds is sodium, twenty to thirty pounds is potassium, and the balance of the loss is organic matter.

As far as can be determined, few reports have appeared in the literature to date, specifically outlining the loss of nutrients due to leaching of plants under the mist method of rooting.

The fact that mist propagation has been slow to develop may account for the absence of information on the leaching of nutrients from cuttings. Although Raines (19) mentioned the unsuccessful efforts of Spencer to root cacao cuttings under mist in 1936, it was not until O'Rourke (18) and Watkins (24) introduced suitable apparatus for the use of mist that this method of rooting was brought to the attention of propagators.

The present method for the rooting of cuttings under mist does not differ widely from the humidification system which has been in use for many years. Both methods require similar equipment. The mist treatment involves the use of an atmosphere of excessive moisture beyond saturation, and provides a constant film of water to the leaf surface. The mist prevents excessive transpiration from the cuttings, and also induces a cooling effect upon the foliage. This mist treatment may permit greater exposure to light

than would be possible by humidification which requires a closed system.

Consequently, the physical conditions involved in mist spray may be similar to the soakings, immersions, and soft rain applications which have been reported previously in the literature to be the causes of nutrients lost from the foliage of plants.

Evans (6) reported that cacao cuttings under mist developed a yellowish green appearance. He associated it with a slow breakdown of chlorophyll. Analysis of the leaf tissues showed that nitrates and phosphates were leached during the first two weeks, while potassium was lost constantly from the foliage.

Snyder (20) mentioned a yellowish-green appearance on the new growth of Taxus cuttings rooted under mist; that less leaching occurred when mist was applied intermittently.

Wells (25) noted that excess water used with constant mist did not appear harmful to cuttings. It caused accelerated rooting and improved the root system, provided that good drainage was secured to remove the excess water. However, he points out that various plants respond differently to the mist treatment. Varieties differ in their ability to remain alive under extreme moisture conditions.

Recently, many modifications in the application of mist to cuttings have been developed, such as intermittent systems (3), timing devices (8),

and the electronic leaf (9) for improving the rooting of cuttings. Little effort has been made to analyze the plant parts histologically to determine the effects that excess applications of water in the form of mist may produce in the cuttings during the rooting period.

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MATERIALS AND METHODS

A. Preliminary Trials

During the summer of 1953, an open-air mist propagation system was installed at the Michigan State University horticultural farm at East Lansing, Michigan. Cuttings of East Malling II, VII and IX apple rootstocks and *Prunus mahaleb* cherry clones were placed in beds to observe rooting conditions under continuous mist.

Probelms such as air circulation, exposure of the cuttings to full sunlight under mist, drainage, and the leaching of nutrients were studied. Side screens were erected to control wind currents which caused drifting of the mist away from the cuttings, while exposing them to the full sunlight. Under these conditions, leaf burning and defoliation of the cuttings resulted. Drainage of excess water from the mist beds was accomplished by installing propagation boxes containing quarter-inch hardware cloth on the under side, allowing the water to drain from the medium.

As a result of the mist treatment, nutrient deficiency symptoms were observed on the leaves of cuttings during the period of rooting. Brownish spots appeared on apple foliage, which in severe cases developed into black necrotic areas covering one-third of the entire leaf blade (Figure 1). Defoliation resulted when the cuttings were left under mist conditions for extended periods. Removal of the new plants from the mist to pots of soil immediately

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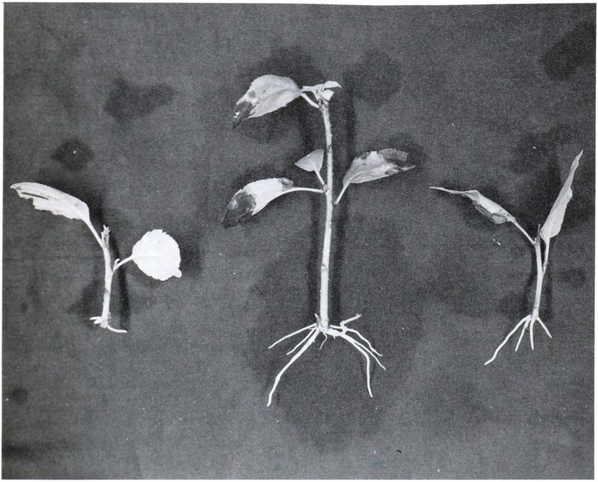
Figure 1. The toxic effect of a prolonged mist treatment on the leaves of Malling II, VII, and IX rootstocks.



after rooting controlled this condition. If the transplanted cuttings were not protected from full sunlight for a period of three days after potting, the leaves wither and turn brown. Defoliation did not always occur in these cases; the dried leaves sometimes remained on the stems. When the transplants were shaded from the full sunlight for three days, they initiated new growth and the old leaves remained green and intact upon the stems in high light intensities.

Other trials of Prunus serrulata (variety Kwanzan) and Rosa odorata (variety Better Times), which were rooted under mist in greenhouse benches in the summer required shading from full sunlight for a three day period after transplanting. When direct sun was allowed to shine on the leaves for a few hours, extreme leaf burn appeared during the first three days. Subsequent sunlight of any intensity failed to cause burning of leaves or necrotic areas. Where the shading technique was employed, the old leaves remained green and intact on the young growing plants.

B. General Procedure

It was suggested that work be conducted with radioactive materials in an effort to determine if certain essential nutrients were leached from the leaves of plant materials under the mist method of propagation.

Certain advantages could be gained by the use of isotopes in the investigation. Comar (4) states that when a tracer is introduced into a plant, it will distribute itself in the proportion to the stable form present in the organs of the plant. Simple measurements of its activity give more information in a comparatively short time than could be obtained in a more difficult and often impracticable chemical analysis. Within this particular study, the fact that the period of entry of the isotope into a plant can be controlled, allows the investigator to determine the leachability of a nutrient in relation to the time and place of absorption. In addition to this, minute quantities of the isotope may be measured with considerable accuracy.

Accordingly, radioisotopes phosphorus^{32}, potassium^{42}, and rubidium^{86} were selected as tracers for the leaching experiments. Additional studies on the histological sections of leaf and stem tissues of the treated and non-treated plant materials were undertaken.

Plant materials used for the investigations were either secured from existing stock in the plant science research greenhouses or were grown from seed and moved into the experimental greenhouses when they were

needed. Softwood cuttings of Rosa odorata (variety Better Times) were taken from flowering wood of greenhouse stock plants. Garden bean seeds Phaseolus vulgaris (variety Cranberry) were germinated and grown in four-inch clay pots filled with washed white quartz sand*. These were fed Hoagland's complete nutrient solution (10) twice each week until ready to use, unless special nutrient solutions were required. When growing plants for specific nutrient cultures, the necessary concentrations were fed to the different groups. Sweet potato, Ipomea batatas (variety Gold Coast) were grown from cuttings. These were planted in five-inch clay pots containing white quartz sand and supplied with nutrient solution once each week. Poinsettia plants, Euphorbia pulcherima (variety Albert Ecke) were rooted from greenhouse stock plants. They were planted in four-inch pots containing quartz sand, and kept in active growth with nutrient solution. The materials used for the histological studies were taken from leaf and stem sections of cherry, Prunus mahaleb (clone 3-16), and apple, Malus sylvestris (East Malling IX) obtained from propagating stock on the University farm.

The misting apparatus for the radioactive work was constructed of chemically-treated wood-frame, three feet wide by four feet long and four feet high. It was covered inside and outside with 0.005-inch polyethylene film. A hinged door, twelve inches high, covered the entire front of the

*American Graded Sand Company, Chicago, Illinois

frame and fitted securely to prevent any mist from escaping. Inside the gable of the mist chamber, a series of three atomizer nozzles were attached to a three-eighth inch galvanized iron pipe line, which was connected to the compressed air supply. The nozzles were directed downward to stainless steel tanks, eight inches wide by twelve inches long and sixteen inches high for housing the treated plant materials. The tanks were open at the top for receiving the mist spray. Pyrex tubing was connected to the drainage nipple on each tank. The tubing passed through the mist chamber to the collecting carboys below. Any mist which was not confined to the steel tanks within the mist chamber was collected in another carboy for inspection.

The leachate was passed through ion exchangers to separate the isotope from the solution. Amberlite IR-120 and Dowex 2X-10, 50 to 100 mesh were used in 10 x 300 mm pyrex chromatographic tubes. The fines were removed by the decanting method and the resin prepared according to company directions. To increase the flow of liquid through the cation and anion tubes, a water suction pump was used to facilitate the movement of the leachate through the columns. A similar apparatus was used for regeneration with six per cent hydrochloric acid and five per cent sodium hydroxide or ammonium hydroxide, depending upon the tracer elution from the columns.

Dry weight of oven-dried leached and unleached plant materials was determined to the nearest tenth of a milligram.

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Wet ashing of the plant materials followed the method described by Toth et al. (23) with certain modifications. Ten milliliters of concentrated nitric acid were added to each beaker containing dried substance. The beakers were covered with watch glasses and were heated on the steam bath under a hood until digestion was complete. Two and one-half milliliters of seventy per cent perchloric acid were added to each sample for further clearing. They were heated gently until the liquid was evaporated to one to three milliliters in volume. The solutions were transferred to numbered twenty-five milliliter volumetric flasks using three per cent hydrochloric acid for washing and bringing the liquid to volume.

Triplicate one milliliter aliquots were lifted from each flask with pipettes into one-inch porcelain planchets. The aliquots were dried on a simmer hot plate under the hood.

All counts were made with a Model SC 1 B Autoscaler (Tracerlab) equipped with a 1.4 mg/cm^2 mica end-window infinite life Geiger-Mueller tube (Nuclear) in a Mark 3 Model 15 (RCL) iron sample chamber. A model P-10 (Tracerlab) preamplifier was used in the imput circuit to the G. M. tube. The counts were taken in triplicate and averaged. The standard deviation was less than ten per cent.

C. Leaching Studies With Radioactive Phosphorus^{32}

Experiments using radioactive phosphorus^{32} were conducted separately on Rosa odorata, Phaseolus vulgaris, and Ipomea batatas plant materials to determine the amount of the isotope which could be removed from the foliage under a water-mist treatment, similar to the mist method used in the rooting of cuttings.

1. Rosa odorata (stem absorption)

Twenty softwood Rosa odorata cuttings (variety Better Times) were taken from flowering wood of stock plants in the greenhouse. They were selected for uniformity and were trimmed to four lateral leaflets on each of two nodes.

Phosphorus^{32} isotope, amounting to 0.01 microcurrie, was added to 100 milliliters of distilled water solution containing 681 milligrams of KH_2P0_4. The solution was placed in a 500-milliliter beaker which was kept inside another container for safe handling. An "airstone" aerator was inserted into the solution. It was attached to the compressed air line by a pyrex tubing for bubbling air through the solution.

The basal portions of the cuttings were immersed the length of two centimeters into the solution contained in the beakers.

The cuttings absorbed the liquid for a period of forty-eight hours, after which they were trimmed. The two lower leaflets were

removed from the lower petiole and three centimeters of the basal stem were cut off to eliminate contaminated parts. The cut surfaces were dried on the open bench for two hours to prevent exudation into the leachate.

The prepared cuttings were divided into two groups; ten were placed in the mist chamber for treatment and the remaining cuttings were secured into paper bags as non-misted control plants.

The mist-treated cuttings were removed after forty-eight hours from the chamber. Each plant was placed in a paper bag. All plant materials were oven-dried for weighing.

The total leachate collected in carboys during the misting period was passed through cation and anion resin tubes to separate the phosphorus^{32} from the collected water. The elution was removed from the resin column with six per cent hydrochloric acid until the monitor failed to record any tracer in the tube. The solution was brought to standard volume in a 200-milliliter flask and amount of radioactive phosphorus^{32} determined.

2. <u>Phaseolus vulgaris</u> (root absorption)

An attempt was made to determine if phosphorus^{32} could be leached from the foliage of bean plants when the active solution was absorbed through the root systems before the cuttings were placed under mist.

Six pots of Phaseolus vulgaris (variety Cranberry) containing two plants in each pot, were selected from plants receiving bi-weekly applications of one-half concentration of nutrient solution. The plants were selected for uniformity of growth with the first trifoliate leaf extending two inches beyond the primary leaf nodes.

The six pots of plants were placed in individual enameled containers on the experimental greenhouse bench. Ninety milliliters of the active solution, containing 0.01 M KH_2PO_4, and 0.19 microcurries of radioactive phosphorus^{32}, was applied to the surface of the sand in each bean pot. The plants had been previously watered, leaving them at approximate field capacity with the addition of the active liquid.

During the absorption period, the sand was moistened with distilled water sufficiently to maintain turgidity in the plants.

Forty-eight hours later, one cutting from each plant was taken from the pots above the cotyledonary nodes. The basal stems were dipped in warmed paraffin to seal the cut surfaces. One cutting from each pot was placed under mist in the chamber for a period of forty-eight hours. An effort was made to secure an even flow of mist upon the plant material at all times.

After the period of misting was completed, the treated plants were removed, bagged, and oven-dried with the non-misted cuttings taken

from the second plant in each pot.

Later, individual plants were weighed, wet-ashed, and triplicate aliquots prepared and counted.

The leachate was passed through resin columns. The elution was checked as before for radioactivity.

3. Phaseolus vulgaris (stem absorption)

It was thought possible that phosphorus^{32} which had previously been absorbed through the cut stem surfaces of cuttings, could later be leached from the foliage of garden beans when placed under mist conditions.

Ten Phaseolus vulgaris (variety Cranberry) seedlings were selected for uniformity in weight and habit of growth. Cuttings were taken one-fourth inch above the cotyledonary nodes and placed immediately in the active solution which contained 0.01 M KH_2P0_4 with 0.2 microcurries of radioactive phosphorus^{32}.

Forty-eight hours later the cuttings were removed. The basal portions were trimmed to prevent contamination of the leachate. The stems were dipped in warmed paraffin to limit exudation of plant juices into the leachate from cut surfaces.

Five cuttings were placed under mist for forty-eight hours. The leachate was collected in glass carboys.

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After the mist period, all plants were secured in paper bags and oven-dried.

The leachate was passed through resin columns and the eluate brought to standard in a volumetric flask.

The plant materials were weighed, wet ashed, and assayed for radioactive phosphorus^{32} in the usual manner.

4. Ipomea batatas (root absorption)

An active solution of 100 milliliters containing 0.2 microcurries of radioactive phosphorus^{32} in 0.01 M KH_2PO_4 solution had been applied to the sand surfaces of two pots of Ipomea batatas (variety Gold Coast) for a period of fifteen days. The G. M. monitor recorded high activity in all leaves and growing points of the plants.

After the absorption period, four mature leaves with petioles attached and five-inch growing point were selected from each plant. The basal portions of the petioles and stems were dipped in warmed paraffin.

The leaves and growing point from one plant were placed under mist for a period of twelve hours. The other plant materials were reserved as controls.

D. Leaching Studies with Radioactive Potassium^{42}

In reviewing the literature on potassium lost from the foliage of plants by the action of rain or dew, it was decided to investigate the loss of potassium from cuttings which were placed under mist with the use of potassium^{42} as the tracer. Because of the short half-life of this isotope (12.4 hours T 1/2), it was suggested that the experiments be limited in duration, and that the analysis be confined to necessary detail. Consequently, the plant materials were treated in groups and the data gathered using shortest possible procedures.

When the active material arrived, the solutions were prepared simultaneously. The three liters of 0.01 M KH_2PO_4 solution contained 42.5 millicurries of potassium^{42}. The reference solution was checked on the Autoscaler to be 325,917 counts per minute of the tracer.

1. *Phaseolus vulgaris* (stem absorption in darkness)

Twenty cuttings of ten-day *Phaseolus vulgaris* plants, variety Cranberry, were selected for uniformity in weight and habit of growth. They were placed in a beaker containing 250 milliliters of potassium^{42} solution as described. The cuttings were allowed to absorb the solution in the dark until the leaves were found to contain high activity*. The basal stems were trimmed and sealed with warmed paraffin. The cuttings

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*10,000 cpm by G. M. monitor reading.

were divided into two equal weight groups, one-half of them were placed under mist for three hours, and the other half reserved as controls.

After the mist treatment, the plant materials were taken to the laboratory for analysis. Each group was placed in 100 milliliter beakers and digested with twenty milliliters of nitric acid. One milliliter aliquots were placed in planchets, dried and counted when cool.

Five liters of the leachate was collected from the chamber. It was passed through ion exchangers, aliquots taken and counted.

2. Phaseolus vulgaris (stem absorption in daylight)

An additional twenty Phaseolus vulgaris (variety Cranberry) cuttings were taken early in the morning, placed in an absorption beaker, and allowed to take up the active solution of potassium^{42} during daylight hours. In the evening, ten cuttings were trimmed and sealed with paraffin in the usual manner. They were placed under mist for a period of three hours.

The treated and the control cuttings were taken to the laboratory for analysis. Plant materials were digested with nitric acid, aliquots prepared and counted. Seven liters of leachate were passed through ion exchangers, and the elution counted in the usual manner.

3. Phaseolus vulgaris (root absorption in darkness)

Ten pots of Phaseolus vulgaris (variety Cranberry), each one containing two plants, were placed in a dark room in porcelain containers. Fifty milliliters of the radioactive potassium^{42} solution were applied to the sand surfaces of each pot. After sixteen hours, cuttings from the plants were taken above the cotyledonary nodes. The basal stems of ten cuttings were sealed with liquid paraffin and placed in the mist house for three hours. Eight liters of the leachate were collected and taken to the laboratory with the plant materials for analysis. The two groups of cuttings were digested with nitric acid, aliquots prepared and counted. The cations were collected in the resin and the elutate brought to volume. One milliliter aliquots were taken and counted in the usual manner.

4. Euphorbia pulcherima (root absorption in darkness)

Eight Euphorbia pulcherima plants were selected for uniform size and shape. They were placed in porcelain containers in a dark room. Fifty milliliters of the active potassium^{42} solution were applied to the sand surfaces of each of the pots. The plant roots remained in the tracer for eighteen hours.

Four cuttings from the treated plants were taken of equal size, their basal stems sealed in warmed paraffin and divided by weight into two groups. Two cuttings were placed under mist for three hours.

All cuttings were prepared for analysis. One milliliter aliquots were placed in planchets, dried and counted. The eight liters of leachate collected were passed on cation resin, and the elution was brought to volume. Aliquots were taken, dried and connted.

5. Ipomea batatas (root absorption in daylight)

Ipomea batatas plants (variety Gold Coast) growing in five-inch pots were used for the studies. Two pots were placed in porcelain containers to prevent the radioactive potassium^{42} from contaminating the greenhouse bench. One hundred milliliters of potassium^{42} solution were applied to the sand surface of each pot.

When 10, 000 cpm activity was monitored in the leaves of the growing plants, leaves with petioles attached and growing points were selected for their physiological age and uniformity and cut from the vines. All cut surfaces were sealed with warm paraffin.

Cuttings were divided into two equal groups, based on their green weight, and selected for the experiment. One group was placed under mist for three hours, and the other remained as controls. Eight liters of leachate were collected in carboys.

All materials were removed to the laboratory for analysis. The cuttings were digetsted with nitric acid, aliquots taken and counts recorded. The leachate was passed through a cation column. The cations were removed and brought to volume. One-milliliter aliquots were placed in porcelain planchets, dried and counts recorded.

E. Leaching Studies with Radioactive Rubidium^{86}

In reviewing the results of the previous experiments, it was considered probable that the amount of nutrients lost from plant parts subjected to mist treatment may be associated with environmental conditions, such as the absorption of the isotope by the plant while growing under normal daylight conditions, or placed in continuous darkness, and also, may be dependent upon the concentration of minerals taken up in the soil solution.

Accordingly, an experiment was planned in which plants could absorb two levels of nutrient solution both in normal daylight and in continuous shade for forty-eight hours prior to placing the cuttings under mist.

Because of the difficulty of working with radioactive potassium^{42} over an extended period, radioactive rubidium^{86} (19.5 days T 1/2) was selected for the tracer. It was assumed that the metabolic reactions involving either of the two univalent cations were of a similar nature within the plant.

1. Phaseolus vulgaris (different environmental conditions)

Phaseolus vulgaris (variety Cranberry) were germinated and grown without nutrients added in clay pots filled with white quartz sand until the primary leaves were fully expanded. The plants were divided into two groups on the greenhouse bench. One group was fed a complete Hoagland's nutrient solution. The other pots received one part Hoagland's solution to nine parts distilled water.

When the plants were fourteen days old, the first trifoliate leaf began to expand rapidly. Their roots were washed free of sand and the plants were placed in water-culture containers which held seven liters each of their corresponding levels of nutrient solution. Sixteen crocks containing four plants each were placed in two rows of eight crocks on the experimental bench. All cultures were equipped with aerators. The plants were fitted in the top plate with styrofoam fasteners, allowing the root systems to be completely covered by their solutions.

The crocks were randomized in each row which contained four solutions of high level nutrients and four of low level water cultures. A black shade cloth was drawn on wires over one row to produce continuous darkness. The other row of bean plants received the normal daylight.

All plants were allowed to adjust to their solutions for twenty-four hours. After the period, 100 milliliters aliquots of radioactive

$rubidium^{86}$, containing Rb_2CO_3 as a carrier, were added to each solution. The absorption period continued for forty-eight hours.

Eight cuttings were taken from each of the four treatments. The cut surfaces were dipped in warmed paraffin to prevent exudation into the leachate. Each group received four hours of mist spray and the separate leachates were collected in glass carboys.

Similar groups of cuttings were taken from the remainder of the plants at the same time as controls.

The leachates were passed through resin columns and the cations were removed to volumetric flasks. Three aliquots of each sample were placed in porcelain planchets for counting.

All groups of plant materials were oven-dried, weighed, and wet-ashed with nitric acid. Aliquots were taken in triplicate for counting.

F. Histological Studies

Samples were taken for histological preparations of treated and control plants which were identical in physiological age. Two cuttings of garden bean, Phaseolus vulgaris (variety Cranberry) were placed under constant mist. Two cuttings of the same group of seedlings, selected for like habit of growth, were placed in a beaker of distilled water under the same temperature and light environment. After five days, two square centimeter samples of treated and non-treated leaves were placed in formalin, acetic acid, and alcohol (F. A. A. 1:1:18) killing and fixing solution. Similarly, samples of misted and non-misted apple leaves, Malus sylvestris (Malling IX), and cherry leaves, Prunus mahaleb (clone 3 - 16) were placed in F. A. A. solution after a mist period of twenty-five days.

All solutions were immediately taken to the laboratory and aspirated to remove air from the tissues. Dehydration was accomplished through the ethyl-tertiary-butyl alcohol series and the tissues were infiltrated and imbedded in Fisher's tissuemat.

The paraffin blocks were sliced at sixteen and twenty microns thickness on a hand-operated rotary microtome. Sections of misted and non-misted tissues of each plant species were mounted on the same glass slides. The infiltrated agent was removed. The slides were passed

through Conant's quadruple staining procedures. Permanent mountings were accomplished with clarite under cover glass.

Microphotographs were made from Adox KB - 17 film using Ethol ultra fine grain developer. Contrast of tissues was improved with the aid of an orange gelatin filter.

RESULTS

I. Radioactive Phosphorus^{32}

a. Rosa odorata (stem absorption)

It was evident that radioactive phosphorus^{32} was leached from rose cuttings which were placed under a mist of distilled water for a period of forty-eight hours. Upon examination, the leachate was found to contain the isotope which was originally supplied to the cuttings before treatment (Table I).

A wide variation existed in the amounts of the tracer actually absorbed by individual cuttings regardless of their individual weights (Table I). However, when the amount of phosphorus^{32} recovered from the leachate was compared with the total amount of tracer absorbed by the plants for forty-eight hours before the mist treatment, the result indicated a considerable loss of the nutrient. The radioactive material recovered in the leachate amounted to 12.8 per cent of the total isotope absorbed by the treated plants. This was obtained by adding the tracer recovered in the leachate to the amount remaining in the plant materials (Table II).

TABLE I

The Leaching of Non-root* Absorbed Phosphorus^{32} from Softwood Cuttings of Rosa odorata (Variety Better Times) During a Forty-eight Hour Water-Mist Treatment.

Misted				Non-Misted			
No.	Dry weight grams	cpm cuttings	cpm g. wt.	No.	Dry weight grams	cpm cuttings	cpm g. wt.
1	0.751	5530	7364	1	0.906	6166	6806
2	0.733	720	983	2	0.672	4494	6688
3	1.099	6116	5565	3	0.794	8919	11233
4	0.730	2954	4047	4	1.038	7065	6806
5	0.917	4860	5300	5	1.055	7692	7291
6	1.343	4751	3538	6	0.981	5136	5235
7	1.301	7611	5850	7	1.049	5622	5359
8	0.822	4304	5236	8	0.676	3113	4605
9	0.759	8872	11689	9	0.768	2491	3244
10	1.785	10817	6060	10	1.567	11506	7343
Total	10.240	56535	55632**	Total	9.506	62204	64610**

*All plant cuttings absorbed radioactive phosphorus^{32} through the cut surfaces of their stems for forty-eight hours prior to being placed under mist for a similar period of time.

**t value equals 0.8134.

The Leaching of ... of Rosa odorata (... Mist Treatment.

No. Day	
1	[illegible]
2	[illegible]
3	[illegible]
4	[illegible]
5	[illegible]
6	[illegible]
7	[illegible]
8	[illegible]
9	[illegible]
10	[illegible]
Total	[illegible]

* [illegible]

** [illegible]

TABLE II

The Percentage of Non-root Absorbed $Phosphorus^{32}$ Leached from Softwood Cuttings of Rosa odorata (Variety Better Times) during a Forty-eight Hour Water-Mist Treatment.

	cpm	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Control cuttings	62, 226	1, 555, 650		
Misted cuttings	56, 568	1, 414, 200		
Leachate (44 1.)	1, 039		207, 892	
Initial absorption				12. 8

b. Phaseolus vulgaris (root absorption)

Analysis of seventy-two liters of leachate collected from plant cuttings which were held under mist for forty-eight hours indicated that the loss of phosphorus^{32} was negligible, when the isotope was absorbed by the root systems of garden bean plants previous to mist treatment (Table III).

The average of one milliliter aliquots of a 100 milliliter solution of the elution from the anion resin column amounted to approximately two counts per minute over background (Table V). At the same time, one milliliter aliquots of the reference solution contained an average of 12, 467 counts per minute over background.

Variations occurred in the amounts of absorbed phosphorus^{32} in relation to their gram weights of both treated and non-treated cuttings (Table III).

c. Phaseolus vulgaris (stem absorption)

The analysis of the eluate of forty liters of leachate collected from the mist spray falling on five bean cuttings over a period of forty-eight hours indicated that radiophosphorus^{32} was leached from the foliage, when the cuttings had absorbed the isotope through their cut stem surfaces previous to treatment.

TABLE III

The Leaching of Root-Absorbed Phosphorus^{32} from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Forty-eight Hour Water-Mist Treatment.

Misted				Non-Misted			
No.	Dry weight grams	cpm cuttings	cpm g. wt.	No.	Dry weight grams	cpm cuttings	cpm g. wt.
1	.3090	1714	5547	1	.2105	1345	6390
2	.2532	1563	6173	2	.2085	1651	7918
3	.2108	1709	8107	3	.2435	1946	7992
4	.3358	1860	5539	4	.2048	1632	7969
5	.3635	1878	5166	5	.2443	1630	6672
6	.1985	1234	6217	6	.2860	1752	6126
Total		9958	36749*	Total		9956	43067*

*t value equals 1.5320

The actual amount of isotope collected in the leachate was 1.54 per cent of the total absorption of the tracer in the misted plant materials (Tables IV and V).

d. Ipomea batatas (root absorption)

When sweet potato, Ipomea batatas (variety Gold Coast) cuttings, which had previously absorbed radioactive phosphorus^{32} through the root system of the mother vine, were placed under mist, none of the isotope could be found in the leachate.

One milliliter aliquots, taken from the eluted solution of the leachate, were monitored by a count rate meter (Nuclear model no. 1615). No significant activity above background was noted.

TABLE IV

The Leaching of Non-Root Absorbed Phosphorus^{32} from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Forty-eight Hour Water-Mist Treatment.

Misted				Non-Misted			
No.	Dry weight grams	cpm cuttings	cpm g. wt.	No.	Dry weight grams	cpm cuttings	cpm g. wt.
1	0.399	7099	17, 792	1	0.361	9605	26, 607
2	0.413	6584	15, 942	2	0.363	6525	17, 975
3	0.315	5654	17, 949	3	0.302	6651	22, 023
4	0.333	5570	16, 727	4	0.336	10055	29, 906
5	0.417	7468	17, 909	5	0.369	11345	30, 745
Total	1.877	32375	86, 319	Total	1.769	44181	127, 276

TABLE V

Comparative Leaching of Root Absorbed and Non-Root Absorbed Phosphorus[32] from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Forty-eight Hour Water-Mist Treatment (Percentages).

	cpm	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
		Root Absorbed		
Control cuttings	43, 067	1, 076, 675		
Misted cuttings	36, 749	918, 725		
Leachate (72. 1)	0		0	
Initial abosrption				0. 0*
		Non-Root Absorbed		
Control cuttings	44, 181	1, 104, 525		
Misted cuttings	32, 375	8 09, 375		
Leachate (40. 1)	505		12, 625	
Initial absorption				1. 54

*0. 0 value is not necessarily zero, but is not significant above background count rate.

II. Radioactive Potassium^{42}

a. Phaseolus vulgaris (stem absorption in darkness)

When radioactive potassium^{42} solution was absorbed through the cut stems of ten day garden bean seedlings for five and one-half hours in the dark, fifty-five per cent of the absorbed tracer was leached from the foliage within a three hour period of mist spray treatment (Table VI).

b. Phaseolus vulgaris (stem absorption in daylight)

Ten day garden bean seedlings which had absorbed potassium^{42} solution through the cut stems under normal daylight conditions for a twelve hour period before the mist was applied to the foliage, leached thirty-five and one-half per cent of the tracer from the cuttings during three hours of mist (Table VII).

c. Phaseolus vulgaris (root absorption in darkness)

Phaseolus vulgaris (variety Cranberry) which had absorbed radioactive potassium^{42} through their root systems for sixteen hours in the dark, lost nine and two-tenths per cent of the total tracer in the cuttings during three hours of mist treatment (Table VIII).

d. Euphorbia pulcherima (root absorption in darkness)

Poinsettia plants in active growth, which had absorbed radioactive potassium^{42} solution through their root systems for eighteen hours

TABLE VI

The Leaching of Potassium^{42} Which was Non-Root Absorbed in the Dark from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Three Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Non-misted cuttings	1580		
Misted cuttings	840		
Leachate (5 l.)		1050	
Initial absorption			55.5

TABLE VII

The Leaching of Potassium^{42} which was Non-Root Absorbed in the Light from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Three Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Non-misted cuttings	48, 475		
Misted cuttings	34, 560		
Leachate (7 1.)		19, 062	
Initial absorption			35. 5

The Leaching ...
Prof. Hawkinson ...
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Non-selected ...

Mixed ...

Leachate (...)

Initial sorption ...

TABLE VIII

The Leaching of Potassium^{42} which was Root Absorbed from Herbaceous Cuttings of Phaseolus vulgaris (Variety Cranberry) During a Three Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Non-misted cuttings	528, 240		
Misted cuttings	412, 920		
Leachate (8 1.)		41, 750	
Initial absorption			9.2

in the dark, lost thirty-one and four-tenths per cent of the tracer from the foliage when placed under mist for three hours (Table IX).

e. Ipomea batatas (root absorption in daylight)

When leaves and growing points of sweet potato vines were subjected to mist spray for three hours, it was found that they lost two and eight-tenths per cent of the radioactive potassium^{42} which had been taken up by the roots during the absorption period a few hours before leaching (Table X).

TABLE IX

The Leaching of Potassium^{42} which was Root Absorbed in the Dark from Softwood Cuttings of Euphorbia pulcherima (Variety Albert Ecke) During a Three Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Non-misted cuttings	3520		
Misted cuttings	2875		
Leachate (7 l.)		1316	
Initial absorption			31.4

TABLE X

The Leaching of Potassium^{42} which was Root Absorbed in the Light from Herbaceous Cuttings of *Ipomea batatas* (Variety Gold Coast) During a Three Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
Non-misted cuttings	47, 100		
Misted cuttings	46, 320		
Leachate (8 1.)		1, 300	
Initial absorption			2. 8

III. Radioactive Rubidium^{86}

a. Phaseolus vulgaris (different environmental conditions)

The greatest loss of rubidium^{86} when mist was applied for four hours to Phaseolus vulgaris (variety Cranberry) occurred from those plants which were grown in high nutrient levels in darkness for forty-eight hours prior to the mist. The loss amounted to fourteen and four tenth per cent of the total tracer absorbed. The plants growing under low nutrient conditions in darkness for forty-eight hours prior to the mist treatment lost only six and six tenths per cent of the isotope (Tables XI and XII).

The plants grown under normal daylight during the experimental period had synthesized more dry matter and absorbed more isotope than those grown in darkness. However, they lost only five per cent of the active element when their cuttings were placed under a four hour mist. There was no apparent difference in the amount of leaching between plants grown in high or low levels of nutrients under normal light conditions (Tables XI and XII).

There were visual differences between the plants growing under the various environmental conditions. The plants growing under low nutrient levels in normal daylight grew rapidly in size, although

TABLE XI

The Leaching of Root-Absorbed Rubidium^{86} from Softwood Cuttings of Phaseolus vulgaris (Variety Cranberry) which had Received Different Environmental Conditions Prior to Forty-eight Hours of Water-Mist Treatment.

Misted			Non-Misted		
Dry weight grams	cpm (8) cuttings	cpm g. wt.	Dry weight grams	cpm (8) cuttings	cpm g. wt.
1. Complete Hoagland's Solution under Continuous Darkness					
1.207	75,000	62,138	1.313	112,600	85,758
2. One-tenth Hoagland's Solution under Continuous Darkness					
1.065	124,800	117,174	1.190	147,600	124,034
3. Complete Hoagland's Solution under Normal Daylight					
1.850	164,800	89,081	1.814	189,300	104,355
4. One-tenth Hoagland's Solution under Normal Daylight					
2.026	1,321,200	652,122	1.832	1,257,500	686,408

The Leaching [illegible]
of Phenolic [illegible]
and Environme[illegible]
Note: Treatme[illegible]

[illegible]	Dry weight gram
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]

TABLE XII

Comparative Percentages of Leaching of Root-Absorbed Rubidium ^{86} from Softwood Cuttings of *Phaseolus vulgaris* (Variety Cranberry) which had Received Different Environmental Conditions Prior to the Forty-Eight Hour Water-Mist Treatment.

	Total cpm cuttings	Total cpm leachate	Per cent isotope leachate
1. Complete Hoagland's Solution under Continuous Darkness			
Non-misted cuttings	112, 600		
Misted cuttings	75, 000	10, 475	14. 4
2. One-tenth Hoagland's Solution under Continuous Darkness			
Non-misted cuttings	147, 600		
Misted cuttings	124, 800	8, 275	6. 6
3. Complete Hoagland's Solution under Normal Daylight			
Non-misted cuttings	189, 300		
Misted cuttings	164, 800	4, 575	4. 9
4. One-tenth Hoagland's Solution under Normal Daylight			
Non-misted cuttings	1, 257, 500		
Misted cuttings	1, 321, 200	33, 275	4. 9

the trifoliate leaflets were light green in color. The plants growing under high nutrient levels and normal daylight were darker green, and exhibited less elongation than the low nutrient plants. The plants growing under continuous darkness grew less rapidly than those under normal daylight.

Some curling of the primary leaves occurred, perhaps indicating a loss of carbohydrates.

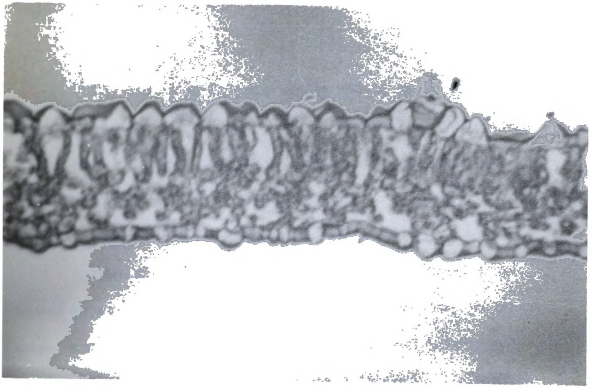
IV. Observations from Histological Studies

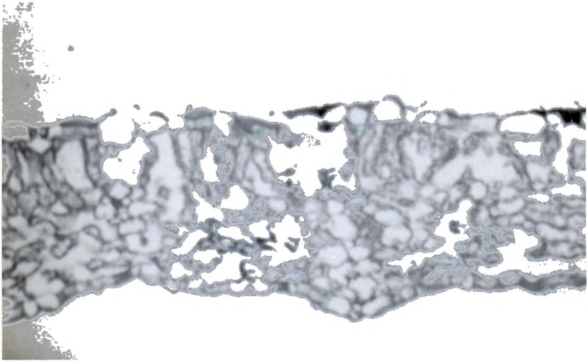
Cross-sections of bean leaves of plants placed under a continuous mist were compared with those from non-misted plants. The sections used for the investigation were selected from tissues of the same physiological age (Figure 2). Observations were made with special reference to the relationship of size and number of cell types within a given radius, special arrangement of respective tissues, and general thickness of leaf blades.

The general size of the various types of cells in the mist-treated samples were found to be considerably smaller than the cells in the non-misted control samples. This was especially noticeable in the epidermal tissue. However, the leaf blade thickness of mist-treated leaves was approximately thirty per cent greater than the non-misted leaves. Upon close examination, this apparent difference may be explained by the greater number of intercellular spaces in the interior of the leaves of the misted plant. This effect of mist-treated leaves resembled the effect which is produced when a seed swells from the imbibition of water.

The average number of cells of the respective tissues were compared, and it was found that within a given area the control section contained twenty per cent more cells than the treated leaves.

Figure 2. Microphotographs of cross-sections of <u>Phaseolus vulgaris</u> (variety Cranberry) leaves of the same physiological age. Top: non-misted leaf. Bottom: leaf given five days of water-mist treatment.





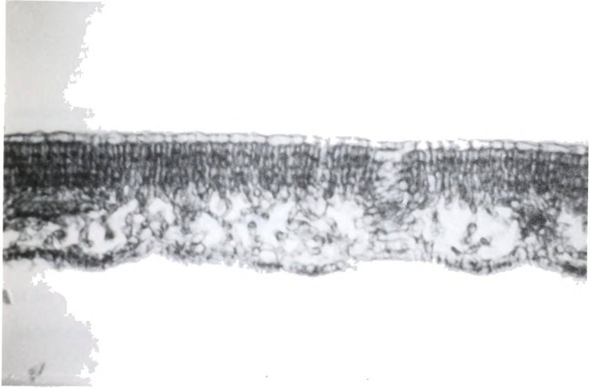
The mist-treated sections did not stain as deeply as the control sections. This was not alone due to the general density of the controls. When viewed under high power lens, the individual cells were highly stained in the untreated tissues, particularly in the palisade layers. The misted tissues were more translucent with the nuclei clearly outlined.

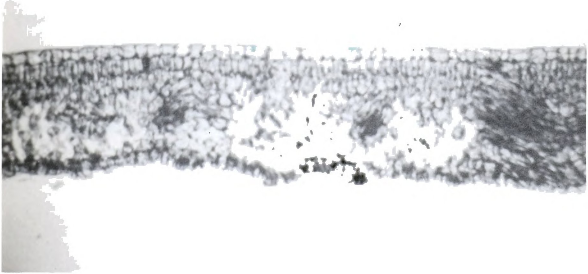
When slides of apple and cherry leaf sections, which were misted for a period of twenty-five days, were examined under the microscope, a similar depth of color was noted in the control sections as compared to the treated tissues. The palisade layers of apple sections of non-misted leaves were dense in color and were regular in form. Similar layers of mist-treated sections were lightly stained, nuclei prominent, and the form irregular. The cells appeared swollen and crowded with few intercellular spaces in the misted sections.

In the non-treated material, the mesophyll cells were well-spaced, orderly, and distinctly visible. Similar tissue which was subjected to mist appeared swollen, indistinct, and occupied all of the intercellular spaces.

In general, the thickness of the mist-treated leaves were equal to the controls. However, with the increase in cell size, the misted tissues apparently filled the intercellular spaces causing an irregular pattern in the leaf section (Figure 3).

Figure 3. Microphotographs of cross-sections of Malus sylvestris (Malling IX) leaves of the same physiological age. Top: non-misted leaf. Bottom: leaf given twenty-five days of water-mist treatment.



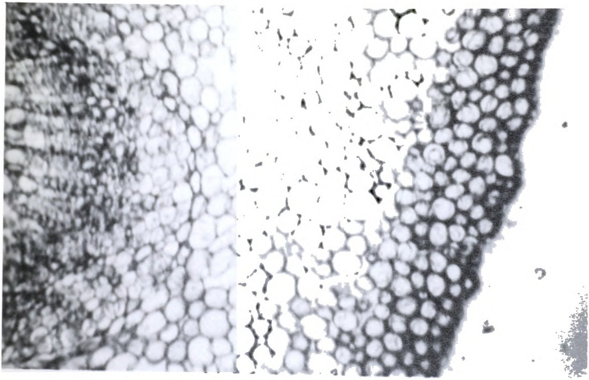


This filling of the intercellular spaces in the mesophyll by swollen cells was especially noticeable in the sections of mist-treated leaves of cherry. Palisade tissues in the control sections were deeply stained as compared with the lightly stained misted tissues. It will be recalled that both treated and controlled sections were passed through the staining procedures on the same slide. Also, the phloem of the veins of the mist-treated materials were generally stained deeply. This was not evident in the control tissues.

Cross-sections of apple stems of identical physiological age of misted and non-misted plant materials are illustrated in (Figure 4). A higher degree of maturation was found in the misted stem sections than in the non-treated stems. This was apparent by the thickening of fiber cells and outer cortical parenchyma tissue. The phloem cells in the vascular bundles and the vascular ray cells were deeply stained in the mist-treated material suggesting additional storage of carbohydrate products in the stems.

Cross-sections of misted cherry stems showed maturation and heavy carbohydrate accumulation in the vascular tissues and the surrounding cells as compared to non-misted sections which remained in a general meristematic condition.

Figure 4. Microphotographs of cross-sections of _Malus sylvestris_ (Malling IX) stems of the same physiological age. Top: non-misted stem. Bottom: stem given twenty-five days of water-mist treatment.



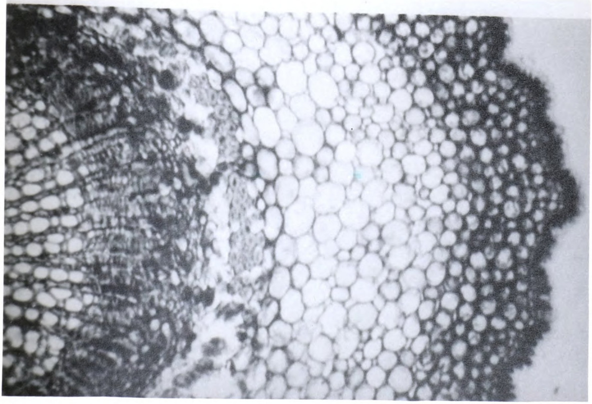
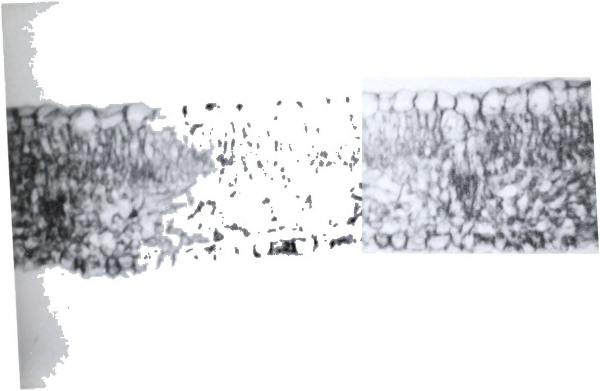


Figure 5. Microphotograph of cross-sections of Prunus mahaleb (clone 3-16) leaves of the same physiological age. Top: non-misted leaf. Bottom: leaf given twenty-five days of water-mist treatment.



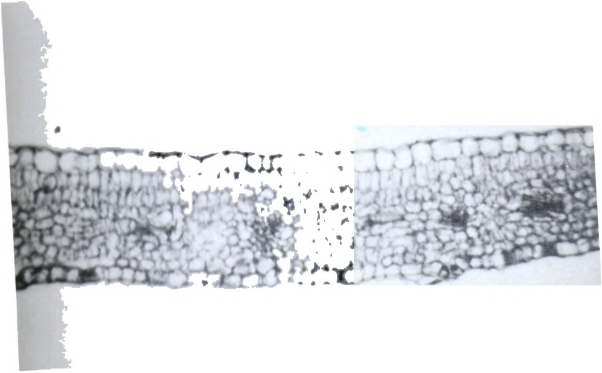
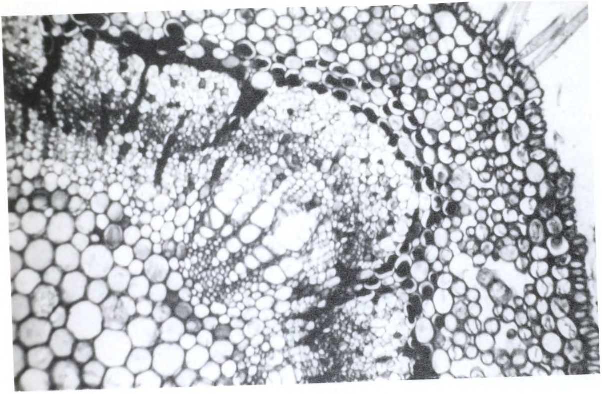


Figure 6. Microphotographs of cross-sections of *Prunus mahaleb* (clone 3-16) stems of the same physiological age. Top: non-misted stem. Bottom: stem given twenty-five days of water-mist treatment.



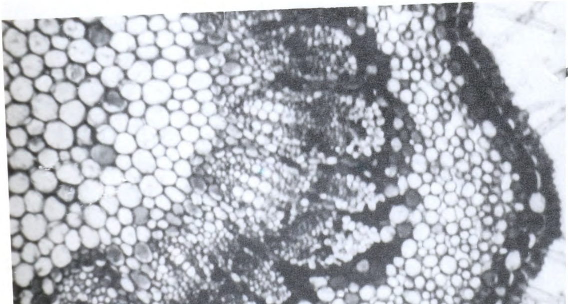
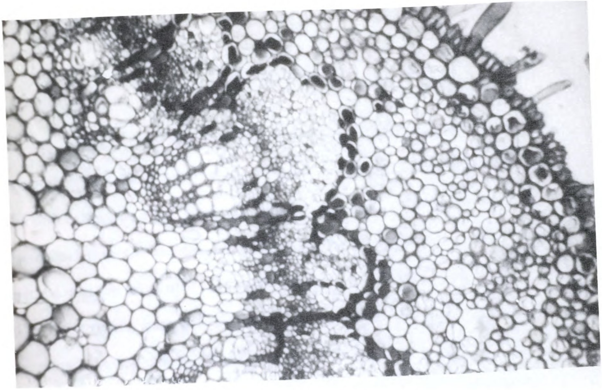
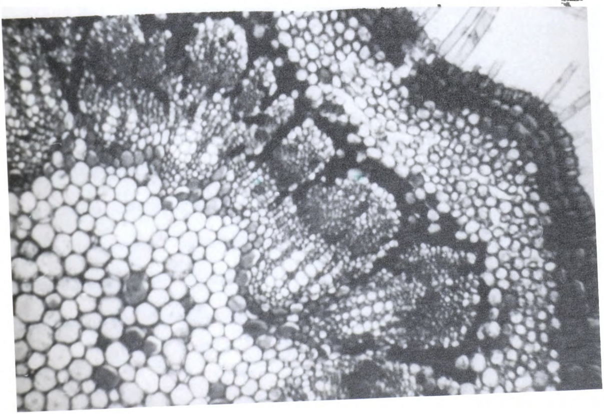


Figure 7. Microphotographs of cross-sections of *Prunus mahaleb* (clone 3-16) stems of the same physiological age. Top: non-misted stem. Bottom: stem given twenty-five days of water-mist treatment.





DISCUSSION

Much of the evidence reported on the leaching of nutrients from plants by the action of water on plant foliage has been based on the differences on a per gram dry weight basis of an ash constituent between the treated and non-treated materials. One exception to this method was the work with radioactive phosphorus^{32} reported by Mes (17). Her values were based upon the per cent of tracer leached from the plant with that originally absorbed by the plant. She was unable to show any appreciable loss of phosphorus^{32} by this method regardless of the length of time of the action on the plant. However, in other experiments, using differences based on per gram dry weight between leached and non-leached materials, considerable loss was evident. Other workers have concluded similar losses of many nutrients based on the differences in the plant materials in dry weight measurements after exposures (Table XIII).

The differences in per cent between the amounts of the element found present in the leached and non-leached materials on the per gram dry weight basis is demonstrated to be a poor criterion for measurement probably due to the differences in absorption of the tracers by individual plants. It has been mentioned previously that LeClerc and

TABLE XIII

Comparative Percentages of the Leaching of Phosphorus^{32}, Potassium^{42}, and Rubidium^{86}, which had been Absorbed by the Plants under Various Environmental Conditions, from Softwood Cuttings Prior to Water-Mist Treatment. The Difference in the Amount of the Isotope in Per Cent per Gram of Dry Tissue Between Misted and Non-Misted Plants is Compared to the Per Cent Recovered from the Leachate.

Isotope	Plant Materials	Environmental Conditions			Per cent	Per cent
		Absorption		Treatment	g. dry wt.	in leachate
Phosphorus^{32}	*Rosa odorata*	Stem	Dark	Light	10	12.8
	Phaseolus vulgaris	Root	Light	Light	14	0.0
	Phaseolus vulgaris	Stem	Dark	Light	27	1.5
	Ipomea batatas	Root	Light	Light	--	0.0
Potassium^{42}	*Phaseolus vulgaris*	Stem	Dark	Dark	56	55.5
	Phaseolus vulgaris	Stem	Light	Dark	29	35.5
	Phaseolus vulgaris	Root	Dark	Dark	20	9.2
	Euphorbia pulcherima	Root	Dark	Dark	20	31.4
	Ipomea batatas	Root	Dark	Light	1.5	2.8
Rubidium^{86}	*Phaseolus vulgaris*	Root	Dark*	Light	28	14.4
	Phaseolus vulgaris	Root	Dark**	Light	5	6.6
	Phaseolus vulgaris	Root	Light*	Light	15	4.9
	Phaseolus vulgaris	Root	Light**	Light	5	4.9

*Complete Hoagland's solution

**One-tenth Hoagland's solution

Breazeale had concluded that the ash constituents of plant materials were not an exact estimate of the minerals used by the plant.

The amount of rubidium^{86} absorbed by the plants depends on the concentration of the nutrient solution applied to the root systems (Table XI).

The environmental factors which surround the plant during growth, the stage of development, the time of year, and the amount of nutrients available to the plant have each been pointed out in the literature as playing a role in determining the amount the loss of nutrients sustained by a particular plant during periods of rain or dew, or mist.

Living plants, which have absorbed their isotopes through the cut stem surfaces, show a greater per cent of loss due to the mist treatment than those plants which have absorbed the tracer only through their root systems. These data may support the theories of Mes and Strugger, that nutrients traveling through the intermicellular spaces of a plant may be simply washed from the surfaces of plants without any physiological process involved. In this particular case, travel through the plant may have been accomplished by diffusion or inhibition into the cut surfaces.

In the histological observations reported, the more deeply stained tissues of the control plants were taken as evidence of storage

materials metabolized from carbohydrates manufactured in the leaves and of minerals accumulated in the surrounding tissues.

Evidence of nutrient losses from cuttings under mist has been established by radioactive tracers. However, the rooting of cuttings has been accelerated and improved under the mist method as shown in the literature.

Histological observations of leaf and stem sections of cuttings rooted under mist have shown accumulation of storage products in the stem tissues and a lack of nutrients in the palisade layers. The vascular system was deeply stained indicating that perhaps the carbohydrates are moving out of the leaf into the stem. Leaf and stem sections rooted under non-misted methods show carbohydrate compounds in the temporary storage layers of the leaf with lesser amounts collecting in stem tissue.

Other evidence of advanced maturation of stem tissues and axillary flower bud initiation is shown in previous work with cherry cuttings rooted under mist. When the cuttings were held under mist for extended periods after the necessary rooting time, flower initiation was evident. The initiation of flower primordia was not necessarily due to maturity as rooted cuttings from the same stock later in the season developed good vegetative growth when transplanted to the soil.

Lees noted that the previous season's rainfall may affect flower bud initiation in the apple. Other investigators have found that certain mist-rooted cuttings when transplanted have a tendency to develop shoots from the transitionary zones of the plant; never again do they initiate growth from the old stem.

Kobel (12) has pointed out that flower bud initiation in fruit trees is associated with the presence of increased amounts of carbohydrates in the immediate vicinity of the axillary buds.

The increased transport of storage materials from the leaves into the stem tissues of cuttings placed under mist may have a direct bearing on the early initiation of roots and also the large number of roots per stem. It is generally accepted that auxin travels with carbohydrates. These may in turn be carried with the sugars into the stem areas where root initials may originate.

The fact that plant parts with mature leaves root well under the mist system would seem to preclude that the amount of nutrients lost by leaching have not impaired the ability of the cuttings to form roots. Biddulph (2) shows accumulation of minerals within the leaf in excess of the quantities used in the plant's metabolism. The possibility of surplus nutrients and organic matter not being vitally connected with the plant processes which motivate root formation and future

growth of the new plant, and which may reside in the intermicellular spaces, as Mes and others point out, may be leached from the leaves of plants without vital injury to them, provided certain precautions are taken.

The observations of the behavior of newly transplanted cuttings rooted under mist in the preliminary trials required a definite shading for a period before they were exposed to the direct rays of the sun in order to prevent leaf burning. These conditions suggested the necessity for renewal of the food supply in the leaf which was depleted of its carbohydrates and minerals to low levels by the action of mist.

Mist-treated transplants growing under reduced light intensities may synthesize a small reserve of food sufficient to withstand the added demands made upon it with increased respiration due to high temperature within the leaf under direct sunlight.

In addition to this, the accumulation in the leaf of certain essential elements such as potassium and phosphorus may be required for fundamental growth processes. The loss of these elements has occurred as demonstrated in the experiments with the leaching of radioactive isotopes under the mist method of propagation.

SUMMARY

1. The effect of a mist treatment on the leaching of root and stem absorbed radioactive phosphorus^{32}, potassium^{42}, and rubidium^{86} from plant foliage was investigated.

2. Different quantities of radioactive phosphorus^{32}, radioactive potassium^{42}, and radioactive rubidium^{86} were independently obtained from collected water that was allowed to fall in the form of mist on cuttings which had absorbed the isotope through their cut surfaces, or which were taken from plants that had absorbed the tracer through their root systems.

3. Greater quantities of potassium^{42} were leached from cuttings under mist when the isotope was absorbed through their cut surfaces than through the root systems of the parent plant.

4. Phosphorus^{32} was not recovered from the mist which fell on bean and sweet potato cuttings when the tracer had been absorbed previously by the root systems of the parent plants.

5. Phosphorus^{32} was found in the leachate from rose and bean cuttings which had previously absorbed the isotope through the cut surfaces of their stems.

6. Garden bean cuttings leached greater quantities of potassium^{42} and rubidium^{86} under mist when the respective isotopes were absorbed in the dark at full nutrient levels.

7. The per cent of a nutrient leached from plants under mist based on the difference per gram dry weight between the leached and the non-leached materials did not correspond with the per cent of the tracer actually lost from the cuttings due to the difference in the relative absorption rates of the individual plants.

8. Prepared slides of thin cross-sections of young bean leaves which remained under mist for five days were twenty per cent larger in size and the cells appeared stretched as compared to sections of non-misted leaves of the same physiological age.

9. Evidence of the transport of reserve substances from the leaves of cuttings under mist to the stem tissues and also evidence of early maturation was observed in the histological sections of apple and cherry cuttings which remained under mist for twenty-five days, as compared to sections of non-misted plant materials of similar physiological age.

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Date Due
