EFFECT OF STOCK-SCION INTERRELATIONSHIPS AND GRAFT UNIONS UPON NUTRIENT ABSORPTION AND TRANSPORT IN HIGHER PLANTS AS INDICATED BY RADIOACTIVE ISOTOPES

By

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AN ABSTRACT

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MARTIN JOHN BUKOVAC ABSTRACT

Budded apple and variously grafted tomato plants, grown in solution and soil cultures, were used to determine the effect of the graft union and stock-scion interrelationships upon foliar and root absorption of nutrients and their subsequent distribution. Radioactive analysis and autoradiography of plant tissues were employed to ascertain absorption and subsequent distribution of isotopically labeled nutrients. Rooted cuttings of East Malling (EM) VII and IX (dwarf apple rootstocks) absorbed less P^{32}, applied to the root medium, than EM XVI, a vigorous apple rootstock. Irrespective of foliar or root applications, McIntosh apple scions on EM VII or IX rootstocks (dwarf) absorbed and accumulated less P^{32} than when budded on EM XVI or Delicious seedling (vigorous) rootstocks. Similarly, less Ca^{45} was recovered in the McIntosh scion 144 hours following treatment, when applied to the medium in which roots of McIntosh scions on EM VII or IX were growing than in scions on EM XVI or Delicious seedling rootstocks. No marked accumulation of P^{32} or Ca^{45} was noted at the bud union.

Genetically dwarf (var. MacArthur 741) and non-dwarf (var. Stokesdale) tomato plants absorbed equal quantities of foliar applied P^{32}, however, less of the absorbed P^{32} was transported to the roots of the dwarf. Tomato plants (var. Stokesdale) chemically dwarfed with maleic hydrazide absorbed less foliar applied P^{32} than non-dwarfed, but no differences were observed

in the subsequent distribution of the absorbed isotope. As in the apple, no significant differences occurred between the P^{32} recovered in the graft union or a comparable stem segment on a non-grafted tomato plant following absorption of P^{32} by the roots. Likewise, no differences were observed in the P^{32} accumulated in the scions of grafted and non-grafted tomato plants 3, 12, 24, 48 and 96 hours after applying P^{32} to the root medium. The graft union, *per se*, did not impede the transport of root absorbed P^{32}. Well formed graft unions in tomato stems did not impede the acropetal transport of Na^{22}, S^{35}, Cl^{36}, Ca^{45}, Mn^{52-54}, Fe^{55-59}, Cu^{64}, Zn^{65}, Rb^{86}, Sr^{89}, Mo^{99} and Ba^{140} following root absorption of the isotopes. However, when laterals of vigorous tomato plants (var. Stokesdale) were grafted "apex to apex" P^{32} transport was significantly reduced and Ca^{45} transport was almost completely blocked, following the absorption of the isotope by the roots of one of the grafted plants.

When two one-centimeter phloem segments were removed from the stems of year old apple trees (EM I) and replaced in an inverted or natural position, more foliar applied P^{32} accumulated above the inversions than above non-inverted rings. Lesser amounts of foliar applied P^{32} accumulated above the non-inverted rings. Approximately 60 percent more adventitious buds grew below the inverted than non-inverted phloem rings. Swellings immediately above the upper phloem ring and reduced top growth were evident on trees with inverted compared to trees with non-inverted phloem rings. Growth of the scion in terms of dry weight and terminal growth was significantly influenced by its orientation. Less radiocalcium absorbed by the roots accumulated in the inverted scion two or six days following treatment than with non-inverted or non-grafted plants. However, when the calcium content was expressed as counts per minute per gram dry weight, no significant differences were observed, thus indicating that growth and not the union was the determining factor. In contrast to the many experiments illustrating the absence of downward transport of foliar applied Ca^{45}, anesthetized bean and radish leaves following treatment with Ca^{45} transported considerable quantities out of the treated leaf to all other parts of the plant. Chelation of radiocalcium with disodium ethylenediamine tetra-acetate, picrolinic acid, 2, 5-dichloro-3, 6-dihydroxy-p-quinone and 1-amino-2-napthol-4-sulfonic acid did not effect its basipetal transport.

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TO

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John and Sadie Bukovac

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I. INTRODUCTION

The art of grafting has been known to mankind since the era before Christ, yet the intricate relationships between stock and scion remain obscure. The dwarf response of scion to rootstock is only one of the many complex manifestations of stock-scion interrelationship. Rootstock effect upon scion, scion on rootstock and interstem on both, with their many marked physiological and morphological changes, have intrigued the scientific mind for many years.

In the past decade or two, the American horticulturist has become extremely interested in utilizing some of these physiological responses to control the production of commercial fruit crops. As an example, the needs of a rapidly expanding horticultural industry for uniform, earlier fruiting, dwarf or semi-dwarf apple trees has materially added to the popularity of the Malling rootstocks[1].

In view of this increased demand the need for a more complete understanding of stock-scion relationship has acquired a greater importance. The work which follows was undertaken, with the aid of radioactive isotopes, to ascertain the nutritional role of the graft union and rootstock in the physiology of dwarfing. Special consideration has been given to absorption and subsequent transport of essential nutrients.

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[1] Apple clones originating from the Paradise or Doucin groups of apples (classified as Malus sylvestris, Mill, by Bailey 1949) were used as rootstocks in a graft combination showing various degrees of dwarfism on the growth of the scion; hereafter, synonymous with East Malling or E. M.

II. REVIEW OF LITERATURE

Stock-Scion Interrelationships

General considerations: It is not unusual that complex interactions arise as a result of grafting, when one considers that two separate independent individuals are brought together, which unite and grow as one. These interactions are of great practical importance to the fruit grower and have been realized as such for many years. As early as 1818 Thomas Andrew Knight (1818) reported that plum stocks were best adapted to the peach, and apricot stocks to Moor's Park apricot. Furthermore, he noted a smooth highly polished bark and a healthier more vigorous tree when Moor's Park was budded on apricot stock than on any other rootstock.

Many grafting failures have been attributed to stock-scion incompatibility. Attempts have been made to explain incompatibility on the basis of anatomical differences (Daniel 1894, Guignard 1907, Crafts 1934, Beakbane and Thompson 1939, and Beakbane 1941) and defective graft unions (Waugh 1904 and Bradford and Sitton 1926). Biochemical differences have been suggested by Green (1926), Milbraith and Zeller (1945), and De Stigter (1956), and an antigen antibody phenomenon has been proposed by Kostoff (1928, 1929, 1931). Vyvyan (1934) suggested a differential growth rate between stock and scion, whereas Swarbrick et al (1946) suggested a differential absorption and subsequent transport or a combination of the two as contributing factors in stock-scion incompatibility.

Stock-scion interactions: The physiological effect of stock on scion or scion on stock is not localized in any one component of a grafted combination. Hatton (1927) studied the effect of a number of apple rootstocks upon the scion and concluded that growth, vigor and fruitfulness of the scion was influenced by rootstock. Trees grown on Malling VIII or IX were small and usually produced swellings at the graft union, while those grown on Malling II and XVI were more vigorous. Amos et al (1936) concluded after twenty years of observations at East Malling, that Malling VIII and IX rootstocks resulted in dwarfing and earlier fruiting was associated with the scions grafted thereon. The dwarfing response of scion to stock was only one of the many manifestations observed. Malling VII, which is considered a semi-dwarf rootstock, imparts vigorous growth to the scion in the early years. However, the growth rate falls off once the tree begins to fruit.

That a stock effect may be exerted by a stem piece was clearly demonstrated by Swarbrick and Roberts (1927) and Roberts (1929). These workers, using various interstems, showed that the dwarfing effect of a stock could be produced by a stem portion of the stock. This was later confirmed by Hatton (1930), Vyvyan (1934), Tukey and Brase (1933, 1943) and more recently by Sax (1953) and Thomas (1954). In the years that followed, the relative importance of the stock and stock-stem became the object of extensive experimentation. Tukey and Brase (1933) concluded after extensive investigations that no one part dominated the entire plant. They showed that all had a marked influence at one time or another, however, the rootstock exerted the greatest influence. This finding has been confirmed by Knight (1935) and Vyvyan (1938, 1955) who agreed, in part, that the rootstock exerted the greater influence. Beakbane, Hatton and Amos (1937) showed that scions grafted on a long stock-stempiece were influenced to a greater degree by the rootstock than scions grafted directly to a root-piece. More recently, Beakbane and Rogers (1956) concluded that the root portions of clonal apple rootstocks are capable of exerting an influence characteristic of the clone in the absence of any rootstock stem. Furthermore, the presence of a stock-stem on the rootstock intensified the rootstock effect.

That physical characteristics of roots of dwarf and vigorous apple trees did not differ materially was reported by Rogers and Vyvyan (1927), Rogers (1939a) and Hatton (1935). Also, roots of a dwarfing rootstock penetrated the soil to depths equal to those of vigorous rootstocks (Hatton 1935). Rogers (1939b) studied, in detail, root growth as related to rootstock, soil, seasonal and climatic factors. Root growth closely followed the above ground vigor, that is, Malling IX least vigorous, Malling I intermediate, and Malling XVI most vigorous. No appreciable differences in appearance were apparent between roots of Malling IX, I and XVI. Root hairs of all stocks looked similar. However, when secondary thickening occurred, Malling IX roots showed a more yellowish color than either Malling I or XVI. Rogers concluded;

> "the different rootstocks, so contrasting in their effect on growth and cropping, showed no dramatic contrast in absorbing root type and conformation. The most striking feature was the degree of similarity between the individual roots and between their reactions to physiological factors"

The effect of stock upon the development of the scion has been well established. On the other hand, there is evidence that the scion may influence the development of the stock (Swarbrick and Roberts 1927, Roberts 1929, Tukey and Brase 1933, Argles 1937, and Rubin 1955).

Tukey and Brase (1933) write;

> "American horticultural literature records observations as early as 1846 that the cion may affect the development of the rootstock, and other instances have been recorded since. It has been shown that the cion may affect the root in other ways than in quantity, direction, and form of roots, such as longevity, hardiness, chemical composition, and color.........."

Recently, Vyvyan (1955) reported that rootstock has a greater influence on tree size than scion, however, scion variety had some effect particularly when Malling XII was the scion. Occasionally one finds that the scion dominates the stock, although this seldom is the limiting factor. Hodgson and Cameron (1943) working with citrus found that when the vigor of the scion is less than that of the rootstock, it was the scion which determined the total growth of the tree.

There is no clear cut growth response of scion to stock or of stock to scion. One variety used as a stock may respond quite differently when used as the scion. Also, the source of the scion may influence its behavior when grafted on the same rootstock. Tukey and Brase (1944) reported a difference in the performance of McIntosh budwood taken from the same horticultural variety when budded on rootstock USDA 227. This led the authors to suggest that the compatibility or incompatibility was due to some factor other then genetic, e.g. virus or physiological disturbance.

The physiological aspect of stock-scion effect has been considered by Vyvyan (1955). He stressed that the stem/root ratio is relatively constant within any one scion/rootstock combination, and is independent of tree size or age. This merits considerable attention if we recognize that the scion and stock must mutually adjust to each other's physiological processes.

Anatomical aspects of stock-scion relationships: The internal structure of the dwarfing rootstock Malling IX was studied in an attempt to explain its dwarfing effect (Beakbane and Renwick 1936). They concluded that Malling IX had smaller vessels and a higher percent of medullary rays than the rooted scion. More detailed studies (Beakbane and Thompson 1939) revealed a correlation between the bark/wood ratio and vigor. The greater the vigor the lower the bark/wood ratio. Thus, the ratio of bark to wood for Malling IX was 3.1, and 1.0 for Malling VII, a more vigorous rootstock. Microscopic observations of the roots revealed that the dwarfing stock (Malling IX) had a greater percent of parenchyma and ray cells and a lower percent of fibers and vessels than the vigorous stock (Malling II). Furthermore, the vessels were generally larger in Malling II, however, no correlation was found between size and vigor. Beakbane and Thompson attached special significance to the higher percent of ray tissue and lower percent of vessels in dwarfing rootstock. They concluded that a larger volume is available for carbohydrate accumulation and storage in the dwarfing stocks. Thus, resulting in earlier fruiting and a reduction in mineral salt transport through the smaller vessels. This may be responsible for the reduced growth observed in the scions grafted on dwarfing stocks. In a more recent paper, Beakbane (1941) found larger quantities of starch, fats, oils, oxidases and peroxidases associated with increased parenchyma cells in the rootstocks of Malling IX than in Malling II or XII.

<u>The role of the graft union in stock-scion relationship--effect on nutrient transport</u>: The uniting of the scion and stock to form a graft union introduces a number of variables, any of which alone or in combination may influence the stock-scion relationship. Considering the graft union from purely a mechanical viewpoint, structural weaknesses, anatomical and physiological differences may all materially affect the stock-scion relationship. These possibilities were recognized by Daniel (1894), Waugh (1904), Bradford and Sitton (1926) and Proebsting (1926, 1928). Waugh observed that in imperfect

unions the continuity of new growth was interrupted by deposition of loose scar tissue formed during the healing process. Bradford and Sitton, on the other hand, stated that dwarfing was associated with phloem discontinuity. They felt the effect was reciprocal, that is, the stock was dwarfed first for lack of elaborated foods, and, in turn, it dwarfed the scion. Proebsting visualized a layer of periderm forming between the scion and stock in the case of apple and pear. These factors alone may account for the many reports on impeded transport through the graft union (Vöchting 1894, Guignard 1907, Chang 1937, Warne and Raby 1939, Herrero 1951, and Glushchenko and Drobkov 1952).

The effect of the graft union on mineral or water transport has not been fully evaluated in intact plants. Warne and Raby (1939), using ten-centimeter segments containing the graft union, measured the quantity of water which passed through in ten minutes under a pressure of 16 centimeters of mercury. They found that unions in which Malling IX was one of the components were less efficient in water conduction than those in which IX was combined with IX, or in which II was combined with itself or another scion. More detailed investigations revealed that at the graft union there was less conducting tissue than in the scion or stock. This led the authors to suggest that the graft union introduced an additional resistance to the flow of water. Chang (1937) reported that the reduced transport of water across the union and the accumulation of starch above the union resulted from mechanical obstruction. He found that only one-fifth as much water passed through graft unions of incompatible combinations of pear and quince as through compatible combinations. Laurent (1908) concluded that the bourrelet (callus or periderm layer arising between the scion and stock) may be differentially permeable, thus permitting the passage of certain glucosides and not of others. Phosphorus, on the other hand, was found (Glushchenko and Drobkov 1952) unevenly distributed within graft combinations of tomato and nightshade. Some accumulation of phosphorus occurred at the graft union.

In contrast to many reports showing the interference of transport by graft unions, Colby (1935), Friedrich (1939) and Herrero (1951) have demonstrated little or no obstruction by the graft union under their conditions. There was no measurable obstruction to the downward movement of carbohydrates by the graft union in Colby's work. However, upward movement may be limited in dwarf trees due to earlier suberization of their roots. This is particularly true when Malling IX is used as a rootstock. Herrero (1951) failed to correlate the starch content above or below the graft union with structural continuity of the union. He found differences only at certain seasons and these were not apparent during the remainder of the year.

That rings of phloem from the stock can produce a stock effect has been shown by Swarbrick (1927a, 1927b) and Roberts (1934, 1935). Also, phloem rings from the same tree if inverted will produce a dwarfing effect (Janse 1914, Sax 1954, and Sax and Dickson 1956). Sax and Dickson (1956) feel that the inverted phloem checks the downward flow of organic nutrients. They postulate that by virtue of reversed polarity (due to inverting the phloem) movement of organic nutrients and auxins were blocked. Furthermore, foliar applied phosphorus was shown to accumulate immediately above the inverted ring (Dickson and Samuels 1956). It is interesting to note that ringing alone will result in the accumulation of starch and reducing sugars and in the swelling of the stem above the ring (Knight 1803, MacDaniels and Curtis 1930, and Swarbrick 1927a, 1927b). Thiamin, pyridoxine, pantothenic acid, riboflavin, sucrose, total nitrogen and non-protein nitrogen were all found to accumulate in the vicinity of the girdled stem in tomato plants (Bonner 1944). This occurred as a result of the limited quantity of conducting tissue present after the ringing or girdling operation. These effects were minimized as new conducting tissue was generated.

<u>Biochemical specificity</u>: Immunity reactions between specific proteins have been used for a number of years in animal physiology to differentiate between species of animals. Green (1926) suggested that this same reaction may be effectively used in grafted plants. Plants of the genus Citrus, which intergrafts easily, gave a uniform positive precipitin reaction with an immune serum obtained from one of them. Also, species of the sub-family

Prunoideae, known to intergraft successfully, gave uniform positive precipitin reactions. However, when serum from this group was added to extracts from species of the sub-family Pomoideae negative results were obtained. Green concluded that the absence of a precipitin between these two sub-families may be associated with the difficulty observed in intergrafting species of these two sub-families. Green's evidence would only explain incompatibility when plants are grafted outside of their species, however, many combinations within the same species are incompatible (Tukey and Brase 1943). Kostoff (1928, 1929, 1933) postulated that positive precipitin reactions may be induced by grafting. Precipitins not normally detectable can be detected after grafting a scion (giving a positive precipitin reaction) on a stock, which gave a negative precipitin reaction. This Kostoff (1929) attributed to production of antibodies which exerted an influence on the scion. Generally a greater degree of specificity is associated with the precipitins acquired through grafting. Kostoff's conclusions have been questioned a number of times (Chester 1932a, 1932b, Chester and Whitaker 1933, Whitaker and Chester 1933, and Jones 1934). Chester (1932a) and Chester and Whitaker (1933) reported that the precipitate is not protein in nature, but chiefly calcium oxalate. They did not agree that this so called immunology can be graft induced. They further conclude that published immunological interpretations of precipitin reactions in plants are not tenable in view of the lack of homology between plants and animals and secondly of the large number of simple non-protein reactions present in plants. Furthermore, there is no indication that as a result of grafting there is an acquired immunity of stock to scion or scion to stock detectable by the present precipitin technique.

More recently, Milbraith and Zeller (1945) and DeStigter (1956) suggested latent virus and specific substance as factors which may be associated with incompatibility. Dwarfed and rosetted peach trees resulted when virus free buds were placed on virus infected stocks (Milbraith and Zeller 1945). For successful grafts of muskmelon on Malabar gourd, one leaf must be present on the gourd stock (DeStigter 1956). In the absence of foliage the sieve tubes disintegrated. Growth and other physiological processes responded directly to the number of leaves on the stock. DeStigter concluded that the presence of stock leaves is required to provide the stock with some "specific substance", which enabled the phloem of the stock to function normally. In the absence of this "specific substance" some enzymatic complex is altered in the sieve tubes which results in phloem disintegration and starch accumulation.

Physiological or individuality aspects have been proposed. Daniel (1894, 1923) noted that plants of the same family can generally utilize the storage reserves of the rootstock as its own. Along these same lines, Vöchting (1894) illustrated the individuality of the graft component by reciprocal grafts of sunflower and artichoke. Irrespective of the combination, starch was found in the sunflower and inuline in the artichoke. With reciprocal grafts of tomato and tobacco, Dawson (1942, 1945) demonstrated that nicotine was produced in the roots of the tobacco plant, and accumulated in the tomato scion. Thus, the synthesis mechanism was localized, and it was shown that the graft union permitted passage of the alkaloid.

Rootstock effects on nutrient absorption and accumulation: In the early part of this century Daniel and Thomas (1902) suggested that grafting may modify the physiology of the newly formed plant so as to alter nutrient absorption and subsequent utilization. Differences in organic and inorganic composition of scions on various rootstocks has been illustrated on a number of occasions. Moreover, certain rootstocks (e. g. peach when used as a rootstock for almond) have been shown to accumulate specific ions to the extent that they are toxic to the scion, therefore, making them undesirable as understocks (Hansen 1955).

Roach (1931) studied the elements absorbed from the soil by spectographic analysis of apple scions (var. Prince Albert) on Malling IX and XII rootstocks. Twenty-one elements were recovered namely: K, Ca, Mg, Na, P, Fe, Al, Mn, Ba, Sr, Li, Cu, Pb, Ti, V, Si, N, Cr, Sn, Mo and Ag. Lead was restricted to the roots, molybdenum occurred only in the stock portion of Prince Albert/Malling IX combination, and was totally absent in the scion and stock of Malling XII. The physiological significance of these observations have not been established.

Using potassium bromide to indicate quantitative absorption, Berry (1938) reported 1.425, 0.999 and 0.933 milligram equivalents of bromine absorbed by Malling XIII, II and IX respectively. Thus, he suggested that vigor of rootstocks may be related to their capacities for absorbing mineral solutes. During the early part of the season, trees on Malling XII absorbed one-third more nitrogen and potassium than those on IX, but the growth rate was proportional, therefore, no differences were observed in composition on a fresh weight basis (Pearse 1939). Analysis of the ash constituents of the terminal shoots of Prince Albert on Malling IX and XII revealed higher quantities of lime and a lower content of potassium in the scions on Malling IX. The content of phosphoric acid showed a negative correlation and magnesium a positive correlation with vigor (Vaidya 1938). Warne and Wallace (1935) studied the influence of rootstock upon the mineral composition of the scion. These authors showed few differences in composition and concluded that the dwarfing effect of the rootstock could not be explained on the basis of chemical composition. In citrus, no significant differences in mineral composition have been demonstrated among cuttings, seedlings, or self budded trees of a given species (Wallace et al 1952). In the absorption of radiophosphorus from the soil, Fuller and Hilgeman (1955) showed no differences in navel orange trees on sour orange or lemon rootstocks, nor did they find a difference in leaf nitrogen.

Accumulation of specific ions as a result of rootstock has been demonstrated. Boron accumulated in the scion leaves of Valencia orange on Cleopatra madarin stock, whereas chloride and potassium accumulated in the scions on sour orange rootstocks (Cooper, Gorton and Olson 1952, Gorton and Cooper 1954). Eaton and Blair (1935) demonstrated that the graft components directly influenced boron accumulation. When sunflower was grafted on its own roots, 684 parts per million of boron was recovered in the leaves, compared to 1026 parts per million when grafted on artichoke. Reciprocal grafting revealed 893 parts per million of boron for artichoke on artichoke, compared to 663 parts per million for artichoke on sunflower. More recently, almond rootstocks have been recommended for almonds in locations where excess boron is a problem, since almond on peach resulted in severe boron injury (Hansen 1955).

Organic chemical analysis has failed to explain the dwarfing effect of certain rootstock other than the accumulation of starch above the graft union in a few instances. Quantitative differences existed in reducing substances. The leaves of the dwarfing apple rootstocks had a consistently higher content of reducing substances (Lennard 1938). Smyth (1938) reported a uniform cycle in reducing sugars, sucrose, starch, hemicellulose, reserve carbohydrates, cellulose and lignin for all stocks tested. Starch and hemicellulose accumulated, beginning about the end of June, more rapidly in the apple scions on Malling IX than in scions on Malling II, V or VII. Similar results were reported for nitrogen fractions. In the case of Malling IX there was a tendency of scions to accumulate acid amide. However, Kench (1938) concluded that the total concentration of the various nitrogen constituents was independent of rootstock.

Rao and Berry (1940) investigated the carbohydrate relations of a single apple scion (var. Crawley Beauty) on rootstocks Malling IX and XIII. Higher percentages of reducing sugars, di-saccharides and starch were observed in the scions on Malling IX. Accumulation of starch took place earlier in the stock of Malling IX, and also in the scion grafted thereon. They postulated that the excessive accumulation of starch may be the cause of growth cessation. Starch accumulated as a result of a low water content caused by early suberization of Malling IX roots. The stock is unable to maintain an adequate water supply, therefore, the scion continues to grow at the expense of the water in the stock, which in turn promotes starch accumulation and further restricts root growth. They found no evidence that the graft union, _per se_, interferes with the downward transport of carbohydrates. From these results they suggest that there was a lack of metabolic unity between the scion and stock, with water deficiency appearing first in the stock and then in the scion.

The above results appeared to take on added impetus when one considers the work of Hartwell (1916), Harvey (1923) and Hooker (1926), who agree that the retardation of growth, as a result of any cause, was accompanied by an increase in starch. It has never been determined whether this increase in starch is the causative factor, or the resulting factor. On the other hand, Rao and Berry (1940) do not attempt to explain the dwarfing effect produced by Malling IX interstem alone, which is independent of the rootstock used.

The ability of water to move into the scion has been considered as a possible source of stock influence in dwarf apple trees. Transpiration studies (Knight 1925, Hafekost 1933) showed that dwarf and vigorous stocks had different transpiration rates, but that a single scion variety grafted on dwarf and vigorous stocks showed no differences in transpiration. This indicated that water movement to the top was not a factor limiting growth in dwarf trees. Pearse (1939) using trees grafted on dwarf and vigorous rootstocks, grown in water cultures, reported that trees on vigorous rootstocks absorbed more water per unit leaf area than those on dwarfing rootstocks. Trees on dwarfing rootstocks, however, absorbed more water per unit increase in fresh weight than those on vigorous rootstocks.

Translocation Studies

General considerations on transport: Since the classical study of Hevesy (1923) showing the absorption and subsequent transport of thorium B by plants, increasing emphasis has been given to transport studies in biological systems. This indicates that these transport processes are equally important to the multitude of reactions taking place in various plant tissues. Transport studies, in relation to stock-scion effect, were given a tremendous impetus when Swarbrick and Roberts (1927) demonstrated that a dwarfing interstem placed between a vigorous scion and stock would dwarf the scion. Later, Roberts (1934) demonstrated that a ring of bark from the stock could also produce a dwarfing effect. The physiological significance of these findings were not ascertained.

Early in the eighteenth century Hales (1727) reported that water moved upward through the wood and downward through the bark. Knight (1803, 1830) confirmed these results and attempted to account for sap movement. This long standing belief was questioned by Curtis (1920), Zimmerman and Connard (1934), Gustafson and Darkin (1937), and Gustafson (1939). Gustafson and Darkin (1937) were convinced that phosphorus passed upward through the phloem as well as the xylem. They maintained that the phloem and xylem functioned in the upward movement of phosphorus in willow and geranium. Zimmerman and Connard (1934), using inarched tomato and coleus plants, concluded that elaborated foods as well as mineral salts moved upward and downward through stems. The orientation of the interveining stem was considered of no importance, since some activity at a distant point was the determining factor of transport. Dixon (1922) and Dixon and Ball (1922) demonstrated that the xylem functioned in downward transport of organic materials. Eosin dye applied to the cut tip of potato and chrysanthemum appeared throughout the plant within 24 hours, and was found in the vessels. They concluded that reverse transpiration resulted in the downward movement of water in the vessels, and this effected the downward transport of solutes. The classical work of Stout and Hoagland (1939) using radioisotopes of P, Na, Br and K, illustrated clearly that the xylem was the primary upward pathway for transport in actively growing plants when the bark and xylem were separated. However, if the bark was left intact, lateral movement rapidly occurred, and the isotope was present in both xylem and phloem. Chen (1951) showed simultaneous movement of P^{32} and C^{14} in the phloem of willow and geranium cuttings. More recently, both basipetal and acropetal transport of phosphorus in the phloem of cotton was demonstrated by Biddulph and Markle (1944). Autoradiograms prepared of the bean stems just below the node of S^{35} and P^{32} treated leaves, indicated that the first pathway of downward movement of sulfur and phosphorus was through the phloem. There was then diffusion into the xylem (Biddulph 1956, and Biddulph, Cory and Biddulph 1956).

Transport of phosphorus: Hevesy et al (1936) suggested that the phosphorus atoms in plants are in a continuous interchange between the different plant parts, thus concluding that phosphorus is a very mobile element. These findings have been confirmed by a large number of workers (Arnon et al 1940, Colwell 1942, Silberstein and Wittwer 1951, Norton 1954 and Biddulph 1955). Biddulph (1941) showed a diurnal migration of P^{32} injected into the leaves of bean plants. The greatest downward migration was at or near 10:00 A.M. with the least at or near 10:00 P.M. Furthermore, phosphorus applied to the foliage of plants is readily absorbed and utilized in the metabolic processes of the plant. This has led to the utilization of supplemental feeding of plants, by foliar applications, during critical stages in their ontogeny (Silberstein and Wittwer, 1951, Eggert et al 1952, Asen et al 1953, Kursanov 1956). Temperature (Swanson and Whitney 1953), transpiration (Wright and Barton 1955), metabolic inhibitors (Kendall 1955), and general health (Stankovic and Becarevic 1954) of the plant are all factors which influence the absorption, transport and distribution of phosphorus. These effects must be considered in the interpretation of results.

Transport of calcium: In contrast to phosphorus which is mobile in plants, calcium is immobile. Very little, if any, downward transport of calcium occurs. The necessity of calcium in the root media was demonstrated by Haynes and Robins (1948), Ririe and Toth (1952) and Bledsoe (1953).

Growing half of the root system of a single tomato plant in a complete nutrient solution and the other half in a nutrient solution minus calcium, Ririe and Toth demonstrated the inability of calcium to move into the roots growing in the calcium deficient solution. These roots died. Studying the absorption of calcium by the peanut fruit, Bledsoe _et al_ (1949) concluded that the fruiting peg was dependent on the peg medium for its calcium supply.

Bean plants grown in a nutrient solution containing Ca^{45} and then grown in a solution containing non-radioactive calcium, showed no appreciable movement of Ca^{45} into the newly developed tissue. Calcium ascends through the xylem to the leaves, but there is no evidence that it is re-exported from the leaf via the phloem (Mason and Maskell 1931). In fact, these authors concluded that the immobility of calcium within the plant is associated with its inability to move in the phloem. Once calcium is in the leaf it is either precipitated or combined with tissue material so that few free calcium ions are left in solution. Smith (1944) suggested that boron is associated with the solubility of calcium particularly in the cytoplasm and cell wall. However, Wittwer (1956) found that boron did not facilitate the basipetal movement of calcium in bean plants. There is considerable evidence that the transport of organic compounds in plants may be regulated by boron (Gauch and Dugger 1954, Sisler _et al_ 1956), however, the mechanism remains obscure.

Transport and polarity: Polar transport of naturally occurring auxins in plants has been known for a number of years (Went 1932, Clark 1937, and Leopold and Guernsey 1953). Furthermore, there is a report that polarity can be temporarily suspended by mild anthesia (Went and Thimann 1937), which results in the non-polar movement of auxin. Niedergang-Kamien and Skoog (1956) reported a modification of auxin transport when tobacco stem cylinders are pretreated with triiodobenzoic acid. No attempt has been made to explain the failure of certain ions to move on the basis of polarity. However, Mason and Maskell (1931) lead one to suspect that calcium may behave in such a manner when they concluded that the immobility of calcium in plants is associated with its inability to move in the phloem. Went (1932) attached significance to polar transport when he studied the movement of organic dyes in plants. Acid dyes moved more rapidly toward the base than toward the apex, whereas basic dyes moved more rapidly to the apex than the base. This suggested that direction of movement is determined not merely by chemical constitution but also by the nature of the dissociating groups.

III. GENERAL METHODS AND MATERIALS

Preparation of apple stions: Stions (combination of stock and scion, Tukey 1937) were prepared by "T" budding clonal McIntosh buds on year-old East Malling (EM) VII, IX and XVI and Delicious seedling rootstocks. The stions were lifted from the nursery row in November and placed in a nursery storage (40-50° F) until used.

Budded and grafted tomato plants (var. Stokesdale and Moreton Hybrid) were prepared by budding a shield containing one bud to tomato stocks of the same variety. The grafted plants were prepared by grafting a two-inch scion on stocks of the same variety, using the saddle or whip and tongue method (Jones 1934). The newly placed bud or scion was securely wrapped in place with a latex[1] band. Water was withheld from herbaceous plants used as stocks for 24 hours before grafting. This facilitated the grafting operation by permitting closer contact of the cambium areas of the stock and scion, and resulted in stronger unions.

Growing plants: Apple stions were removed from the nursery storage cellar in February. The stock above the newly placed bud was removed and the stions were grown in solution cultures. A modified Hoagland solution (Hoagland and Arnon 1950) with ferric ethylenediamine tetra acetate as the iron source was used as suggested by Jacobson (1951).

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[1] Manufactured by the Sealtex Company, Chicago, Illinois.

Plants were started in a half strength Hoagland solution, and as soon as the buds broke the solution was changed to a modified (full strength) Hoagland, and replaced weekly thereafter. When plants were grown in soil cultures high nutrient levels were maintained by frequent fertilization with diammonium and monopotassium phosphates.

Isotope studies: Radioactive materials, both processed and irradiated units, were obtained either from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, Nuclear Science and Engineering Corporation, Pittsburg, Pennsylvania, or Abbott Laboratories, Chicago 36, Illinois. The P^{32} was received as ortho-phosphoric acid ($H_3P^{32}O_4$) and Ca^{45} as $Ca^{45}Cl_2$ in weak hydrochloric acid. Other isotopes were obtained as the chloride in weak hydrochloric acid with the exception of Cu^{64}, Cl^{36} and S^{35}, which were received as $Cu^{64}NO_3$, HCl^{36} and $S^{35}O_4$ respectively.

In studies involving downward transport, a definite quantity of the isotopic solution was applied to the midvein of a leaf. A tuberculin syringe with a number 27 stainless steel needle or a micropipette was used. For root absorption studies, a definite quantity of the isotopically labeled solution was applied to the root media. Results from a preliminary study were used to establish suitable isotope and carrier concentrations and time interval between treatment and harvest of specific plant tissues.

Plants treated as described above were harvested at various time intervals after treatment, separated into the desired samples, and dried to a constant weight in a forced air oven at 70° C. Dry weights were determined, the samples cut into small segments, and ashed by standard dry or wet procedures.

Radiocalcium determinations were made by ashing the plant samples in 50 milliliter crucibles at 550° C for 12 hours in a muffle furnace. No loss of radioactivity was detectable under these conditions. The resulting ash was dissolved in 2N hydrochloric acid, transferred to a 25 milliliter volumetric flask and brought up to volume. One or five milliliter aliquoits were transferred to Coor milk ashing capsules[1], and evaporated to a uniform film under a bank of 250 watt infrared heat lamps. All samples were counted with a mica end-window Geiger-Müller tube using a Tracerlab Model SC-1B Autoscaler or Nuclear Instrument and Chemical Corporation, Model 172 Ultrascaler. Both instruments were equipped with shielded counting chambers.

Radiophosphorus determinations required pre-digestion of plant tissue and fixation of the phosphorus prior to ashing. Samples were treated according to the procedure described by A. O. A. C. (1950); first, 5 N magnesium nitrate then concentrated hydrochloric acid were added to the samples while on a steam bath. The concentrated hydrochloric acid served to pre-digest the tissue permitting closer contact between the tissue

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[1] Coor milk ashing capsules, manufactured by Coor's and Company, Golden, Colorado.

phosphorus and the added magnesium nitrate. The magnesium nitrate formed magnesium pyrophosphate with the phosphorus in the plant tissue, which is thermally stable at 550°C, thus permitting complete ashing in a muffle furnace. The ash was dissolved in 2 N hydrochloric acid and treated as described for calcium above.

It was found more convenient, in working with woody material, to wet ash the samples. Stem segments and graft union segments were cut into small pieces and treated with five milliliters of concentrated nitric acid per gram of tissue. After the initial reaction subsided, an additional five milliliters were added and heated on a steam bath. The temperature was gradually increased until all of the plant tissue was oxidized and the solution had taken on a light yellow color (approximately 12 hours). After cooling, the samples were transferred to a 25 milliliter volumetric flask and treated as described previously for Ca^{45}.

Most samples were counted until 4096 or 2048 counts had registered on the counter. However, in case of low radioactivity each sample was counted for three minutes, or until 400 counts had registered. Counts twice the existing background were deemed necessary for significance. Radioactive analysis were expressed as total counts per minute (cpm) or counts per minute per gram (cpm/gm) dry weight.

Calculations were made for radioactive decay when necessary.

The following equation was used:

$$A_t = A_\circ e^{-\lambda t}$$

Where:

A_t = activity at time interval t
$A_\circ$ = activity at initial time
e = base of natural log. (2.3026)
λ = decay constant for the isotope used
t = time elapsed

No corrections were made for self-absorption since all but two experiments were counted as an infinitely thin sample layer. Direct counting of dried plant material was successfully employed in two experiments after no differences could be observed on a comparative basis. Self-absorption was only a factor where plant specimens differed greatly in weight.

Experiments were completely randomized, randomized block or split plot designs. Estimates of variability were by the analysis of variance method described by Snedecor (1948). Where significant "F" values occurred, calculations were made for least differences necessary for significance at the probability of five and one percent levels (designated L.S.D. 5% and 1% respectively). Where "F" values were insignificant, the designation "not significant" was used.

Autoradiography was used to show gross absorption and subsequent transport of radioisotopes from the site of treatment (Wittwer and Lundahl 1951 and Boyd 1955). Satisfactory autoradiograms were obtained of woody plant material by rapidly drying at 80° C, cutting into five millimeter longitudinal sections, and planeing to obtain a smooth surface. If all operations were made in the direction of an increasing activity gradient, little or no contamination occurred. The specimens were fixed on a mounting board, covered with Saran wrap (Dow Chemical Company, Midland, Michigan) and placed in contact with X-ray film for exposure. Herbaceous specimens were prepared for autoradiography, as described by Wittwer and Lundahl (1951).

Kodak X-ray no-screen or blue-brand film was used, depending on the sensitivity desired. Exposure time was determined by preliminary studies considering the isotope in question, and radiation intensity of the mounted specimen. The exposed film was developed using Kodak X-ray developer and fixed with Kodak X-ray fixer and replenisher.

IV. EXPERIMENTAL RESULTS

Section A

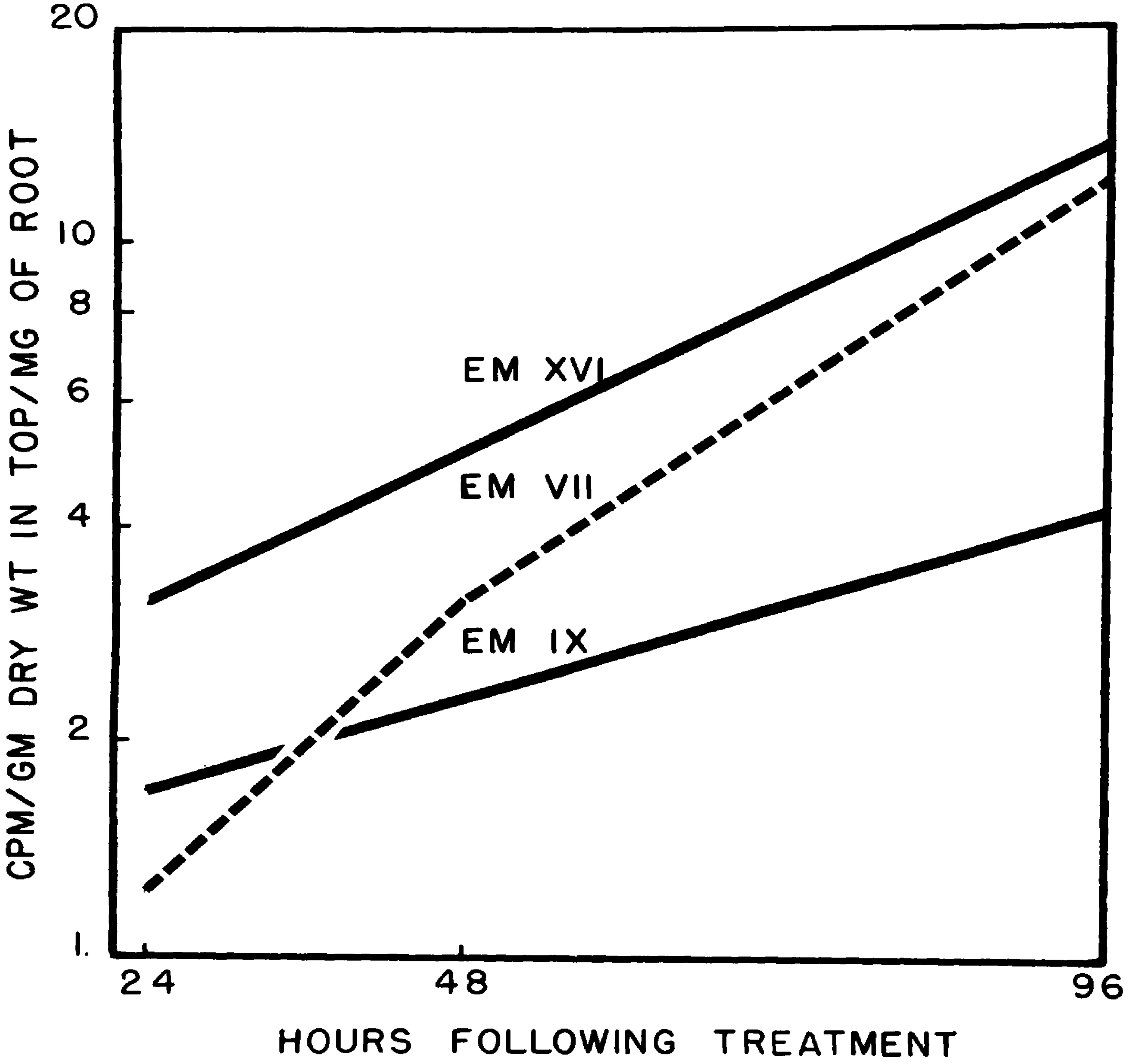
Effect of Stock-Scion Interrelationship Upon Nutrient Absorption and Transport

Experiment 1

Relative absorption and transport capacities of rooted cuttings of Malling VII, IX, and XVI were studied. Nine uniform rooted cuttings of East Malling (EM) VII, IX, and XVI were selected from the nursery storage on March 24, 1955. They were thoroughly washed, transferred to solution cultures (6 liter glazed crocks) and cared for as described under methods. Three days after the initiation of growth all but one of the new shoots were removed. On April 27, 0.5 microcurie (uc) of P^{32} per milligram of P^{31} was added to the solution cultures, (approximately 93.0 uc/ solution culture). This was below the range which resulted in radiation injury according to Blume et al (1950) and Blume (1952). The new shoot and roots were harvested 24, 48 and 96 hours after treatment. The harvested shoots (tops) were dried, weighed, wet ashed and assayed for radioactivity. Dry weights of the roots and tops were recorded. The radioactivity was expressed as counts per minute per gram dry weight of top per milligram of root. This constituted a correction for difference in quantity of roots.

Figure 1

Accumulation of radiophosphorus (P^{32}) in the tops of rooted cuttings of East Malling (EM) VII, IX and XVI, 24, 48 and 96 hours after applying P^{32} to the root medium.


20
10
8
6
4
2
1.
CPM/GM DRY WT IN TOP/MG OF ROOT
24
48
96
HOURS FOLLOWING TREATMENT
EM XVI
EM VII
EM IX

The data show (Figure 1) that the tops of EM VII, IX and XVI accumulated increasing quantities of the root applied P^{32} with time. The greatest quantity of root applied P^{32} was absorbed and accumulated by the vigorous rootstock EM XVI, and the least by the dwarfing rootstock EM IX. The semi-dwarf rootstock EM VII was intermediate. No explanation can be offered as to why the tops of EM VII contained less radioactivity at 24 hours than the more dwarfing EM IX.

Experiments 2, 3 and 4

The following experiments were designed to observe the foliar absorption and transport of P^{32} and root absorption and transport of P^{32} and Ca^{45} by apple scions (var. McIntosh) budded on dwarfing (EM IX and VII) and vigorous (EM XVI and Delicious seedling) rootstocks.

Thirty-six (nine of each rootstock) uniform trees, which were budded in August, were removed from the nursery storage cellar and the stock above the newly placed bud was removed. The roots were thoroughly washed and uniformly pruned. They were placed in 3.5 liter glass jars containing a half-strength Hoagland solution. The jars containing the plant specimens were then set in controlled temperature tanks maintained at 55° F ± 2.0° by a circulating water bath and thermostatically controlled refrigerator and heating coils (Figure 2). The design of the controlled temperature tanks

Figure 2

Solution cultures used in growing the plants. Root medium maintained at 55° F in each tank by refrigerator compressors and heating units. A Brown automatic potentiometer continuously recorded temperature of water-bath, nutrient solution and air.

has been described in detail by Roberts (1953). Temperature of the water-bath, nutrient solution and air were recorded automatically by a Brown Potentiometer[1]. The nutrient solutions were vigorously aerated from a central air supply which was filtered and distributed to each jar through aerating stones[2].

Within a week after placing in solution cultures, a fungus was observed on the roots of EM XVI and Delicious seedling. It appeared to be localized on the old roots; new roots were not affected. The fungus was isolated and identified as a Mucor strain saprophyte. After removing the stions, the roots were washed and the nutrient solutions were replaced with standard Hoagland. The fungus was not a serious problem once the buds broke and the stions were actively growing. Also, in a number of cases the newly placed bud appeared as a mixed (blossom and shoot) bud rather than a shoot bud. Approximately four or six weeks after scion growth began, trees showing poor vigor or containing mixed buds were replaced by more normal plants similarly grown for such a purpose.

In Experiment 2, the desired isotopic phosphorus was placed in 0.2 percent ortho-phosphoric acid carrier, and the pH of the solution was adjusted to 2.0 with potassium hydroxide. Fifteen drops of the isotopic treating solution (30 uc/ml) were applied to the fourth and fifth leaves of the

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[1] Minneapolis Honeywell, Brown Instruments, Industrial Division, Philadelphia, Pennsylvania.

[2] Wards Natural Science Establishment, 3000 Ridgewood East, Rochester, New York.

scion. There were no visual symptoms which would indicate leaf injury.

Plants were harvested 24, 48 and 96 hours following treatment, and separated as follows: (a) treated leaves, (b) shoot above treated leaves, (c) shoot below treated leaves, (d) five centimeter phloem segment containing the bud union, and (e) roots.

In root absorption studies of P^{32} (Experiment 3) the treating solution was prepared by adding phosphorus labeled ortho-phosphoric acid to distilled water so that ten milliliters would deliver 108.5 microcuries of P^{32}. Ten milliliters of the treating solution were then added to each culture jar with a resulting specific activity of one microcurie of P^{32} to one milligram of P^{31}. The stions were harvested 24, 48 and 96 hours after the addition of P^{32} to the root medium, and separated into the following samples: (a) scion, (b) five centimeter segment containing the bud union, (c) stock below the bud union, and (d) roots.

Radiocalcium in weak hydrochloric acid was applied carrier free to the nutrient medium (Experiment 4) at the rate of 0.5 microcurie of Ca^{45} to one milligram of Ca^{40}. The stions were harvested 48, 96 and 144 hours after treatment by separating into (a) scion, (b) bud union, and (c) stock below the bud union. There was no visible injury apparent as a result of treatment of the root medium with either P^{32} or Ca^{45} under the described conditions and times studied.

Within the short duration (4 or 6 weeks) of the experiments little differences were visible in the vigor of the scion except on EM XVI. In Experiments 2 and 3 McIntosh on EM XVI was slower to initiate roots, break bud and produced the least terminal growth (Table I). Radioactive analysis of the scion stem above the treated leaves, following foliar application of P^{32}, showed no significant differences irrespective of the rootstock. However, scions on EM XVI contained more P^{32} than those on EM VII, IX or Declicious seedling rootstocks. Comparative amounts of foliar applied radiophosphorus recovered in the scion stem portion immediately above the bud union and below the treated leaves are illustrated in Figure 3 (Top). The quantity recovered in this segment closely paralleled the scion vigor observed in the field. Less of the foliar applied P^{32} was recovered in this segment on dwarfing stocks EM VII and IX than vigorous stocks EM XVI and Declicious seedling. There was no extensive increase of the isotope in this region from 24 to 96 hours after treatment. This suggested that the bud union did not offer an obstruction to the downward transport of foliar applied phosphorus. With McIntosh scion on EM XVI, the decrease in P^{32} at 48 hours, as compared with 24 hours, in the stem immediately above the bud union was correlated with a higher concentration of P^{32} in the stem segment above the treated leaves. This decrease was evident in all samples below the treated leaves in this rootstock.

TABLE I

LENGTH OF TERMINAL GROWTH OF MCINTOSH SCIONS FOUR AND SIX WEEKS AFTER BUD BREAK ON DWARFING AND VIGOROUS ROOTSTOCKS RECORDED IN THREE EXPERIMENTS

McIntosh Scion on	Experiment		
	2[2]	3[1]	4[1]
		(centimeters)	
EM VII	26.5	15.8	13.1
EM IX	21.3	12.3	18.4
EM XVI	15.8	9.9	18.2
Delicious Seedling	23.1	15.2	----
L.S.D. 5%	4.3	3.0	3.4
1%	5.9	4.0	4.1

[1] Measurement taken 4 weeks after bud break.

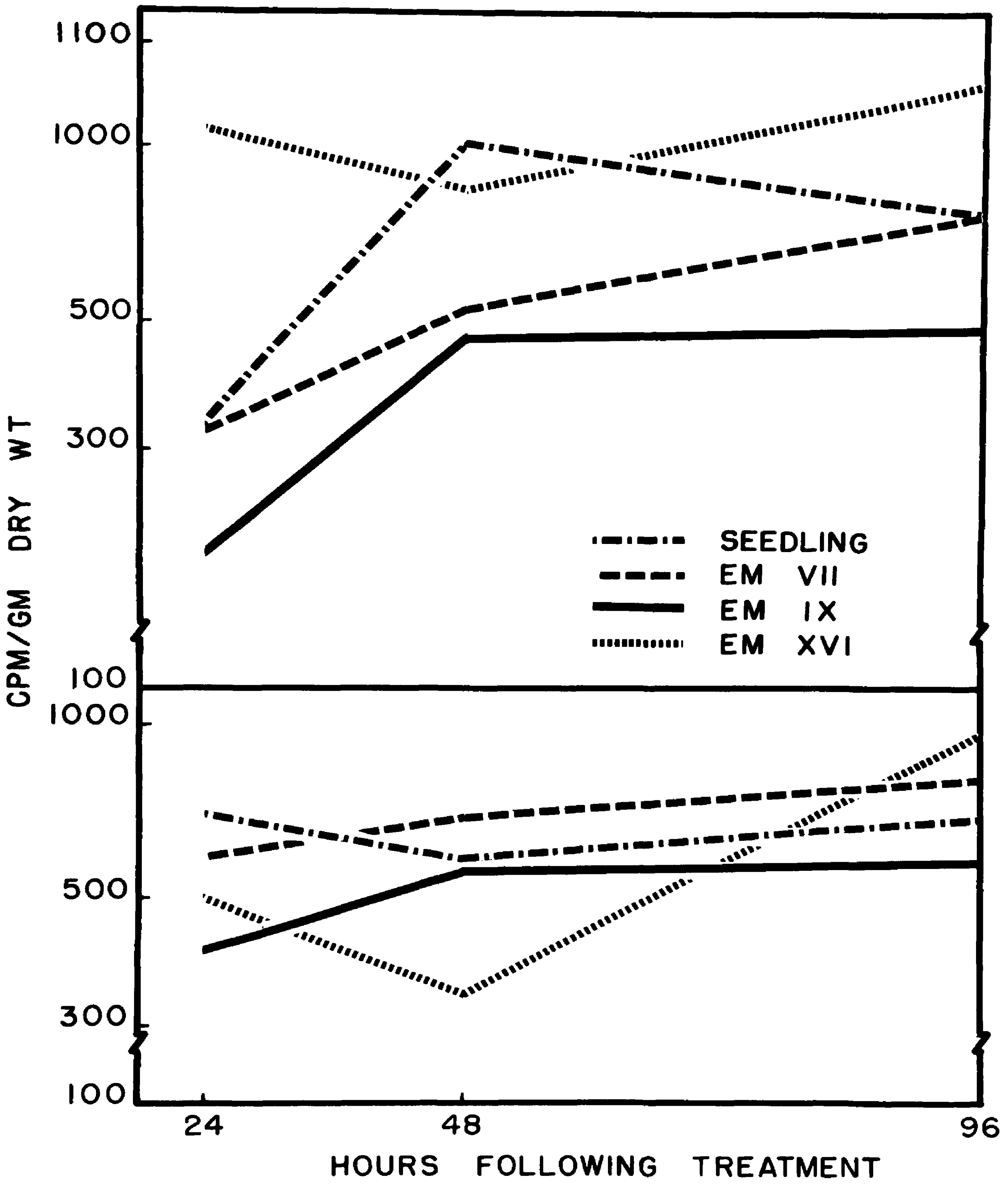
[2] Measurement taken 6 weeks after bud break.

Figure 3

Radiophosphorus (P^{32} cpm/gm dry wt.) recovered in various tissues at 24, 48 and 96 hours following foliar application of P^{32} labeled ortho-phosphoric acid (0.2%) to McIntosh scions budded on East Malling (EM) VII, IX and XVI and Delicious seedling rootstocks.

(Top) The McIntosh scion segment immediately above the bud union.

(Bottom) The five centimeter phloem segment containing the bud union.


1100
1000
500
300
100
1000
500
300
100
CPM/GM DRY WT
SEEDLING
EM VII
EM IX
EM XVI
24
48
96
HOURS FOLLOWING TREATMENT

The quantity of foliar absorbed P^{32} recovered in the phloem segment containing the bud union formed by McIntosh scions on EM VII, IX and XVI and Delicious seedling rootstocks did not differ significantly among rootstocks (Table II). At 48 hours all, with the exception of EM XVI, had attained a plateau and remained relatively constant under these conditions (Figure 3, Bottom). It is interesting to note that only in McIntosh/EM IX does the phloem segment containing the bud union contain more P^{32} per gram dry weight than the stem (Figure 3, Top) immediately above it. This may indicate that there is an accumulation at the bud union, but if so, the magnitude is very small within the first 96 hours, and does not differ significantly from the stem above it.

Significantly different quantities of foliar absorbed P^{32} were recovered in the roots of the various rootstocks (Table III). Radioactive analysis revealed that the least amount of P^{32} was transported to the roots of EM IX and XVI, the greatest quantity in Delicious seedlings with intermediate values for EM VII. The foliar applied P^{32} transported to the roots of EM IX increased proportionately with time (Figure 4, Bottom). East Malling VII and Delicious seedling accumulated P^{32} at a greater rate during the first 48 hours and then leveled off. While EM XVI again showed less activity at 48 hours than at 24 hours. No explanation can be offered to account for the behavior of EM XVI at 48 hours.

TABLE II

RADIOACTIVITY IN BUD UNIONS 96 HOURS FOLLOWING FOLIAR APPLICATION OF P^{32} AND ROOT APPLICATIONS OF P^{32} AND Ca^{45}

Bud Union Formed by	Foliar Absorbed P^{32} Recovered in Bud Union	Root Absorbed P^{32} Recovered in Bud Union	Root Absorbed Ca^{45} Recovered in Bud Union
	(cpm/gm dry wt - mean of 3 replications)		
McIntosh/EM VII	799[1]	78	518
McIntosh/EM IX	574	106	447
McIntosh/EM XVI	950	39	322
McIntosh/Delicious Seedling	754	8	---

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[1] Differences among bud unions formed by McIntosh scions on various rootstocks not significant at 5%.

TABLE III

TRANSPORT OF RADIOPHOSPHORUS (P^{32}) AND RADIOCALCIUM (Ca^{45}) IN MCINTOSH APPLE SCIONS BUDDED ON EAST MALLING VII, IX, AND XVI AND DELICIOUS SEEDLING ROOTSTOCKS

Rootstock	Foliar Absorbed P^{32} Recovered in Roots	Root Absorbed P^{32} Recovered in Scion	Root Absorbed Ca^{45} Recovered in Scion
	(cpm/gm dry wt.)		
East Malling VII	224[1]	194[1]	1187[2]
East Malling IX	151	148	621
East Malling XVI	169	457	2028
Delicious Seedling	308	297	----
L.S.D. at 5%	82	77	1020
L.S.D. at 1%	113	106	1395

[1] Each value represents the mean of three times (24, 48 and 96 hours) of harvest.

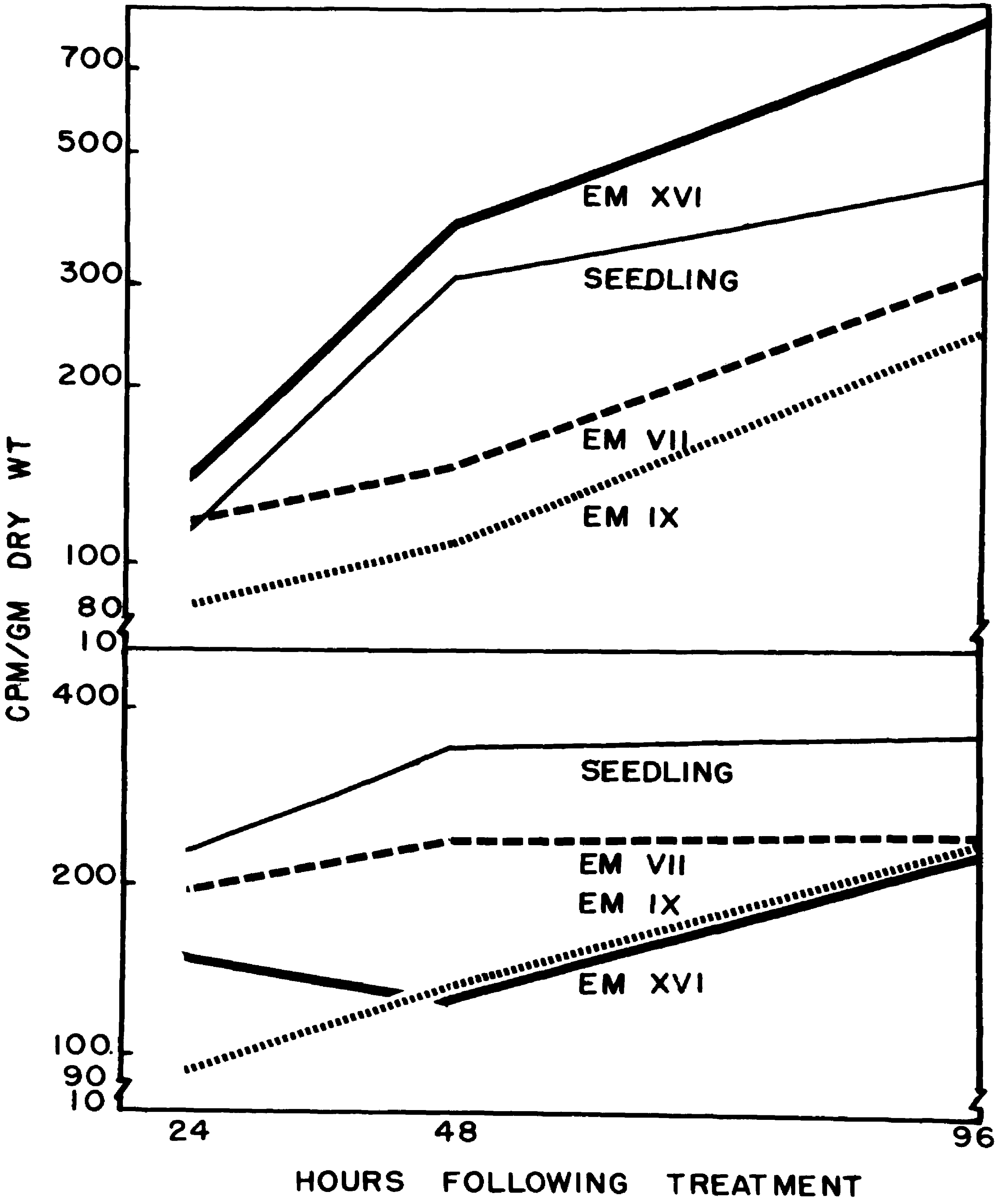
[2] Each value represents the mean of three times (48, 96 and 144 hours) of harvest.

Figure 4

Radiophosphorus (P^{32} cpm/gm dry wt.) recovered in various tissues of McIntosh scions budded on East Malling (EM) VII, IX and XVI and Delicious seedling rootstocks, 24, 48 and 96 hours following treatment with P^{32}.

(Top) Radiophosphorus content of McIntosh scions following application to the root medium.

(Bottom) Radiophosphorus content of roots following foliar application.


700
500
300
200
100
80
10
400
200
100
90
10
CPM/GM DRY WT
EM XVI
SEEDLING
EM VII
EM IX
SEEDLING
EM VII
EM IX
EM XVI
24
48
96
HOURS FOLLOWING TREATMENT

It appears from these data that the rootstock in some way modifies the physiology of the scion. This modification probably expresses itself in the foliar absorption or transport mechanism to more closely accommodate the requirements of the stock. If it were a question of reduced transport through the stock, then one would expect an accumulation of the foliar applied P^{32} above the bud union. This is not the case, however, since on a comparative basis a similar distribution of P^{32} existed between the stem segment above the bud union and roots in the various stocks.

When P^{32} was applied through the roots no significant differences could be detected in a five-centimeter stem segment containing the bud union, on the various rootstocks (Table II).

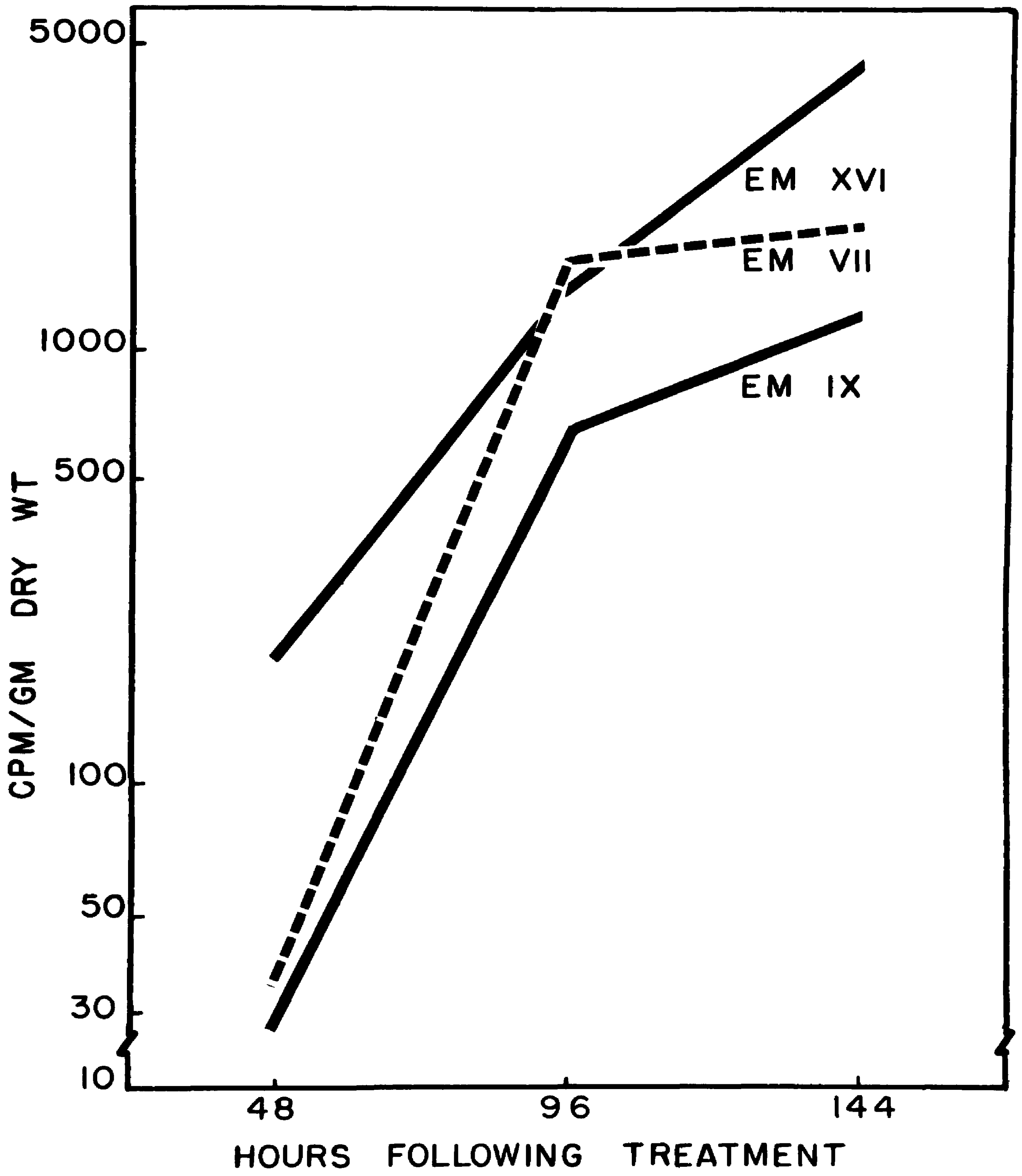
However, a clear distinction was apparent between the dwarfing (EM VII and IX), and vigorous (EM XVI and Delicious seedling) rootstocks in their ability to absorb and translocate P^{32} to the scion. The least amount of P^{32} was recovered in the McIntosh scion on EM IX, with an intermediate value for EM VII and the greatest on Delicious seedling and EM XVI (Table III). At 24 hours the most vigorous combination (McIntosh/EM XVI) differed from the most dwarfing (McIntosh/EM IX) by only 100 counts per minute. These differences were accentuated at 48 and 96 hours after the addition of the isotope to the root medium. It is interesting to note that the time activity curves for dwarfing stocks (EM VII and IX) were almost parallel (Figure 4, Top). The vigorous stocks (EM XVI and Delicious seedling) also closely paralleled each other. The order of P^{32} absorption and transport in stions (McIntosh/EM IX, VII and XVI and Delicious seedling) closely followed the vegetative growth observed in McIntosh scions on EM IX, VII and XVI and Delicious seedling under commercial practice. These data show that each stion has a characteristic time absorption curve, which is closely correlated with vigor.

When Ca^{45} was applied to the root medium of McIntosh scions on EM VII, IX and XVI rootstocks, significantly greater quantities of Ca^{45} were recovered in the McIntosh scions on EM XVI (Figure 5). The least quantity of the root absorbed Ca^{45} was found in the scions on the dwarfing stock EM IX. McIntosh scions on EM VII (semi-dwarf) were intermediate in Ca^{45} content (Figure 5). As with root absorbed P^{32}, the dwarfing rootstocks (EM VII and IX) exhibited similar time absorption curves. Radioactivity in the scions on the vigorous stock (EM XVI) increased with time and larger amounts were present than in the scions on dwarfing stocks (EM VII and IX). Consequently, the inherent capacity of EM XVI rootstocks to absorb calcium was greater than either EM VII or EM IX.

There was no apparent relation between stion vigor and Ca^{45} concentration in the stem segment containing the bud union (Table II). No marked accumulation of root applied Ca^{45} was observed at the bud union (Table II), indicating that the union did not materially impede Ca^{45} transport during the times studied.

Figure 5

Radiocalcium (Ca^{45} cpm/gm dry wt.) detected in the McIntosh scions on EM VII, IX and XVI rootstocks, 48, 96 and 144 hours following its addition to the root medium.


5000
1000
500
100
50
30
10
CPM/GM DRY WT
48
96
144
HOURS FOLLOWING TREATMENT
EM XVI
EM VII
EM IX

Experiment 5

The transport of foliar applied radiophosphorus by genetically dwarfed and non-dwarfed tomato plants was studied. Seedlings of genetically dwarf (var. MacArthur 741) and non-dwarf (var. Stokesdale) tomato plants were started in vermiculite and then transferred as small seedlings to solution cultures using a standard Hoagland formulation. A randomized block design with five replications was used. After ten days, one-half of the plants of each variety were treated by applying 0. 1 microcurie of P^{32} to the first true leaf. At the time of treating, both dwarf and vigorous plants had the same number (4-5) of leaves, thus their physiological development was comparable.

Plants were harvested after 6 and 24 hours and separated into the following parts: (a) treated leaf, (b) stem plus non-treated leaves, and (c) roots. The samples were placed in 50 milliliter beakers, dried in a forced air oven (70° C) for 96 hours, and then counted directly. No correction was made for self absorption.

The total P^{32} absorbed and translocated from the treated leaf was almost identical for the dwarf (1627. 7 cpm) and non-dwarf (1626. 5 cpm) tomato plants (Table IV). This indicated that no differences existed between the dwarf and non-dwarf tomato plants in their ability to absorb foliar

TABLE IV

ABSORPTION AND TRANSLOCATION OF FOLIAR APPLIED RADIOPHOSPHORUS (P^{32}) BY GENETICALLY DWARF (MACARTHUR 741) AND NON-DWARF (STOKESDALE) TOMATO PLANTS

Time After Treatment[1] (Hours)	Total P^{32} Translocated Out of the Treated Leaf		Total P^{32} Translocated to the Stem and Non-treated Leaves		Total P^{32} Translocated to the Roots		Percent of P^{32} Absorbed Translocated to Roots	
	Dwarf	Non-dwarf	Dwarf	Non-dwarf	Dwarf	Non-dwarf	Dwarf	Non-dwarf
	(cpm)		(cpm)		(cpm)			
6	887.2	877.9	673.1	573.6	214.1	304.3	0.95	1.33
24	2368.1	2375.0	1965.3	1673.3	402.8	698.7	1.81	3.17
Mean	1627.7	1626.5[2]	1319.2	1123.5[2]	308.5	515.0[3]	1.38	2.25[3]

[1] Means for time significant (1%) for all values.

[2] Differences between dwarf and non-dwarf plants not significant.

[3] P^{32} values significantly (1%) greater for non-dwarf plants.

applied radiophosphorus. However, there were significant differences in the distribution of the absorbed phosphorus. More P^{32} was recovered in the stems and non-treated leaves of the dwarf than the non-dwarf plants, but the differences were not significant (Table IV). Significantly more of the foliar applied P^{32} was recovered in the roots of the non-dwarf than the dwarf tomato plants, indicating that less foliar applied P^{32} was translocated to the root of the dwarf tomato plant. Thus, with tomatoes, the absorption capacity of leaves of dwarf and non-dwarf plants did not differ, but transport to the roots of the dwarf plant was reduced.

Experiment 6

Previous studies (Experiment 5) indicated that dwarf and non-dwarf tomato plants absorbed equal quantities of foliar applied P^{32}, but less P^{32} was transported to the roots of the dwarf than the non-dwarf plant. The following experiment was designed to observe the absorption and subsequent transport of foliar applied P^{32} in chemically dwarfed and non-dwarfed tomato plants.

Tomato (var. Stokesdale) seedlings were grown as in the previous experiment. After ten days of growth in standard Hoagland solution cultures, the foliage was dipped in solutions (0, 1000 or 4000 ppm) of maleic hydrazide (formulated as a water soluble diethanolamine salt of 1, 2-dihydro-3, 6-pyridazinedione). Four days after treatment with maleic hydrazide, 0. 1 microcurie of P^{32} (as $H_3P^{32}O_4$) was applied to the first true leaf. The plants were harvested 6 and 24 hours later, and samples prepared and counted as described in the previous experiment.

New top growth was greatly reduced in the maleic hydrazide treated plants. Tomato plants treated with 4000 parts per million showed burning of the leaf edges. However, no injury occurred with 1000 parts per million. The percent of the foliar applied P^{32} recovered in the stem or roots did not vary significantly with chemical treatment at six hours. Significantly less foliar applied radiophosphorus was found in the stems and non-treated leaves at 24 hours of plants treated with 4000 parts per million of maleic hydrazide. No differences were observed in the P^{32} content of the roots due to chemical treatment (Table V). The total amount of foliar applied P^{32} absorbed and transported out of the treated leaf did not differ significantly with chemical treatment at six hours (Table V), however, at 24 hours considerably less P^{32} was transported out of the treated leaf in tomato plants treated with 4000 parts per million of maleic hydrazide. This indicated that chemically dwarfed tomato plants absorbed less foliar applied P^{32}, transported less of the absorbed P^{32} from the treated leaf, or both.

TABLE V

TRANSPORT OF FOLIAR APPLIED RADIOPHOSPHORUS (P^{32}) IN CHEMICALLY DWARFED[1] TOMATO PLANTS (VAR. STOKESDALE)

Maleic Hydrazide Concentration	Time After Treatment					
	6 Hours			24 Hours		
	Stem	Root	Total	Stem	Root	Total
(ppm)	(means of 8 replications - % recovered)					
0 (control)	.42	.24	.66	1.79	.49	2.28
1000	.40	.20	.60	1.56	.39	1.95
4000	.39	.30	.69	.53[2]	.42	.95[2]

[1] Maleic hydrazide applied 4 days prior to treatment with P^{32}.

[2] Differs significantly at 1% level from control.

Section B

Effect of the Graft Union and Scion Orientation on Nutrient Transport

Experiment 7

The effect of the graft union on the transport of phosphorus was studied by following the acropetal movement of P^{32} under short time intervals. Vigorous tomato plants (var. Stokesdale) with 8-10 leaves, grown in soil cultures, were saddle grafted. The scion consisted of a two-inch stem piece with one growing point in the leaf axil. To minimize plant variability the scion was of the same clone as the stock. For controls, other plants were decapitated at approximately the same height as the top of the scion of the grafted plants. Immediately after grafting, the plants were placed in a humidity (95-100% R. H.) chamber for four days. They were then grown in a greenhouse under the normally long days of late spring and early summer with high nutrient levels in the root medium. Fifty-two days after grafting, 30 milliliters of a .05 M potassium di-hydrogen phosphate solution containing one microcurie of P^{32} per milliliter was applied to the soil in a small trench around the base of each plant.

The plants were harvested 3, 12, 24, 48 and 96 hours following the addition of P^{32} to the root medium. The grafted plants were separated into the scion and graft union. In the non-grafted plants, the new shoot was harvested as a scion and a comparable stem segment as the graft union.

Dried plant tissue samples were ground to pass through a 40 mesh screen and then wet ashed prior to radioactive assay. Each sample was counted three times and the average count per minute per gram dry weight of tissue recorded.

Radiophosphorus (P^{32}) recovered in the scions and graft unions of grafted plants and corresponding tissues (new shoot and stem segment comparable to the graft union segment) of non-grafted plants are shown in Table VI. The radioactivity found in the scions and graft unions generally increased with time in both grafted and non-grafted plants. No explanation is offered for the low radioactivity of non-grafted scions at 24 hours and the grafted scions at 48 hours. There were no consistent differences in the P^{32} concentration of the scions or graft unions of grafted or comparable portions of non-grafted plants at any of the time intervals after the isotope was added to the root medium. Likewise, no significant differences were observed between the means for all times (3, 12, 24, 48 and 96 hours) for grafted versus non-grafted tomato plants. No obstruction, associated with grafting, to the upward transport of root absorbed radiophosphorus was observed. Furthermore, the presence or absence of the graft union in tomato plants did not affect the quantity of radiophosphorus absorbed and transported to the scion.

TABLE VI

EFFECT OF THE GRAFT UNION ON ACROPETAL TRANSPORT OF P^{32} IN TOMATO (VAR. STOKESDALE) STEMS

Hours After Applying P^{32} to the Root Medium	Scion		Graft Union	Segment Comparable to Graft Union
	Grafted	Not Grafted		
	(cpm/gm dry weight - means of 6 single plant replicates)			
3	41	693	44	105
12	1112	1221	173	143
24	1143	565	190	173
48	773	1018	205	225
96	1468	1753	380	281
Mean[1]	907	1050	198	185

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[1] Differences between grafted and non-grafted scions and between the graft union and segments comparable to graft unions were not significant (5%).

Experiment 8

Nutrient distribution in grafted tomato plants was ascertained by the autoradiographic technique. Six week old tomato plants (var. Moreton Hybrid) were grafted as described in the previous experiment. After four weeks, a solution of the desired isotope was applied to the soil in a band around the base of each plant. The isotopes; Na^{22}, Cl^{36}, Ca^{45}, Mn^{52-54}, Fe^{55-59}, Zn^{65}, Rb^{86}, Sr^{89} and Ba^{140} were used in their chloride form, P^{32} as ortho-phosphoric acid, S^{35} as sulfate, Cu^{64} as copper nitrate, and Mo^{99} as ammonium molybdate. The soil in which each plant was growing received 100 microcuries of one of the respective isotopes except in the case of chlorine where 15 microcuries were used.

The plants were harvested 48 hours following the addition of the radioactive isotopes to the root medium. In harvesting, a 20-centimeter segment of the stem minus appendages (except in the case of Mo^{99} the appendages were retained) but including the graft union, was removed and pressed between botanical drying paper in a forced air oven at 70° C. Upon drying, the samples were transferred to new 8 x 10 inch sheets of botanical drying paper, covered with Saran wrap (Dow Chemical Company, Midland, Michigan) and exposed to Kodak X-ray film (Blue brand). Three stem specimens from three plants respectively, for each radioisotope, were utilized for procuring the autoradiograms.

In order to obtain a desirable image the pressed and dried stem sections were exposed as follows:

(a) 24 Hours Na^{22}, P^{32}, Cl^{36}, Ca^{45}, Rb^{86}, Sr^{89}, Mo^{99}, Ba^{140}

(b) 7 Days S^{35}

(c) 15 Days Mn^{52-54}, Cu^{64}

(d) 21 Days Fe^{55-59}, Zn^{65}

All autoradiograms show a similarity in that there was no accumulation of the isotope at the site of the graft union (Figures 6, 7, 8, 9). No accumulation of any of the isotopes above or below the graft union occurred with the possible exception of chlorine (Cl^{36}). This was of little significance since chlorine tends to accumulate in the vascular system of tomato plants (Broyer 1956).

Experiment 9

Distribution of root absorbed radiocalcium was next studied through a dwarfing apple interstem involving two graft unions. Three two-year-old McIntosh trees[1] containing a Clark's dwarf interstem were started in sand cultures with standard Hoagland solution. Two weeks after the initiation of bud activity, and while the trees were actively growing, 100 microcuries of Ca^{45} were applied to the root medium of each tree. A 20 centimeter

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[1] Trees supplied by Stark Brothers Nursery, Louisiana, Missouri.

Figure 6

Autoradiograms of grafted tomato stems showing the distribution of Ca^{45} (left), Ba^{140} (center), and Sr^{89} (right), 48 hours following the addition of the isotopes to the root medium. (Arrows indicate site of the graft union).

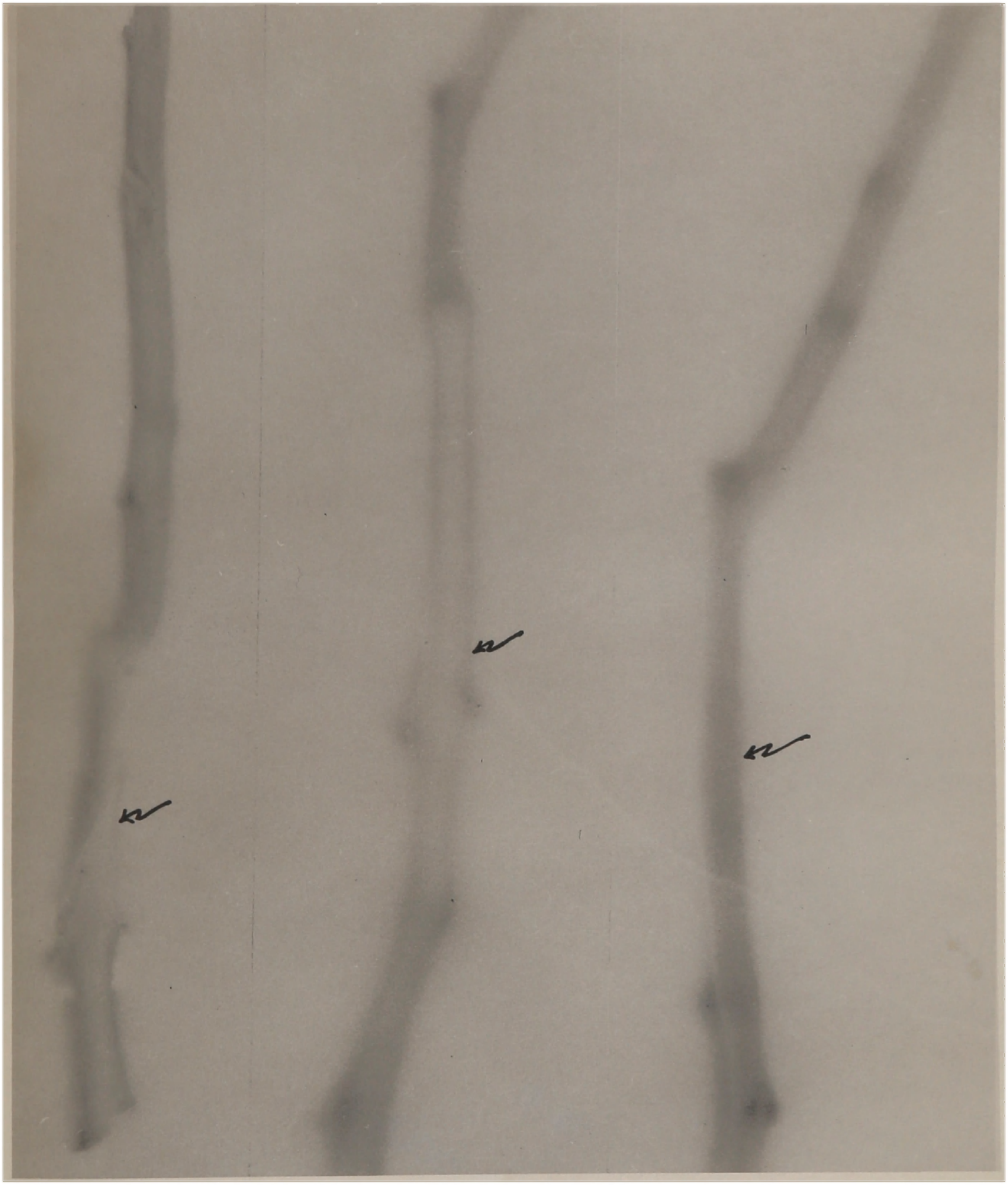


Figure 7

Autoradiograms of grafted tomato stems showing the distribution of Cl^{36} (left), S^{35} (center) and Na^{22} (right), 48 hours following the addition of the isotopes to the root medium. (Arrows indicate site of the graft union).

Figure 8

Autoradiograms of grafted tomato stems illustrating the distribution of P^{32} (left), Rb^{86} (center) and Mn^{52-54} (right), 48 hours following soil application. (Arrows indicate site of the graft union).

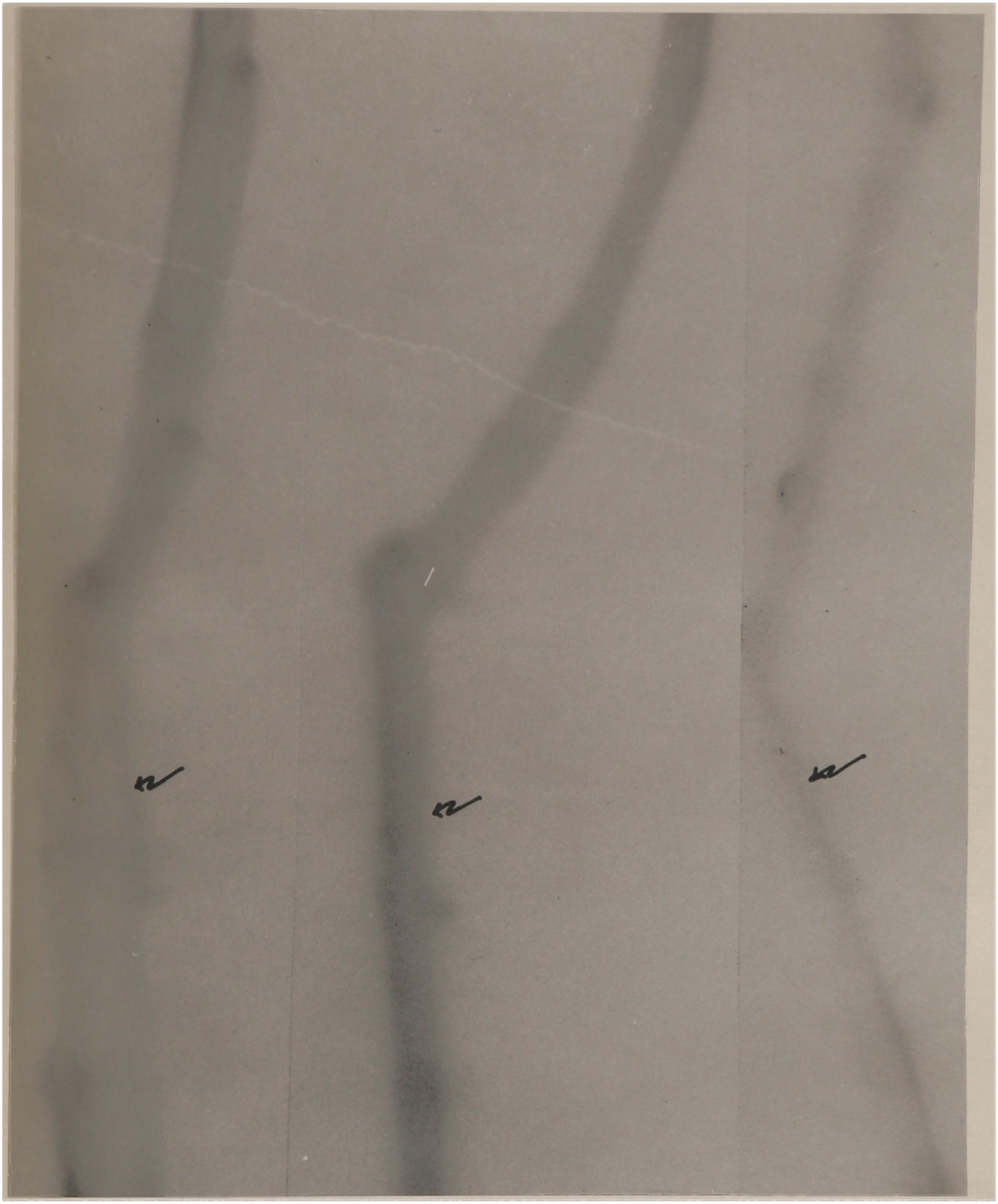
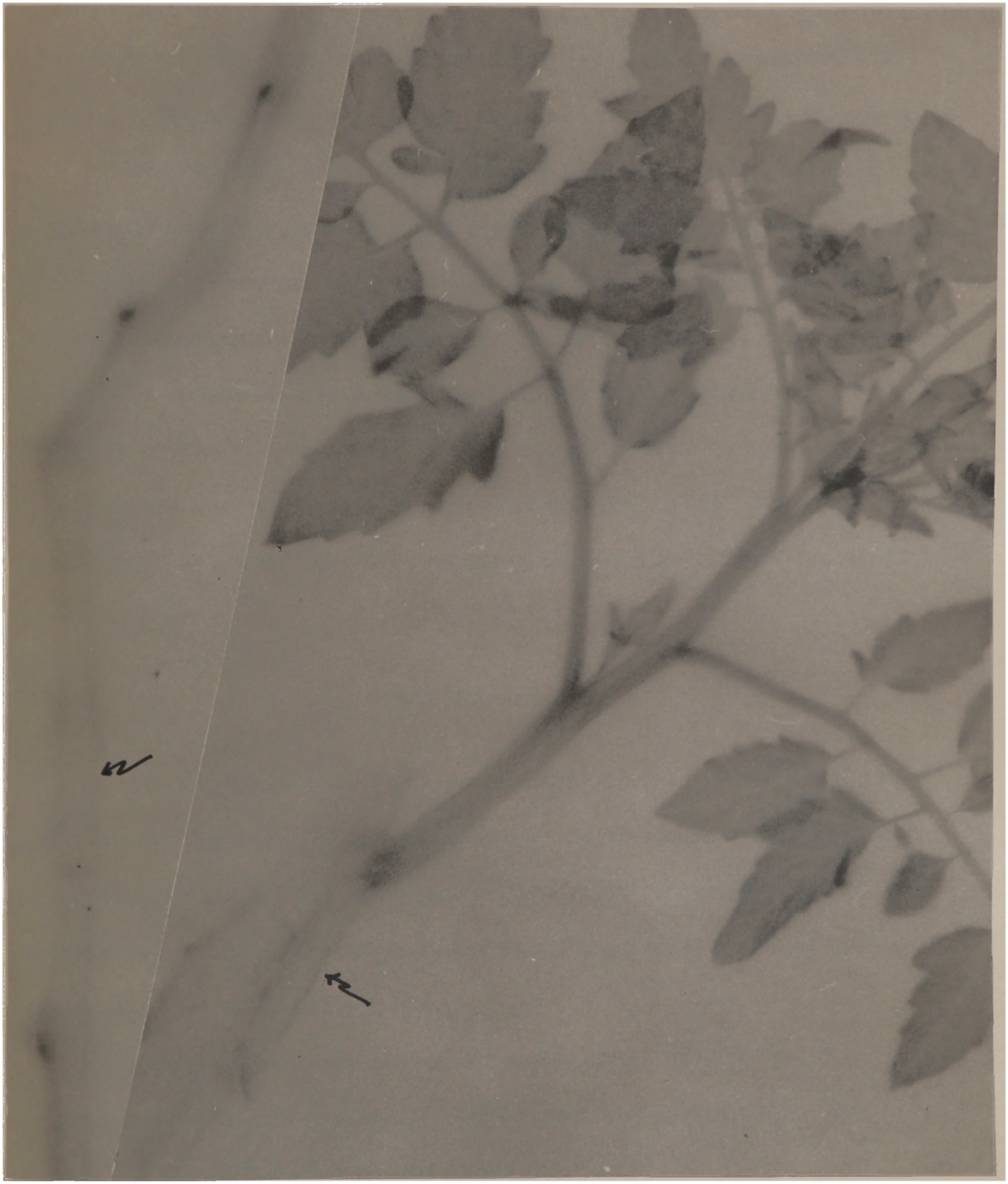


Figure 9

Autoradiograms of grafted tomato stems illustrating the distribution of Zn^{65} (left) and Mo^{99} (right), 48 hours after treating the root medium. (Arrows indicate site of the graft union).



segment of the stem containing the interstem was removed 24 hours after treating. The segments were dried, sectioned and autoradiograms prepared.

The dwarfing interstem was larger in diameter than either the stock or the scion (Figure 10). The graft unions between the scion and interstem were well formed, whereas those between the interstem and stock showed evidence of internal breakdown. There was, however, no external evidence of structural weakness. The primary and initial pathway for the transport of root absorbed radiocalcium during the first 24 hours appeared to be the newly formed xylem (Autoradiogram, Figure 10). No interruption of transport was noted, but for conclusive evidence serial sections would be necessary.

Experiment 10

During the course of earlier experimentation it was observed that Ca^{45} failed to move out of the treated leaf. Furthermore, when applied to the stem Ca^{45} moved only acropetally. The "polar" transport of Ca^{45}, in comparison with P^{32}, was next studied by utilizing graft unions of tomato plants involving reversed polarity.

Uniform five-week-old tomato plants (var. Stokesdale) were decapitated above the second true leaf. Two laterals were permitted to grow and four weeks later the lower lateral of one plant was grafted (apex to apex) into the upper lateral of another plant (Figure 11).

Figure 10

Photograph (left) illustrating a longitudinal section through a Clark's dwarf interstem with a McIntosh scion and Hibernal stock, and autoradiogram (right) showing the distribution and pathway of transport of Ca^{45}, 24 hours after application to the root medium.

Figure 11

A union formed by grafting together (apex to apex) the forced laterals of two tomato plants.

Three weeks after grafting, and when the first flowers were open, 100 microcuries of Ca^{45} or 50 microcuries of P^{32} were added to the soil in which the roots of one of the grafted plants were growing. The grafted laterals were severed from the two plants 6 or 12 days after treatment and segmented as follows: (a) five centimeter segment containing the graft union, (b) five centimeter segment adjacent to the graft union and from the plant receiving the radioactive isotope, and (c) five centimeter segment adjacent to the union but from the plant receiving no radioactive isotope. Twelve days following treatment young immature fruits were harvested from both plants. Stem segment and fruit samples were prepared and assayed for radioactivity. Three apex to apex unions with approaching laterals were used to obtain a representative autoradiogram (Figure 12).

Values for radioactivity in the grafted laterals and young fruits are given in Table VII. For P^{32} no significant differences were observed between the five centimeter lateral segment on the treated side and the graft union segment, or between the graft union segment and the five centimeter lateral segment on the non-treated side. However, a significantly higher value was observed for the five centimeter lateral segment on the treated side than for the comparable segment on the non-treated side. Radiophosphorus was also recovered in the immature fruits occurring on the second lateral of the non-treated plants. These data clearly show that phosphorus

Figure 12

Photograph (top) and autoradiogram (bottom) of an "apex to apex" graft union between a lateral from one tomato plant and that of another. Ca^{45} was supplied to the roots of the plant from which the lateral originated on the left.

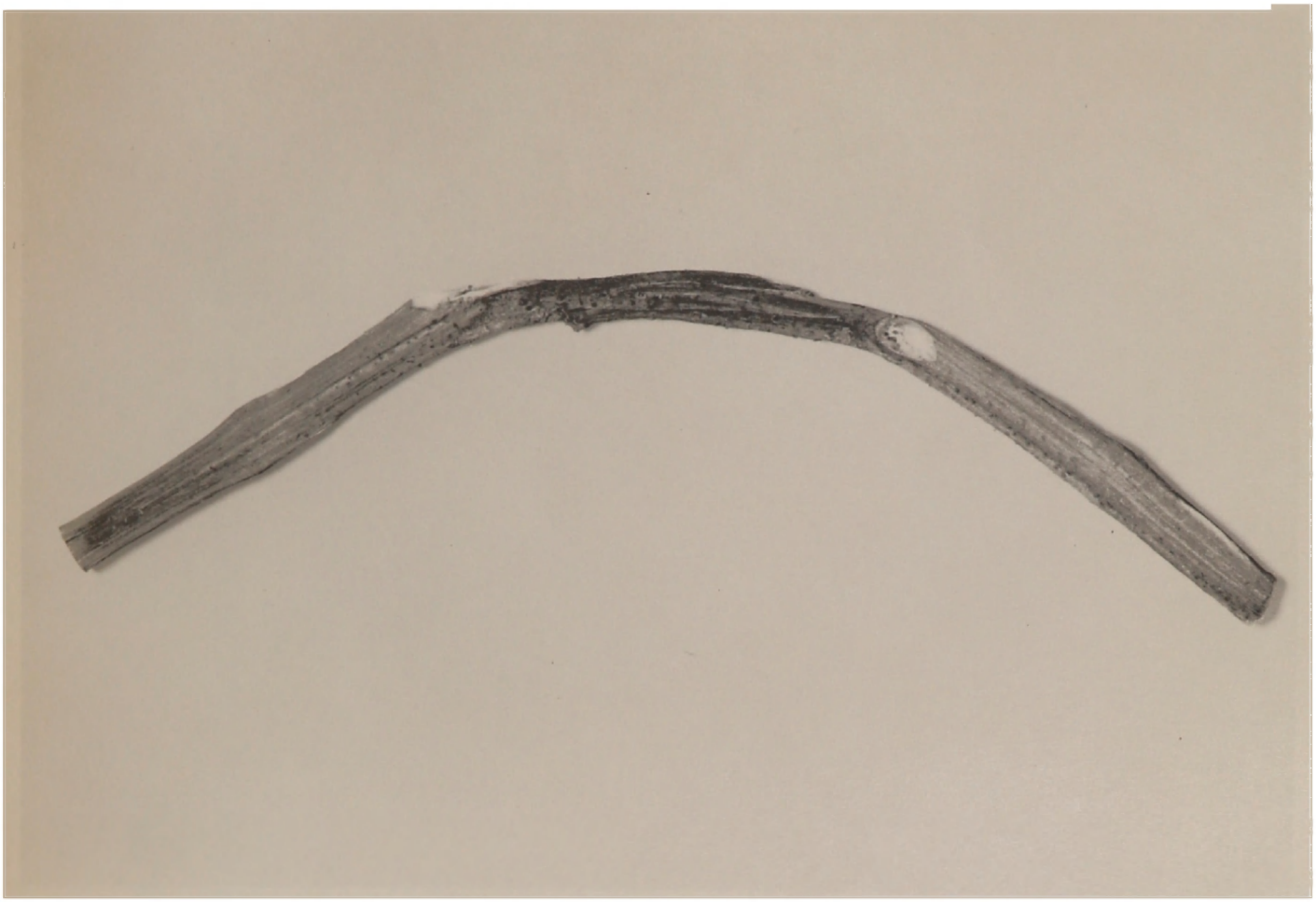


TABLE VII

TRANSPORT OF P^{32} AND Ca^{45} ACROSS GRAFT UNIONS (APEX TO APEX) INVOLVING REVERSED POLARITY IN THE TOMATO

Isotope	Days Following Treatment	Part Analyzed			
		Five Cm Segment of the Lateral of the Treated Plant	Graft Union	Five Cm Segment of the Lateral of the Non-Treated Plant	Fruit of the Non-Treated Plant
		(cpm/gm dry wt. - means of 4 single plant replications)			
P^{32}	6	682	470	302	
	12	1462	1075	609	129
Mean		1072	772	456	
L. S. D. mean of plant part 5%			375		
L. S. D. mean of plant part 1%			519		
Ca^{45}	6	4012	669	58	
	12	12659	2929	34	0
Mean		8336	1799	46	
L. S. D. mean of plant part 5%			2611		
L. S. D. mean of plant part 1%			3611		

can be absorbed by the root system of one plant, transported acropetally through its lateral, across a graft union and basipetally through a lateral of a second plant, and utilized in the development of its fruit. Graft unions involving reversed polarity offer little resistance to the transport of P^{32}.

Radiocalcium, on the other hand, does not readily transgress a graft union formed by apically grafting forced laterals together. Highly significant differences occurred between the lateral segment on the treated side of the graft union and the graft union segment or the lateral segment on the non-treated side of the graft union. A large accumulation of Ca^{45} was apparent by 12 days on the side of the graft union formed by the plant supplied the isotope and in the graft union (Table VII). That Ca^{45} transport was not completely blocked is shown in Figure 12 (Autoradiogram). No Ca^{45} was recovered in the immature fruits on the plant not receiving the isotope.

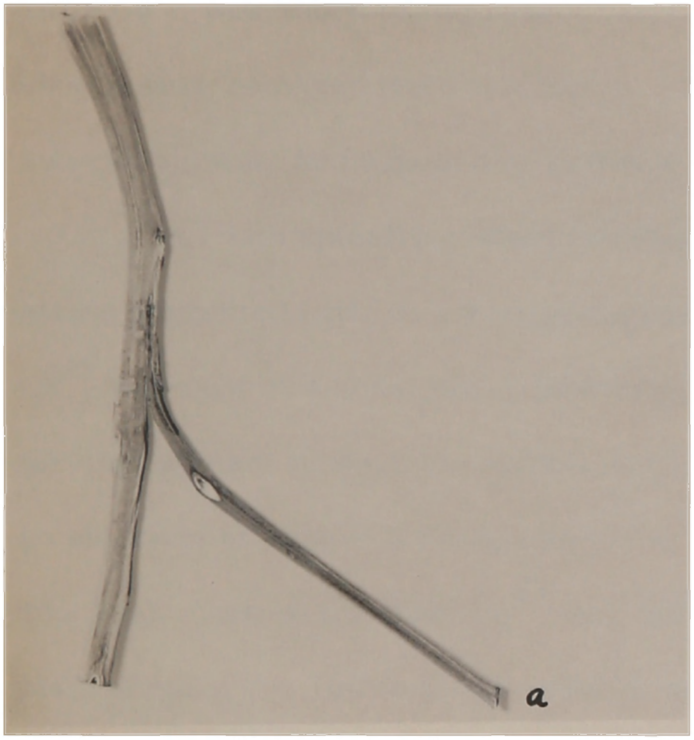
In a second series the stem of one tomato plant was inarched into the stem of another. In the discussion which follows, the stock will be considered as the plant being grafted, and the scion as the second plant, which supplied the top (scion), however, also retained its own root system. Thus, presumably the scion had the benefit of its own roots as well as those of the stock (Figure 13). The inarched plants were separated into two groups, A and B. Group A was treated by adding 100 microcuries of Ca^{45} to the soil in which the stock plant (a) was growing. In group B the roots of the scion plant (b) absorbed the isotope. Plants were harvested 48 hours

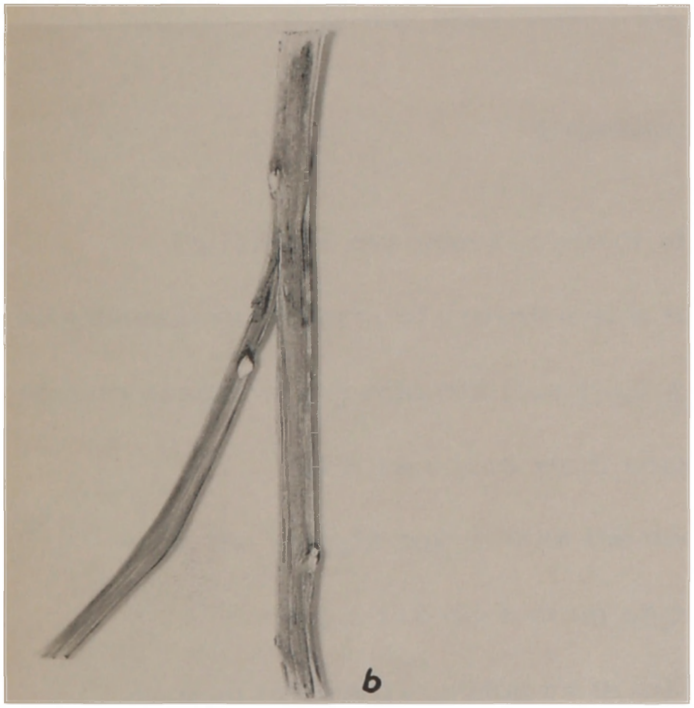
Figure 13

Photographs (left) and autoradiograms (right) of inarched stems of tomato plants showing the distribution of root absorbed radiocalcium (Ca^{45}) 48 hours after treatment.

(Top) Radiocalcium applied to the soil in which the stock plant (a) was growing.

(Bottom) Radiocalcium applied to the soil in which the scion plant (b) was growing.


a


b

after Ca^{45} was added to the respective root cultures and all leaves and side-shoots were removed from the stems. The stems were then prepared for autoradiography and exposed to Kodak X-ray film (Blue Brand).

As with apically grafted laterals, results with inarched tomato plants suggested little or no basipetal transport of Ca^{45} (Figure 13). When Ca^{45} was supplied to the stock root system the calcium followed the transpiration stream through the approach graft and into the scion. There was no evidence of basipetal transport in the stem of the scion plant, (Figure 13, Top). Likewise, when Ca^{45} was applied to the root system of the scion plant (Figure 13, Bottom), the calcium moved up the vascular system of the scion and very little was observed in the stock.

Experiment 11

To further evaluate the effect of graft unions on Ca^{45} transport and to separate the effects of growth and scion orientation vigorously growing tomato plants were prepared (see Figure 14) as follows:

(a) A two-inch stem segment containing one bud was grafted in the upright position on the decapitated stem of the same plant.

(b) A two-inch stem segment containing one bud was grafted in an inverted position as in (a) above.

(c) Plants were decapitated at the approximate height of

Figure 14

Top: Photograph showing growth of scions on decapitated tomato plants 21 days after budding. (Left) bud inverted, (center) bud in normal position, and (right) control (not budded).

Bottom: Photograph showing growth of scion on grafted tomato plants 11 days after grafting. (Left) scion inverted, (center) scion in normal position, and (right) control (not grafted).

the scion in (a) and (b) above. (No graft union).

(d) A shield with one bud was budded to the stock in the upright position and the stock decapitated above the shield.

(e) A shield with one bud was budded in an inverted position and the stock was decapitated above the shield.

(f) Plants were decapitated at heights comparable to the budded plants (d) and (e) above.

In an attempt to reduce plant variability in the above series, the scion and stock were of the same clone.

Upon completion of grafting or budding the plants were placed in a humidity chamber (95-100% R. H.) for four days after which they were grown under long days of early summer with frequent fertilization (weekly applications of 100 ml of 10-52-17).

Terminal growth of the grafted scions were recorded 0, 11 and 24 days after grafting. Eighteen days after grafting and 21 days after budding, when the various unions were well formed, 20 milliliters of a $Ca^{45}Cl_2$ solution (2.5 uc/ml) was applied to the soil in which each plant was growing. Two and six days after the plants were supplied with Ca^{45} they were harvested. The scions and graft unions in grafted plants and the scions and bud unions in budded plants were separated and dried in a forced air oven at 70° C. Dry weights were determined and the tissue assayed for radiocalcium.

Radioactivity was expressed as counts per minute per gram dry weight or total counts per minute per plant part. Autoradiograms were prepared of some of the treated plants and are illustrated in Figure 15.

Terminal growth and dry weights of the scions are recorded in Table VIII. From these data it is apparent that the inverted scion was slow to initiate growth, as compared to the normally grafted scion or the control. This may indicate that a longer period of time was required for an adequate union to form, or that by inversion some essential factor for growth was temporarily excluded from the scion. It appears (Table VIII, Bottom) that the slow formation of the union was the causative factor. The scions on the non-inverted grafted and control plants elongated 5. 5 to 6. 7 centimeters in 11 days, whereas the inverted scion elongated only 0. 6 centimeters. However, from the 11th to the 24th day both scions and control trippled their heights. The rate of growth from the 11th to the 24th day of the inverted scion approached that of the non-inverted scion and control. There were no significant differences in dry weight or terminal growth of the normally grafted scions and the controls (Table VIII, Top). The reductions in dry weight and terminal growth of the inverted scions were highly significant when compared to the normally grafted or non-grafted (control) plants.

Inverting a bud did not affect the scion growth as drastically as inverting the scion (Figure 14). The dry weight of the scion of an inverted

Figure 15

Autoradiograms illustrating the distribution of radiocalcium as influenced by scion orientation in budded and grafted tomato plants 48 hours following application of Ca^{45} to the root medium.

Top: Budded plants, (left) bud not inverted; (right) bud inverted.

Bottom: Grafted plants, (left) scion not inverted; (right) scion inverted.

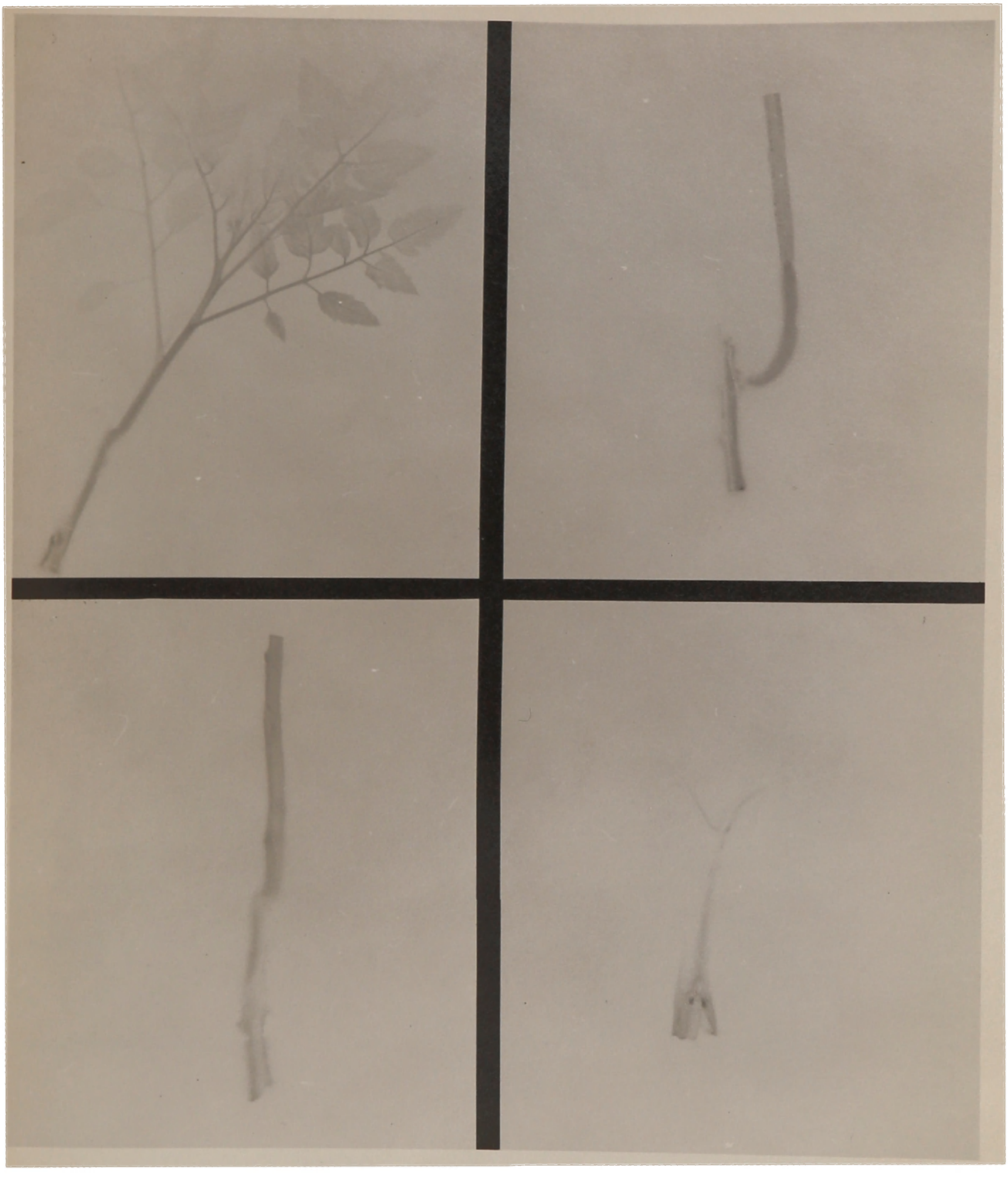


TABLE VIII

GROWTH OF SCION (TOMATO/TOMATO) AS INFLUENCED BY ORIENTATION OF SCION

Days After Grafting	Treatment of Scion: Inverted	Not Inverted	Control (Not Grafted)	Mean For Time
	(Grams dry weight - means of 6 replications)			
20	0.02	2.20	2.42	1.55
24	0.23	4.49	5.01	3.24
Means	0.13	3.35	3.72	
L.S.D. treatment: 1%		0.67		
L.S.D. time: 1%		0.54		
	(Length of terminal growth in centimeters)			
0 (initial length)	1.5	1.6	1.5	1.5
11	2.1	7.1	8.2	5.8
24	6.6	24.2	28.3	19.7
Means	3.4	11.0	12.7	
L.S.D. treatment: 1%		2.3		
L.S.D. time: 1%		1.9		

bud was only one-half of the normally budded scion and one-third of the control (Table IX). As time progressed the growth rate of the inverted buds approached that of the normally budded plants and non-budded controls.

Analysis of the graft union for radiocalcium derived from root absorption showed no significant differences among the unions formed by the inverted scion, non-inverted scion or a comparable segment on the non-grafted plant (Tables X and XI). Likewise, no differences occurred in the bud union of the budded plants (Tables XII and XIII).

Less radiocalcium (total cpm) was recovered in the inverted scion than the normally grafted or the scions of the non-grafted plants (Table X); yet the graft unions of inverted, non-inverted and a comparable segment in the control plant contained about equal quantities of Ca^{45}. This would indicate that Ca^{45} is being transported to, but not as rapidly across the graft unions of the inverted scions.

In order to fully evaluate the influence of the graft union in relation to transport, the data were expressed in counts per minute per gram dry weight. The quantity of Ca^{45} (cpm/gm dry wt.) recovered in the graft unions or scions did not differ significantly among the inverted, non-inverted or control plants (Table XI). This suggested that the rate of growth was the determining factor in calcium transport and not the nature of the graft union. Polarity of the tissue was still an important factor in calcium transport, for as soon as growth commenced the polarity was reoriented in the scion.

TABLE IX

GROWTH OF SCION (TOMATO/TOMATO) AS INFLUENCED BY ORIENTATION OF BUD

Days After Budding	Inverted Bud	Non-Inverted Bud	Control (Not Budded)
	(grams dry weight - means of 4 replications)		
23	0.73	1.82	2.44
29	1.99	2.70	3.99
Mean	1.36	2.26	3.22
L.S.D. for differences in bud orientation: 5%			0.75
L.S.D. for differences in bud orientation: 1%			1.04

TABLE X

TOTAL ROOT ABSORBED RADIOCALCIUM (Ca^{45}) RECOVERED IN THE SCION AND GRAFT UNIONS OF VARIOUSLY GRAFTED TOMATO PLANTS

Days After Treatment of Root Medium with Ca^{45} [1]	Scion			Graft Union		
	Inverted	Not Inverted	Control (Not Grafted)	Inverted Scion	Not-Inverted Scion	Comparable Stem Segment
			(Total cpm)			
2	23	1192	1071	313	407	263
6	123	3005	2468	1370	1373	1203
Mean	73	2099	1770	842	890	733
L. S. D. for means: 5%		268			Not Significant	
L. S. D. for means: 1%		381			Not Significant	

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[1] Time significant at 1% for all treatments.

TABLE XI

ROOT ABSORBED RADIOCALCIUM (Ca^{45}) RECOVERED IN THE SCION AND GRAFT UNIONS OF VARIOUSLY GRAFTED TOMATO PLANTS

Days After Addition of Ca^{45} to the Root Medium	Scion			Graft Union[1]		
	Inverted	Not Inverted	Control (Not Grafted)	Inverted Scion	Not Inverted Scion	Comparable Stem Segment
			(cpm/gm dry weight)			
2	673	480	500	209	386	233
6	870	693	600	927	1118	1037
Mean	772	587	550	490	690	587
L.S.D. means: 5%		Not Significant			Not Significant	

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[1] All values significant for time: 1%

TABLE XII

TOTAL ROOT ABSORBED RADIOCALCIUM (Ca^{45}) RECOVERED IN THE SCIONS AND BUD UNIONS OF VARIOUSLY BUDDED TOMATO PLANTS

Days After Addition of Ca^{45} to the Root Medium[1]	Scion			Bud Union		
	Bud Inverted	Bud Not Inverted	Control (Not Budded)	Bud Inverted	Bud Not Inverted	Comparable Stem Segment
			(Total cpm)			
2	788	2288	2178	278	473	260
6	2410	4465	5835	1533	1663	1388
Means	1599	3377	4007	906	1068	824
L. S. D. means: 5%	1338				Not Significant	
L. S. D. means: 1%	1853				Not Significant	

[1] Values for time are significant at 1% for all treatments.

TABLE XIII

ROOT ABSORBED RADIOCALCIUM (Ca^{45}) RECOVERED IN THE SCION AND BUD UNIONS OF VARIOUSLY BUDDED TOMATO PLANTS

Days After Addition of Ca^{45} to the Root Medium	Scion			Bud Union[1]		
	Bud Inverted	Bud Not-Inverted	Control (Not Budded)	Bud Inverted	Bud Not-Inverted	Control (Not Budded)
	(cpm/gm dry weight)					
2	1093	2107	1053	1563	2218	1715
6	2147	2750	1760	2083	2933	2813
Means	1620	2428	1407	1823	2576	2264
L. S. D. means: 5%	Not Significant			Not Significant		

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[1] Values for time are significant at 1% for all treatments.

Similar transport trends were indicated in budded plants. Radiocalcium recovered as total counts in the scion was significantly less for the inverted bud (Table XII). However, the bud unions did not differ significantly (Table XII). The quantity of Ca^{45} (cpm/gm dry wt.) recovered in the scions and bud unions of the budded tomato plants is recorded in Table XIII. When the data were expressed as counts per minute per gram dry weight, no significant differences were observed in the scions or bud unions. It appears that growth of the scion and not the nature of the bud union is the primary factor in the upward transport of Ca^{45}.

Experiment 12

In earlier experiments it was shown that Ca^{45} was not transported through a graft union involving reversed polarity. Conversely, P^{32} readily moved across such a union. It was further observed that the growth rate of the scion was a more important factor in Ca^{45} transport than was the nature of the graft union. The following experiment was designed to evaluate the effect of phloem polarity upon the basipetal transport of foliar applied radiophosphorus in the apple.

In August of 1954, 14 uniform Malling I apple trees (one-year-old) were selected from the nursery at the University farm. Two one-centimeter segments of phloem (bark) approximately two to three centimeters apart and 15 centimeters above the ground level were inverted on seven of the trees. The verticle incisions of the phloem rings were placed opposite one another and held in place by rubber grafting bands. On the seven remaining Malling I trees, two one-centimeter phloem segments were removed and replaced without inverting, and with the verticle incisions opposite one another.

In the following spring the young trees were potted and moved into the greenhouse. Terminal growth was recorded. After ten weeks, 100 microcuries of P^{32} were applied to a side shoot at approximately comparable distances above the upper phloem ring. Plants were harvested 48 hours after treatment by removing a five-centimeter phloem segment immediately above the phloem rings and a second segment containing the phloem rings. The samples were then prepared and assayed for radioactivity.

Within two weeks after performing the phloem orientation treatments, it was observed that many adventitious buds began to grow below the phloem rings in the nursery; however, no significant differences were observed. In the greenhouse, the following spring, the number of new shoots arising on trees below the inverted phloem rings was 70 percent greater (Table XIV). Similar results were demonstrated on tobacco within four weeks after phloem orientation treatments (Table XIV). The total new top growth which occurred on Malling I trees, having two inverted phloem rings, was only 50 percent of that which occurred on the trees not having the phloem inverted (Table XV). This agrees with the work of Sax (1954)

TABLE XIV

NUMBER OF BUDS FORCED BELOW TWO PHLOEM RINGS ON APPLE AND TOBACCO PLANTS AS A RESULT OF REMOVING, INVERTING AND REPLACING, REMOVING AND NOT REPLACING, OR REMOVING AND REPLACING IN THE NORMAL POSITION TWO RINGS OF PHLOEM

Treatment of Phloem Rings	Apple	Tobacco
	(means of 6 replications)	
Removed, inverted and replaced	7.4	2.4
Removed and not replaced	---	5.0
Removed and replaced in normal position	4.4	1.0
Phloem not altered	0.7	---
L.S.D. 5%	1.2	1.5
L.S.D. 1%	1.7	2.1

TABLE XV

SIZE OF STEM SWELLING AND TERMINAL GROWTH OF ONE-YEAR-OLD APPLE TREES (EAST MALLING I) AS INFLUENCED BY THE REMOVAL AND INVERSION OR REMOVAL AND NORMAL REPLACEMENT OF TWO PHLOEM RINGS

Treatment of Phloem Rings	Size of Stem Swelling (mm in diameter)			
	Above Upper Phloem Ring	Above Lower Phloem Ring	Ratio of Swelling Above Upper and Lower Ring	Terminal Growth (cm)
		(Means of 6 replications)		
Removed, inverted and replaced	7.25	3.41	2.13	43.1
Removed and replaced in normal position	1.61[2]	0.98[1]	1.64	90.1[2]

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[1] or [2] Significantly different from values for phloem rings inverted at p .05 or p .01 repectively.

where he suggested the possibility of dwarfing vigorous trees by inverting phloem rings. Furthermore, a four fold increase in the size of the swelling was observed above the upper inverted phloem ring (Table XV) compared to non-inverted phloem rings.

The effect of the phloem rings on downward transport of P^{32} are shown in Figure 16. The quantity of P^{32} (cpm/gm dry wt.) recovered above the inverted phloem rings was greater than in a comparable segment above the non-inverted phloem rings. This suggested that basipetal transport of P^{32} was reduced by inverted phloem. Considerable amounts of P^{32} passed through the upper segment of inverted phloem (Figure 17, Bottom), particularly in the region of the verticle incision where the xylem and phloem were regenerated most rapidly. The autoradiograms (Figure 17) portray the distribution of P^{32} in only one plane through a given region of the stem. If one closely observes the pattern in which the new xylem was formed, the regeneration which took place at the verticle incision of the top phloem ring followed around to the opposite side of the stem to the verticle incision of the lower phloem ring (see photograph, Figure 16, Bottom). This suggested the downward transport of nutrients was channeled through the verticle incisions. Such would account for the accumulation of P^{32} below the second phloem inversion.

Figure 16

Top: The comparative amount of foliar applied radiophosphorus (cpm/gm dry wt.) recovered in the various phloem segments in the apple (EM I). (A) Five cm segment immediately above the upper inversion, (B) five cm segment containing the inverted rings, (C) five cm segment immediately above the upper non-inverted phloem ring, and (D) five cm segment containing non-inverted rings.

Bottom: Photographs showing a segment of the apple stem with the phloem removed illustrating xylem regeneration in vicinity of phloem rings. (Left) Phloem rings were inverted. Note: Swelling and regeneration of xylem at site of verticle incision of inverted phloem rings. (Right) Phloem rings were not inverted.

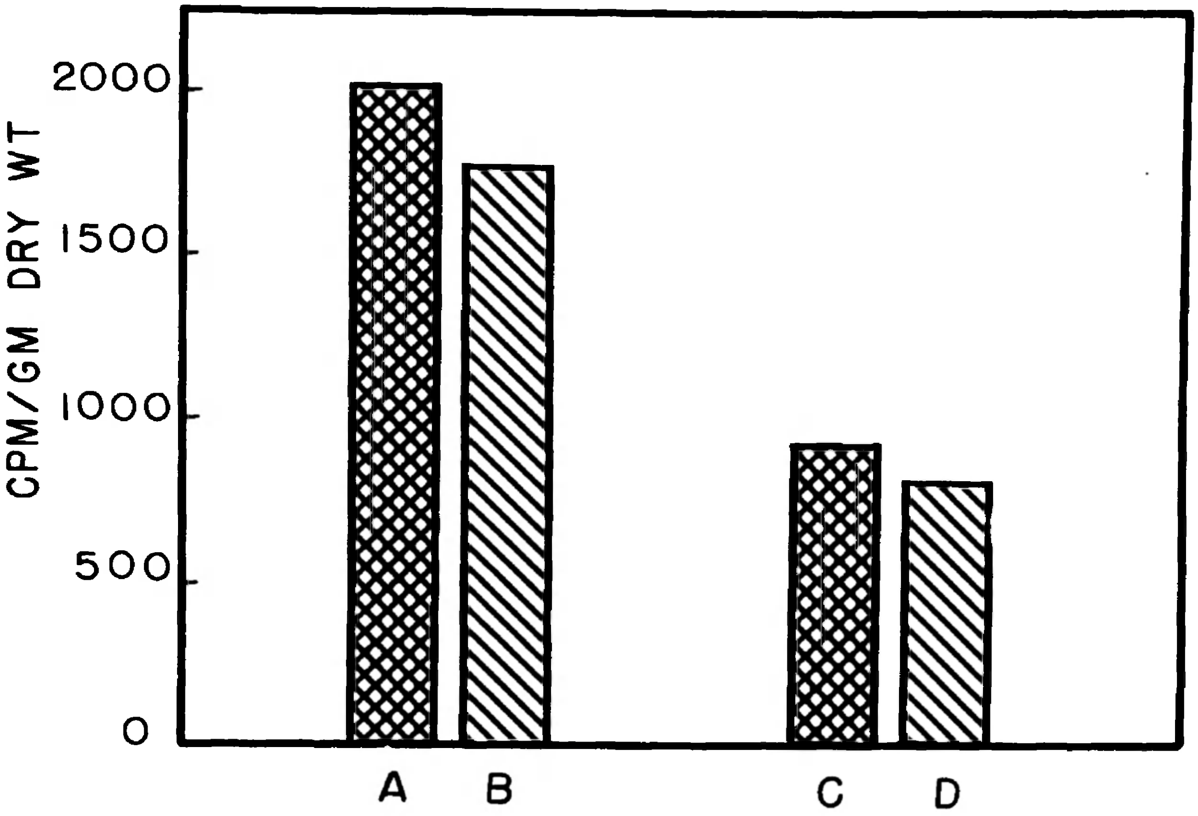
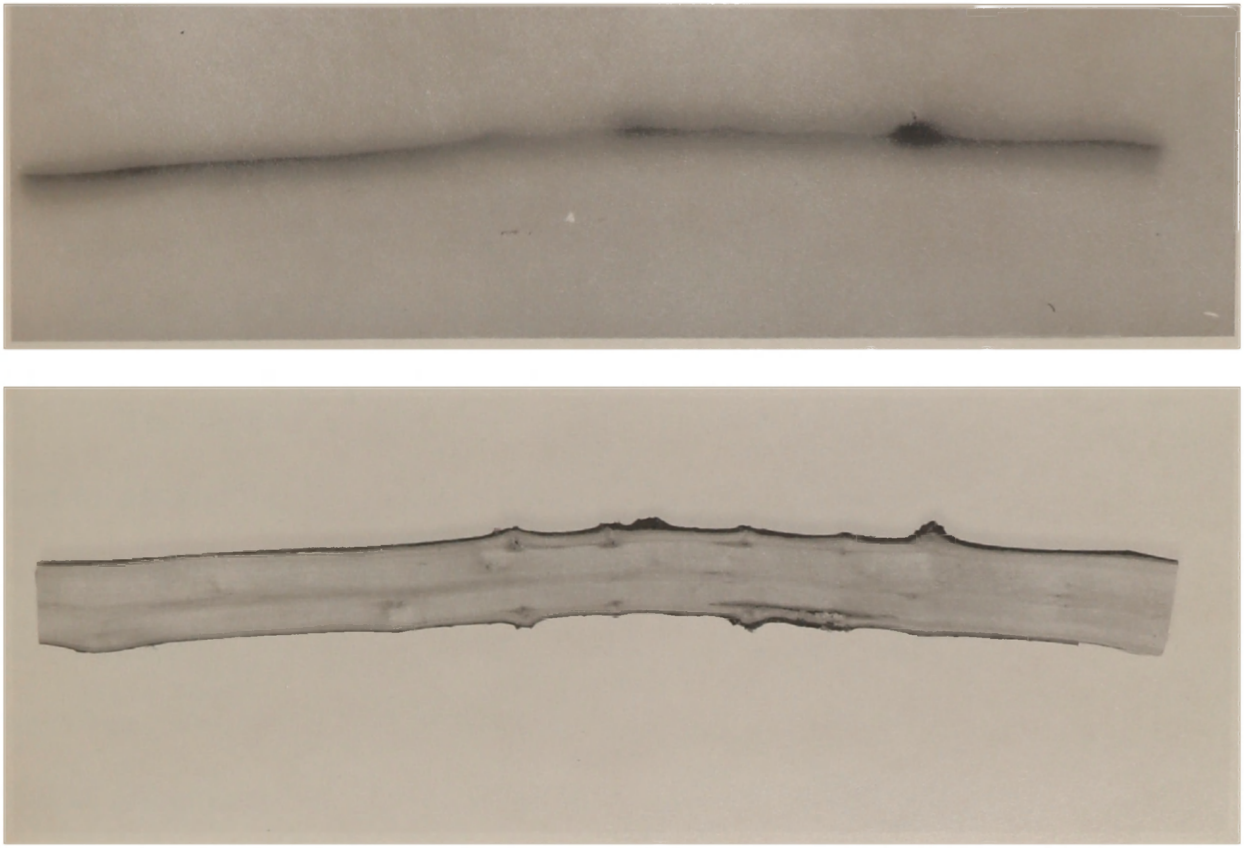
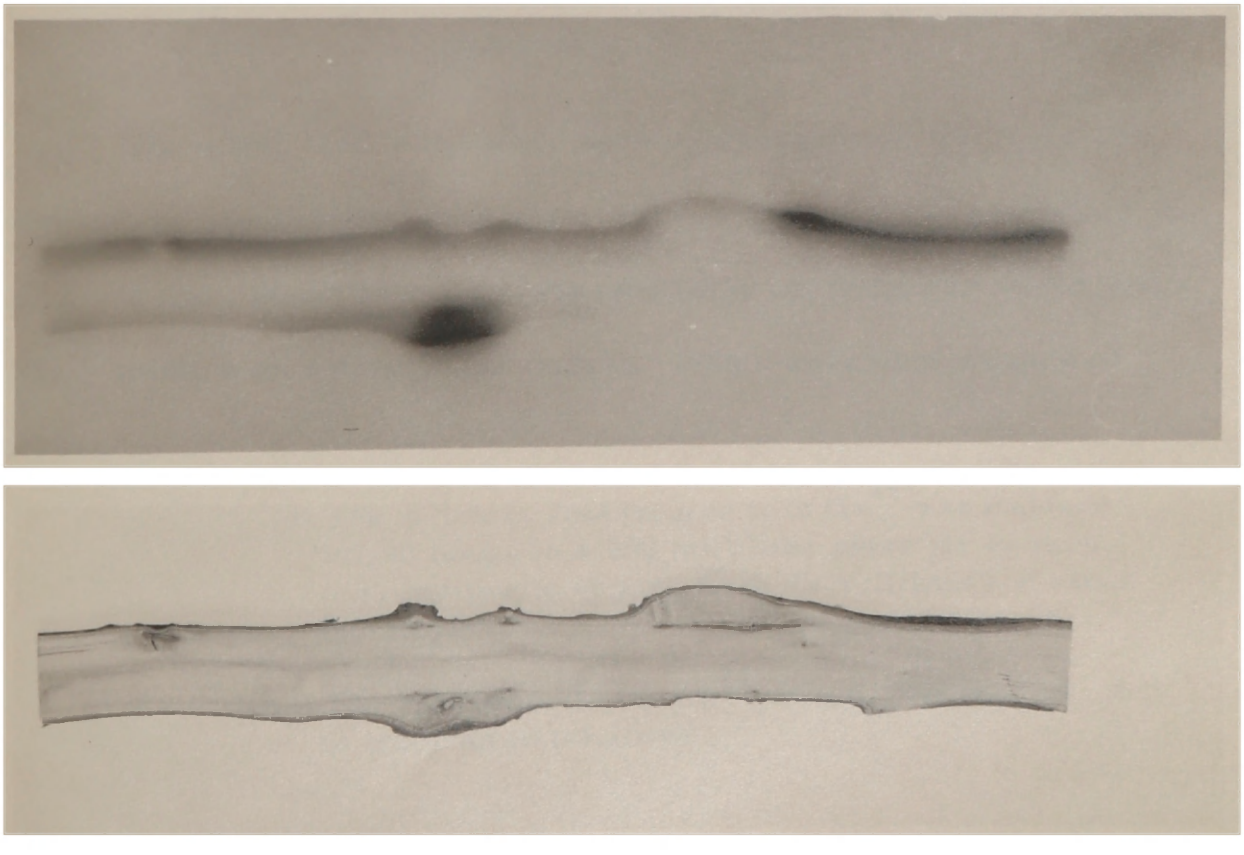

CPM/GM DRY WT
2000
1500
1000
500
0
A
B
C
D

Figure 17

Left: Photograph and autoradiogram showing the distribution of foliar applied P^{32} through inverted phloem rings, and swelling associated herewith.

Right: Photograph and autoradiogram showing the distribution of foliar applied P^{32} through non-inverted phloem rings, and the absence of the swelling above the phloem ring.





Section C

Some Factors Affecting Transport in the Phloem

The lack of basipetal transport of calcium in cotton has been attributed to its inability to move in the phloem (Mason and Maskell 1931). Previous experiments (10, 11) on calcium mobility suggested the possible role of polarity in controlling the transport of calcium. Went and Thimann (1937) reported that polarity was temporarily suspended by low concentrations of ether vapors. Accordingly, the following experiment was designed to ascertain the role of polarity in calcium transport.

Experiment 13

The effect of di-ethyl ether upon the transport of foliar applied radiocalcium was studied. One of the fully expanded primary leaves of the bean plant (var. Black Valentine) was treated by applying four microcuries of Ca^{45} solution as a drop on the midrib. Plants were then grouped as follows:

(a) The primary leaf treated with Ca^{45} was exposed for 96 hours in a 500 milliliter glass jar at room temperature to vapors from 25 milliliters of ether (di-ethyl).

(b) The leaf opposite the Ca^{45} treated leaf was exposed to ether as in (a) above.

(c) The Ca^{45} treated leaf was placed in a comparable glass jar with no ether.

(d) The leaf opposite the Ca^{45} treated leaf was placed in a comparable glass jar with no ether.

Plants were harvested 96 hours after treatment with Ca^{45} and autoradiograms prepared.

When the Ca^{45} treated leaf was subjected to ether considerable basipetal transport of Ca^{45} occurred out of the treated leaf and into other parts of the plant (Figure 18). When the leaf opposite the Ca^{45} treated leaf was subjected to ether, limited transport occurred within the Ca^{45} treated leaf. No basipetal transport occurred in the leaves of groups (c) or (d) above.

Similar results were obtained with radish plants (var. Icicle) subjected to treatments (a) and (c) described above. When the Ca^{45} treated leaf was exposed to ether vapors, considerable basipetal transport occurred. Photographs and autoradiograms (Figure 19) of cross and longitudinal sections of the swollen hypocotyl of radish suggested that Ca^{45} was transported basipetally. In treatment (c) where the Ca^{45} treated leaf was not subjected to ether vapor, no basipetal transport of Ca^{45} occurred. Under the conditions described above no visible injury from anesthetization occurred to the treated plants.

Figure 18

Autoradiograms showing distribution of foliar applied Ca^{45} in the bean plant. (Left) Anesthetized Ca^{45} treated leaf showing considerable transport out of the treated leaf; (upper right) Ca^{45} - treated leaf opposite an anesthetized leaf in which very little transport of Ca^{45} occurred; (lower right) Ca^{45} - treated leaf showing no basipetal transport of Ca^{45} from a plant which received no anesthetization.

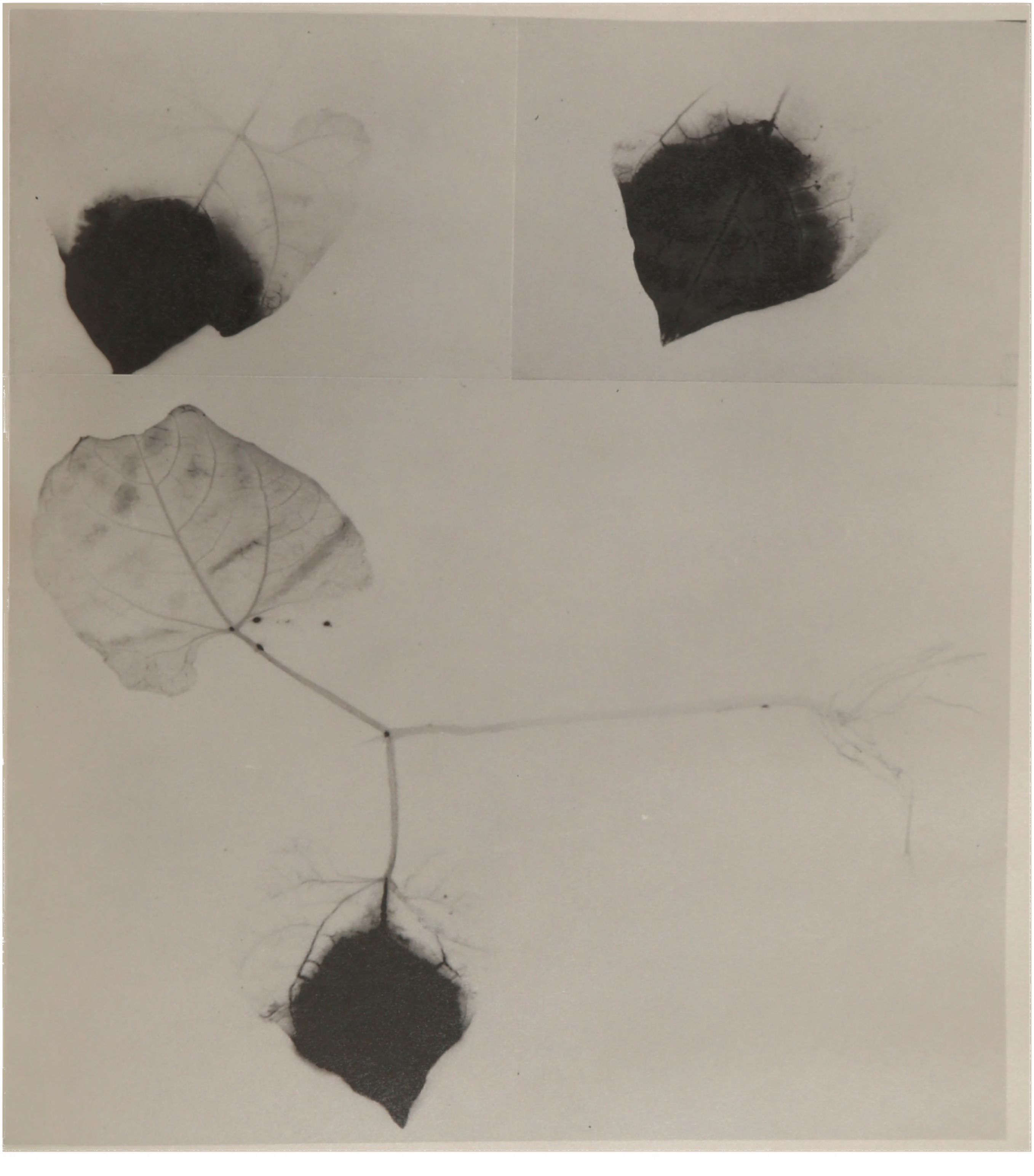
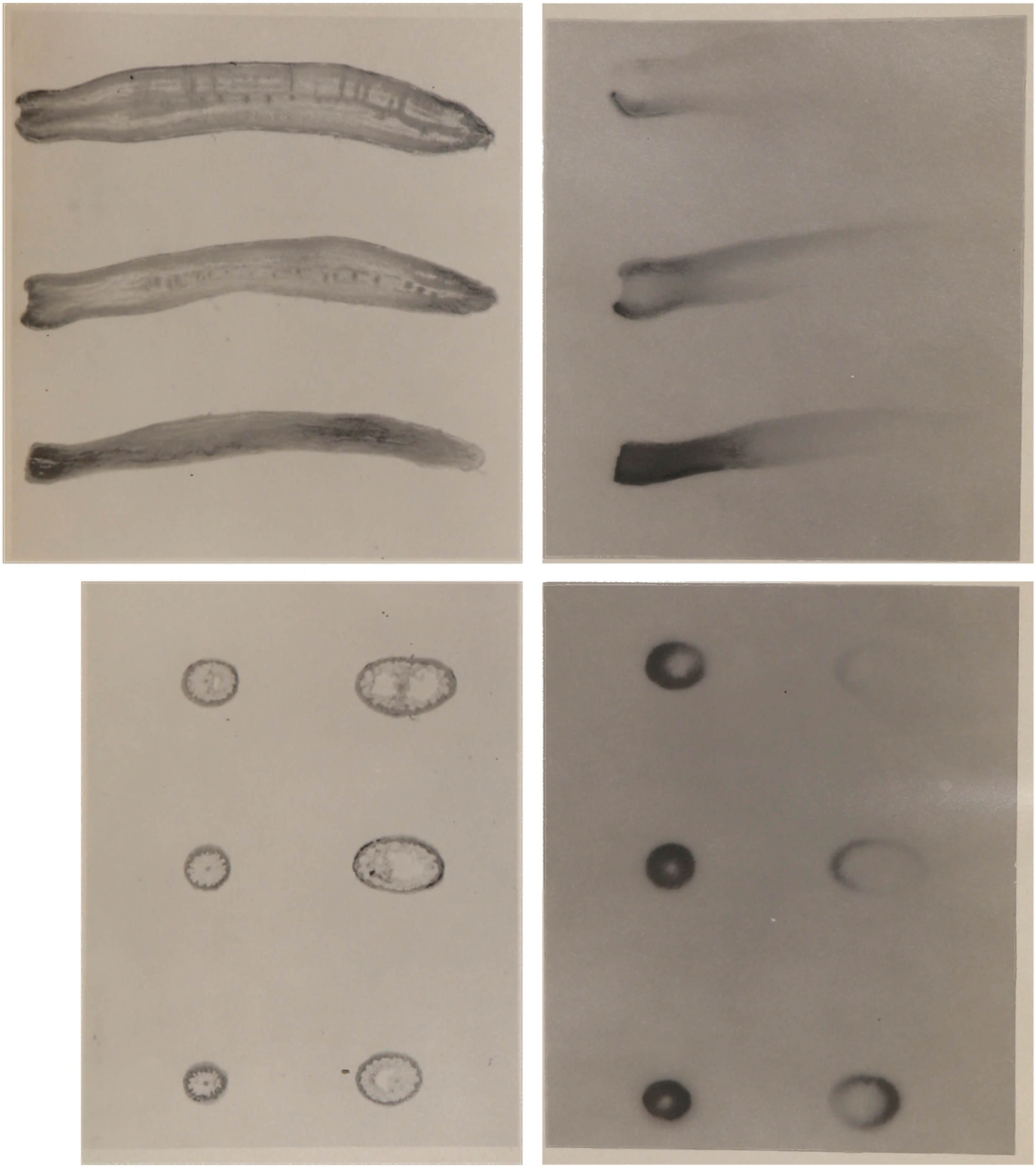


Figure 19

Photographs and autoradiograms of mounted radish hypocotyl specimens showing distribution of foliar applied Ca^{45} following anesthetization of the Ca^{45} treated leaf. (Left) photograph (top) and autoradiogram (bottom) showing distribution of radiocalcium in cross sections of the hypocotyl; (right) photograph (top) and autoradiogram (bottom) of longitudinal serial sections through the hypocotyl.



Experiment 14

The absorption and subsequent transport of foliar applied chelated radiocalcium was studied in bean plants. Radiocalcium (408 uc) was added to ten milliliters of a ten percent solution of di-sodium ethylenediamine tetraacetate[1] and saturated solutions of picrolinic acid, 2, 5-dichloro-3, 6-dihydroxy-p-quinone, and 1-amino-2-napthol-4-sulfonic acid. The pH of the solutions was adjusted to 8.0, 1.5, 1.5 and 2.0 respectively with potassium hydroxide or hydrochloric acid and aerated for 24 hours. The control solution consisted of 408 microcuries of $Ca^{45}Cl_2$ in ten milliliters of distilled water with the pH adjusted to 2.0 with hydrochloric acid. After 24 hours a one-milliliter aliquot of each of the above four chelated preparations was removed, and an excess of 2N oxalic acid added. The absence of a precipitate (calcium oxalate) indicated chelation of the Ca^{45}.

The chelated radiocalcium (0.01 ml) was applied to the midrib of a fully expanded primary leaf of the bean (var. Bountiful). After 24 hours plants were harvested and separated as follows: (a) treated leaf, (b) stem plus non-treated leaf, and (c) roots. Other plants were prepared for autoradiography.

Picrolinic and 1-amino-2-napthol-4-sulfonic acids resulted in severe burning of the treated leaf. This likely resulted from the low pH required for chelation. Radioactive assay of the various plant parts revealed that no Ca^{45} was transported out of the treated leaves irrespective of chelation. The results were substantiated by autoradiograms. All solutions entered the leaves but were transported only toward the periphery.

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[1] Recrystalized di-sodium ethylenediamine tetraacetate was supplied by the Alrose Chemical Co., Providence, R. I.

V. DISCUSSION

Stock-Scion Interrelationship

With the advent of radioisotopes and related techniques it has been possible with certain horticultural plants to study the role of the rootstock, scion and graft union in absorption and subsequent transport of nutrients. Both quantitative and visual results have been obtained for the upward and downward transport of nutrients. These studies have been concerned chiefly with phosphorus and calcium; phosphorus, because of its importance in the metabolic processes of the plant, and calcium, because of restriction of growth when absent.

It is believed that the critical time in the growth of the scion on any rootstock is in the spring when growth is rapid and the metabolic processes of the plant are at a maximum. If at this time, for some reason, the rootstock cannot supply the necessary metabolites, it is conceivable that scion growth will be affected. For these reasons experiments reported, herein, were of relatively short duration and during periods of high metabolic activity.

It was shown that rooted cuttings of Malling (EM) VII, IX, and XVI have different capacities to absorb radiophosphorus (P^{32}) from nutrient solutions. Absorption was closely related to the vigor observed in these stocks under field conditions. The results presented here for P^{32} absorption agree, in part, with those of Pearse (1939) for nitrogen and potassium. Pearse showed a differential absorption of potassium for dwarfing and vigorous rootstocks early in the season, but as the season progressed this difference disappeared. Berry (1938) measured the absorption of potassium bromide and concluded that rootstocks differed in their absorption capacities. He found the greatest absorption with EM XII, EM II was intermediate, with the lowest values for EM IX. Swarbrick et al (1946) suggested that the dwarfing effect may be the result of a differential rate of absorption or translocation, or a combination of the two.

In a similar manner, phloem transport of foliar applied P^{32} in McIntosh scions on EM VII, IX and XVI and Delicious seedling rootstocks very closely parallel the scion vigor observed on these stocks under field conditions. No marked accumulation occurred at the bud unions indicating that P^{32} passed freely through the phloem portion of the graft. There was no significant accumulation above the bud unions.

It appeared then that the scion (McIntosh) was somehow modified, either through its absorption or transport mechanism, by the stock. That reduced transport in the stock of EM IX can account for the lesser quantity of P^{32} recovered from the foliar application in the roots is not tenable. If this be the case, then a marked increase of P^{32} would have resulted in the scion. The form in which the applied phosphorus was transported has not been established.

In contrast to phosphorus, Ca^{45} applied to apple foliage failed to move basipetally from the site of application.

In root absorption studies of P^{32} and Ca^{45} using scions of McIntosh on EM VII, IX and XVI and Delicious seedling rootstocks consistent differences were observed. The most dwarfing stocks (EM IX and VII) absorbed and transported the least quantity of both P^{32} and Ca^{45} to the scions budded thereon. Vigorous stocks showed increased absorption and transport to the scion of both P^{32} and Ca^{45}. In general, a qualitative comparison of the P^{32} and Ca^{45} recovered in the scions on EM VII, IX, and XVI or Delicious seedling was closely related to vigor.

As with the downward transport of P^{32} from foliar application, no significant accumulation of P^{32} or Ca^{45} were observed at the bud union from root applications. This indicated that the bud union, <u>per se</u>, did not substantially interfere with the upward or downward transport of P^{32} or the upward transport of Ca^{45}. The lesser quantity of P^{32} and Ca^{45} recovered in the scions on dwarfing stocks can then be attributed either to reduced absorption or transport, or a combination of the two. In view of the data on phloem transport and absorption with rooted cuttings, it is highly suggestive that the major contributing factor responsible for the lesser quantities of P^{32} or Ca^{45} recovered in the McIntosh scions on dwarfing rootstocks is the lower absorbing capacity of the rootstock. Transport may also be a contributing factor since the absorption and transport processes are interdependent upon one another. No distinction, however, was made between the two processes.

In studies of foliar absorption and transport using dwarf and non-dwarf tomato plants, the lesser quantity of P^{32} recovered in the roots of the genetically dwarfed tomato plants was definitely attributed to reduced transport. Dwarf and non-dwarf plants absorbed approximately equal quantities of foliar applied P^{32} (Table IV). However, more P^{32} was recovered in the tops (minus the treated leaf) of the dwarf than in a comparable sample of the non-dwarf plant. Accordingly, less P^{32} was recovered in the root of the dwarf than non-dwarf tomato plant.

Effect of the Graft Union and Scion Orientation on the Transport of P^{32} and Ca^{45}

In the preceeding studies with compatible combinations of apple scions and rootstocks, the graft union did not appear to restrict calcium or phosphorus transport. Studies designed to more critically evaluate the role of the graft union indicated no apparent restriction of phosphorus transport. Considerable variation, however, was evident following root absorption of P^{32}. This undoubtedly resulted from uncontrollable factors existing in the soil media, particularly phosphorus fixation. Also, it is difficult to obtain a constant specific activity when the radioisotope was added to soil cultures.

An investigation of the acropetal transport of Na^{22}, P^{32}, S^{35}, Cl^{36}, Ca^{45}, Mn^{52-54}, Fe^{55-59}, Cu^{64}, Zn^{65}, Rb^{86}, Sr^{89}, Mo^{99}, and Ba^{140} through graft unions was performed by means of autoradiography. In no case did the graft union, per se, impede the upward transport. Similarly, it may be assumed that it offered no resistance to the transport of the non-radioactive elements.

In contrast to normally grafted scions, graft unions which involved reversed polarity severely reduced Ca^{45} transport and to a lesser degree transport of P^{32}. When forced laterals of vigorous tomato plants were grafted together terminally, a union was formed involving the apexes of two plants. Radiocalcium (Ca^{45}) applied to the roots of one of the two plants accumulated in the grafted lateral of the treated plant. Radiophosphorus on the other hand, accumulated to a lesser degree and was eventually distributed throughout both plants. The fact that calcium is not mobile in the phloem as suggested by Mason and Maskell (1931) may account for the reduced transport across a graft union involving reversed polarity. To be translocated to an adjacent plant attached by grafting, Ca^{45} must be transported basipetally through either the phloem or xylem. Since calcium is presumably immobile in the phloem and little evidence exists for the downward transport of ions or more complex compounds in the xylem, the results reported herein appear consistent. The termination of the transpiration streams of apex to apex grafted laterals can be visualized at the graft union; thereby each lateral obtains its calcium requirement from its parent plant. It is clearly evident that different mechanisms are operative for calcium and phosphorus transport under these conditions.

The data herein reported suggested that polarity as a physio-electrical phenomenon may, under certain conditions, govern calcium transport. This was further supported by recent work of Lowenhaupt (1956), who, using aquatic plants (Polamogeton crispus L.) reported that calcium moved into the abaxial leaf surface when in contact with the aqueous media and out of the adaxial surface when the latter was in contact with the aqueous media. Essentially, the direction of calcium movement under lighted conditions was into the lower surface of the leaf and out of the upper surface. In the dark the direction was reversed.

When Ca^{45} treated bean leaves were subjected to low concentrations of ether (di-ethyl) vapor, the foliar applied Ca^{45} was transported out of the treated leaf and to all other parts of the plant (Figure 18). Similar results occurred with radish (Figure 19). This may have resulted from a temporary suspension of polarity as a result of anesthetization, as reported by Went and Thimann (1937). On the other hand, a change in cell membrane permeability has not been completely eliminated. It would appear, however, that under the conditions of localized anesthesia only, the cell membranes in close proximity would be affected. Still, local anesthesia is sufficient providing the Ca^{45} tissue is anesthetized. It is conceivable that as a result of treatment with ether, a translocatable compound is formed, which is capable of affecting the permeability of the membranes through which it passed. If this be the case, then one could explain the transport of foliar applied Ca^{45}, in the presence of di-ethyl ether, on the basis of cell membrane permeability. The author is not, however, aware of such a compound.

When a tomato scion is inverted on a stock, the total root applied Ca^{45} transported to the scion was less than in normally grafted or non-grafted plants. Linear growth and dry weight of the inverted scions were also less. If, however, the Ca^{45} recovered in the inverted scion is expressed as per unit of dry weight (cpm/gm dry wt.) then no differences were observed. This suggested that the rate of growth of the scion was the factor determining calcium transport and not the nature of the graft union. There was no marked accumulation at or below the unions. The fact that Ca^{45} transgressed the union formed by an inverted scion does not make the polarity hypothesis untenable. A longer time was required for the graft union to form between the inverted scion and the stock, but once it was established and growth of the scion commenced, the growth rate approached that of the normally grafted plant. This suggested that the polarity of the inverted scion was re-established as the new pathways of transport were established. Conversely, when two laterals were grafted together terminally, there was no need for re-establishment of the transport pathways. Zimmerman and Connard (1934) suggested that elaborated foods, as well as salts, moved upward and downward through stems. They concluded that living cells take an active part in translocation, and that protoplasmic streaming should have the same effect whether the direction be backward or forward. This, however, was not the case as herein reported for calcium.

The orientation of the phloem has a pronounced effect upon the downward transport of metabolic products in the apple. Sax (1954) suggested that by inverting rings of phloem the tree can be dwarfed, presumably by preventing the movement of nutrients and auxins through the inverted phloem cells. Sax (1954) and Sax and Dickson (1956) attributed this to phloem polarity. However, no quantitative data were presented. In the present experiments, the growth of the top was definitely restricted as a result of inverting two one-centimeter phloem rings (Table XIV). That the inverted phloem rings interfered with auxin transport can be assumed as a result of the growth of the latent buds below the rings. One year after ringing adventitious buds continued to break on those trees with inverted phloem rings. This may have resulted from the inability of auxin, which tends to suppress lateral bud development, to pass across the inverted phloem rings.

More foliar applied P^{32} accumulated above the inverted than above the non-inverted phloem rings. The nature of the accumulation product was not determined. Dickson and Samuels (1956) reported similar results on a single tree, and concluded that the dwarfing response observed may have resulted from inpeded transport of organic nutrients. The assumption being that P^{32} was translocated in an organic form. Tolbert and Wiebe (1955) have isolated three translocation forms of phosphate compounds (inorganic phosphate and two unknown products) in xylem sap of barley. More recently Maizel et al (1956) has characterized one of the unknowns as phosphoyrl choline.

From the data presented herein, it is not possible to attribute the dwarfing effect of the stock on the scion totally to differences in the nutritional capacities of the stock. Transport is important, but has not been fully evaluated. The differences observed in these two processes may only be manifestations of some other factor (s) more directly concerned but unknown to the investigator. A better understanding of the metabolic processes of the stock and scion are required to more fully understand and evaluate the stock-scion interrelationships. These aims may be achieved by an evaluation of the metabolic activities (enzyme and respiration) of the stock and scion and auxin relationships. A critical study of the rate of nutrient transport, especially P^{32}, across graft unions would also be informative.

VI. SUMMARY

The effect of the graft union and stock-scion interrelationships, in certain horticultural plants, on foliar and root absorption and distribution of nutrients was studied with the aid of radioactive isotopes and autoradiography.

The quantity of root absorbed radiophosphorus (cpm/gm dry wt/mg root) recovered in the tops of East Malling (EM) IX, VII and XVI rooted cuttings closely followed scion vigor on these stocks observed in the field. Tops of EM XVI contained twice as much P^{32} as EM IX, EMVII was intermediate.

Irrespective of foliar or root application of P^{32} the most dwarfing rootstock (EM IX) absorbed the least, EM VII intermediate, and EM XVI or Delicious seedling the largest quantity of P^{32}.

In no case were significant differences of P^{32} or Ca^{45} concentration observed in the bud unions formed by McIntosh on EM VII, IX and XVI and Delicious seedling rootstocks.

No differences were observed between genetically dwarfed (Mac Arthur 741) and non-dwarf (Stokesdale) tomato plants in their ability to absorb foliar applied P^{32}. Significantly less P^{32} was transported to the roots of the dwarf than the non-dwarf plant.

Tomato plants chemically dwarfed with maleic hydrazide absorbed less foliar applied P^{32}, but no differences in transport were observed within

24 hours after treatment with P^{32}.

There were no significant differences in the soil applied P^{32} recovered in the tops of grafted or non-grafted tomato plants. Furthermore, there was no indication that P^{32} accumulated at the graft union, or that the union itself interfered with the upward transport of phosphorus. Likewise, no marked accumulation of root absorbed Na^{22}, S^{35}, Cl^{36}, Ca^{45}, Mn^{52-54}, Fe^{55-59}, Cu^{64}, Zn^{65}, Rb^{86}, Sr^{89}, Mo^{99}, and Ba^{140} was observed at or below the graft union in tomato plants.

In apple, Clark's dwarf interstem pieces offered no resistance to the upward transport of Ca^{45} applied to the root media within 24 hours after treatment.

When two one-centimeter phloem segments were inverted on one-year-old Malling I apple trees, foliar applied P^{32} accumulated above the phloem inversions, with lesser amounts above the non-inverted phloem rings. Swellings above the inverted phloem rings and reduced growth of the tops were evident. Approximately 60 percent more adventitious buds broke on trees with phloem inversions than on those not having inverted phloem rings.

Graft unions formed by apically grafting together forced laterals of vigorously growing tomato plants significantly reduced the transport of P^{32} and almost completely blocked the transport of Ca^{45}.

With tomato, scions grafted in an inverted position grew less in terms of dry weight and terminal growth. Also, less radiocalcium was recovered in the inverted scions than on normally grafted scions or on a comparable segment on a non-grafted plant. However, when expressed on a dry weight basis there were no significant differences in the amounts of root absorbed Ca^{45} recovered in the scions.

Bean and radish leaves treated with Ca^{45} and subsequently anesthetized with di-ethyl ether transported considerable quantities of Ca^{45} from the site of application to all other parts of the plant.

Chelation of Ca^{45} by di-sodium ethylenediamine tetraacetate, picrolinic acid, 2, 5-dichloro-3, 6-dihydroxy-p-quinone, and 1-amino-2-napthol-4-sulfonic acid did not favor the basipetal transport of Ca^{45}. The chelated calcium was absorbed by the leaf, but transported only toward the periphery.

VII. GLOSSARY

Acropetal	In the direction from the transition zone toward the stem or root apex.
Basipetal	In the direction from the shoot or root apex toward the transition zone.
Carrier free	A radioisotope is said to be carrier free if it contains only radioactive atoms of the element. However, this ideal is usually only approached and "carrier free" is used in this text to mean no carrier added.
Clone	A group of individuals produced from a single original individual by asexual means.
Inarch	Inarching is a process of grafting in which the scion unites with the stock while it is still attached to the parent plant.
Interstem	A stem which is used to separate and connect a rootstock and a budded or grafted top, yet different from both.
L. S. D.	Least significant difference.
Microcurie (uc)	A unit of measurement of radioactivity equaling 3.7×10^4 disintegrations per second.
Root-piece	A root when used as a stock. The scion is grafted directly to a root.
Rootstock	A plant used as the root for a grafted or budded plant.
Scion	A plant used as the top for a grafted or budded plant.
Scion effect	A modification of the rootstock character induced by the scion.
Stem-piece	See interstem above.
Stion	Term used to designate the new individual formed by grafting or budding a scion to the stock.
Stock effect	A modification of the scion character induced by the rootstock.
T Budding	Method of budding deriving its name because the two cuts made on the stock intersect so as to form a "T".

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