NUTRITION OF THE STRAWBERRY (Fragaria spp.) WITH SPECIAL REFERENCE TO FOLIAR ABSORPTION OF RADIOPHOSPHORUS AND CALCIUM

Thesis for the Degree of Ph. D.

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Robert Alan Norton

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This is to certify that the

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NUTRITION OF THE STRAWBERRY (*Fragaria spp.*) WITH SPECIAL REFERENCE TO FOLIAR ABSORPTION OF RADIOPHOSPHORUS AND CALCIUM

By

Robert Alan Norton

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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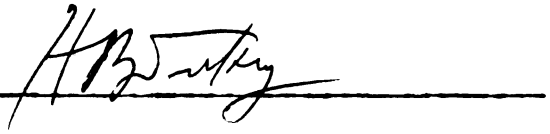
Approved 

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Robert Alan Norton

ABSTRACT OF THESIS

Nutrition of the Strawberry with Special Reference to Foliar Absorption of Radiophosphorus and Calcium

The Robinson strawberry was used to study the comparative efficiency of utilization of radiophosphorus (P^{32}) and radiocalcium (Ca^{45}) when applied to the foliage and to the roots. Plants were grown in sand culture in the greenhouse, using variations of the Hoagland nutrient solution. For foliage treatments individual leaves were dipped, and root treatments consisted of pouring the fertilizer solution around the base of the plants at least two inches from the crown. Through the use of autoradiography and isotope dilution analysis to determine changes in relative specific activities of P^{32} and Ca^{45} it was shown that P^{32} was readily translocated to non-treated parts, especially developing leaves, fruits, root tips and runner plants from both foliage and root applications at a similar rate of movement. In contrast to P^{32}, translocation of Ca^{45} from foliage application was negligible. When applied to the roots Ca^{45} was readily absorbed and translocated to all plant parts including runners but there was little movement into the roots of the attached runner plants. With respect to the efficiency

of leaves and roots in nutrient absorption, the uptake of P^{32} from the leaves was less affected by the phosphate level at which the plants were grown than was absorption from the roots. The rate of foliar uptake of three nitrogen-phosphorus carriers -- mono- and di-ammonium phosphate and phosphoric acid plus urea -- was studied using radiophosphorus and it was found that the phosphoric acid-urea combination was most readily absorbed and utilized. In a study of the influence of the temperature of the root medium on plant growth and absorption of radiophosphorus by leaves and roots it was found that dry weight production by leaves was greatly reduced as the root temperature decreased from 75° F. to 45° F. while dry weight of roots was reduced very little. A highly significant reduction in P^{32} uptake by the roots occurred as the temperature was decreased from 55° to 45° F. while absorption and translocation of foliar-applied P^{32} appeared to be less influenced by the temperature of the root medium. Similar long-term experiments with the roots of the strawberry maintained at various temperatures showed no advantage of foliar applications of nitrogen and phosphorus to plants growing at low (50° F.) root temperatures as compared to the same nutrients applied to the root medium.

In limited field experiments in southwestern Michigan foliage applications of urea, phosphoric acid, di-ammonium

phosphate and a commercial product, Bonro (10-50-10) were tested on a number of strawberry plantings. Pre-bloom and post-bloom foliar applications of fertilizer were made to both the Robinson and Premier varieties in addition to the growers regular fertilizer program. A yield increase was obtained in only one location of low fertility, with no increase on plantings receiving adequate soil fertilization. It was concluded that foliage applications of fertilizer to strawberries in early spring would be advantageous only when an inadequate soil fertility program is followed.

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INTRODUCTION

Until recently, biologists were generally of the opinion that plant leaves were covered with an impermeable layer of cutin broken only by small "pores" or stomata distributed over the surface. It was also believed that the passage of chemical substances into the interior tissues occurred invariably through these structures, if it occurred at all. Since the stomata occupy only a very small proportion of the surface area of leaves, only extremely minute quantities would be capable of absorption in this manner.

Recent investigations, however, have shown that substantial quantities of both the so-called major and minor nutrient elements may be taken in through the leaves and in certain cases the entire nutrient requirement of the plant satisfied. In this connection Roberts, Southwick and Palmiter (93) have suggested that McIntosh apple leaves are not covered with a continuous cuticle. They have shown microchemically that highly absorptive pectinaceous substances are interspersed in the cutin permitting the penetration of all types of materials including plant nutrients, growth regulators and systemic insecticides.

The numerous limitations of soil fertilization have recently led plant scientists to devote considerable attention to the efficiency of leaves as nutrient absorbing

organs. Results have been encouraging. In many areas where trace element deficiencies are known to exist, they are often corrected by leaf feeding. The importance of urea as a foliage spray for apples (41, 42) is an example of the practical significance of leaf feeding.

One of the major problems in fertilizer utilization by plant roots is that of phosphorus fixation. It has been demonstrated repeatedly that much of the fertilizer phosphorus applied to the soil is unavailable to plants. The rest is usually fixed very rapidly by the soil colloids, organic matter or other nutrient elements such as iron or calcium. It has been shown also that many plants require a rather high proportion of their phosphorus during the early stages of growth. Plants which are grown for their fruit such as tomatoes, corn or any of the small fruits are examples of such crops. The tree fruits, however, may be able to accumulate sufficient stored reserves of this element during the previous growing season so that, except in a few instances, little direct response to phosphate fertilizers has been noted.

The use of all soluble forms of phosphate fertilizers both for soil and foliage application may offer a partial solution to this important problem. The investigations presented herewith are an attempt to evaluate the significance and extent of foliar absorption utilizing radioactive isotopes and soluble forms of phosphorus and calcium.

REVIEW OF LITERATURE

A. Nutrition of the Strawberry

1. General Considerations: Some of the most successful growers consider that the strawberry does not require liberal fertilization while others believe that the application of comparatively large quantities of fertilizer is a necessary part of successful culture. Inconsistancy still exists regarding the relative importance of various nutrients, the optimum timing of fertilizer application, and their effect on fruit quality.

In 1919, Mooers (83) stated "Acid phosphate may be the best single fertilizer material for the strawberry grower to rely upon, but acid phosphate, together with some such material as nitrate of soda, is generally needed to give best results". Evelin (40) in Arkansas agrees with this statement but cautions that the use of too much nitrogen at a single application might cause excessive vegetative growth and poor fruit quality. Evelin believed potash to be important though less so than nitrogen and phosphorus.

In contrast, disclaiming the benefits of fertilizer, Latimer and Wentworthy (71) in New Hampshire were unable to derive any benefit from nitrogen or potash and only a slight possible benefit from phosphorus when used in combination with manure. Manure alone gave the greatest benefit by far.

Similarly Hartman, White-Stevens and Hoffman (56) on a sandy soil on Long Island, New York found that the recommended application of 50 pounds per acre of urea in the spring of the fruiting year actually depressed yield. Furthermore, no response was obtained from fertilizers applied the previous season regardless of the time of application or the fertilizer used. They noted that strawberries did not respond to superphosphate when it was required for good growth of vegetable crops.

2. Effect of fertilizers on fruit quality: Strawberry fruit-quality generally is measured by moisture content, sugar content, color and firmness, the latter being the one most commonly determined. While some workers (74, 75, 90) have claimed that nitrogen applications have reduced firmness, others have failed to detect any reduction (29, 110). According to Webster (110) "....nitrogen applications up to 200 pounds per acre did not significantly alter the firmness of the berries". Darrow (29), though finding no significant effect on firmness, did find that applications of nitrogen increased vegetative growth and fruit decay. Most significant in this respect is the statement of Kimbrough (68) that "there is a much greater difference in quality (moisture content, sugar content, and firmness) due to rainfall and variations in soil moisture than to fertilizer treatments".

3. Time of application of fertilizers: From the considerable amount of literature on the optimum time for applying nitrogenous fertilizers, only a few reports (27, 100) favored the application of liberal quantities in the spring prior to harvest. Taylor (100) in Alabama suggested nitrogen application in the winter (January) which corresponds to a spring application in the sorthern areas. Two years results on Klondike led him to conclude that, "The increase in the number of berries resulted chiefly from an increase in the number of flowers produced rather than from a high percentage of flowers set."

Many workers (3, 25, 96, 108) have claimed adverse effects from nitrogen applied in the spring before harvest, as a result of the overstimulation of vegetative growth. Both Waltman (108) and Hartman (56) report reductions in yield as compared with plots not fertilized and Shoemaker (96) and Collison (25) found no benefit. In addition to the aforementioned workers, extensive tests (45, 76, 77, 78, 96, 104) have stressed the importance of maintaining an optimum fertility level during the previous summer and fall when fruit buds are normally differentiated. Results of some of these tests follow:

Long (76): "The fruit production of a strawberry plant (in Missouri) is determined by the food reserves that accumulate during the growing season and are made available in the spring." In addition, Long (77) found that summer fertilization

resulted in more flowers formed and a greater percent set than the check while spring fertilization in addition to summer treatment proved of little value, causing increased leaf area and poor ripening.

Loree (78): "....Applications of fertilizer (Michigan) in the spring of the fruiting year have no effect on the number of clusters, or the number of flowers per cluster". These results were obtained by means of carefully controlled pot culture experiments in which the actual number of clusters and flowers was related to fertilizer treatment.

Tucker (104): "....Repeated tests (in Virginia) have demonstrated that a fall application (in late August or early September) is more suitable than a spring application."

Gardner (45) in an extensive nutrition study in Missouri states: "It is clear, however, that the nutrition question as it relates to strawberries, is a late summer and fall question to a much greater extent than has been generally suspected."

4. Controlled nutrition studies: The knowledge of deficiency symptoms and nutrient interrelationships, developed by means of controlled culture techniques, is important in a study of crop nutrient requirements. Such investigations have been made by numerous workers (32, 33, 34, 73, 117). Deficiency symptoms in the strawberry of nitrogen, phosphorus, potassium, calcium and magnesium have been described by Davis and Hill (32, 33) and later by Lineberry

and Burkhart (73) and Iwakiri and Scott (66) as follows:

Nitrogen: Early stages -- reddening of the serations at the margins of older leaves; young leaves, yellowish green; petioles become red and brittle. In later stages older leaves become necrotic. Fruit small.

Phosphorus: Early stages -- blue-green coloration of the foliage with reddening at the margins. Later the entire surface of the leaves becomes bronzed and purpled, suggesting physiological nitrogen deficiency.

Potassium: Marginal necrosis of the leaf margins which fold upward and inward. Dead calyx on the fruit is common as well as wilting and drying up of the pedicels and peduncles. Symptoms differ greatly with variety. Bronzing or purpling may be present on the undersurface of the leaves, also bronzing and necrosis of the petioles.

Calcium: Early stages -- slight marginal necrosis and partial interveinal chlorosis of fully expanded leaves. Later the unopened leaves exhibit tip burning, becoming deformed and crinkled when fully expanded.

Magnesium: Interveinal chlorosis of older leaves developing into necrosis accompanied by twisting and downward cupping of the leaf margins. In later stages there may be either a reddish brown or chlorotic, grayish band around the margins of the leaflets.

Of the so-called minor elements, boron deficiency has received the most attention, being studied by Hoagland and Snyder (65), Gilbert (46) and Iwakiri and Scott (66). Boron deficiency symptoms as described by these workers include severe deformation of the leaves being "dwarfed, cupped upward, puckered and generally brown at the tips", as well as retarded growth, deformation of runners and fruits, and pronounced stunting of the rootlets.

An attempt has been made to develop manganese deficiency (66) in sand culture but no symptoms appeared. It is believed that more refined solution culture techniques would be necessary for the development of other trace element deficiencies. In addition, Hoagland and Snyder (65) have investigated the role of sodium in strawberry nutrition. They have pointed out that injury from excess sodium salts probably exists in the field. Such a sodium toxicity could arise from continuous use of sodium nitrate on a soil already high in this element.

By means of controlled culture techniques, true nutrient element deficiencies or excesses may be observed. In the field, however, nutritional disorders commonly arise from a lack of balance or antagonism of two or more elements. Such investigations of nutrient element interrelationships in the strawberry may be found in papers by Overholzer and Claypool (90), Davis, Hill and Johnson (32) and Wallace (107). The former studied the effect of various fertilizer

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elements on the respiration rate and firmness of the strawberry fruit. They found that the higher the respiration rate the softer the fruit and that nitrogen applications increased the respiration rate while phosphorus decreased it. Thus they postulated that the "application of phosphorus may lower the respiration intensity by tending to depress nitrogen intake". Wallace (107) in England suggested a possible nitrogen-potassium relationship by showing that strawberry fruit had a higher nitrogen content when potassium was deficient in the nutrient medium than when it was not. Davis, Hill and Johnson (32), analyzing the ash of Parson Beauty variety strawberries grown under various fertilizer treatments, reported a negative correlation between potash and either calcium oxide, magnesium oxide and phosphoric acid. They also obtained a positive correlation between magnesium oxide and phosphoric acid.

Nutrient element interrelationships and plant growth in general are substantially influenced by the hydrogen ion concentration of the nutrient medium. The effect of the pH on the growth and composition of the strawberry has been investigated in some detail (24, 72, 84). Sand culture experiments with the Premier variety by Clark (24) demonstrated that at a pH of 4.6 best growth was obtained when nitrate-nitrogen was used while at a pH of 6.4 ammonium nitrogen proved to be the better source.

5. Nutritional aspects of the strawberry runner plant: The strawberry is propagated readily vegetatively by stolons which give rise to new plantlets at its distal end. The exact nutritional relationship of a new runner plant to its parent before and after the runner becomes rooted has aroused the interest of many, including the author. When does the runner plant become physiologically independent of the parent? Is the nutrition of the runner plant different from that of its parent? Are the runner plants of any benefit to the parent plant before or after they become rooted? These and other questions have been studied in some detail by White (116) and later by Darrow (30). White in his "Studies of the Physiological Anatomy of the Strawberry" (116) has shown that the stolon is a true stem with extensive xylem tissues specialized for free conduction of water and nutrients. Thus it would appear that the physiology of the runner plants before they become independent would be identical with that of the parent. However, White also points out that the large adventitous roots which arise from the crown of the young runner plants are relatively inefficient absorbing organs compared to the small fibrous branch roots which form later.

Darrow (30) in a similar study reported that the mother plant can support an infinite number of unrooted runners and vice versa -- rooted runner plants can support the mother plant from which the roots have been removed,

indicating free movement of water and nutrients in both directions through the stolon.

B. The Use of Radiophosphorus in Plant Nutrition

1. General considerations: The discovery by Hevesy (59) in 1923 that radioactive mineral elements could be traced throughout plants opened an entirely new and fruitful field of research in plant nutrition. Hevesy and his co-workers (60, 61) are also credited with the first plant studies using radiophosphorus, which they prepared by bombarding elemental phosphorus with neutrons from a beryllium-radon mixture. Their early studies demonstrated the high mobility of phosphorus, a finding which has been substantiated repeatedly thereafter (7, 8, 26, 62, 120). The development of the cyclotron in 1931 made radiophosphorus available and resulted in renewed investigations in California (48, 49, 99). Finally in 1942 the construction of the nuclear chain reactor (47) assured the availability of inexpensive P^{32} for all types of chemical and biological research.

2. Basis for tracer studies: The tracer technique is based on two fundamental assumptions. First, the behavior of the radioactive isotope of the elemtnt must be physiologically identical with that of the non-radioactive or stable form (21, 67). Secondly, there must be no radiation effect on normal plant processes. The latter assumption has been questioned (95) and radiation injury has been demonstrated

in higher plants (79) but the general opinion is that no damage to plant tissues from P^{32} occurs at usual tracer levels (13, 15, 36, 58).

3. Uptake and translocation by the roots: Absorption of phosphorus through plant roots as indicated by P^{32} is influenced markedly by the reaction of the nutrient medium (2, 94) and by the presence of antagonistic ions (9, 124). Arnon, Fratzke and Johnson (2) have shown that although phosphorus absorption varies with the plant, generally, it decreases as the pH decreases from 7 to 3 and as the pH increases from 7 to 9. In some plants such as Bermuda grass, there was little difference in phosphorus absorption between pH 4 and 7.

Closely related to the influence of soil reaction, and perhaps the basic cause for differences in phosphorus absorption is the antagonistic effect of other ions such as iron, aluminum and calcium. Recently Robertson, Neller and Bartlett (94) using soils of different sesquioxide contents adjusted to various pH's, gave evidence of close correlation between phosphorus availability and sesquioxide content. When iron and aluminum were plentiful in the soil, phosphorus availability (as indicated by the P^{32} taken up by the plant) increased with an increase in soil pH up to 7.2, then decreased. However, when iron and aluminum were low, phosphorus availability was reduced with a decrease in soil reaction. Other isotope studies using P^{32} have explained phosphorus

unavailability in terms of precipitation of calcium (14), aluminum (124) and/or iron (9) phosphates on the surface of or within the plant roots. Tracer studies by Biddulph and Woodbridge (9) with bean plants indicate that when the ratio of P:Fe in the nutrient medium is greater than 10:1 uptake of phosphorus is not reduced by iron regardless of the pH. Below this ratio precipitation on or inside the roots may occur.

In addition to nutrient absorption studies, radiophosphorus has been employed to show the pattern of distribution and mobilization of phosphorus in a wide variety of crops (7, 48, 98, 121). The channels through which phosphorus travels up and down the plant were not clearly understood before the inception of the tracer technique. Gustafson and co-workers (48, 49) in early tracer studies (1937 and 1939) gave evidence for upward movement of labelled phosphorus in the phloem. Other studies by Stout and Hoagland (99) in which they separated the xylem and phloem of willow and geranium gave strong evidence that upward movement of solutes was almost entirely confined to the xylem. Recent conclusive studies by Chen (23) have substantiated the latter view, stating that P^{32} moves upward through the xylem in the inorganic form while moving both up and down the plant in the phloem when applied to the leaves, probably in an organic form associated with carbohydrate fractions.

4. Radiophosphorus in soil fertility studies:

Following the development of the nuclear chain reactor (47) which made available sufficient quantities of P^{32}, a nation-wide program was initiated in cooperation with the Atomic Energy Commission to investigate the complex problem of soil phosphorus availability (57, 58). Over the course of about four years a wealth of information was accumulated regarding phosphorus fixation in the soil, placement and timing of fertilizer application, and the efficiency of various phosphate carriers.

The general technique of experiments such as these has been described by Hendricks and others (57, 58). Various methods of preparing the samples for radioactive assay are available (53) but in almost every case efficiency of utilization is determined by comparing the specific activity of the plant sample with that of the original fertilizer material (22), according to the formula (121): Percent of nutrient in plant derived from fertilizer equals:

$$\frac{\text{Specific activity of the nutrient recovered from plant} \times 100}{\text{Specific activity of the nutrient in applied fertilizer}}$$

The specific activity is essentially the ratio of the amount of the radioactive isotope to the total quantity of the element present. This is the method of so-called isotope dilution analysis. A brief summary of a few of the important results obtained in these experiments is given below.

5. Timing of phosphate application: It has been found that grain crops have a high phosphorus requirement early in their growth while the differences in phosphorus uptake at the end of the season are much less (37, 85). Potatoes, on the other hand, absorb phosphorus at a somewhat constant rate throughout the season (85). Thus, the best time for fertilizer application varies with the crop grown.

6. Fertilizer placement studies: The pattern of soil and fertilizer phosphorus utilization has been determined for many crops and under a variety of climatic and edaphic conditions (14, 35, 36, 51, 57, 106). For most crops placement of the fertilizer in bands close to the seed is much better than broadcast application (57). Ulrich, Jacobson and Overstreet (106) found that less than one percent of the phosphorus in grape vines was derived from a 2300 pound per acre suface application of phosphoric acid. Hall (51, 52) has very effectively utilized labelled phosphorus in placement studies and in addition has found P^{32} useful in studying root distributions.

7. Phosphate carrier: The tracer technique has provided the means of conclusively ascertaining the efficiency of the various sources under a wide range of environmental conditions. A few examples of these experiments are cited:

A group of Canadian workers in Saskatchewan (36, 37) have tested various calcium, ammonium and sodium phosphate sources in their alkaline soils (pH 7.1) and have found

more P^{32} (in the plant) to be derived from monoammonium phosphate than from any of the other carriers tested. In Colorado, however (89), monoammonium phosphate was not superior to superphosphate.

Isotope studies by Fried and MacKenzie (43) have shown conclusively that rock phosphate is a more efficient source of phosphorus than superphosphate at pH 4.9 but that superphosphate is more effective in soils at pH 5.8 or above. Superphosphate when compared with manure has been shown to be more available in the early growth cycle but the differences diminished later in the season. Green manure was found to supply 70 percent as much phosphorus to succeeding crops as did superphosphate (44).

The use of phosphoric acid as a carrier of phosphorus applied either directly to the soil or in the irrigation water is a relatively new development which has been furthered by tracer experiments. Olsen and his co-workers in Colorado (88, 89) have shown that it has supplied more phosphorus (as P^{32}) to alfalfa than superphosphate and was about equal to superphosphate for sugar beets. He claims that when phosphoric acid is applied with the irrigation water, deeper penetration can be obtained than a dry phosphate application with the seed. However, cost and the need for special transporting and application equipment may limit its usefulness.

8. Uptake through stems: In the last few years interest has been renewed in the application of fertilizer materials to the dormant bark of fruit trees (54, 102, 105). Although some entry has been noted, tracer experiments with P^{32} have shown the amount to be rather small and of possible practical value only under certain conditions of growth, temperature and plant species (102). Absorption through woody stems (102) and into excised dormant branches (38) appears to be most pronounced just before and during bud swell in early spring.

9. Uptake and translocation by leaves: Increasing interest in the nourishment of plants through foliar absorption has been due in part to isotope research which indicates that "on the basis of quantities applied, the efficiency of utilization (of nutrients) is several times higher from applications to the leaves than from soil treatments" (119). In these studies the efficiency of foliar and root uptake of P^{32} by the tomato has been compared in relation to light, temperature and phosphorus source (62). Although "leaves are efficient organs for phosphorus absorption," Wittwer (119) cautions that "with the tomato it seems practical to supply but a small part of the total phosphate needs of the crop by leaf feeding".

Foliar applications using P^{32} have also been studied in connection with solute translocation (26) and the efficiency of foliar absorption by apple trees in the field and greenhouse (39).

10. Leaf feeding: Considerable evidence has been accumulated recently to support the concept that nutrients may be absorbed by the leaves of plants by direct entry through the cuticle (93, 121). Although the work with urea applied to the leaves of the McIntosh apple (41, 42) has perhaps received the widest recognition, other nutrients and crops have produced results which may be of equal or greater economic value (16, 55, 97, 123). Trace element sprays were applied to plants long before urea (82) and in some areas they are used to supply the entire requirement for a particular element (101).

On the other hand, several investigators (17, 28, 111) have been unable to obtain significant increases in yield from the use of nutrient sprays when a standard soil fertilization program had been practiced. A review of the literature (87) on the utilization of urea-nitrogen by the peach exemplifies the wide differences in results which can be obtained in leaf feeding experiments. The prevalent opinion in regard to leaf feeding of the so-called major elements is that only a part of the total plant needs can be feasibly supplied by this method (119). Leaf feeding is therefore to be considered primarily as a supplementary measure for use in special situations in which nutrient uptake from the soil may be inadequate or when close control of the nutrient level is of great improtance for optimum vegetative or reproductive development.

C. Factors affecting absorption of nutrients through leaves and roots

Tracer experiments (35, 85, 122) have demonstrated that the rate of phosphorus absorption from the soil is dependent upon its supply and upon its requirement by the plant. Thus, when a phosphate fertilizer is added to the soil already well supplied with this element, less phosphorus will be absorbed from the fertilizer than when the soil phosphate content is initially low.

The influence of the fertility of the root medium on the rate of nutrient uptake by leaves has received little attention primarily because leaf feeding has just recently gained practical importance. In one such investigation by Hinsvark and Wittwer (62) it was found that both leaf and root uptake were affected similarly by changes in the phosphorus concentration of the nutrient solution. This suggests a similar mechanism of absorption by these two organs.

1. Effect of phosphorus source: The development of the isotope tracer technique provided an excellent means of comparing the efficiency of various phosphate materials for foliage and root application (39, 62, 97). Some of these experiments have already been discussed on page 15. Comparisons of the yield response to soil applications of various phosphate carriers have been made by McCall (81) and later by Bennett and his co-workers (6). Their results favored

the use of soluble materials such as sodium and ammonium phosphate rather than the calcium or rock phosphates.

Weissflog and Mengdehl (112) have made one of the few critical studies on the uptake of organic phosphate compounds by roots and noted growth responses from organic acids such as phytic, nucleic, glycerophosphoric and hexose phosphoric acid.

In regard to foliage application of phosphate materials, Silberstein and Wittwer (97) tested a large number of inorganic and organic compounds and reported that phosphoric acid was the most effective phosphate carrier for foliage application on the tomato.

2. Effect of temperature on phosphorus absorption: While considerable literature is available on the influence of temperature on growth (4, 5, 50, 86, 91, 92, 115), water (70), nutrient (4, 5, 18, 19, 20, 64, 109, 114) and carbohydrate (115), absorption and translocation, few reports relate specifically to phosphorus uptake as influenced by temperature. In this connection, Roberts (92) found that root temperature had no appreciable effect on the phosphate content of strawberries. On the other hand, Mayberry (80) showed that the rate of P^{32} absorption by the roots of the tomato plant decreased with a lowering of the night temperature from 75° to 45° F. However, foliar uptake was "practically unaffected by temperature variations". Whether this reduced uptake was actually due to a temperature effect on

absorption or whether it may have resulted from reduced translocation or utilization is not known. Later studies by Hinsvark and Wittwer (62) described similar temperature coefficients (Q_{10} relationships) for both foliar and root uptake and translocation of P^{32} to the developing fruit in tomato. Broyer and Hoagland (20) showed that temperature had a much greater influence on nutrient uptake than it did on water absorption indicating that ion absorption itself may be affected more by reduced temperatures than either translocation or utilization. In contrast, Went (113) and others (91, 109) have suggested that only when growth conditions such as aeration, nutrient supply, and other factors are sub-optimal is growth influenced by the temperature of the root system.

The effects of temperature on solute absorption are well summarized by Kramer (71) as follows:

"Many demonstrations have indicated that the absorption of solutes is reduced by low temperature. Presumably this is, to a great extent, because reduced respiration releases less energy for that part of the absorption process which requires the expenditure of energy; but doubtless other factors, such as increased viscosity of protoplasm and reduced mobility of ions, are also operative. It is difficult to separate the effects of low temperature on the absorption process from its effect on translocation and on utilization of the nutrients in the plant. Little is

known concerning the extent to which reduction of nutrient absorption by low soil temperature hinders plant growth. It may be a limiting factor on the growth of some crops in the spring and on alpine or arctic plants; but low temperature retards growth in a number of ways, and it is difficult to distinguish them from one another."

THE PROBLEM FOR INVESTIGATION

The purpose of this study was to investigate the uptake and consequent utilization of certain plant nutrients, particularly phosphorus, through the foliage of the strawberry. The experimental data presented are divided into two sections. The first deals with the use of calcium and phosphorus radioisotopes as a tool to evaluate the efficiency of foliar and root absorption and utilization. The techniques of autoradiography and isotope dilution analysis were employed to demonstrate visually and quantitatively the flow of radionuclids throughout the strawberry plant. Part two presents data on studies conducted in the greenhouse and field on actual growth and yield response to foliar applications of soluble fertilizers, particularly nitrogen and phosphorus.

METHODS AND MATERIALS

The strawberry (Fragaria spp., variety, Robinson) was selected for several reasons. First, being an herbaceous plant it is more adaptable to isotope tracer studies than woody plants and yet the results may be more applicable to pomological research than would those from vegetable crops. The strawberry is easily grown in the greenhouse in bench or pot culture at almost any time of the year and by the adjustment of the photoperiod can be maintained vegetative or induced to become reproductive. Since it is clonally propagated by runners, genetic variability can be eliminated. The Robinson variety produces runners profusely, enabling one to select uniform plant material.

A characteristic of the strawberry useful in nutrition studies is its self propagation by means of runner plants. As is demonstrated in the isotope investigations to follow, translocation of nutrients can be easily studied not only from tops to roots and roots to tops, but also through the stolon from the mother to the runner plants and the reverse.

Finally, the strawberry was selected because it flowers and fruits in the field early in the spring when edaphic conditions are often unfavorable for fertilizer uptake from the soil. It was theorized that foliar application of phosphorus might be of particular advantage to this crop.

The chief disadvantage in regard to isotope studies of foliar feeding proved to be the lack of any well defined stem separating the leaves from the roots. Some of the early experiments had to be discarded because of the extreme variability between replicates often introduced by dipping the entire tops of the plants which included leaves at all stages of development and absorptive efficiency. This was later circumvented by treating individual leaves of comparable maturity.

Methods of growing plants: Plants of the Robinson variety were obtained from the Michigan State College Experimental Farm during the fall and spring of 1951-52. Additional plants were propagated clonally as needed.

In the greenhouse investigations sand culture techniques were employed. Various grades of sand were tried including Flint Shot[1/], a fine, uniform quartz sand and other grades of a coarser nature[2/]. The A.G.S. No. 7, a coarse grade providing good aeration and ease of removal from the roots, gave the best results. Variations of the basic Hoagland nutrient solution (63) were employed. A reduced nutrient concentration proved to be more suitable in experiments carried on during the winter season. Periodic leaching of the cultures with tap or distilled water prevented excessive salt accumulation.

1/ Flint Shot is produced by the Ottawa Silica Company, Ottawa, Illinois.

2/ Other quartz sands used were obtained from the American Graded Sand (A.G.S.) Company, Chicago 13, Illinois.

Statistical evaluation: Because of detailed cultural, treating and harvesting methods, single plant replications were used in most greenhouse investigations. Both the chemical and radioactive analyses were performed on single plant replicates and in many cases duplicate determinations were made as a check on the procedures. The data were evaluated by analysis of variance according to the methods of Snedecor (98) and suggestions of members of the Michigan Agricultural Experiment Station staff. Differences necessary for significance are designated by L.S.D. 5% or 1%. Where F values were found to be insignificant, the designation of N.S. is given.

Whenever possible replications were completely randomized in the experimental area. An exception to this occurred in the root temperature experiments in which only one tank at each temperature was available. However, the treatments were randomized within each temperature tank.

ISOTOPE STUDIES

All radioactive materials were obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee. The P^{32} was received as ortho-phosphoric acid, $H_3P^*O_4$ in dilute HCl and the Ca^{45} as calcium chloride, Ca^*Cl_2.

Methods of treating plants: Initially the foliage was treated by dipping the entire tops into the solution

contained in a large beaker or other vessel of convenient size. It was found, however, that rather large errors were introduced by this method and it was replaced by individual dipping of three to six uniform leaves from each plant. In certain experiments the remaining leaves were counted and weighed to determine the proportion of the foliage which was treated. In all cases root applications were made by pouring the solution around the base of the plant at least two inches from the crown.

For foliage applications a 25 millimolar solution of phosphoric acid was generally used containing from one to five microcuries per milligram phosphorus. Similarly solutions of calcium chloride were prepared having a specific activity of approximately two microcuries per milligram of calcium. DuPont Spreader-Sticker at the rate of .15 milliliters per liter was added to all solutions applied to the foliage to insure uniform coverage.

Preparation of samples for analysis: Samples were dried in a well insulated over at 120° F. Dry weights were then recorded and the tissues ground in a micro-Wiley mill to pass through a 40 mesh screen. Ashing of the samples was accomplished by either the wet or dry methods. Wet ashing was done with nitric and perchloric acids according to the method of Toth, Prince and Mikkelson (103). In dry ashing the samples were placed in 45 millimeter milk ashing

capsules[1/] in a muffle furnace held at 600° C. for 12 hours in accordance with standard A.O.A.C. procedures (1). Prior to dry ashing, the weighed samples were pre-digested with hydrochloric acid in the presence of magnesium nitrate. After ashing (dry or wet) the residues were dissolved in a few drops of concentrated hydrochloric acid and made to volume.

Radioactive and chemical assay: Following wet-ashing, aliquots were transferred to one ounce tin boxes[2/] and evaporated to dryness on a hot plate. The size of the aliquot depended on the approximate activity of the samples. The boxes were then placed in a shielded chamber and the radioactivity of the samples determined by a Nuclear model 172 Ultra scaler. For tissues ashed in the muffle furnace, the ash was dissolved in concentrated hydrochloric acid and evaporated to dryness leaving a thin film over the bottom of the dish. Counting was done directly on the residue, thus avoiding loss by transfer to other containers. With no dilution, this method was not well adapted to samples of high activity. Direct counting of the ground tissue without ashing was employed in one experiment with good results.

1/ Coors porcelain milk ashing capsules. Coors & Co., Golden, Colorado.

2/ Labelstick gold laquered tin boxes. George D. Ellis & Sons, Inc. Philadelphia, Pennsylvania.

Total phosphorus was determined using the standard A.O.A.C. molybdate method (1). A monochromatic colorimeter with a 630 millimicron filter was used to measure transmission density which was then converted to percent phosphorus from a standard curve. Total calcium was determined also by the standard A.O.A.C. procedure (1).

Autoradiography: For qualitative estimation of movement of radioisotopes from the point of application, frequent use was made of the technique of autoradiography, as described by Wittwer and Lundahl (120). A pressed mount of the treated plant material was covered with pliofilm to prevent contact of the specimen with the X-ray film placed over it. Close contact of the film against the plant, necessary for good resolution and for shielding against adjacent samples was obtained by pressing during exposure with one-quarter to one-half inch steel plates. In order to prepare autoradiograms of entire plants or a series of runner plants, a special exposure box was constructed to hold specimens and film 14 x 36 inches in size. Following the exposure period, which varied with the nature and degree of radioactivity of the isotope used, the film was processed in Kodak X-ray developer and fixer.

Calculations: In tracer studies differences due to treatment may be expressed in a number of ways. Expressing results as counts per minute, or milligrams of phosphorus was often found insufficient to give a clear picture.

For this reason calculations were made of the total amount of phosphorus (or calcium) per gram of dry tissue or per fruit which was derived from the treatment applied. In other instances the percent of the total amount of the nutrient found in the plant which was derived from the treatment is given.

Formulae used for the above calculations are given below:

1/ Total P derived from treatment (micrograms/gm. tissue) =

$$\frac{\text{counts per min.}}{\text{gm. tissue}} \div \frac{\text{counts per min.}}{\text{microgram total P}}$$

2/ Total P derived from treatment (micrograms/fruit) =

$$\frac{\text{counts per min.}}{\text{fruit}} \div \frac{\text{counts per min.}}{\text{micrograms total P}}$$

3/ % of total P derived from treatment =

$$\frac{\text{micrograms treat. P}}{\text{gm. tissue}} \div \frac{\text{micrograms total P}}{\text{gm. tissue}} \times 100$$

EXPERIMENTAL

SECTION I -- THE UPTAKE AND UTILIZATION OF RADIOPHOSPHORUS AND RADIOCALCIUM BY THE LEAVES AND ROOTS OF THE STRAWBERRY

I. Determination of the Amount of Solution Retained after Dipping Strawberry Leaves

To compare the efficiency of foliar and root absorption of nutrients, it is necessary to know how much material is applied to each. While the amount applied to the roots may be readily ascertained, the amount of liquid which adheres to the leaves is not so easily measured. The isotope technique is useful in this respect, enabling one to determine accurately the amount of material which adheres to a particular leaf.

The leaf blades of twelve fully expanded strawberry leaves were individually immersed momentarily in a 0.3 percent calcium chloride solution containing approximately one microcurie of Ca^{45} per milligram. For convenience Ca^{45} was used in this test although it is believed that similar results would have been obtained using P^{32}. The leaves, still attached to the plants, were maintained in an inverted position until dry and were then excised from the plants. The leaf blade area was then measured with a planimeter in order to compare adherence on an area basis with adherence

per unit dry weight. Then the leaves were dried at 70° C., weighed and ashed in milk ashing dishes. The ash was dissolved in hydrochloric acid, evaporated to dryness and assayed for radioactivity. Small aliquots of the treating solution used as a reference standard were similarly counted.

Results: The results are given in Table 1. Milliliters of liquid adhering to the foliage was calculated as follows:

$$\text{No. of ml. adhering} = \frac{\text{Activity of sample (counts per minute)}}{\text{Activity of treating solution per ml.}}$$

Considerable variation existed in the amount of liquid retained by a strawberry leaf, ranging from about 0.1 to 0.3 ml. per leaf. This variation was not unusual since a range of different leaf sizes was selected in order to achieve an average value which could be used for calculation of the amount of liquid adhering to the entire leaf area of a plant. As shown in Table I both the adherence per unit dry weight or per unit area was rather constant. However, considering all factors, the adherence of the liquid per gram dry weight of leaves was adopted. Accordingly, 0.56 ml. per gm. of dry leaf tissue appeared to be a representative and fairly accurate basis for estimating the amount of liquid adhering in a uniform film to strawberry foliage. This factor was used as a standard in later studies.

Table 1. Retention of Calcium Chloride Solution by Fully Expanded Leaves of the Robinson Strawberry.

Leaf No.	Leaf Area (sq. cm.)	Dry Wt. (mgms.)	Activity counts per min.	Adherence (ml.) per leaf	per sq. cm.	per gm.
1	48.7	234	2180	.171	3.5	.58
2	38.1	281	2264	.177	4.6	.63
3	54.0	428	2165	.250	4.6	.58
4	43.1	379	2085	.161	3.7	.43
5	50.6	406	2225	.174	3.4	.43
6	58.4	469	3697	.290	4.9	.62
7	48.3	333	2285	.179	3.7	.54
8	39.5	284	2097	.164	4.1	.58
9	50.0	437	2737	.215	4.2	.49
10	34.8	245	2161	.169	4.8	.69
11	35.0	246	1926	.151	4.3	.61
12	24.6	188	1329	.104	4.2	.56
Averages				.170	4.2	.56

II. Distribution of P^{32} Throughout the Plant as Shown by Autoradiography from Foliage and Root Applications of Ortho-phosphoric Acid

Seventy-five Robinson strawberry plants, removed from the field the previous fall, were planted January 10, 1952 in coarse quartz sand in six-inch clay pots. Hoagland's minus-phosphorus solution was used with occasional leaching of the cultures with distilled water.

Phosphorus deficiency symptoms, characterized by a reddish-purple coloration of the mature leaves, developed in some of the cultures within about three weeks. Some phosphate was then added to insure growth but still maintain low phosphate status.

On March 1, twenty uniform plants were selected for treatment, each having several flowers and at least one developing fruit. Half of the plants were given a foliage application of a 25 millimolar solution of orthophosphoric acid containing one microcurie of radiophosphorus, P^{32}, per milligram. The entire tops of the plants were dipped in the solution and supported in an inverted position until dry. Corrugated cardboard was fitted around the base of plants to prevent loss of the root medium when inverted and also to separate the foliage from the flowers and developing fruits which were not treated.

The remaining ten plants received 100 ml. of a solution of one-tenth the concentration of the foliage treating

solution or containing 0.1 microcurie per milligram. Thus 10 microcuries of P^{32} was applied to the roots of each plant as compared to about two microcuries applied to the leaves[1/].

Two plants each from the leaf and root applications were harvested at intervals of 2, 6, 24, 96, and 384 hours after treatment. In order to prepare an 8 x 10 mount of the entire plant, each was pruned to two to three leaves. The fruits and crown were sliced so as to retain only the center section of tissue, thereby obtaining a uniformly flat pressing of the entire plant. Autoradiograms of the mounted specimens were then prepared.

Results: Figure 1 shows the distribution of radio-phosphorus from foliage and root applications at various time intervals after treatment. Very little movement occurred after two hours, while within six hours, P^{32} was translocated to all portions not treated, especially meristematic regions such as developing fruits, leaves and root tips. Furthermore, radiophosphorus apparently moved at a similar rate from both the treated leaves and the roots into the developing fruits and leaves. Within 24 hours, most of the translocation of radio-phosphate had occurred since there was little increase in the intensity of the

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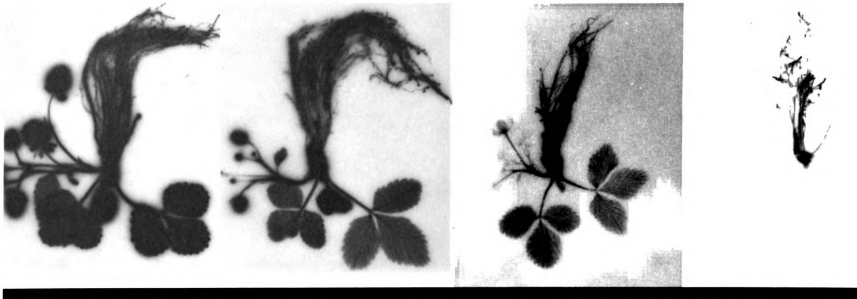
1/ Each plant in this experiment had 3 to 4 grams of leaves calculated to retain approximately 2 ml. of solution.

Figure 1. Distribution of P^{32} in the Robinson strawberry plant following foliage and root applications.

Top: Foliage application 2, 6, 24 and 96 hours after treatment.

Bottom: Root application 2, 6, 24 and 96 hours after treatment.



radiation after 96 hours. It should be emphasized that the foliage treatments received approximately one-fifth as much P^{32} of similar specific activity as was applied to the soil. This explains the somewhat greater intensity of the latter autoradiograms taken at 24 and 96 hours after treatment.

III. Distribution of P^{32} and Ca^{45} Throughout an Interconnected Series of Rooted Runner Plants from Foliage and Root Applications.

Previously, phosphorus was shown to have been readily translocated to all plant parts from both foliage and soil applications. There is some question, however, as to the mobility of calcium (11, 12). The Robinson variety of strawberry is ideally suited to translocation studies since it produces runner plants profusely. These runner plants can be treated separately from the parent plant to study translocation between the two.

Robinson strawberry plants were allowed to produce runners during the summer in the greenhouse. However, the young runner plants were prevented from coming in contact with any medium favorable for rooting. When long chains of unrooted runner plants were formed, 32 parent plants were selected, each having a series of four to five runner plants at equivalent stages of development. Each of the runner plants were allowed to root separately in six-inch pots of coarse sand watered with complete Hoagland nutrient solution. After about two months, when the oldest runner plants were firmly established and the others had roots at various stages of development, treatments were applied as follows: Successive runner plants along a series were designated for either foliage or root treatments such that only

one plant of a runner series received the fertilizer application. The treatments were replicated twice. Thus, a total of 16 runner series were used for treatment with P^{32} and a similar number with Ca^{45}.

The foliage treatments consisted of dipping six fully expanded leaves in the radioactive solutions (.3% $H_3P^{32}O_4$, or $Ca^{45}Cl_2$, one microcurie per milligram) and maintaining the plants in an inverted position until dry. The root treatments consisted of pouring 10 ml. of the appropriate solution into the sand around the base of the plant taking care to avoid contamination of either foliage or crowns. The solutions were prepared so as to provide a greater quantity of isotope for the root application since all of the material applied would not likely come in contact with the complete absorbing area as it does with foliage feeding.

Since no information on the rate of movement was desired, no attempt was made to harvest the plants at designated time intervals as was done in the previous tests. Instead the progress of the nutrient transport was checked daily by means of a stationary monitoring instrument. After one week, sufficient translocation from the treated plants had been detected and the plants were harvested. The roots and tops of all plants were thoroughly washed in tap water, taking care to wash treated portions separately. An attempt was made to keep the complete runner series intact for

the autoradiograms but this was not possible in some cases because the point of attachment of the stolon was sometimes removed in the crown sectioning operation.

Results: Representative autoradiograms demonstrating the pattern of translocation of phosphorus and calcium throughout the interconnected runner plants are shown in Figures 2, 3 and 4. It may be clearly seen that radiophosphorus may move in all directions from the point of application regardless of whether applied to the leaves or the roots. In some cases it moves preferentially toward the later-formed runners; in others, toward the parent plant (Figure 2). Observations of all of the plant specimens indicated that when P^{32} was applied to either the roots or the foliage of runner plants which had not yet become firmly established, most of the radiophosphorus remained in this developing plant. Translocation of phosphorus out of this plant occurred almost entirely toward the younger developing runner plants. It also appeared from this qualitative evidence that uptake of phosphorus from leaves was at least equally as efficient as root absorption. In this particular experiment, ten times more P^{32} was applied to the roots than to the leaves. Yet, the activity in non-treated plant parts was similar.

In contrast to phosphorus, translocation of radiocalcium from a foliage application (Figure 3) appears to be negligible,

Figure 2. Distribution of P^{32} throughout an attached series of runner plants from foliage and root application.

Top: Foliage treatment of first runner plant (a) showing preferential movement back to parent plant (b) rather than to later-formed runners (c).

Center: Soil application to third runner plant (a) showing preferential movement to later-formed runners (b,c,d) rather than back to second runner plant (e).

Bottom: Foliage treatment of third runner (a) showing preferential movement toward younger runners (b,c). Note the presence of P^{32} in both tops and roots.

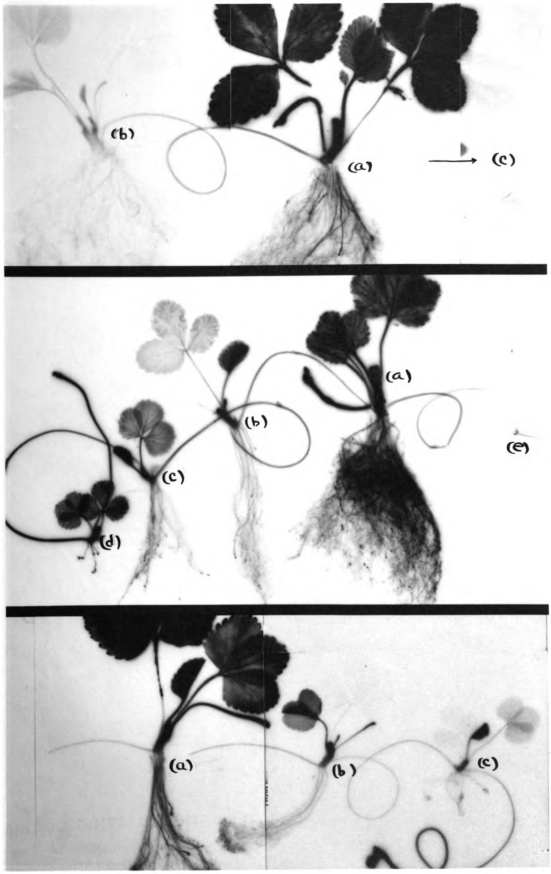

(b)
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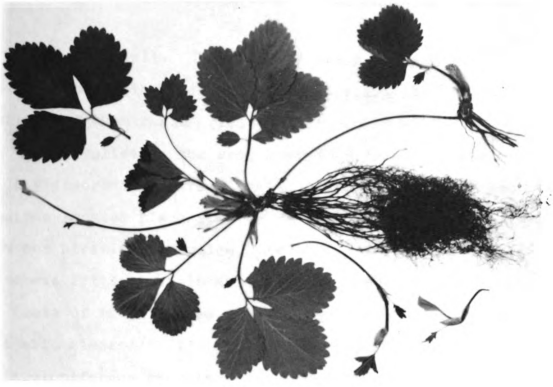
Figure 3. Distribution of Ca^{45} from a foliage application of $Ca^{45}Cl_2$.

Left: Autoradiogram of foliage treated plant showing no translocation away from the dipped leaves.

Right: Photograph of plant specimen from which autoradiogram (left) was made.



if it occurs at all. This lack of movement from the foliage was characteristic of all treatments regardless of the position of the treated plant in the runner series.

When applied to the root system (Figure 4), Ca^{45} was readily absorbed and translocated in both directions away from the treated plant similar to the movement of phosphorus, with one striking exception. As with the foliar treatments there was little translocation of Ca^{45} down the plant into the roots of the attached runner plants. This is in agreement with Bledsoe's (11) findings with the peanut and with the stoloniferous Pangola grass (Digitaria decumbens) (10). From these investigations it appears that the parent plant may not be able to completely satisfy the calcium requirements of the roots of a developing runner plant and that the necessary calcium must be derived from the rooting medium of the new plant.

Figure 4. Distribution of Ca^{45} from a root application to the second runner plant.

Upper: Autoradiogram of entire runner series showing translocation of Ca^{45} from the roots of the treated plant (a) back toward the first runner and parent plant (b and c) and out to younger runners (d,e,f,g).

Lower: Photograph of entire plant mount from which the autoradiogram was prepared. Dark leaves had upper surface exposed.

a
b
c
d
e
f
g

IV. Translocation of P^{32} and Ca^{45} from the Leaves and Roots of Rooted Runner Plants as Affected by Their Nutritional Status.

In the previous experiment, the translocation of phosphorus and calcium throughout the various parts of the strawberry plant was studied qualitatively by means of autoradiograms. The present objective was to study more quantitatively the efficiency of uptake from leaves and roots as affected by the nutrient status of the plants.

Thirty-two Robinson strawberry plants were selected in June 1952 from about 75 plants which had been potted in washed quartz sand on February 23, 1952 and grown with Hoagland's complete nutrient solution. Plants were selected that had one runner with four to five mature leaves and a later formed one of lesser development. All other runner plants originating from the parent plant were removed. None of the runner plants had been allowed to root prior to June 21, at which time the larger runner plants were set in four-inch clay pots of Flint Shot sand. Distilled water was added as needed to the rooting medium until June 23 when the differential nutrient treatments were started. The plants designated to receive Ca^{45} were similarly treated excluding calcium instead of phosphorus.

The runner plants were allowed to root in the differential media for two weeks during which time considerable root growth occurred.

On July 8 the P^{32} and Ca^{45} applications were made as follows: Half of the first runner plants in each nutrient group received a foliage treatment and half a root application. The entire foliage consisting of four to five leaves was momentarily dipped in the fertilizer solutions and the plants then maintained in an inverted position until dry. The solution supplied to the foliage contained 0.3% H_3PO_4 assaying approximately one microcurie P^{32} per milligram of phosphorus. A 0.3% calcium chloride solution of similar radioactivity was used for the calcium foliage treatments. Root application of the respective nutrients consisted of pouring onto the sand around the base of the plant 5 milliliters of the foliage treating solution contained in 50 millliliters of water. Based on about 3.5 grams of leaves treated and an adherence of .56 ml. per gram (Experiment I), it was estimated that about 2 millliliters of treating solution adhered to the leaves. Thus, approximately $2\frac{1}{2}$ times the amount of P^{32} and Ca^{45} was applied to the roots as to the leaves.

The experiment was terminated after a 48 hour absorption period. The plants were divided into (1) leaves and roots of the treated runner plants, (2) crowns (including immature leaves) of the parent plants, and (3) developing runner plants, including stolon tissue connecting them to the rooted runner plants. All portions except the treated

leaves were carefully washed separately in tap and distilled water to facilitate removal of sand and other foreign matter. The leaves were not washed since a measure of adherence of the solution (as calculated in Experiment I) was desired.

All tissue samples were dried, weighed, and ashed by the nitric-perchloric wet ashing method. Radioactive and chemical analysis of the samples was determined as described under Methods and Materials.

Results: The results of the chemical and radioactive analyses of the phosphorus-treated plants are given in Table II. Data for the crown tissue of the parent plants are not given as the activity of this portion was not significantly above background, thus indicating little or no movement into this tissue from the treated runner plant.

The results indicate that (1) the treated plants were definitely at two different phosphorus levels when treated, (2) the differential nutrient treatments probably affected dry weight production though the difference was significant only with the non-rooted runner plants, (3) uptake of P^{32} from the roots was much more affected by the nutrient status of the plant than was absorption and translocation of P^{32} from the leaves. Both the actual amount (micrograms per gram dry weight), (Figure 5) and the percentage of fertilizer phosphorus which came from the root treatment were greatly

Table II. Effect of Phosphorus Status on Absorption and Translocation of Radio-phosphorus by the Leaves and Roots of the Robinson Strawberry.

Treated Portion	Portion Analyzed	P Status[1/] of Plant	Dry Wt. (gms.)	Total P (%)	P derived from Treat. ug per gm. dry wt.	P derived from Treat. % of Total
Leaves	Roots	low	1.18	0.215	49.0	2.30
		normal	1.21	0.344	44.7	1.30
	L.S.D.	5%	N.S.[2/]	0.027	N.S.	0.55
	L.S.D.	1%		0.041		0.83
Roots	Leaves	low	3.38	0.232	62.6	2.70
		normal	3.97	0.356	17.8	0.50
	L.S.D.	5%	N.S.	0.037	18.8	0.71
	L.S.D.	1%		0.057	28.5	1.08
Leaves	Runner Plant	low	2.05	0.325	6.5	0.20
		normal	2.65	0.439	7.5	0.17
	L.S.D.	5%	N.S.	0.030	N.S.	N.S.
	L.S.D.	1%		0.045		
Roots	Runner Plant	low	1.59	0.324	16.8	0.52
		normal	2.56	0.438	4.8	0.11
	L.S.D.	5%	0.60	0.034	3.1	0.12
	L.S.D.	1%	0.84	0.051	4.7	0.18

1/ Treatments designated as "low" phosphorus status indicate that P was withheld for two weeks prior to the application of the P^{32} treatments. "Normal" indicates half-Hoagland nutrient solution applied throughout.

2/ F value not significant.

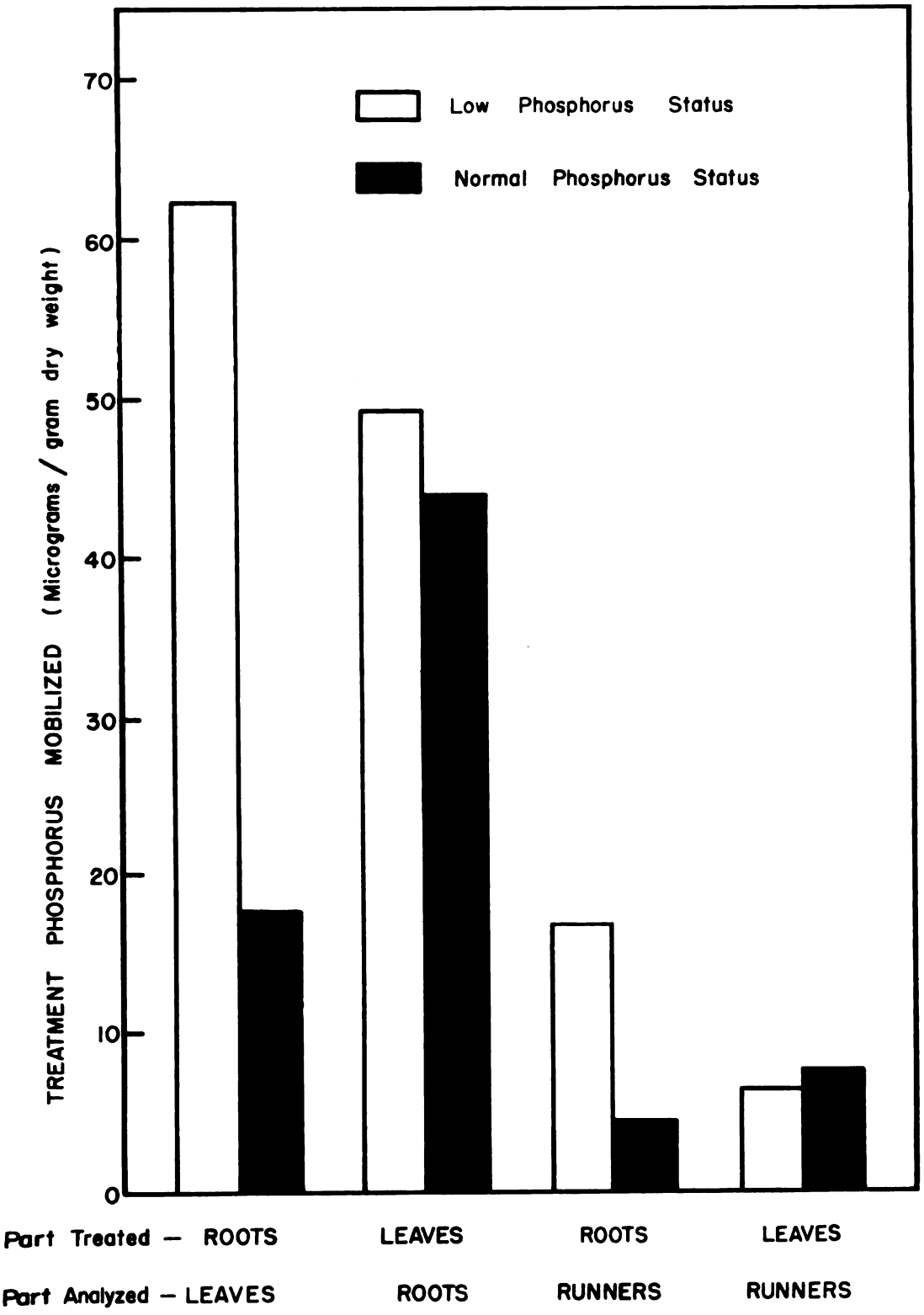


Figure 5. Effect of Phosphorus Status of the Strawberry plant on Uptake of P^{32} by the Leaves and Roots (see footnote 1, Table II).

influenced by the phosphorus status of the plant, whereas leaf uptake was affected to a lesser extent.

It should be emphasized that numerical comparisons between values for foliage and root applications may not be accurate and indeed favor root applications since approxi- $2\frac{1}{2}$ times as much P^{32} was supplied to the roots as to the foliage. Accordingly, the relative differences as a result of the nutritional status rather than the actual quantities of phosphorus mobilized are emphasized.

The data presented for Ca^{45} absorption by the leaves and roots (Table III) also indicate a possible growth response caused by the differential calcium fertilization prior to the application of isotope treatments. Though no chemical analysis for calcium are given, it would appear that two different plant calcium levels existed. The data show that twice as much root-applied calcium has been derived from the Ca^{45} treatment at the "low" level than at the "normal" level. In no instance was there any observable activity in non-treated portions as a result of foliage applications. This was in agreement with the results obtained by autoradiography.

It should be stated that although the absolute amounts of phosphorus and calcium derived from the single application were very small, these values are considerable when one considers that the total absorption of the labelled nutrient occurred over a period of only 48 hours.

Table III. Effect of Calcium Status on Absorption and Translocation of Radio-Calcium by the Leaves and Roots of the Robinson Strawberry.

Treated Portion	Portion Analyzed	Ca Status of Plant	Dry Wt. (gms.)	Micrograms Ca Derived from Treatment
Leaves	Roots	low[1/]	0.81	None[2/]
		normal	0.67	None
	L.S.D.	5%	N.S.[3/]	
Roots	Leaves	low	2.36	19.0
		normal	2.58	9.5
	L.S.D.	5%	N.S.	9.5
	L.S.D.	1%		14.9
Leaves	Runner Plant	low	0.76	None
		normal	1.35	None
	L.S.D.	5%	0.55	
	L.S.D.	1%	0.85	
Roots	Runner Plant	low	0.85	7.5
		normal	2.61	3.8
	L.S.D.	5%	1.14	7.8
	L.S.D.	1%	1.74	11.8

1/ Treatments designated as "low" calcium status indicate calcium was withheld for two weeks prior to the application of the Ca^{45} treatments. "Normal" indicates normal fertilization (half-Hoagland nutrient solution) throughout.

2/ No radioactive calcium detectable

3/ F value not significant

V. Effect of Root Temperature on the Mobilization of Foliar and Root Applied Phosphorus by the Fruit of the Strawberry.

In previous experiments, mobilization of radioelements in non-treated portions was studied with respect to the rate of utilization and to the influence of the nutritional status of the plant. Absorption by the roots, subsequent translocation and eventual utilization of nutrients is affected also by temperature, both of the entire plant (80) and of the roots specifically (92). Nutrient intake through leaves as influenced by temperature, especially the temperature of the root medium has received very little attention. Whether absorption and translocation from above ground portions is reduced by low temperatures to the same degree as root uptake was the subject of the present investigation. This test was of a preliminary nature since a rather small number of plants were found suitable for treatment. A subsequent experiment was planned to study the phenomenon in more detail.

In June of 1952, 16 uniform strawberry plants growing in six-inch pots of coarse washed sand were selected from more than 50 plants potted the previous winter. Each of these plants were of comparable vigor, having about ten normal, fully expanded leaves and several flower clusters. The plants had received weekly applications of half-Hoagland nutrient solution and were otherwise watered with tap water as needed. Prior to treatment the plants were pruned to a single flower cluster containing the primary and two

secondary buds, with the primary flower in full bloom and the secondary flower ready to open. By this method of selecting similar fruits, variations in the rate of mobilization of phosphorus by the fruit from foliar and root applications was greatly reduced.

Following the selection and pruning process, the cultures were leached thoroughly with distilled water and inserted to the level of the pot in two-gallon crocks of wet sand. The crocks in turn were placed in special root temperature tanks (Figure 7) in which the temperature of each root medium was maintained within two degrees Fahrenheit by means of thermostatically controlled refrigeration and heating coils. The construction of the temperature baths has been described in detail by Roberts (92).

For this experiment the root temperatures were adjusted to 50° and 70° F. Within a few hours, the temperatures in the root medium had reached the prescribed levels, but the isotope treatments were withheld until the following day.

P^{32} treatments -- Foliage application: Five fully expanded leaves from each plant were dipped in a 25 millimolar 0.3% $H_3P^*O_4$ solution containing about one microcurie per milligram of phosphorus. The pots containing the foliage treated plants were removed from the temperature tanks to facilitate the dipping operation and to avoid contamination of the fruit and roots, but were returned before appreciable

changes in the temperature of the root medium could take place. A total of eight plants, four at each temperature, were treated in this manner. It was estimated, as in Experiment I, by measuring the dry weight of the treated tissue that about one milliliter of liquid or one microcurie of P^{32} adhered to the treated leaves.

Root Treatments: One hundred milliliters of a phosphoric acid solution prepared by diluting the solution used for treating the foliage tenfold with water, was poured around the base of the plants as in the previous experiments. Thus, while there was no change in specific activity in the root treatments, approximately ten microcuries of P^{32} were applied to each plant as compared with approximately one in the foliage applications.

After treatment the plants were maintained in the tanks for a period of two weeks with applications of Hoagland's minus phosphorus solution as needed. During this period the flowers were hand pollinated so that normal fruit development occurred. The two week post-treatment period was afforded in order to determine whether temperature effects on absorption and mobilization would be maintained throughout the fruit development period. Ideally, harvests of fruit should have been made at various time intervals after treatment but this was not possible because of the small number of plants available at the proper stage of development.

Results: The data (Table IV) show a general increase in fruit phosphorus derived from both foliage and soil applications with root temperature of 70° F. To check the effect of slight differences in fruit development, the micrograms of phosphorus mobilized per fruit was determined but the measurements produced the same trend as when calculated in micrograms per gram dry tissue. The differences observed in the phosphorus content of the fruit probably developed during the two week fruit development period as a result of the single fertilizer application.

It appears from these preliminary data that root temperature had some effect on absorption or radio-phosphorus through both the leaves and the roots. Phosphorus uptake from both roots and leaves appeared to be depressed at the lower temperature. However, in spite of precautions to achieve uniformity of plant material, variations among replicates were sufficiently great to make differences among treatments not significant statistically.

Table IV. Effect of Root Temperature on the Mobilization of Foliar- and Root-applied Radio-phosphorus by the Fruit of the Strawberry.

Root Temp.	% P in Fruit	Treatment P Mobilized in Fruit micrograms per gram (dry wt.)	micrograms per fruit	% total P in fruit
		Root Application		
50° F.	0.277	26.4	19.9	.95
70°	0.321	34.9	24.6	1.09
L.S.D.	5%	N.S.	N.S.	N.S.
		Foliage Application		
50°	0.242	10.6	7.6	.45
70°	0.271	13.9	8.6	.51
L.S.D.	5%	N.S.	N.S.	N.S.

VI. Translocation and Mobilization of Foliar and Root Applied Phosphorus in Non-treated Portions of the Strawberry Plant Grown at Different Root Temperatures.

In the previous experiment, plants were treated with P^{32} within one day after being subjected to the differential root temperature environments. Thus, root temperature was the predominant factor influencing phosphorus uptake. However, under field conditions plants are often exposed to prolonged periods of low or high temperature causing morphological changes which could directly influence nutrient uptake through leaves and roots. It was the purpose of this experiment to study absorption through leaves and roots of plants which had been subjected to different root temperatures for a prolonged period before treatment.

Regulation of the root temperature within very close limits was accomplished by means of the tanks previously mentioned. Ninety-six apparently uniform plants, dug from the field during early March and stored at 35° F. until needed, were set two plants per crock in acid-washed quartz sand in the greenhouse in July 1952. The 2-gallon crocks were immediately placed in the four tanks, 12 crocks per tank (Figure 7) and the four temperatures established the following day. One unit was maintained at 45°, another at 55°, a third at 65° and a fourth at 75° F.

The experiment was originally designed to study mobilization of the fertilizer phosphorus in the fruit, an easily isolated non-treated plant part. It was apparent, however, after about a week that normal flowers were not being produced. Most of the flowers that appeared had abortive stamens. In many cases the filaments failed to elongate and the anther sacs turned brown. This condition may have been caused by prolonged storage, high day temperatures in the greenhouse or other unknown reasons. The flower abnormalities were not correlated with the root temperature treatments.

Since it became apparent that the fruit could not be used as a mobilization index for both leaf and root applications, the leaves and crowns for the root treatment and the roots and crowns for the foliage applications were used.

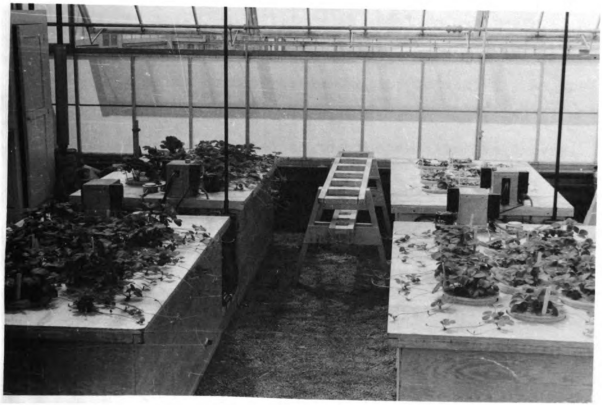
During the five week pre-treatment period, striking differences in growth became evident as a result of the various root temperatures (Figures 6 and 7). Full Hoagland solution was supplied throughout with weekly leaching of the cultures with distilled water followed by nutrient solution. Conductivity and pH of the nutrient solution leachates from each crock were measured weekly to detect the accumulation of excess salts or unfavorable soil reaction.

On August 15, 1952, foliage and soil treatments of $H_3P^{32}O_4$ were applied as follows: Half the plants (twelve) at each root temperature received a foliage application and

Figure 6. Effect of root temperatures on growth of Robinson strawberry plants.

Figure 7. General view of the tanks used for growing plants at different root temperatures. Left background, 75°; left foreground, 65°; right foreground, 55°; right background, 45°.


45
55
65
75



half a root treatment. Three fully expanded normal leaves from each plant were dipped in a 25 millimolar phosphoric acid solution having a specific activity of approximately 2.1 microcuries P^{32} per milligram of phosphorus. The treated leaves were separated from the non-treated portions until dry by means of a one-inch layer of absorbent cotton. It was calculated that from 0.2 to 0.8 millliliters of liquid adhered to the treated leaves. Thus, on an average of less than two microcuries of P^{32} were applied to each plant.

For the root treatments, a total of 20 microcuries of P^{32} contained in 200 millliliters of solution was poured around the base of each plant at least one inch from the crown. This solution had the same specific activity as the foliage treating solution. Thus, more than ten times as much radiophosphorus was applied to the roots as to the leaves. From these approximations an accurate comparison between the efficiency of foliar and root uptake could not be made.

At intervals of 24, 48, 96 and 144 hours after treatment, one representative plant from each temperature and method of treatment was selected and prepared for autoradiography as described (Methods). Of the remaining plants 24 (12 plants per method of treatment) were harvested at 48 hours and the remaining 24 at 144 hours. All were separated into their various parts, dried, ashed and otherwise prepared for

radioactive and chemical assay. Dry weight measurements of all parts were also recorded.

Results -- Growth response: Visual and quantitative differences in growth as a result of root temperature were evident (Table V, Figures 6 and 8). Runner production was most significantly influenced, followed by leaf growth. Dry weight production of roots was affected very little. However, at the lower temperatures the roots were of a more fleshy nature as compared with the more fibrous roots at the higher temperature. There was a marked decrease in the leaf:root ratio as the temperature was decreased from 75° to 45°. Crown growth was not significantly influenced by root temperature.

Effect on phosphorus uptake: It may be seen (Table VI, Figure 9) that a highly significant reduction in P^{32} uptake from the roots occurred as the temperature was decreased from 55° to 45°. This reduction was reflected in both the non-treated leaves and crowns. There was a tendency approaching significance, for a decrease in absorption and translocation of P^{32} from the roots as the temperature increased from 55° to 75°.

Translocation of radiophosphorus from the leaves of plants grown at different root temperatures presents a somewhat different picture, especially when compared with the roots. Translocation from the leaves to the crown

decreased both above and below 65° while transport to the roots was not significantly affected by root temperature. It should be emphasized again that actual amounts of labelled phosphorus applied to the leaves and roots were not the same, but at least ten times more P^{32} was applied to the roots. No attempt was made to adjust the data to base it on equivalent amounts of P^{32} applied as excessive errors might thereby be introduced. Comparisons are thus made between temperatures only and not with respect to the relative efficiencies of the foliar and root feeding methods.

The autoradiograms, similar for all time periods from 48 to 144 hours after treatment, visually substantiated these results. The plants harvested at 48 hours after treatment, shown in Figure 10, demonstrate the reduced uptake of phosphorus by roots which have been growing at 45°. Above this temperature little difference in uptake on a unit weight basis is evident, judging from the similarity of exposure of the X-ray film. However, the P^{32} concentration in the crowns and roots of the foliage treated plants appeared to be very similar regardless of root temperature, indicating that absorption and translocation from leaves to roots is not as greatly influenced by the root temperature as is absorption through roots and translocation to the leaves.

Table V. Effect of Root Temperature on Dry Weight Accumulation in the Various Plant Parts of the Robinson Strawberry. (Means of 12 single plant replicates).

	Dry Weight (gms. per plant)				Runners	
Temperature	Leaves	Roots	Leaf/Root Ratio	Crowns	Number	Dry Weight
75° F.	4.52	2.21	2.05	0.85	8.3	5.91
65°	3.61	2.07	1.74	0.71	6.3	2.73
55°	3.26	1.97	1.65	0.61	5.2	1.46
45°	1.63	1.66	0.98	0.63	1.2	0.13
L.S.D. - 5%	1.04	0.54		0.22	1.2	1.26
L.S.D. - 1%	1.39	0.72		0.29	1.7	1.68

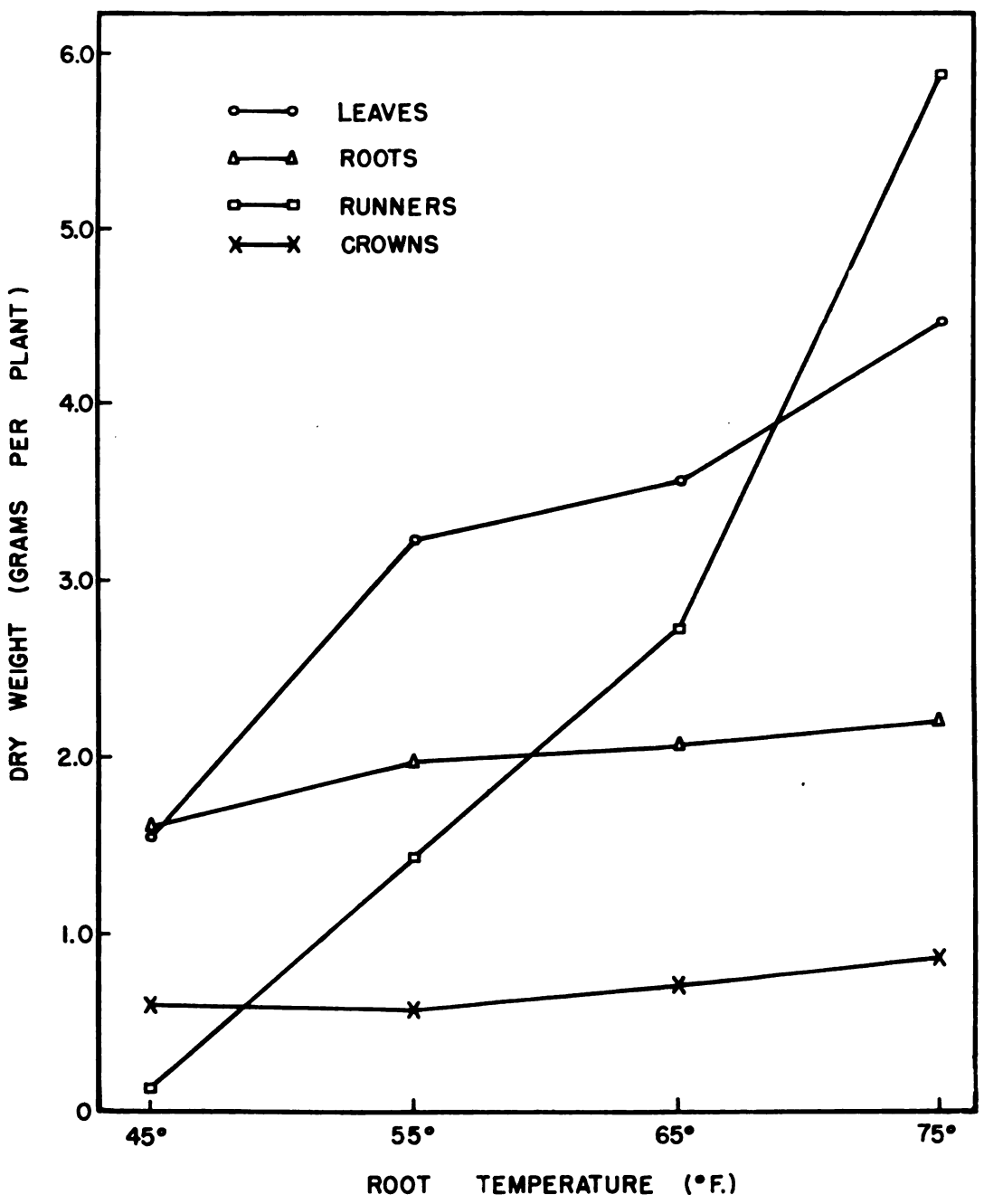


Figure 8. Effect of Root Temperature on Dry Weight Accumulation in the Robinson Strawberry.

Table VI. The Effect of Root Temperature on Translocation of Foliar and Root Applied Phosphorus to non-treated Portions of Robinson Strawberry Plants. (Means of six single plant replicates[1/]).

Root Temperature	Micrograms P Mobilized per gm. dry tissue			
	Root Application[2/]		Leaf Application	
	Leaves	Crowns	Roots	Crowns
75° F.	231.6	161.0	11.2	21.2
65°	253.9	175.5	7.1	37.5
55°	326.0	200.3	8.0	25.9
45°	135.7	92.4	7.9	14.6
L.S.D. - 5%	88.6	56.5	N.S.	17.1
L.S.D. - 1%	129.0	77.7	N.S.	24.9

1/ Values from 48 and 144 hour harvests are combined since the trends in both were similar

2/ At least ten times more radio-phosphorus was applied to the roots than to the foliage.

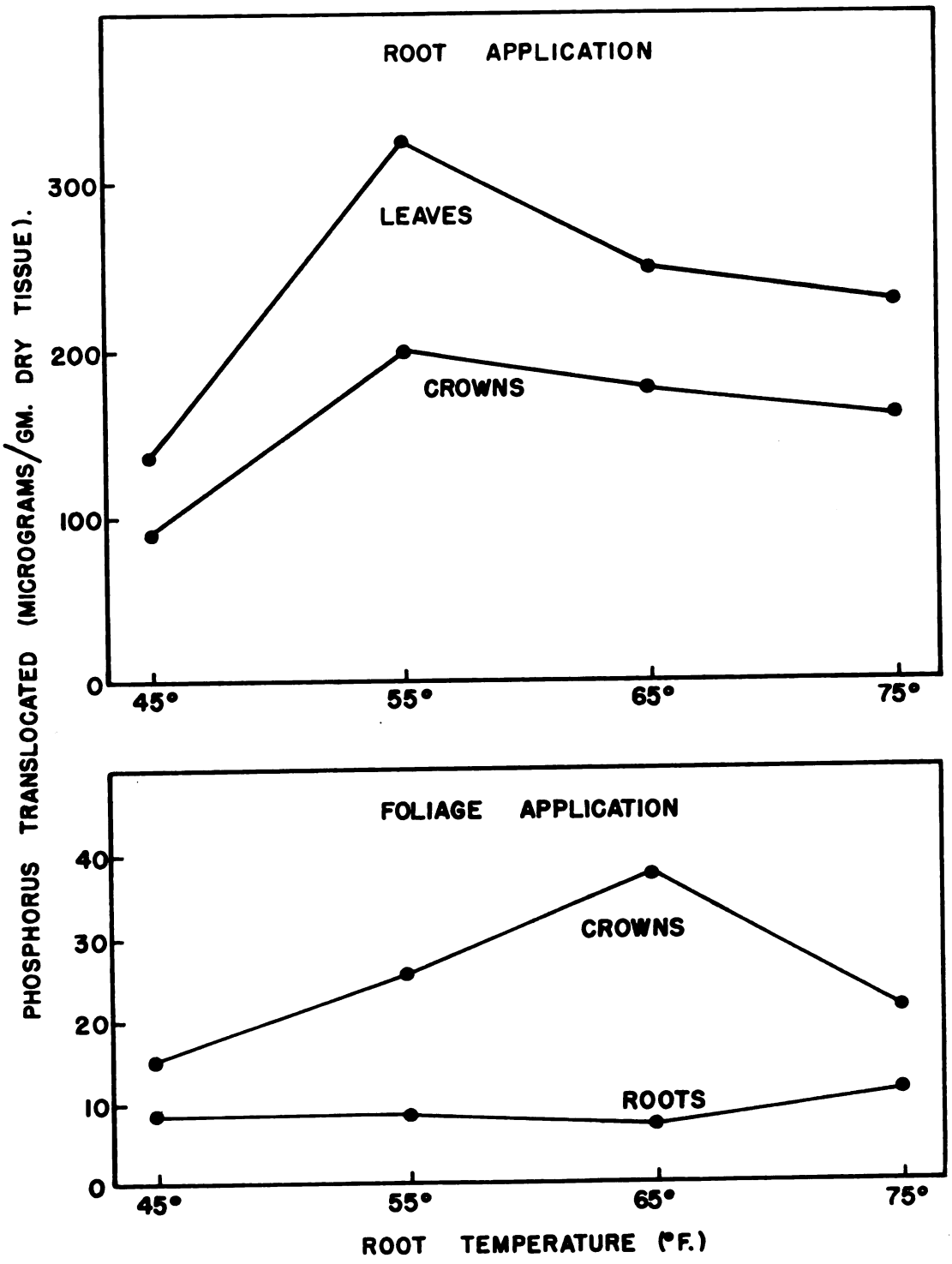


Figure 9. The Effect of Root Temperature on Translocation of Foliar- and Root-applied Phosphorus to Non-treated Portions of the Strawberry Plant.

Figure 10. Effect of root temperature on uptake of P^{32} from leaves and roots, 48 hours after treatment. The small numbers indicate the position of the runner plant in relation to the treated plant. 1 means first runner; 2, second runner, etc.

Top: Left to right: Foliage application to Robinson strawberry plants grown at root temperatures of 45°, 55°, 65° and 75° F. Note little difference in radiation intensity in the crown, indicating that root temperature had little effect on the translocation of P^{32} from the leaves.

Bottom: Left to right: Soil application to Robinson strawberry plants grown at 45°, 55°, 65° and 75° F. Note reduced uptake at 45° root temperature.

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VII. Effect of Phosphate Carrier on P^{32} Uptake by Leaves and Mobilization in the Fruit.

Four water soluble phosphate carriers are mono-potassium phosphate (KH_2PO_4), mono- and di-ammonium phosphates ($NH_4H_2PO_4$ and $(NH_4)_2HPO_4$) and ortho phosphoric acid (H_3PO_4). All have been used in starter solutions with generally good results. There are, however, indications that differences in availability may exist especially in regard to foliage application. Previous tests of numerous organic and inorganic phosphatic compounds by Silberstein and Wittwer (97) showed that phosphoric acid was the best source of phosphorus for foliage application on tomato. More recently Wittwer (119) using the more accurate isotope technique found that pH as well as carrier was important. It was the purpose of this experiment to compare the efficiency of three nitrogen-phosphorus carriers: Mono- and di-ammonium phosphate and phosphoric acid plus urea, using the isotope dilution analysis technique.

Twelve uniform Robinson plants were selected for the test in April 1953 from over 50 similar plants growing in six-inch pots of quartz sand. As in Experiment V, each plant contained an inflorescence at comparatively the same state of development. Instead of retaining only three flower buds, five were selected including the primary, two

secondary and two tertiary buds. All others were removed as they appeared. A diagram of the typical strawberry inflorescence is given in Figure 11.

P^{32} Treatments: The treating solutions were prepared as follows: A known volume of 0.25% phosphoric acid solution containing approximately one microcurie per milliliter of P^{32} was titrated against a concentrated ammonium hydroxide solution on a Beckman pH meter until the ionization point of mono-ammonium phosphate was reached. This occurred at a pH of 4.4. A second volume of phosphoric acid was then titrated to the second ionization point, that of the di-ammonium phosphate, which occurred at 7.8. Twice the amount of ammonium hydroxide was required. To a third portion of labelled phosphoric acid, urea (NuGreen)[1/] was added equivalent to six grams per liter or five pounds per 100 gallons. Finally all solutions were made up to the same volume with distilled water. Thus each of the solutions contained the same amount of P^{32} and P^{31} and had the same specific activity.

Five uniform leaves on each plant were designated for treatment with four single plant replicates. The leaves were treated by immersing them in the respective solutions twice at weekly intervals. The developing flowers and

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1/ NuGreen is a commercial product of the E. I. duPont de Nemours Company containing 43% nitrogen.

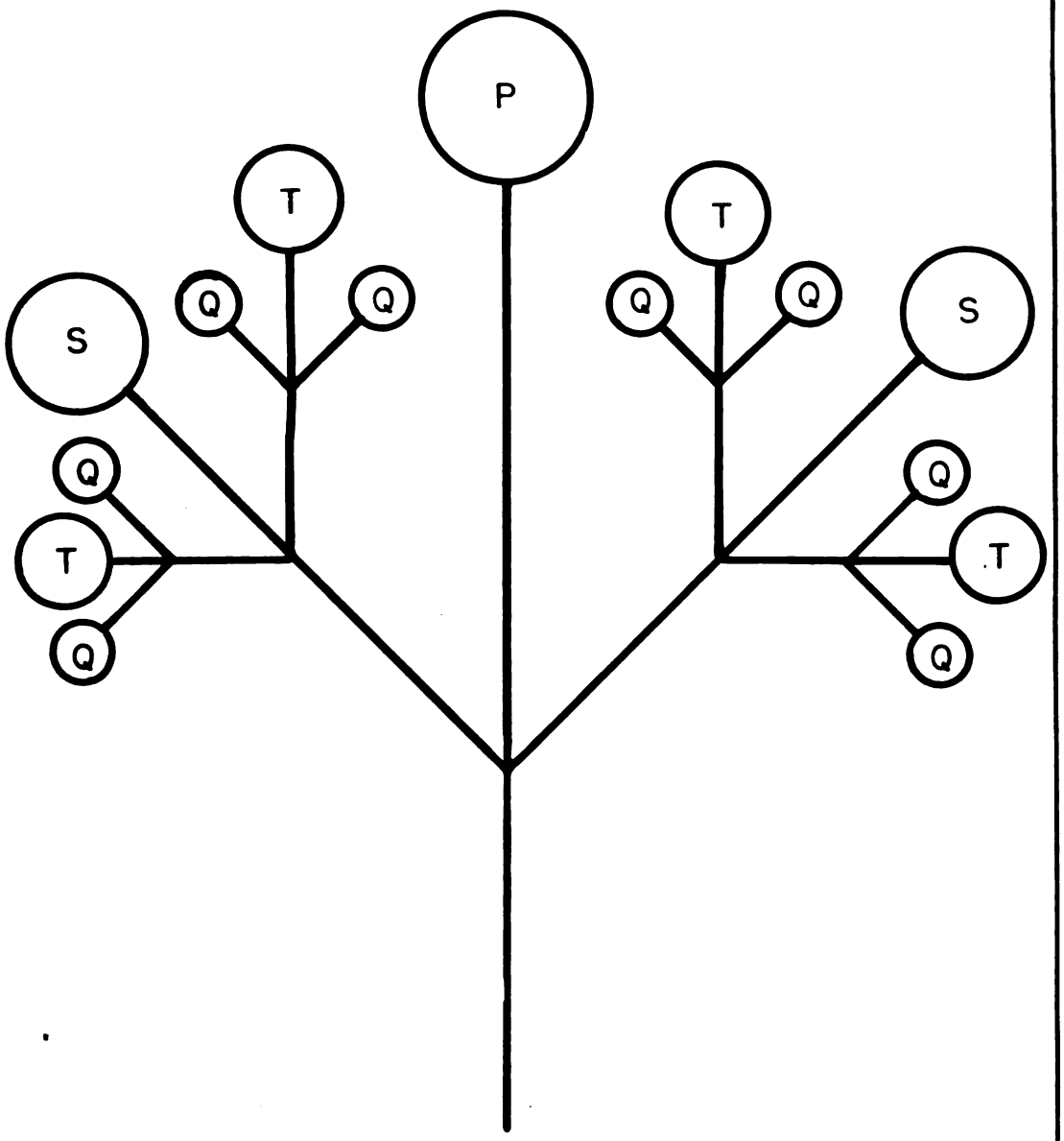

FIGURE 11. DIAGRAM OF TYPICAL STRAWBERRY INFLORESCENCE (CYME).

P = Primary

S = Secondary

T = Tertiary

Q = Quaternary

fruits were protected from contamination by means of Vinylite plastic covers. All flowers were hand pollinated.

Rather than harvesting the fruits at specified time intervals after treatment, it was thought preferable to harvest the fruits just prior to maturity. A rather sharp indication of the approach to maturity of the Robinson variety is the change of the achenes or "seeds" from green to red. This occurs a day or two before the first coloring of the flesh. All the fruits at each fruiting position were harvested as they reached this stage. As a result of careful selection of plants at the beginning of the experiment, all fruits at each fruiting position turned color almost simultaneously. The primary fruits were harvested fifteen days after the initial foliage treatments and the final collection was made eleven days thereafter.

Throughout the course of the experiment the plants received half-Hoagland nutrient solution at each watering.

Subsequent to harvest, the fruits were individually dried, weighed and ashed in porcelain milk ashing dishes. The ash was taken up in HCl and evaporated to dryness, leaving a uniform deposit suitable for counting. Total phosphorus content of the fruits was determined from the ash by the Standard A.O.A.C. molybdenum blue method (1). From the chemcial and radioactive analyses, the phosphorus mobilized in the fruit as a result of the foliage application was determined.

Results: A summary of the data obtained is given in Table VII. By comparing the treatment averages for each of the factors measured (micrograms per gram, micrograms per fruit, etc.) it may be seen that the phosphoric acid-urea combination resulted in a highly significant increase in phosphorus uptake and utilization as compared with the other carriers tested. Expressing the data in micrograms per fruit as well as in micrograms per gram dry weight may reveal differences in uptake which are correlated with variations in fruit size. In other words, a small developing fruit may accumulate phosphorus in its early development at the same rate as a larger one but differences in the mass of the two fruits would make it appear that the smaller fruit were accumulating much more phosphorus than the larger ones. In this experiment the fruits at each position in the cyme were exceptionally uniform in size as reflected by the similarity in the trend of the results by the two methods of representation (Figure 12). Similar also were the results when expressed as percent of the total phosphorus in the fruit derived from the foliar applications. A rather small percent of the total phosphorus in the fruit came from the radiophosphorus application to the foliage regardless of the carrier used. It is possible that a large proportion of the phosphorus found in the fruit may have been accumulated in the plant during the previous season. The data further

Table VII. Effect of the Phosphate Carrier on the Mobilization of Foliar-applied Phosphorus by the Strawberry Fruit.

Fruit Position in Inflorescence	H_3PO_4 + urea	$(NH_4)_2HPO_4$	$NH_4H_2PO_4$	Fruit Position Average
		Treatment Phosphorus Mobilized (ug/gm. dry weight)		
Primary	75.3	39.8	41.8	52.3
1st secondary	82.1	43.3	48.0	57.8
2nd secondary	70.2	52.2	45.4	55.9
1st tertiary	62.9	39.0	39.5	47.1
2nd tertiary	80.6	48.7	39.7	56.4
Treatment Aver.	74.2	44.6	42.9	
L.S.D. between treatment		5% - 11.2	1% - 14.9	
L.S.D. between fruit position		5% - N.S.	1% - N.S.	
		Treatment Phosphorus Mobilized (Micrograms per Fruit)		
Primary	83.7	43.3	46.1	57.7
1st secondary	63.7	35.5	35.9	45.0
2nd secondary	53.9	38.6	29.0	40.5
1st tertiary	26.5	20.7	17.9	21.7
2nd tertiary	39.0	24.6	18.7	27.4
Treatment Aver.	53.4	32.5	29.5	
L.S.D. between treatment		5% - 6.4	1% - 8.6	
L.S.D. between fruit position		5% - 8.3	1% - 11.1	
		Treatment Phosphorus Derived from Fertilizer Applied (% of total P)		
Primary	2.64	1.56	1.57	1.92
1st secondary	2.94	1.67	1.51	2.04
2nd secondary	2.78	1.87	1.52	2.05
1st tertiary	1.34	1.30	1.10	1.25
2nd tertiary	3.00	1.63	1.40	2.01
Treatment Aver.	2.54	1.61	1.42	
L.S.D. between treatment		5% - 0.52	1% - 0.69	
L.S.D. between fruit position		5% - 0.67	1% - 0.89	

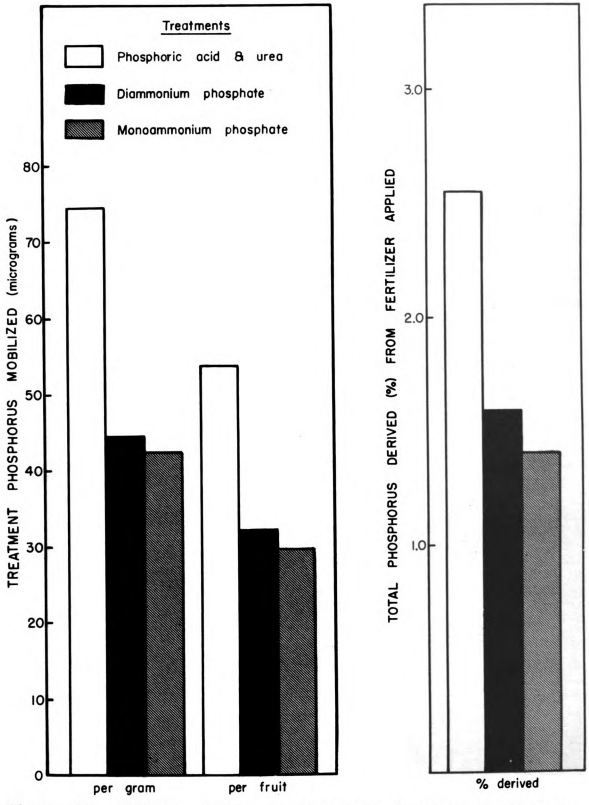

Figure 12. Effect of Various Phosphate Carriers Applied to the Leaves on the Mobilization of Phosphorus by the Strawberry Fruit.

indicate no significant difference between mono- and di-ammonium phosphates as carriers of phosphorus for foliar application.

The results obtained regarding the effect of fruit position on phosphorus uptake are difficult to evaluate since a time factor is also involved. In every case regardless of method of representation of the data, the lowest values were invariably obtained at the fourth harvest, that is, the first tertiary fruit. Also differences between carriers were generally not significant at this fruit position.

Results of phosphorus analyses of fruits: Although, as shown above, the isotope technique gave indication that certain phosphate carriers appeared to be more efficient than others for foliage application, the differences involved in relation to the total phosphorus content of the fruit were too small to be detected by chemical means. Thus, as shown in Table VIII, there were no significant differences in phosphorus contents of the fruits as a result of using different carriers. It may be of interest, however, to know whether any priority exists in regard to the nutrient distribution to the various members of the fruit cluster. That is, does the primary berry (the first and largest fruit) accumulate phosphorus to any greater extent than the later developing fruits such as the secondary or tertiary berries? It may be seen from Table VIII and Figure 13 that the dry

Table VIII. Effect of the Fruit Position and Phosphate Carrier on the Dry Weight and Total Phosphorus Content of the Fruit. (Mean values of four replications).

Fruit Position in Inflorescence	Phosphate Carrier H_3P*O_4 + urea	$(NH_4)_2HP*O_4$	$NH_4H_2P*O_4$	Fruit Position Average
		Dry Weight in Grams		
Primary	1.1327	1.0838	1.2362	1.1509
1st secondary	0.8329	0.8373	0.7793	0.8165
2nd secondary	0.8369	0.7252	0.6302	0.7308
1st tertiary	0.4708	0.5308	0.4398	0.4804
2nd tertiary	0.5332	0.5190	0.4692	0.5071
Treatment Aver.	0.7163	0.7392	0.7109	
L.S.D. between fruit position		5% - 0.1206	1% - 0.1823	
L.S.D. between treatment		5% - N.S.	1% - N.S.	
		Milligrams Phosphoruc per Gram Dry Weight		
Primary	2.85	2.60	2.66	2.70
1st secondary	2.74	2.58	3.20	2.84
2nd secondary	2.55	2.81	3.03	2.80
1st tertiary	3.16	3.02	3.60	3.26
2nd tertiary	2.64	2.63	2.95	2.74
Treatment Aver.	2.79	2.73	3.09	
L.S.D. between fruit position		5% - 0.44	1% - N.S.	
L.S.D. between treatment		5% - N.S.	1% - N.S.	
		Milligrams Phosphorus per Fruit		
Primary	3.23	2.80	3.30	3.11
1st secondary	2.23	2.15	2.49	2.29
2nd secondary	2.06	2.03	1.88	1.99
1st tertiary	1.41	1.60	1.60	1.54
2nd tertiary	1.33	1.34	1.23	1.30
Treatment Aver.	2.05	1.98	2.10	
L.S.D. between fruit position		5% - 0.28	1% - 0.42	
L.S.D. between treatment		5% - N.S.	1% - N.S.	

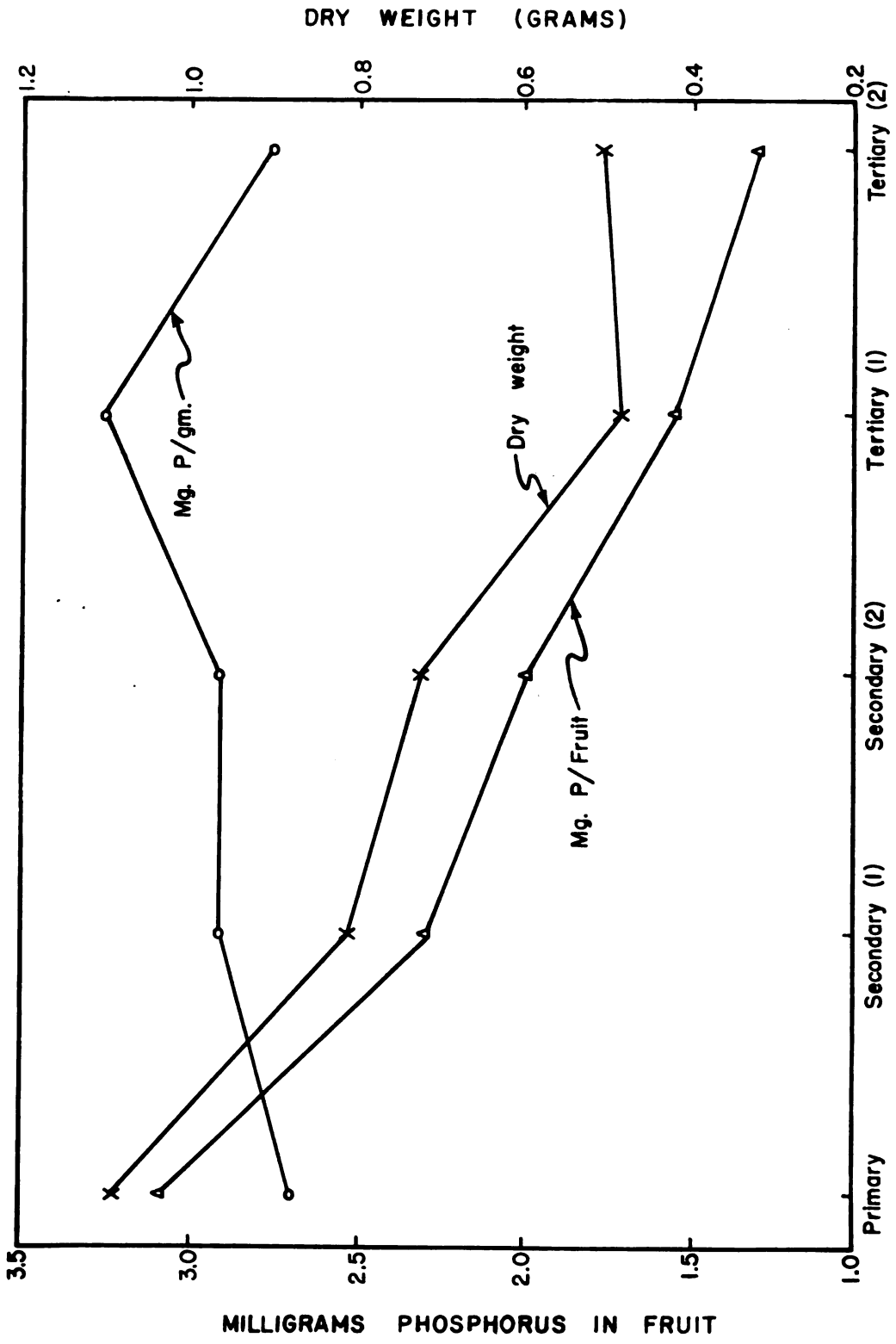

Figure 13 Influence of Fruit Position on the Dry Weight and Phosphorus content of the Strawberry Fruit.

weight of the fruits decreases in proportion to their secondary and tertiary positions. The primary berry was invariably the largest followed by the first secondary and so on down to the tertiary fruits which were about alike in dry weight (Figure 11). When phosphorus content was expressed in milligrams phosphorus per fruit there was a parallel decrease as might be expected. Most important, however, is the fact that, with one exception, the phosphorus content on a unit dry weight basis was rather constant for all the fruits regardless of their position in the cluster.

SECTION II

INFLUENCE OF FOLIAR SPRAYS OF NITROGEN AND PHOSPHORUS ON GROWTH AND FRUITING OF THE ROBINSON STRAWBERRY -- GREENHOUSE STUDIES

I. Study of the Tolerance of Strawberry Foliage and Fruit to Sprays of Urea and Phosphoric Acid.

On February 23, 1952, 25 plants, similar to those used in the experiments discussed in Section I, were potted in six-inch pots of a good greenhouse potting soil. One month later, on March 24, 16 of these, each having flowers and fruits at various stages from bloom to white-fruit stage, were treated with urea (NuGreen) and phosphoric acid at the following rates (2 plants per treatment):

(1) Phosphoric acid at .025 M., .05 M., .1 M., and .2 Molar[1/].

(2) Urea (NuGreen) at 6.0, 12.0, 18.0 and 24.0 grams per liter[2/].

The solutions were applied with an electric Handi-Sprayer[3/] to all surfaces of the leaves and fruit. The pH of the phosphoric acid solutions ranged between 1.5 and 2.3 and of the urea, 7.5 to 8.0.

1/ A .025 molar solution (approximately 0.25%) is equivalent to about 2 1/3 pounds of concentrated phosphoric acid (85%) per 100 gallons.

2/ 6.0 grams per liter is equivalent to five pounds of urea per 100 gallons.

3/ Varley and Sons, St. Louis 15, Missouri.

Results: A brief summary of the results is given in Table IX.

Phosphoric acid injury from all concentrations was detected within a day after the spray applications. It was characterized by brown necrotic areas particularly on the leaf margins and on the younger immature leaves. The youngest leaf immerging from the crown was the most susceptible to injury. A necrotic spot usually occurred wherever a drop of solution had dried and an upward cupping of the leaves was often associated with the injury. The fruit of the strawberry, particularly as it approached maturity, was highly susceptible to injury, which appeared as a browning of the epidermis. Urea injury, while somewhat similar to that of phosphoric acid, was more inclined to affect older mature leaves where it was seen as a marginal necrosis. The younger developing leaves were much more resistant to injury than the older leaves. No evidence of injury to the fruit was noted.

These tests were repeated in the field with the Robinson strawberry and other varieties. Similar trends and injury patterns were observed except that somewhat higher concentrations were found to be safe. It was concluded from these tests that urea may be safely used on field grown strawberries up to about eight pounds per 100 gallons and phosphoric acid (85% H_3PO_4) up to 2.5 pounds per 100 gallons.

Table IX. Tolerance of Strawberry Foliage to Solutions of Phosphoric Acid and Urea.

Phosphoric Acid		Urea (NuGreen)	
Concentration	Injury Factor	Concentration	Injury Factor
.025 Molar	1	6.0 gms./liter	0
.05	2	12.0	1
.10	3	18.0	3
.20	4	24.0	4

0 = no injury
1 = very slight injury
2 = slight injury
3 = moderate injury
4 = severe injury

II. Effect of Foliage Applications of Nitrogen and Phosphorus on Fruit Size of Robinson Strawberry.

This test, like the previous one, was designed to provide information which might be of use in proposed field trials.

It has been generally considered that the fruiting process depends almost entirely on foods and nutrients stored in the crowns and buds the previous growing season, and that the materials accumulated during the early spring just before fruiting are of minor importance in the current season's crop. No critical studies of the nutrient needs of the strawberry during the fruiting period have been found in the literature. The object of this experiment was to determine the effect of foliar applied nutrients applied during the fruiting period on the weight of individual fruits produced by Robinson strawberry plants.

Fifty plants were established in five-inch pots containing washed quartz sand in the greenhouse on February 23, 1952. Throughout the experimental period (April 23 - May 25) the plants received a basic Hoagland solution low in nitrogen (56 ppm) and minus phosphorus. Extra plants receiving complete Hoagland's solution were grown for comparison. At no time were visible symptoms of deficiency or growth differences between plants in evidence.

On April 9, when the primary flowers were in full bloom, twenty-eight uniform plants, seven per treatment (Table X), were selected from the original fifty for the foliage sprays. Each plant selected was pruned to one flower cluster with four buds at an equivalent stage of development. Pruning not only resulted in more uniformity but also decreased the possibility of the occurrence of a nutrient response. Thus, any significant treatment difference which occurred would likely be accentuated with unpruned plants.

The spray solutions shown in Table X were applied with the small electric Handi-Sprayer previously described, thoroughly wetting the entire leaf area, about 20 cc. being applied to each plant.

No precaution was taken to prevent the spray solutions from contacting the rooting medium, although it is believed that most of the liquid applied adhered to the leaves. Two sprays were applied at weekly intervals, with a total of 0.36 grams of urea and 0.15 grams of phosphoric acid (100%) applied per plant.

Prior to each of the weekly spray treatments the pots (but not the foliage) were submerged in a distilled water bath to thoroughly leach out any solute accumulation in the root medium. After each spraying the plants were completely randomized in the experimental block.

Table X. Effect of Foliage Sprays of Urea and Phosphoric Acid on the Weight and Rate of Maturity of Robinson Strawberry Fruits[1/].

Spray Treatments	Ave. Wt. per Fruit (gms.) Primary	Secondary	Tertiary	Quaternary[2/]	Ave. Yield per Plant	Bloom to Maturity (days) Primary	Quaternary
Urea (NuGreen) 6.0 gms./liter	19.7	10.9	8.8	7.2	46.6	22.7	33.7
Phosphoric acid 0.25%	20.6	12.6	10.2	8.1	51.5	23.7	36.0
Urea (6.0 gms.) + phosphoric acid 0.25%	21.0	11.8	10.4	7.6	50.8	22.1	33.1
Control (water)	18.4	9.0	6.5	5.9	39.9	23.9	37.3
L.S.D. between treatment weight totals (yield)					5% - 8.0 1%- 11.5		
L.S.D. detween maturity averages						5% - N.S.	1% - N.S.

1/ Each value given is the mean of seven single plant replicates.

2/ Indicates position of fruit in inflorescence (see Figure 11).

The fresh weight of fruit from each plant at each of four pickings was recorded as well as the harvest date. These data are presented in Table X.

Results: When the data were analyzed statistically for total weight of fruit produced in each treatment, a significant increase was noted in both treatments containing phosphoric acid. The presence of urea in addition to phosphoric acid did not result in an increase over phosphoric acid alone. It should be mentioned in this connection that some nitrogen (56 ppm) was present in the nutrient solution in which the plants were grown while phosphorus was entirely excluded. The data indicate, however, that the nutrients accumulated the previous growing season might not be sufficient to allow maximum fruit growth and that an additional amount supplied through foliar applications during the fruiting season could possibly be of benefit in increasing fruit production.

It may be seen from the data also that while the nutrient treatments increased the weight of each of the fruits harvested, they apparently had little effect on the maturity of the first and last fruits. It is interesting to note, however, that urea nitrogen did not delay maturity.

A graph of the fruit weight data (Figure 14) shows rather strikingly that the decrease in size of the later maturing fruits greatly overshadows the differences due to the fertilizer treatments.

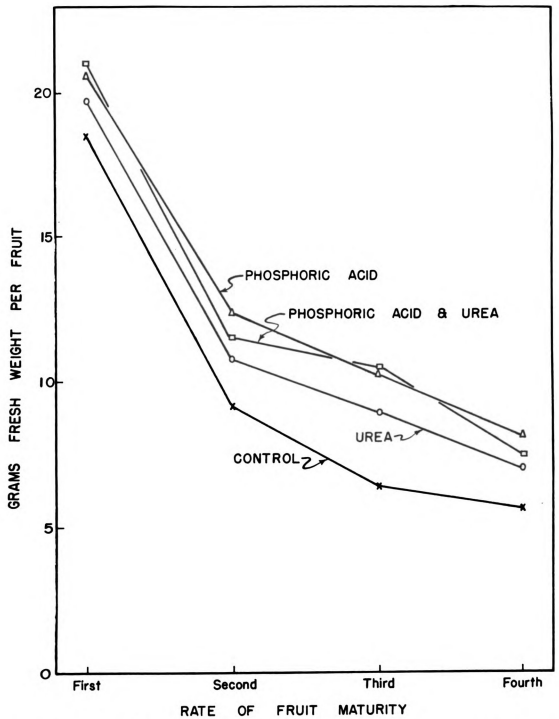

Figure 14. Effect of Foliage Sprays of Nitrogen and Phosphorus on the Weight of Robinson Strawberry Fruits Maturing at Progressively Later Dates.

III. Effect of Foliar Applications of Nitrogen and Phosphorus on the Growth and Phosphorus Content of Strawberry Plants Grown at Different Root Temperatures.

In experiments V and VI of Section I, the results showed a marked effect of the temperature of the root medium not only on growth but also on the absorption of phosphorus through leaves and roots of the strawberry plant. In the Northern states, soil temperatures of 50° or below may commonly prevail during the spring when strawberries are starting to renew growth and fruit production. Whether the low root temperature affects growth to the same degree as it affects nutrient absorption is still the subject of some question. If the reduced growth may, in part, be caused by decreased nutrient uptake, it may be possible to affect the growth, fruitfulness and maturity of the strawberry by means of leaf feeding. Thus, in this experiment the response of Robinson strawberries growing at 50° and 70° root temperatures to various nitrogen and phosphorus materials applied to the foliage was compared with different concentrations of these elements supplied in the root medium.

Well-rooted runner plants, dug from the field on August 25, 1952, were washed and were planted three per pot in two-gallon crocks filled with Flint Shot and No. 7 quartz sand. The plants were shaded until established and watered daily with half-Hoagland nutrient solution until September

5 and with distilled water from September 6 to 16 at which time the crocks were transferred to 50 and 70 degree experimental temperature tanks and the differential nutrient treatments started. No visible symptoms of nutrient deficiency appeared throughout the experimental period. The prevailing daylength was prolonged four hours with fluorescent lighting to maintain vegetative growth.

The spray treatments listed in Table XI were applied on September 18 and 26 and October 2 and 9. On each of these occasions the crocks were removed from the tanks and placed in wooden racks designed for this purpose. In this way, no spray drift between treatments occurred. Furthermore, a layer of absorbent cotton was placed completely around the base of all plants to prevent contamination of the root medium. In order to check this, a small amount of P^{32} (0.1 microcurie per milliliter) was included in the phosphorus spray treatment. Since no activity was detected in the leachate from the crock it was concluded that no contamination had occurred. The plants not receiving foliar treatments were similarly surrounded with the cotton so as to avoid possible variation due to difference in the evaporation rate from the surface of the sand.

It was shown by Roberts (92) that the half-Hoagland nutrient solution produced best growth of the Robinson strawberry in sand culture. Therefore, this solution was used as a basis for the addition of all elements except

Table XI. Treatments Applied to Strawberry Plants Growing in Sand Culture at 50° and 70° Root Temperatures.

Spray Treatment	Nutrient Solution[1/]
1. Urea (NuGreen) 6.0 gm./liter	0.1 NP
2. Phosphoric acid 0.3%	0.1 NP
3. Urea (6 gms./l.) + H_3PO_4 (0.3%)	0.1 NP
4. Control - not sprayed	0.1 NP
5. Control - not sprayed	0.5 NP
6. Control - not sprayed	1.0 NP

1/ A basic half-Hoagland solution in regard to all elements except nitrogen and phosphorus was used for all treatments. Nitrogen and phosphorus were adjusted to the one-tenth, one-half and full Hoagland levels as shown in Table XII which follows.

nitrogen and phosphorus. The nitrogen and phosphorus levels given in Tables XI and XII were based on the contents of these nutrients in a full Hoagland solution. For example, the 0.1 NP solution contained one-tenth the nitrogen and phosphorus present in a normal Hoagland solution. The 0.5 NP was a one-half Hoagland solution throughout and the 1.0 NP contained the full compliment of nitrogen and phosphorus.

At weekly intervals during the course of the experiment the crocks were leached with their respective nutrient solutions and a small quantity of this first leachate analyzed for conductivity (Solubridge Conductivity meter) and pH (Beckman pH meter). If any difference existed between the leachates and the nutrient solutions the cultures were again leached and samples taken until the two were alike. In this manner possible solute accumulations or adverse pH levels were avoided.

The experiment was terminated on October 20, five weeks after the initiation of the treatments and 11 days subsequent to the last nutrient spray. The plants were separated into leaves, roots, crowns and runners each of which was washed thoroughly in tap and distilled water. Fresh and dry weights of all portions were obtained. After drying, the leaf and root tissues were ground in a Wiley mill to pass through a 40 mesh sieve and one gram samples were ashed in a muffle furnace for analysis of total phosphorus.

Table XII. Salts Used and Composition of Nutrient Solutions.

Salt	ml./l. of Molar Stock Soln. 1.0 NP	0.5 NP	0.1 NP	Nutrient	ppm in Solution 1.0 NP	0.5 NP	0.1 NP
Calcium nitrate	2	2	-	N	210	105	22.4
Potassium nitrate	3	3	1.5	P	31	15.5	3.1
Monoammon. phosphate	1	0.5	0.1	K	69	69	63
Magnesium sulfate	1	1	1	Ca	80	80	80
Ammonium nitrate	3	-	-	Mg	24	24	24
Calcium chloride	-	-	2	S	32	32	32
Potassium chloride	-	-	1.5	Cl	--	--	124.3

Trace elements were supplied at the half-Hoagland level.

Results: The effects of the root temperatures and of the foliar sprays are treated separately in Tables XIII and XIV. From Table XIII it may be seen that in terms of both fresh and dry weights, significantly greater development of leaves occurred at 70 degrees while root growth was greater at the 50 degree root temperature. Fresh weight of the crown tissue was significantly higher at 70 degrees but dry weight was not. It is interesting to note that there was practically no difference in total dry weight of plant material produced at the two temperatures (Table XIII).

Effects of foliar sprays and nutrient concentrations on growth: It is evident from Table XIV that leaf growth of the strawberry was influenced to a greater degree by fertilization that was root or crown growth. Fresh and dry weight of leaves was significantly increased not only by each of the foliage treatments but also by the 0.5 NP and 1.0 NP nutrient solution concentrations while no significant effect on root or crown growth was obtained. A greater response to the foliage sprays was noted at 50° than at 70° (Figure 15).

The 1.0 NP concentration, however, gave no significant increase over the 0.5 level of the two elements. There was no significant difference among any of the foliar sprays. The data produced no conclusive evidence that leaf growth could be increased more by foliar applications of nitrogen

Table XIII. Effects of Root Temperature on Fresh and Dry Weight Accumulation in Robinson Strawberry.

Root Temperature (degrees F.)	Weight in Grams per Three Plants							
	Leaves		Roots		Crowns		Total	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
50	37.5	10.58	57.7	7.21	9.4	2.30	101.6	20.09
70	51.4	12.23	48.2	4.82	10.6	2.11	110.2	19.16
L.S.D. 5%	3.3	0.72	4.1	0.46	0.8	N.S.	7.5	N.S.
L.S.D. 1%	4.5	0.96	5.5	0.61	1.1		10.1	

Table XIV. Effect of Foliar Sprays on Fresh and Dry Weight Accumulation of Robinson Strawberry.

Spray Treatment	Weight in Grams per Three Plants					
	Leaves		Roots		Crowns	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Urea	47.3	11.5	51.1	5.95	10.0	2.06
Phosphoric acid	43.9	10.9	51.1	6.17	10.4	2.25
Urea + phosphoric acid	44.1	10.9	50.9	5.52	10.3	2.15
Control - 0.1 NP	37.4	10.1	49.6	6.37	8.8	2.11
Control - 0.5 NP	46.4	12.1	49.4	6.02	9.6	2.23
Control - 1.0 NP	47.3	12.6	57.0	6.07	10.8	2.40
L.S.D. - 5%	5.8	1.25	N.S.	N.S.	N.S.	N.S.
L.S.D. - 1%	7.8	1.67				

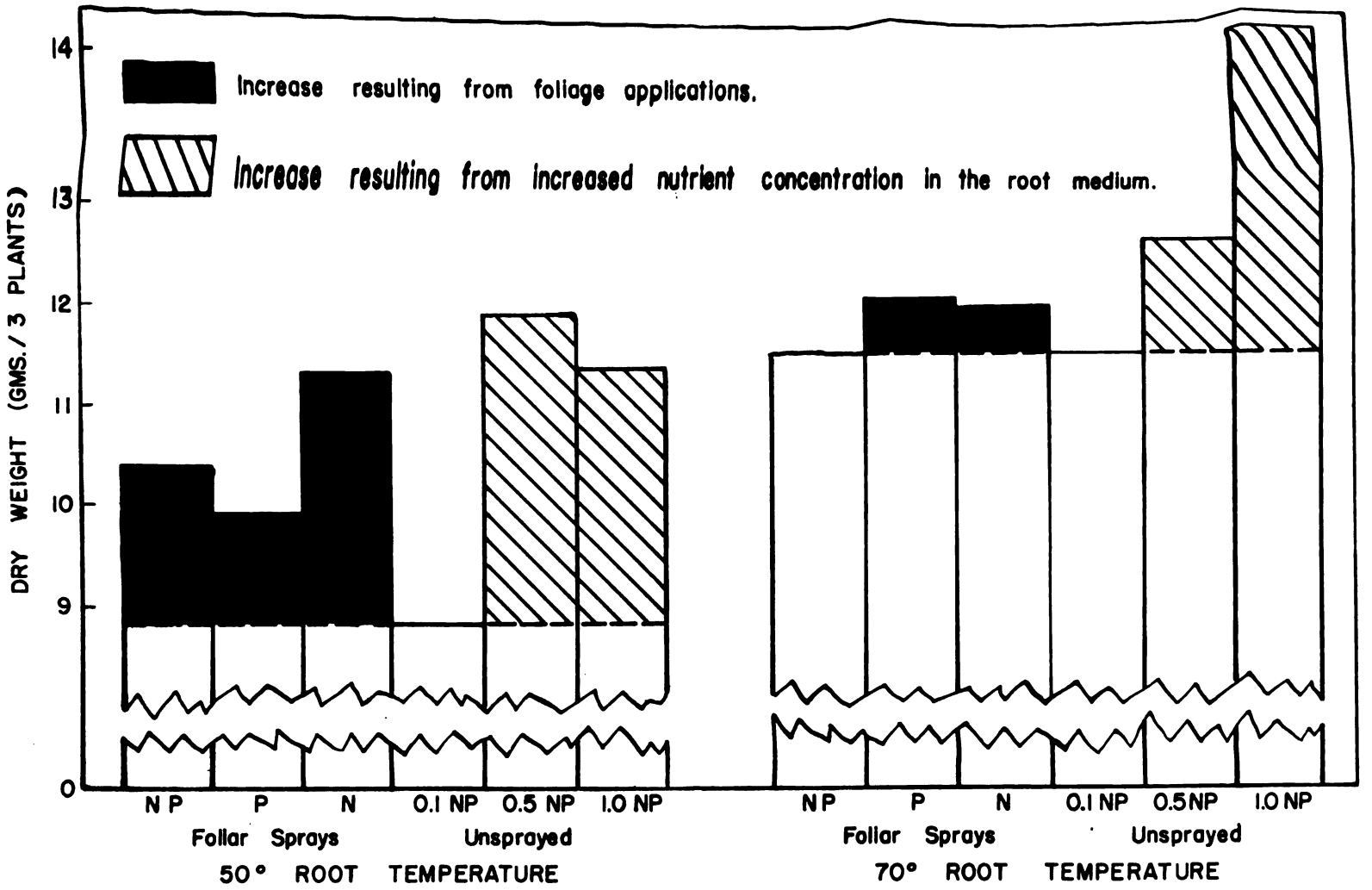

Figure 15. Effect of Root Temperature, Foliar Sprays and Nutrient Concentration on the Dry Weight of Strawberry Leaves (Explanation of treatments in Table XI).

and phosphorus than by increasing the concentration of these elements in the nutrient solution.

Root temperature and nutrient treatments on phosphorus content: The data in Table XV show the effect of root temperature and nutrition on phosphorus uptake and utilization in leaves and roots. It may be seen by comparing temperature averages that phosphorus was accumulated to a significantly greater extent at 70 degrees than at 50 degrees. When the treatment averages are compared, it is seen that the phosphorus content of leaves and roots was also significantly increased by both the foliar sprays containing phosphorus and by increasing the phosphorus concentration in the nutrient solution, especially the latter. The urea spray treatments had no significant effect on phosphorus content of any plant part when compared to the 0.1 NP control. The higher phosphorus content of leaves and roots at the 1.0 NP nutrient solution level was not reflected in an increase in growth over the 0.5 NP concentration (Table XIV).

Table XV. Effect of Root Temperature and Foliar Sprays on Phosphorus Content of Robinson Strawberry (% dry weight).

Root Temperature (degrees F.)	Urea Spray	Phosphoric Acid Spray	Urea + Phosphoric	Control 0.1 NP	Control 0.5 NP	Control 1.0 NP	Temp. Average
			Leaves				
50	0.174	0.276	0.263	0.178	0.331	0.576	0.300
70	0.238	0.345	0.348	0.214	0.381	0.490	0.336
Treatment Average	0.206	0.311	0.306	0.196	0.356	0.533	
L.S.D. between treatment averages				5% - 0.0273		1% - 0.0366	
L.S.D. between temperature averages				5% - 0.0157		1% - 0.0211	
L.S.D. between temperature x treatment				5% - 0.0384		1% - 0.0517	
			Roots				
50	0.149	0.210	0.205	0.143	0.321	0.416	0.240
70	0.149	0.193	0.186	0.125	0.351	0.550	0.259
Treatment Average	0.149	0.201	0.196	0.134	0.336	0.483	
L.S.D. between treatment averages				5% - 0.025		1% - 0.034	
L.S.D. between temperature averages				5% - 0.014		1% - 0.019	
L.S.D. between temperature x treatment				5% - 0.036		1% - 0.048	

FIELD EXPERIMENT

IV. The Effect of Foliage Sprays of Phosphorus and Other Major Nutrients on the Yield and Quality of Strawberries.

The foregoing greenhouse studies which showed (1) ease of absorption of certain nutrients through leaves, (2) depression of uptake by roots at low temperatures and (3) possible yield responses from foliage sprays, led to the desire to test some of these results under commercial conditions. It was believed that nutrients, especially phosphorus, applied to the developing leaves early in the season might significantly increase yield, fruit size and quality of the strawberry.

On April 7, 1953 the experimental areas (hereafter designated as Farms A, B and C) were selected from commercial plantings made the previous year. All the farms were located in the main strawberry-producing area of Van Buren County in Southwestern Michigan, but were situated at least five miles apart. The varieties used, soil type and fertilizer practice are given in Table XVI. At each location, seven uniform 100-foot sections of row were staked off for the treatments which were randomized throughout the experimental area. Because of lack of uniformity in some of the plots, records were taken only on three representative 10-foot sections of row within the 100-foot block.

Table XVI. Varieties, Soil Types and Previous Fertilizers Applied to Experimental Plots.

Farm	Variety	Soil Type	Previous Fertilizer Practice		
			Date	Analysis	Rate
(A)	Robinson	Fox Sandy Loam	1952	3-12-12	150#/acre
			Apr. 1953	2 parts 4-16-16 1 part 6-10-4	500#/acre
(B)	Premier	Plainfield Sand	1952	6-10-4	2000#/acre in 3 applns.
			Apr. 1953	6-10-4	500#/acre
(C)	Premier	Rimer Sandy Loma	1952	6-10-4	Approx. 500#/acre
			1953	none	

All treatments were applied with a 1 3/4 gallon Myers "Pestop" sprayer[1]. One gallon of material was used for each 100-foot plot. The Bonro[2] soil application (Treatment No. 7) was made in solution from a sprayer, the nozzle being removed and the solution being dispensed at low pressure directly over the row. The list of treatments is given in Table XVII.

The concentrations of the treating solutions were calculated so that an equivalent amount of actual phosphorus (approximately .008 pounds per gallon of solution) was applied wherever this element was included in the treatment. Greenhouse experiments had shown that considerably higher rates of some of the chemicals could have been used without foliage injury, but the low tolerance of leaves to phosphoric acid prevented increasing their concentrations.

Data Obtained: For an estimation of yield, the actual weight of fruit to the nearest ounce was measured from each 10-foot sampling plot on each picking date. Whenever possible, the same pickers were used in harvesting the entire experimental areas. Fruit size was determined by counting the number of fruit in 1½ pounds at each picking date.

1/ Myers "Pestop" sprayer. F. E. Myers & Bro. Co., Ashland, Ohio.

2/ Bonro is a product of Swift & Company, Hammond, Indiana.

Table XVII. Fertilizer Formulations applied in Field Experiments.

Treat. No.	Chemical	Concentration	Dates of Application	State of Development when sprayed
1	Control Water only		May 4,8,23	2 pre-bloom 1 post-bloom
2	NuGreen (urea) to foliage	5 lbs/100 gal.	May 4,8,23	2 pre-bloom 1 post-bloom
3	Phosphoric acid to foliage	2.5 lbs/100	May 4,8,23	2 pre-bloom 1 post-bloom
4	Phosphoric acid for foliage	2.5 lbs/100	May 8,23,30	1 pre-bloom 2 post-bloom
5	Diammonium Phosphate to foliage	3.3 lbs/100	May 4,8,23	2 pre-bloom 1 post-bloom
6	Bonro 10-50-10 to foliage	3.5 lbs/100	May 4,8,23	2 pre-bloom 1 post bloom
7	Bonro 10-50-10 to soil	3.5 lbs/100	May 4,8,23	2 pre-bloom 1 post-bloom

As an index of fruit quality, representative samples from each treatment were collected twice a week for analysis of firmness, soluble solids and freezing quality. Firmness was measured according to the method and instrument described by Whittenberger and Marshall (118). Ten fruits from each treatment were individually placed in an upright position on the stage of the instrument and compressing discs were adjusted to hold the fruit lightly. By means of a spring loaded trigger mechanism, one of the discs was then forced against the fruit. After 10 seconds, the compression was recorded to the nearest one-half millimeter. The compression readings for the 10 fruits were averaged to give a single firmness measurement for each treatment.

Soluble solids were determined from a juice extract of 20 fruits from each treatment by means of a Bausch and Lomb hand refractometer. These readings are expressed in per cent soluble solids.

Samples were obtained from each location for freezing both with and without sugar to ascertain whether there was any effect of treatment on freezing quality.

Results: The average yield at the three farms, though measured in pounds per 10 feet of row, was converted to crates per acre in Table XVIII. From the weight of fruit per 16-quart crate (24 pounds) and the total area under treatment (1200 feet of row or about 1/9 acre) it was

Table XVIII. Effect of Fertilizer Treatments on the Yield of Strawberries at Three Farms in Southwestern Michigan.

Treatment[1/]	Yield in Crates per Acre		
	Robinson Farm A	Premier Farm B	Premier Farm C
Control - water only	450	504	243
Urea 5/100	423	576	324
Phosphoric acid early (0.3%)	450	450	333
Phosphoric acid late (0.3%)	423	351	261
Diammonium phosphate 3.3/100	374	459	301
Bonro spray 3.5/100	396	477	301
Bonro soil 3.5/100	486	450	283
L.S.D. 5%	N.S.[2/]	85	51
L.S.D. 1%		119	72

1/ The unabbreviated list of treatments is given in Table XVII.

2/ N.S. - F value not significant.

determined that a yield of 10 pounds of fruit per 10 feet of row was equivalent to 450 crates of strawberries per acre.

Table XVIII shows clearly that yields at Farms A and B were consistently higher than at Farm C. At A and B there was no significant increase as a result of the fertilizer treatments. On the other hand, at Farm C, all the fertilizer treatments except No. 4, the late phosphoric acid spray treatment, gave a significant increase in yield of fruit of from 17 to 37 per cent. A beneficial response was obtained both from the nitrogen and phosphorus formulations. The phosphorus in treatment No. 4 may have been applied too late to give a response in yield or there might have been some invisible foliage injury. The foliage application of Bonro (Treatment No. 6) did not result in yields significantly higher than when Bonro was applied to the soil in solution (Treatment 7).

In explanation of the yield results, it may be recalled from Table XVI that Farms A and B received 500 pounds per acre of a 6-10-4 fertilizer early in the spring of the second season. Also at Farm B, 2000 pounds of a 6-10-4 fertilizer was applied the first season as compared with 500 pounds at Farm C with the same variety. At C, however, no fertilizer was applied to the experimental area in the spring of the second season, though the remainder of the patch

received a single foliar application of a soluble fertilizer (13-26-13) at 5 pounds per 100 gallons of water. This would hardly compare with the nutrients supplied to A and B. Thus the wide difference in yield between Farm C and the other two locations are likely accountable to differences in fertilizer applications. The lower level of fertility at C could easily account for the yield differences noted in Table XVIII.

The most outstanding difference in fruit size shown in Table XIX is notably larger size of the Robinson variety, (Farm A), than of the Premier variety. Throughout the harvesting period the Robinson variety averaged 50 per cent greater size than the Premier.

No consistent effect of treatment was evident on firmness or soluble solids (Table XIX). The Robinson variety tended to be somewhat firmer than Premier. This is in agreement with the experience of growers. The data also indicated that fruit from Farm C was somewhat firmer than that at Farm B. This is logical in light of the differences in fertility programs.

Table XIX. Effect of Fertilizer Treatments on Strawberry Fruit Size, Firmness and Soluble Solids. (Season average).

Treatment	Average number of fruits per 1½ lbs. (one quart)			Firmness Compression in cm. (Aver. of 10 Fruits)			Soluble Solids %		
	Robinson		Premier						
	A	B	C	A	B	C	A	B	C
1. Control - water	57.2	98.7	95.5	.82	1.05	.92	7.4	7.4	7.7
2. Urea 5/100	62.2	86.0	92.0	.87	.99	.93	7.9	7.6	7.0
3. Phosphoric acid early (.3%)	68.4	81.0	113.0	.82	1.06	.97	7.7	7.7	7.2
4. Phosphoric acid late (.3%)	70.6	108.0	107.3	.94	1.17	.90	8.2	8.1	7.2
5. Diammonium Phosphate 3.3/100	63.8	86.7	89.8	.91	1.13	.90	7.9	7.2	7.2
6. Bonro spray 3.5/100	59.4	90.7	102.8	.83	.99	.94	8.2	7.6	7.7
7. Bonro soil 3.5/100	59.0	101.2	102.5	.92	1.10	.88	7.7	7.4	7.2
Average of all treatments	64.4	93.2	100.4	.87	1.07	.92	7.9	7.6	7.3

DISCUSSION

SECTION I - UPTAKE OF RADIO-PHOSPHORUS AND CALCIUM BY THE LEAVES AND ROOTS OF THE STRAWBERRY

Distribution of P^{32} and Ca^{45} throughout the plant from foliage and root applications: Tracer techniques such as autoradiography and isotope dilution analysis yield visual and quantitative results which the most painstaking chemical methods could not duplicate. By the use of these techniques, it has been shown that radiophosphorus is readily absorbed by leaves and roots of the strawberry and within a few hours is transported to all parts of the plant. The rate of translocation from both absorptive organs appeared to be similar. Movement from both leaves and roots is primarily toward meristematic regions such as developing fruits, leaves, runners and root tips. This has been demonstrated also with the bean (7) and tomato (97, 120).

In contrast to phosphorus, the translocation of radiocalcium from a foliage application is negligible, if it occurs at all. It is difficult to ascertain positively whether there was any absorption of calcium through the leaf cuticle, although there is no evidence that would suggest that leaf absorption is selective.

By means of autoradiography, a clear picture of the movement of Ca^{45} from the roots is presented, showing that while Ca^{45} is readily translocated upward to the leaves and laterally to top portions of adjacent runner plants, it moves only in the direction of the transpiration stream. Although reaching the foliage of the adjacent runner plants, very little Ca^{45} was found in their roots. These results are similar to Bledsoe's investigations with the peanut (11) and with the stoloniferous Pangola grass (Digitaria decumbens) (12). Bledsoe found that the fruiting pegs of the peanut plant could not obtain calcium from the mother plant but depended on direct absorption of this element from the soil surrounding the pegs. He also was able to induce a calcium deficiency in the roots of the Pangola grass runner plant although adjacent connected plants were well supplied with this nutrient.

The explanation as to why phosphorus but not calcium moves freely from both leaves and roots is still theory. The opinion in regard to translocation from roots (23, 99) is that upward movement of all solutes occurs primarily in the xylem, each nutrient element being translocated somewhat independently by diffusion gradient rather than being merely swept along with the transpiration stream. Absorption through the leaves of plants appears to occur by simple diffusion through pectinaceous materials in the leaf cuticle

(93) rather than by any complex process such as ion exchange. Thus, it would appear that the relative rate of entry of phosphorus and calcium would depend upon the diffusion gradient exerted. Once inside the leaf both elements are capable of being utilized.

The investigations of Chen (23) have shown that translocation of P^{32} from the leaves occurs both upward and downward in the plant in the phloem tissue, probably in an organic form, such as a sugar phosphate. Calcium, which probably does not complex with soluble carbohydrate fractions, would not become mobile in the organic state. Whether there is any appreciable downward movement of inorganic solutes in the phloem is not known. It is difficult to visualize how P^{32} could move from leaf application as rapidly as it is known to do completely in association with carbohydrates. It would seem that this question could be answered by double tagging certain organic phosphates with C^{14} and P^{32} to determine the relative rate of movement of the two isotopes from a leaf application.

Influence of the nutritional status on P^{32} and Ca^{45} uptake: The importance of an adequate realization of the nutritional status of the plant has been emphasized by Hoagland and Broyer (64) in their classical paper regarding factors affecting salt accumulation. These workers showed that a low salt content in the tissue is generally accompanied by a high sugar content, both of which permit a

very rapid subsequent absorption of salt. The results presented herein are in agreement with those of Hoagland and Broyer in regard to root uptake. Four times as much P^{32} was absorbed through the roots by "low" phosphorus plants than was taken up by "normal" plants. However, the utilization of P^{32} when applied to the leaves was influenced much less by the phosphorus status of the plant. An explanation of this cannot be given according to the diffusion theory. Both leaves and roots at the low phosphorus level had a lower content of phosphorus than the "normal" plants. It would seem that under these circumstances leaf absorption would also be influenced by the phosphorus status. Further investigations are necessary before definite conclusions can be made.

Nutritional relationship of the runner plant: During the early stages of these investigations many questions came to the author's mind in regard to the role of the runner plant in the physiology of the entire clone. For example: When does the runner plant become physiologically independent?; is the nutrition of the runner plant different from that of its parent?; are the runner plants a benefit or hindrance to the parent plant either before or after they become rooted? After completion of these studies and thorough reference to the literature, some definite conclusions can be made.

The strawberry runner plant cannot be considered as a separate individual as long as it is connected to its parent by a functional stolon. According to White (116) the stolon is a true stem which has been specialized for free conduction of water and nutrients. Thus it is no more logical to think that the physiology of the runner would be different from its parent than it is to think that one branch of a tree is physiologically different than another. The autoradiograms substantiate this belief.

Like a cutting which one places to root under suitable conditions, the independence of the runner plant from its progenitress is similarly dependent upon the suitability of the environmental conditions. There is no state beyond which the runner becomes independent. Furthermore, as a branch of a tree in leaf, the runner at first depends upon the parent for carbohydrates and nutrients and so may be a hindrance to the independent growth of the parent. Later, when the runner is rooted and mature leaves are present, it is, according to Darrow (30) capable of returning foods, nutrients, and water to the mother plant from which the roots have been removed. An exception to this, brought out by the studies presented herewith, is with reference to calcium which may not be translocated to the roots of connected plants.

Root temperature in relation to phosphorus absorption and utilization: The data presented on the effect of root temperature on phosphorus absorption (Table 4), though not significant, show a general trend toward reduced uptake of phosphorus from both leaves and roots at 45 or 50 degree root temperature as compared with 70 to 75 degrees. This trend has also been noted by Hinsvark and Wittwer (62). The data also give some indication that the depression of phosphorus uptake by roots at reduced temperature is more pronounced than from the leaves. This is in agreement with Mayberry (80) who found that the rate of root absorption of P^{32} by the tomato decreased as the night temperature was lowered from 75 to 45 degrees but that foliar uptake under these conditions was "practically unaffected".

In the course of the investigations the influence of root temperature on phosphorus absorption and utilization was studied, not only in plants subjected to the differential temperatures for a very short time before treatment but also in plants grown under similar conditions for a period of five weeks prior to the isotope applications. In the latter tests growth was significantly affected as well as nutrient uptake. The results in terms of fresh and dry weight production were very similar to those of Roberts (92) in that lowering of the root temperature greatly retarded leaf and runner growth while it had very little effect on root and crown development (Figure 8).

Phosphorus (as P^{32}) absorption from roots which had developed at different temperatures exhibited a peculiar trend (Figure 9). As the temperature decreased from 75 to 55 degrees there was a significant increase in P^{32} uptake at 55 compared to 75° F. then a very sharp reduction at 45 degrees. Detection of this reversal was not possible using the 50 and 70 degree temperatures.

Uptake of P^{32} from the leaves and consequent translocation to the crowns exhibited a similar trend except that the peak of P^{32} absorption occurred at 65 degrees. Translocation to the roots, however, was not affected by temperature.

It should be noted that the autoradiograms demonstrated reduced phosphorus uptake from the roots at the 45 degree temperature but failed to show any of the other trends brought out by the quantitative measurements.

The author knows of no similar tracer studies which would serve to either substantiate or dispute these results. Furthermore, it is not known the extent to which morphological differences may have influenced the results. Reference is made to the excellent summary of the effects of temperature on solute absorption cited from Kramer (page 21).

Phosphate carrier in relation to P^{32} utilization: Numerous references cited earlier have shown that the particular source of phosphate best for soil application varies

considerably with plant and soil conditions. The literature on the relative efficiency of various phosphorus carriers for leaf feeding is extremely limited. Silberstein and Wittwer (97) tested a number of inorganic and organic phosphates on the growth and yield of tomato and reported that phosphoric acid appeared to be the best source for leaf feeding. The data presented herein (Figure 11) show that a combination of phosphoric acid and urea supplied approximately 80 percent more phosphorus to the developing strawberry fruits than did either mono- or di-ammonium phosphates, containing an equivalent amount of phosphorus. Whether any particular benefit was obtained by the inclusion of urea is not known. An additional test comparing phosphoric acid with and without urea would be necessary.

Isotope studies have given rather conclusive evidence that root and perhaps leaf uptake of P^{32} is retarded at low root temperatures. Broyer and Hoagland (20) have shown that "lowering of the temperature in the culture solution to 10 degrees C. caused a relatively far greater decrease in ion absorption than of water absorption by the transpiring plant". It is still speculative whether nutrient absorption under these conditions is reduced to any greater extent than other factors influencing the final product -- growth. The data presented herein have shown that leaf growth (greatly retarded at low root temperatures) could not be

increased to any greater extent by foliar applications of nitrogen and phosphorus than by increasing the concentration of these elements in the nutrient solution. However, it was found (Figure 15) that a relatively greater response to the foliar sprays occurred at 50 degrees than at the 70 degree root temperature under conditions of low nitrogen and phosphorus nutrition. The fact remains that increases in the nutritional level of the root medium caused a greater growth increase than did any of the leaf sprays. Thus it would appear to the author that nutrition cannot be singled out as being more limiting at low root temperatures than other factors affecting growth.

Limitations of artificiality: Two very important factors must be kept in mind in considering the possibility of applying the results of these investigations to field conditions. First, all of the experiments were performed by means of sand culture, a technique which on one hand allows careful control of the nutrient level and on the other eliminates the influence of the exchange complex, fixation and other normal conditions of the soil affecting nutrient availability. Therefore, the use of sand culture tends to introduce a bias in favor of root absorption since little or no fixation occurs.

A similar bias toward foliage application results through the method of dipping the foliage and measuring the amount

of solution adhering to it. The dipping operation is necessary because of the hazard involved in spraying radioactive materials. Yet it should be considered that under normal field conditions more than half of the material applied as a spray either drips off the leaves or is lost as drift. Thus, while it is possible to compare the efficiency of foliage and root uptake of nutrients under controlled greenhouse conditions such as those described in this paper, it is very difficult, if not impossible, to apply this information directly to plants growing in soil and sprayed by normal methods. Consequently no attempt has been made to make such comparisons.

Section II - FOLIAR FEEDING OF STRAWBERRIES WITH NITROGEN AND PHOSPHORUS

Preliminary greenhouse studies (Section II, Experiment II) indicated that foliage sprays of nitrogen and phosphorus could increase fruit size of plants growing in a nutrient medium low in these elements. Field trials in commercial plantings also gave significant yield increases to leaf sprays of nitrogen and phosphorus compounds when the level of soil fertilization was below that generally used. However, with adequate soil management including the use of sufficient commercial fertilizers, no additional benefit was obtained from leaf feeding. Thus, it would appear that applications of nitrogen and phosphorus foliage sprays to

strawberries should be confined to situations in which a quick temporary supply of plant food is required, which may not be readily available through the soil application.

SUMMARY

The absorption and subsequent utilization of radiophosphorus (P^{32}) and calcium (Ca^{45}) by the leaves and roots of the strawberry (Fragaria spp. var. Robinson) was studied. Horticulturally, the strawberry was selected because of growth habits well adapted to nutritional studies. Visual and quantitative evidence of the efficiency of uptake of P^{32} and Ca^{45} under various environmental conditions was obtained by means of autoradiography and isotope dilution analysis. Data are presented for the growth and fruiting response of the strawberry to foliar sprays of nitrogen and phosphorus in the greenhouse and under field conditions.

The results obtained may be briefly summarized as follows:

(1) Solutions of ortho-phosphoric acid containing tracer amounts of radiophosphorus were readily absorbed by both the leaves and roots of the strawberry, being translocated to all portions of the plant within a period of six hours after treatment. Mobilization of P^{32} occurred primarily in meristematic regions such as developing leaves and fruits (especially the achenes or "seeds"), root tips and runners.

(2) In contrast to P^{32}, translocation of radiocalcium (Ca^{45}) from foliage application appeared to be negligible, if it occurred at all. When applied to the root system, Ca^{45} was readily absorbed and translocated, presumably in the transpiration stream, to all above ground portions of the treated plant and attached runner plants. However, the autoradiograms showed very little movement into the roots of the attached runner plants.

(3) Absorption and utilization of P^{32} and Ca^{45} from the roots was considerably greater in plants growing at low levels of these nutrients than when they were in normal supply. Uptake from the leaves under these conditions was not as greatly affected by the nutritional status of the plants. In agreement with the autoradiograms, no translocation of Ca^{45} from the leaves was detectable by dilution analysis.

(4) Studies on the effect of the temperature of the root medium on growth (fresh and dry weight production) and on phosphorus uptake by leaves and roots, showed that leaf and runner development was greatly reduced by decreasing root temperatures from 75 to 45 degrees while root and crown growth were reduced but slightly. In one experiment there was a significant increase in dry weight accumulation of roots as the temperature decreased from 70 to 50 degrees.

Absorption of P^{32} by the roots and subsequent translocation to crowns and leaves reached a peak at 55 degrees and decreased above and below this root temperature. Translocation to crown tissue from foliar applications of P^{32} followed a similar trend with a peak in absorption occurring at 65 degrees. Absorption of P^{32} by roots was influenced to a greater extent by root temperature than was leaf absorption

(5) Foliar applications of nitrogen (urea) and phosphorus (phosphoric acid) applied to plants growing at 50^{o} and 70^{o} root temperatures, and at different levels of these elements in the nutrient solution, gave no definite evidence that leaf or root growth at either temperature could be increased more by foliar applications than by supplying the nitrogen and phosphorus in the nutrient solution.

(6) Foliage applications, having the same specific activity, of radiophosphorus as mono- and di-ammonium phosphates and phosphoric acid plus urea to strawberry plants at bloom revealed that the phosphoric acid plus urea combination supplied up to 80 percent more phosphorus (as P^{32}) to the developing fruits than either of the other carriers tested. There was no significant difference in the efficiency of phosphate utilization between mono- and di-ammonium phosphates as carriers for leaf feeding. Significant increases in fruit size resulted from foliar applications

of urea and phosphoric acid in the greenhouse on plants growing at a low level of nitrogen and phosphorus.

(7) Significant increases in the yield of Premier strawberries to foliage applications of nitrogen and phosphorus were obtained in a commercial planting which had received inadequate applications of commercial fertilizers. No yield increases from either Premier or Robinson strawberries were obtained from any of the soluble fertilizers tested when sufficient commercial fertilizers were applied to the soil.

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