Physiology of interstem dwarfing in apple

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PHYSIOLOGY OF INTERSTEM DWARFING IN APPLE

by

Earl Walter Scholz

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

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Approved:

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INTRODUCTION

Dwarf fruit trees, for hundreds of years popular in European gardens, are now gaining considerable attention from horticulturists in America. Not only are they desirable from the aesthetic viewpoint but because of their diminutive size they are thought to be ultimately more profitable in the commercial orchard.

Probably one of the most costly operations in commercial fruit production today is that of spraying; small trees may be more easily and effectively sprayed. Without the necessity of large ladders, pruning and harvesting operations are facilitated, too. Windfall damage is obviously reduced. These facts justify the renewed interest in dwarf fruit trees.

Apple trees, dwarfed by graftage, mature earlier, usually bearing their first fruit in three years or less as contrasted to six to ten years for standard trees. They may tend more to annual bearing (Banta, 1955b). Their fruits are often larger and more highly colored than the same variety grown as a standard tree; it has been claimed, also, that the apples ripen earlier (Fowler, 1955), but this is not conceded by all. Higher acre yields have been reported from the few large dwarf apple orchards now in bearing in the United States.

Dwarf apple trees are not without disadvantages. Chief among these is their high initial cost. Not only do dwarf
trees cost more to propagate, but more must be planted per unit area. Certain of the rootstocks used are shallow rooted and trees grown on them must be staked or grown to a trellis. Some varieties which ordinarily tend to biennial bearing are brought to annual bearing by being dwarfed, but it is reported by Fowler (1955) that Starking and Golden Delicious, which are annual bearing varieties as standard trees, tend to lapse into biennial bearing when grown as dwarfs.

European dwarf understocks, the Paradise stocks, are commonly not hardy in the rigorous climate of the midwest; furthermore, they root poorly at prevailing summer soil temperatures (Banta, 1955a, and Nelson and Tukey, 1955). Maney (1943) discovered a more hardy dwarfing stock, which he called Clark Dwarf, which has greatly alleviated this trouble. The disadvantage of shallow rooting, necessitating staking and trellising, has also been obviated by using an interstem of a dwarfing stock, grafted between a vigorous root and a scion of the variety in which the dwarfed growth habit is to be induced. The dwarfing influence may then be derived from this small section of stem.

The use of dwarfing understocks indicates several possible physiological influences which might reduce the growth of the scion, namely: (1) reduced water and mineral absorption, (2) reduced rate of water and mineral movement or reduced translocation of elaborated material, (3) production, destruction
or impeded transport of growth regulators, and (4) incompatible graft unions. The employment of a dwarfing interstem, however, precludes the first possibility as a direct influence.

The induction of dwarfing of apple varieties is only one of the more prominent manifestations of the intricate interactions between stocks and scions of grafted plants. In an art so old as graftage it is amazing that so little has been learned of stock-scion physiology. One is still not able to predict with accuracy the action of a new stock-scion combination; only by experience can it be determined what results will be obtained.

This study was undertaken with the purpose of adding to our knowledge of graft dwarfing and to investigate the physiological influence of dwarfing interstems.
Dwarf stocks have been known for over 2000 years. In the early history of the Greeks, when they were still in the fruit and not the restaurant business, they used dwarf stocks,

wrote T. J. Maney (1942).

Employment of an interstem section to accomplish the dwarfing reaction may be a comparatively new process, however. This method was described in Flora in 1665 by the Englishman, John Rea:

I have found out another expedient to help them forward, that is by grafting the Cyen of the Paradise apple in a Crab, or other Apple-Stock, close to the ground, with one graft, and when that is grown to the Bigness of a finger, graft thereon about eight inches higher, the fruit desired, which will stop the luxurious growth of the Tree, almost as well as if it had been immediately grafted on the forementioned Layers, and will cause the Trees to bear sooner, more and better fruits. (Graves, 1950)

Although knowledge of the interstem method was available in Europe almost 300 years ago, the preponderance of the work to date has been with dwarfing understocks of the Doucin and Paradise stocks. This is not unexpected, considering that the popular Doucin and Paradise dwarfing understocks grow satisfactorily in the mild climates of England, France, and Central Europe.

In recent years Hatton (1917, 1919) at East Malling completed research on the standardization of European dwarf stocks; various types of the so-called Paradise had been
introduced into common use during the seventeenth and eighteenth centuries—until no very clear idea was held as to what these various Paradise stocks were. Duhamel du Monceau in his *Traité des Arbres Fruitiers*, 1768, pointed out that in his comparisons of the Doucin and *Le Pommier nain de Paradis*, degrees of dwarfingness existed (Hatton, 1917). These clonal rootstocks were studied by Hatton and cooperating workers, and finally classified with Roman Numerals from I to XVI. They are now known as East Malling rootstocks. East Malling I (EM I) the Broad-Leaved English Paradise, is slightly dwarfing, the degree of dwarfing increasing to EM IX, the Juane de Metz Paradise or Yellow Paradise, which is used as a standard of comparison of dwarfing today. EM XII and EM XVI and sometimes EM XIII are considered to be standards of comparison for vigorous clones or standard trees.

The clonal rootstock, Clark Dwarf, is probably of Paradise origin. Maney (1942) claims that it is distinct in its characteristics from the standardized English stocks, but that it undoubtedly originated as a seedling from the old English Paradise (EM II or EM VII). According to Maney (1943), Iowa State College acquired dwarf stock in 1924 from H. Walton Clark, a biologist connected with the United States Bureau of Fisheries Biological Station at Fairport, Iowa. He had discovered it in the garden of an old lady in Muscatine, Iowa. The tree was 20-25 years old and had experienced temperatures
of -25°F without showing signs of injury. According to the lady, her sailor son had brought the stock from South America.

Karl Brase (1953) compared Clark Dwarf and EM VIII and found them identical in their characteristics of growth, bark, internodes, lenticels, leaf blade, base, serrations, petiole, stipules, and fruit. He claims Clark Dwarf cannot be distinguished morphologically from the root stock EM VIII and believed them to be identical.

Early in the nineteenth century, 1816, Thomas Andrew Knight (1822), first president of the Royal Horticultural Society of London, discussed the physiology of dwarfing fruit trees:

Whenever the stick, and graft, or bud, are not perfectly well united to each other, an enlargement is well known always to take place at the point of their junction, and generally to some extent both above and below it. This is particularly observable in Peach trees, which have been grafted at any considerable height from the ground upon Plum stocks; and it appears to arise from obstruction, which the descending sap of the Peach tree meets with in the bark of the Plum stock, for the effects produced, both upon the growth and produce of the tree, are similar to those which occur when the descent of the sap is impeded by a ligature, or by the destruction of a circle of bark. ...The disposition in young trees to produce and nourish blossom buds and fruit, is increased by this apparent obstruction of the descending sap; and the fruit of such young trees ripens, I think, somewhat earlier than upon other young trees of the same age, which grow upon stocks of their own species....

Knight also postulates the same explanation for the dwarfing effect of the Paradise apple stocks. He thus
attributes the dwarfing and early fruiting propensities to the 
check of the flow of nutrient sap to the roots, which deters 
vegetative growth, and the resulting accumulation of nutrients 
in the tops, which stimulates flowering, fruiting, and hastens 
maturity and differentiation; the junction then, accordingly, 
is a living, well-controlled girdle.

It is of interest at this point to note Knight's (1824) 
hypothesis on the physiological action of a girdle:

According to that hypothesis the true sap of 
trees is wholly generated in their leaves, from 
which it descends through their bark to the ex-
tremities of their roots, depositing in its course 
the matter which is successively added to the 
tree; whilst whatever portion of such sap is not 
thus expended sinks into the alburnum, and joins 
the ascending current to which it communicates 
powers not possessed by the recently absorbed 
fluid. When the course of the descending current 
is intercepted, that necessarily stagnates, and 
it is repulsed, and carried upwards, to be ex-
aped in an increased production of blossoming 
and of fruit: and, consistently with these con-
clusions, I have found that part of the alburnum, 
which is situated above the decorticated space, 
to exceed in specific gravity, very considerably, 
that which lies below it. The repulsion of the 
descending fluid therefore accounts, I conceive 
satisfactorily, for the increased produce of 
blossoms, and more rapid growth of the fruit upon 
the decorticated branch: but there are other 
causes which operate in promoting its more early 
maturity. The part of the branch which is below 
the decorticated space is ill supplied with 
nutriment, and ceases almost to grow; it in con-
sequence operates less actively in impelling the 
ascending current of sap, which must also be 
impeded in its progress through the decorticated 
space. The parts which are above it must there-
fore be less abundantly supplied with moisture; 
and drought, in such cases, always operates 
powerfully in accelerating maturity.
Dr. F. R. Tubbs, Director of the East Malling Research Station, says, "We do not know of any dwarfing apple rootstock that does not induce the formation of a bulge." (Sax, 1954). This lends credence to Knight's ideas.

Colby (1935) found that roots of trees dwarfed on EM IX were not starved for starch, fat, or nitrogen compounds, and concluded that the graft union did not prevent the downward movement of carbohydrate or soluble nitrogen to the roots. Upward movement of reserve foods in the spring was found to be limited, he thought, because of the early cyclic suberization of the roots resulting in a limited water supply to the tops. Since translocation of organic material is through the phloem and is not directly related to water absorption, this explanation is dubious. Moreover, he found that where an interstem of EM IX was used, the retention of organic materials below the interstem was even more extreme, which seems to deny the validity of the postulate that early root suberization is the cause of organic reserves being retained in the basal parts of the tree.

It is not within the province of this paper to review all of the publications pertaining to stock versus scion influence. Tukey and Brase (1933) have very satisfactorily reviewed the literature to that date, and have concluded that in considering vigorous wood no one part dominates the entire plant, since all have been shown to have marked influence at one time or
another. But where dwarfing stock is used the dwarfing character predominates, whether used as a rootstock, interstem, or top scion. Maney (1942) and Roberts (1929) concur that dwarf stocks are still dwarfs when worked on a vigorous stock.

The carbohydrate relations of Crawley Beauty apple variety on the EM IX and EM XIII rootstocks were investigated by Rao and Berry (1940). They found no indications of translocation interference at the graft union. They found a greater carbohydrate concentration in trees on EM IX than in those on EM XIII. Starch and sucrose accumulated earlier in EM IX stocks than in EM XIII, and much earlier in EM IX stocks than in scions on EM IX. Scions on EM IX accumulated starch much earlier than those on EM XIII. They observed that starch accumulation in EM IX stocks, and in the scion bark, preceded cessation of terminal growth in the scion. But in the scion wood a rise in starch content occurred at the same time with growth cessation, both on EM IX and on EM XIII. Water content of the EM IX stock and its scion was considerably less than that in the EM XIII tree. They believe that this is the primary cause of the dwarfing effect. The water content of EM IX wood fell continuously from the latter part of July, while that of the scion was rising until the first part of September. They explained that this must be because the scion continues to remove water from the stock after the roots cease to maintain an adequate supply; the explanation is based on
Colby's observation of early cyclic suberization of EM IX roots.

Dana (1952) applied $^{14}\text{O}_2$ to the leaves of a Clark Dwarf interstem tree and measured the resulting radioactive photosynthate which was transported down to and through the interstem by harvesting longitudinal strips of bark. He found concentration crests which he was unable to explain. These may have been a result of the anastomosing or wandering of the phloem, the straight strips of bark intercepting the stream of radioactivity at random. Dana was unable to detect any significant buildup of photosynthate above the interstem of the single tree used.

Subsequent to the start of this study, Dickson and Samuels (1956) reported on work in which they introduced radioactive phosphorus into a leaf petiole above the interstem, followed the radioactivity up the shoot, thence down, and found its accumulation in the interstems of both Clark Dwarf and Em IX in 7 days and 3 days, respectively. They also had similar results with young trees dwarfed with knots tied in their trunks and with inverted bark grafts, and therefore compared the dwarfing processes of interstems to that of knotting, inverted bark grafts, and girdles. Their data on the interstem trees were reported as autoradiographs of stem discs. The published film pictures appeared to be of fully saturated exposures, and therefore not necessarily quantitative for the
Clark Dwarf tree. The accumulation in the EM IX interstem did appear to be somewhat greater than in the areas above and below the interstem. They assumed that the radioactive phosphorus material was in an organic form. Inverted bark grafts showed a very high accumulation above the juncture, indicating almost certainly an interference with translocation; no valid evidence was shown that this build-up was present when interstem pieces were used.

Friedrich (1939) found no evidence of accumulation of materials at or immediately above the graft union. He showed that there was usually more dry matter, sugar, nitrogen, and higher osmotic pressures in trees grafted on dwarfing stocks.

Terminal shoots of trees on Malling stocks were sampled through the season at Long Ashton, England. Vaidya (1938) found the ash to contain high calcium and low potassium in trees on EM IX; magnesium was low in trees worked on EM IX and EM VII and higher on vigorous stocks. The seasonal cycles were somewhat earlier in the trees on EM IX. Smyth (1938) reported the carbohydrate data on the same material. There were no significant differences in seasonal cycle of carbohydrate or lignin for trees on EM II, V, VII, or IX, although there was a tendency for starch and hemicellulose to accumulate in trees on EM IX at a higher rate in late summer and autumn than in trees on other stock. Kench (1939) presented the corresponding nitrogen analyses. There was higher total nitrogen in terminal
shoots from trees on EM IX, and a tendency to accumulate amide nitrogen.

Using the spectroscope, Roach (1931) detected molybdenum in the EM IX rootstock in trees on EM IX. Molybdenum was not present in high enough concentration in the soil to be detected and was not found either in EM XII or in scions worked on either of the rootstocks. Mahlstede and Haber (1957) wrote that Jonathan developed molybdenum deficiency when worked on EM IX rootstock and attributed this to the inability of the rootstock to absorb molybdenum from the soil. Tukey (1940) had found Jonathan well suited to EM IX rootstock after three years performance.

Dana (1952) investigated nitrogen increases of potted dwarf Golden Delicious trees treated with ammonium nitrate. The nitrogen starved trees on Clark Dwarf interstem accumulated nitrogen more slowly in their tops than those on Golden Delicious interstems. When the dwarfing interstems were bridged with Golden Delicious wood the effect was intermediate. He evaluated this as showing an impediment to phloem translocation, since upward translocation of nitrogen is through the phloem (Loomis, 1935). He used the same trees to calculate water losses, concluding that there were no indications that the dwarf interstem interferes with water movement.

Water relations were studied in dwarf stocks by Knight (1925, 1926) who pointed out small differences in transpiration
rates between EM I and EM IX. Nevertheless, individuals of any one variety worked upon the different stocks did not show any differences in transpiration.

Transmission of water through the graft unions was investigated by Chang (1938) for pears, peaches, and plums, and by Warne and Raby (1939) for apples. Chang drew water through incompatible combinations, and found them to offer much higher resistance to flow than compatible graft unions. Warne and Raby studied double worked apple combinations containing EM IX and EM XII. Those with EM IX interstems offered greater resistance to flow of water than those combinations containing EM XII. In none of the unions with EM IX, however, was there an almost complete barrier to water transport such as Chang found in certain incompatible unions.

Warne and Raby also determined the conductivity of plain stem pieces 15 cm and 7.5 cm in length, measuring it in grams of water passed in 15 minutes at a negative pressure of 30 cm of mercury. Conductivity of the longer pieces was half that of the shorter. This may explain the increasing dwarfing with increasing lengths of interstems, as indicated by Hatton (1930), Grubb (1939) and later confirmed by Dana (1952).

Roberts (1934, 1935) was able to demonstrate effects on vigor where only ring grafts were made, the xylem being left intact, supposedly showing that faulty xylem unions are not the sole cause of the dwarfing of apple scions. However, the
ring grafts were placed on 1-year nursery trees after the bark would slip readily, and supposedly were healed by the end of the growing season; data were collected during the following two years. It must be taken into consideration that the bark graft, or so-called rind graft, might during this period lay down considerable new xylem of its own kind. That the bark or rind is the portion to regenerate the cambium and new wood, has been concluded in 1808 by Knight (1841) and Sax (1956). However, Roberts says that the new xylem under the bark ring appears to vary in different combinations, being like the ring variety, the stem variety, or resembling neither.

Hafekost (1933) experimented with green shoots of apple by placing them in graded series of sugar solutions and determining their so-called wilting limit. He used a simple measure of transpiration, namely, the calculated differences in green weight before and after suspending cuttings in air for 30 minutes. He states that all vigorous types of apple rootstocks possess a higher wilting limit and a higher intensity of transpiration. He explains that scions of vigorous types on dwarf rootstocks are at a disadvantage because of a greater transpiration rate.

While on the subject of the scion's water supply, it is of interest to note that Daniel (1894) divided grafted plants into two categories: (1) those in which the water absorbed by the stock enters the scion in smaller amounts than the
scion could obtain from its own roots; (2) those in which water reaches the scion in great abundance. Of the first class he says, "Le greffon se développe moins vigoureusement, reste plus petit, fleurit et fructifie plus vite et plus abondamment." and of the second, "Les vaisseaux sont plus développés, les pousses plus vigoureuses, les fleurs plus rares comme dans tout arbre qui s'emporte sous l'influence d'un excès de nourriture."

In a series of articles, Beakbane and co-workers (1936, 1939, 1941, 1947) correlated dwarfing propensities with anatomical differences. Dwarfing stocks were discovered to have smaller cross-sectional area of vessels, smaller volume of xylem fibers, and greater volume of xylem parenchyma and medullary rays. EM IX, compared to the average of 14 vigorous trees, had almost three times the volume of living cells contrasted to lignified cells in the wood of the roots. There was a striking correlation between the bark/wood ratios of the roots and the vigor of the scions of 10 rootstocks of varying dwarfing tendencies; EM VIII was not included. Microchemical tests indicated that EM IX and EM II contained more starch, more oxidases, and more peroxidase than EM XII. It was suggested that the smaller vessel area of dwarfing stocks may reduce water uptake and that increased volume of living cells, parenchyma and ray tissue, may serve as a carbohydrate
reservoir, both tending to throw the balance to differentiation and the precocious fruiting habit.

Graft incompatibility has been studied by several investigators, but there is some disagreement as to the nature of incompatibility. The difference between the terms incompatible and compatible must be one of degree. Webster's lexicon does not solve the question with its definition, "capable of coexisting in harmony," for here there might be varying degrees of harmony or disharmony.

Amos (1936) distinguished between healthy dwarfingness, with which is combined precocity and heavy cropping, and dwarfingness which is accompanied by failure to crop and other undesirable characteristics. Thus the former is considered harmonious or compatible, the latter incompatible.

Chang (1938) points out certain characteristics of incompatible unions: (1) inward curving layers of cells or crevices in the bark or xylem, (2) limited and contorted elements of the union, which are also indicated by impeded passage of dyes or resistance to water flow, and (3) structural weakness.

Incompatibility has been explained in many ways, none of which is universally accepted. If measure of water conductivity under vacuum (Chang, 1938, Warne and Raby, 1939) be used as a criterion of compatibility, then it possibly may be said that dwarf graft unions are to a degree incompatible.
There is a need for some good histological study of graft unions of dwarf apple trees to determine their compatibility status.

According to Swarbrick, et al. (1946), the upper unions in trees double worked with EM IX are very swollen and very weak, whereas the lower unions of the same tree are not swollen and are strong. Tukey and Brase (1933, 1943) observed similarly that certain scion-intermediate-stock combinations make good trees, whereas other trees made up of the same combination but in a different order were undesirable. The idea was advanced that one scion produced a toxic substance so that when it was in top position it caused the death of the intermediate. Swarbrick, et al. (1946), mentioned that plant growth substances may provide a basis for the explanation for this and other similar observations.

As pointed out by Dana (1952), the possible influence of growth regulators in stock-scion relationships has not received adequate attention. He observed that trees with dwarf interstems made a more spreading growth, that secondary branches arose at a smaller angle but grew out at the same angle, whereas, branches on own stem trees started at a greater angle but soon bent upward forming a vase shaped tree. When dwarf interstems were bridged with vigorous wood, the crotch angles characteristic of the dwarf trees were still apparent after
two seasons. More time should have been given to observe these trees, but they were, unfortunately, destroyed.

Verner (1938) and Preston and Barlow (1951) were able to cause crotch angles to develop wider or narrower depending on whether they applied indolebutyric acid to the trees or whether they girdled just above the bud, and thus indicated that crotch angles were likely determined or controlled by auxin.

Dickson and Samuels (1956) have suggested the possibility that auxin may be used or destroyed at a rapid rate in the interstem and/or its transport retarded; the result supposedly would be overgrowth of the interstem and growth reduction of the roots with consequent dwarfing.

Dwarfing in some other plants has been shown to be directly related to auxin. A dwarf type of corn, nana, was investigated by Van Overbeek (1935, 1938). As a seedling, the coleoptile is of normal length, but the mesocotyl is shorter. In the mature plant, the topmost internode and that below the flower are of normal length, but though the number of internodes is the same as in normal corn (Abbe, 1936), they are much shorter. Van Overbeek's experiments conclude that there is sufficient auxin produced but that it is destroyed as it is translocated downward. Although this conclusion has been contested by Gustafson (1946), conclusions similar to those of Van Overbeek have been drawn by DeHaan and Gorter (1936) from experiments with dwarfed varieties of peas.
Von Abrams (1953) demonstrated a 30 percent increase in growth of dwarf pea plants by spraying them with IAA (beta-indole-3-acetic acid) at a rate which repressed the growth of normal peas. His experiments showed, however, that their auxin productivities were similar and their capacities for IAA inactivation were equal. He concluded then that a strong competitive demand for auxin in the dwarf would reduce the level of the hormone below that in the tall pea. Ether extraction and diffusion failed to show this.

Treatment of dwarf plants with auxin has failed to restore them to normal size, perhaps because the auxin inactivating mechanism is auto-catalytic. However, application of gibberellic acid to some of the dwarf mutants of corn by Phinney (1956) has induced fully normal growth. Apparently, gibberellic acid acts as an auxin but is not so subject to inactivation. Nevertheless, all of the types of dwarf corn tried did not respond to gibberellic acid, and Phinney concluded that the reasons for dwarfism can vary.

Normal elongation of non-after-ripened embryos of Malus arnoldiana was caused by gibberellic acid (Barton, 1956). No other chemical has been found to produce normal growth of these dwarfs.

Application of suitable doses of gibberellic acid to dwarf peas by Brian and Hemming (1955) was said to have virtually eliminated the differences in growth rate between
tall and dwarf varieties. IAA had a qualitatively similar but much smaller effect.

The question of auxin relations in dwarf plants is obviously complicated as there are apparently many causes of dwarfing.
Where interstem trees were used in the following experiments, they were all of the variety Golden Delicious. Golden Delicious was chosen as top variety, partly because it was used at Iowa State College in previous experiments and is abundantly available, but chiefly because it is compatible with Clark Dwarf used as an interstem, and because it produces a tree with desirable dwarf characteristics. Two types of interstems were used, Clark Dwarf, and Golden Delicious. Those with Golden Delicious interstems were used as controls. The purpose was to duplicate the number of graft unions, leaving only the differences in action of the two woods. In both cases the understock was Virginia Crab budded on Western Seedling roots. The choice of Virginia Crab may have been a poor one; Tukey and co-workers (1943, 1954) and Miller (1954) have observed trunk disorders indicative of a virus susceptibility. No such troubles were noted on the experimental material, however.

These trees were produced in the spring. A short piece of Golden Delicious was whip grafted to the interstem piece on the bench; the grafted pair was then placed under a greenhouse bench for 3 to 4 days to callus at a temperature of 80-85°F. They were then grafted to the one year whips of Virginia Crab on Western Seedling root which had been planted in the field about one month previously.
This procedure of propagation is similar to that on which Paul Stark (1950) holds a United States patent. The method was used previously by Tukey and Erase (1933), by Vyvyan (1938), and by Grubb (1939).

One hundred twenty-five true dwarf trees were also produced on the bench by whip grafting scions of EM II, EM VII, EM VIII, and EM IX, and Clark Dwarf to root pieces of Hopi Crab or Malus Colombia. These were planted in the field in the spring in a 5 x 5, linear latin square design, each experimental unit consisting of five trees. Graft unions were well covered with soil so that scion rooting could take place. These trees were sampled for an inhibitor study and an anatomical study.

Trees used in the anti-auxin spray trials were 75 two-year cutbacks of Golden Delicious, field planted in the spring in a randomized block design. In the second trial 120 one-year whips were potted into 12-inch clay pots, selected to 100 uniform trees, and placed in two 5 x 5, linear latin square designs in the greenhouse, each experimental unit consisting of two trees.
METHODS

Growth Measurements

Trunk diameter measurements were made during the 1957 growing season on eight-year old interstem dwarf trees to determine if possible at what time in the season a differential in growth of interstem occurred. The growth of five varieties of dwarfing stocks was likewise compared.

Several very complicated dendrometers, such as that of Zeiger and Childers (1954), are described in the literature, most of them designed for trees of large diameter. A simple measuring method was devised by cementing ball bearings in opposite sides of the trunk with Black Miracle adhesive to give a firm and reproducible bearing for measurement with machinists' micrometers. Although ball bearings were used successfully in the greenhouse they rusted badly in the field. Ordinary cap nuts were finally used; they are, of course, flat on one side and hemispherical over the top and are generally made of non-corroding material such as aluminum or nickel-plated brass.

Some trouble was encountered in the measurement of the diameter increase of the Clark Dwarf interstems because of the characteristic sloughing of the bark, which of course carried the cap nuts off with the pieces of bark. To obviate this, it was necessary to anticipate sloughing, to re-lay another
pair of cap nuts, and then to transfer the measurement to the new pair.

Anatomical Studies

Anatomical studies made in England have indicated characteristic differences between the roots of dwarfing and standard stock; studies of the stem included EM IX, EM II and EM XII. EM VIII, which is thought to be similar to Clark Dwarf, was either not included in their investigations or the material used was not comparable. Since stem pieces are used in inter-stem graftage, knowledge of the structure of the stems of dwarfing stocks and especially of EM VIII and Clark Dwarf should be of value.

Small pieces of wood were sawed to 0.5 to 1 cm in length and placed in a formaldehyde-acetic acid-ethanol killing solution and evacuated for rapid penetration of the fixative. They were subsequently dehydrated, embedded in pyroxylin, hardened in chloroform, stored in glycerol-alcohol, and sectioned to 20 with a sliding microtome (Wetmore, 1932). The sections were differentially stained in safranin and fast green and photomicrographs taken and enlarged. The photomicrographs were cut by hand into paper pieces representative of the different tissues; the pieces were then weighed to determine the relative volumes or cross sectional areas of the various tissues. Variation inherent between sheets of paper used was less than 1
percent. All photomicrographs were made at the same magnification and uniformly enlarged.

Sections were also produced by slicing the fresh wood while keeping it wet with water and then transferring the slices into the killing solution. After a day or two the slices were transferred into 70% ethanol and stained as above. Mounted specimens were in this way available within a short time of sampling. As compared to the method of embedding in pyroxylin, this is an amazingly fast and inexpensive way to section materials which are solid enough to be clamped tightly in the microtome; furthermore, the mounts were very satisfactory.

Experiments with Radioactive Phosphorus

One of the most obvious possible causes of dwarfing by interstems is the effect on translocation, both upward and downward. By introducing a radioactive tracer such as $^{32}$P into the tree the movement of materials in the plant and their subsequent accumulation can be estimated.

In the fall of their second growing season, Clark Dwarf and Golden Delicious interstem trees were excavated in pairs, preserving as much of the root system as possible. The trees were stored through the winter at 38° F, wrapped in damp sphagnum and polyethylene film. On 30 March they were planted
in 12-inch pots. Holes were drilled in each pot through which to pass one large root and wires to attach a side pot. Flat-bottomed vases were prepared by cutting down one side to accommodate the protruding root and drilling a drainage hole in the bottom. The protruding root was then established in sand in this side pot.

Treatments were applied to the interstem trees in pairs, one Clark Dwarf interstem tree (dwarf) and one Golden Delicious interstem tree (standard), by knocking off the side pot, washing the root free of sand, cutting the root with a diagonal slice, and quickly applying a small test tube or vial containing the radioactive isotope. Some trees were treated through leaf petioles or green twigs. All applications of radioactive phosphorus were made to greenhouse plants between 12 noon and 3 pm on sunny days; temperatures in the air conditioned greenhouse at this period of the day were generally above 90°F.

High activity $^{32}$P isotope, as $\text{PO}_4$ in dilute hydrochloric acid, was obtained from Oak Ridge, Tennessee. It has a half life of 14.3 days and decays by emission of a negative beta-particle. The dose was calculated and transferred to the vials with a micropipette and diluted with a milliliter of buffer. The buffer was made by adding concentrated acetic acid to a $10^{-2}$ M solution of sodium citrate to bring it to pH 5.0 on a direct reading Beckman pH meter. Whenever the milliliter of
solution was nearly all taken up by the tree, the vial was refilled with the buffer at least once as a rinse.

Measurements of radioactivity were made directly with an integrating Geiger-Müller survey counter, by autoradiography, and by the briquet method.

Direct external measurements with a side window counter are, at best, rather crude. However the conditions of counting were duplicated fairly well by making a lead shield with a 1/2 square centimeter hole and marking white spots on the tree at different positions so that the opening of the counter tube could be replaced on this same area. Thickness of the bark of course was a variable controlled only by choice of position.

Kodak Tri-X or Royal Pan sheet film was used in producing the autoradiographs. The activity in a piece to be autoradiographed was estimated with the survey meter to time the exposure calculated to give a maximum of $10^7$ beta particles per cm$^2$ (Yagoda, 1949).

Samples also were ground and pressed into briquets (MacKensie and Dean, 1950). However, this apple material ground to 20 mesh would not make a solid cake at 12,000 pounds per square inch, so the method was modified. After drying for 2 days at 70°C, it was ground to 40 mesh in a micro-Wiley mill and pressed at 16,000 pounds per square inch. Although these briquets tended to crumble after a few days, they held together satisfactorily during the counting procedure. Briquets were
counted by mounting them rigidly beneath an end window Geiger-Müller tube connected to a binary scaler. The briquets were 1 1/8 inches in diameter and were mounted under a heavy brass shield with a 1 inch opening in order to duplicate the geometry accurately. A series of briquets of different weights were made up of a single well-mixed sample of radioactive apple wood and an infinite thickness curve calculated (Figure 1). As can be seen, infinite thickness falls close to 2.5 g. Consequently, all briquets were made at least 3 g in weight or, in a very few instances where insufficient wood was available, corrections were made for thickness. Thus, the assay result is proportional to the radioactive phosphorus per unit dry weight.

Dead time, 105 microseconds, for the counter was measured by the paired source method and calculated according to the methods used by Bleuler and Goldsmith (1952); corrections in counts were then made using their dead-time correction graph. There was a tendency for the powdered wood and bark to separate as it was poured into the mold to be pressed. To balance this error, which was slight except where samples were appreciably over 3 g, the briquets were counted on both sides, and the two counts averaged.

In all cases the counts were made over a sufficient length of time so that the standard deviation, \( \sqrt{\frac{\text{no. of counts}}{\text{no. of minutes}}} \), was less than 2 percent. This is less than the
Figure 1. Determination of infinite thickness of briquets made by pressing ground apple wood.
variation associated with the counter tube, scaler, circuitry, etc. All counts were corrected for background.

Growth Inhibitors of Apple Bark

Dwarfing in some plants has been shown to be directly related to auxin, auxin destruction, or growth inhibitors. The growth characteristics of increased spread, wider branch angle, and precocious flowering and fruiting suggest that auxin relations in dwarf apple trees may be a cause of dwarfing. Five dwarfing stocks were tested for growth inhibition.

Bark of dwarfing stocks was assayed for growth inhibitors by a straight growth wheat coleoptile method similar to that used by Barlow, et al. (1955).

Wheat seed of the variety Pawnee, a hard red winter wheat, was carefully selected for size, color, shape, and plumpness; this seed had been grown on one 4-acre plot and was fairly uniform. The kernels were soaked in water for two hours and planted in rows on facial tissue in 8 x 12 inch plastic refrigerator crisper boxes. The embryos were all placed the same way so that when the boxes were sloped about 45° they were all directed downward (Nitsch, 1956) resulting in the longest, straightest possible coleoptiles. Where 100 seeds were planted on 9 layers of Kleenex tissues (cut down to fit the box), 90 ml of distilled water was supplied at planting time.
Planted boxes were placed in a dark, ventilated growth chamber in a 23° C temperature controlled, walk-in incubator for the seed to germinate. Subsequent manipulations were carried out at 23° C in the light of a Westinghouse green fluorescent light with a filter made of 0.0225 inch green plus 0.0225 inch amber cellulose acetate window shading (Nitsch, 1956). This gives a light having mostly the wavelength of the 546 μm mercury band. This light does not appreciably inhibit growth and was found by Johnston (1935) to be phototropically inactive. It is in the zone of maximum sensitivity of the human eye; light of a ruby red lamp bothered the author's eyes after one hour, and in one trial gave slightly more coleoptile inhibition under the growth conditions used.

After approximately a 72 hour germinating period the seedlings were selected for those with coleoptiles between 25 and 35 mm in length, discarding all others including those with deformities, doubles, or any in which the leaves were very near to or had penetrated the end of the coleoptile. About 40% of the seeds planted made useable coleoptiles. Selected coleoptiles were placed in a jig (Figure 2), made of soft balsa wood and cut with a double bladed guillotine, taking off 3 mm at the tip and a 1 cm section just below this tip. The 1 cm sections were tapped out of the jig (this jig held 20 coleoptiles) into a chromium channel. Handling the coleoptiles with tweezers was avoided whenever possible as they were easily crushed.
Figure 2. Coleoptile cutting jig. Jig has U-shaped cross section. The center section is notched to hold coleoptile section while being sliced, and the arms of the U have holes. After the coleoptiles of the seedlings are inserted into the jig, they are pushed back from the right with a straight-edge. The two-bladed cutter with razor blades in place can be seen at the right of the picture.

Figure 3. Micrometer mounted for measuring coleoptiles. A tiny shelf shorter than the shortest coleoptiles is mounted between the anvil and the screw of the micrometer. After the coleoptiles are blotted of their excess moisture, they are placed on the shelf. With proper placement of light and white background, the micrometer can be screwed down accurately to just touch the end of the coleoptile section.
Coleoptile sections were floated on a large volume of water for 3 hours to dispel any inhibitor from the cut surfaces. They were then placed into 2 ml vials, 5 per vial, containing 1 ml of growth solution. To prevent geotropic curvature and increase growth the vials were rotated at 1/3 rpm on a clinostat wheel during the growth period (Hancock and Barlow, 1953). Rotation was around the long axis of the vials which, although without caps, were prevented from spilling by the meniscus of the solution and the constriction of the neck.

At the end of the growth period the coleoptiles were measured to 0.001 inch with a machinists micrometer mounted as in Figure 3.

Except where noted the growth solution contained IAA, 2 percent sucrose, and a potassium phosphate-citric acid buffer, pH 5.0, in pyrex distilled water. Nitsch (1956) found that a buffer concentration of $10^{-3}$M gave optimum growth but used $10^{-2}$M for better buffering action. Both concentrations were tried; that of $10^{-2}$M gave slightly better growth so was used in this work.

A series of IAA concentrations was assayed with results as in Figure 4. Growth was approximately proportional to the logarithm of the concentration of IAA between 0.01 mg/l and 1.0 mg/l. Except where otherwise noted, a point midway between
Figure 4. Dilution curve of indoleacetic acid

(Points represent the mean of 25 tests)
maximum and minimum, 0.1 mg/l IAA, was used so as to give an indication of stimulation as well as of inhibition.

A growth-time curve was calculated, Figure 5, by removing samples from the growth chamber at intervals. Growth was found to be linear with time to between 13 and 16 hours. Repeated growth curves were almost invariably linear to 13 hours but sometimes varied uncertainly after this time; consequently, a 12 to 13 hour growth period was chosen.

If the bottles containing the coleoptiles were allowed to stand for a day or two after the growing period they often showed signs of fungal or bacterial growth. It was suspected that early contamination might be responsible for the uncertain variability after the 13 hour period. Potassium penicillin G was added to the cultures at a concentration of $10^{-5}$ M. It was not only ineffective in inhibiting contamination but resulted in a slight overall inhibition of coleoptile growth.

It was desirable, at times, to delay operations in the analysis even though the seed had already been planted. An attempt was made to store the floating, cut sections in darkness at 40° F. This procedure caused unsatisfactory inhibition after as much as 12 hours storage. However, when the seedlings, still in their plastic boxes, were placed at 40° F in darkness for as much as 24 hours, growth of the sections cut subsequently was equal to that of unstored seedlings.
Figure 5. Sample growth-time curves of wheat coleoptile straight growth test at two indoleacetic acid concentrations

(Points represent the mean of 25 tests)
The graph shows the growth of Coleophora in mM/L of IAA over time after pretreatment. Two concentrations are compared:

- 0.5 mg/L IAA (solid line with filled circles)
- 0.1 mg/L IAA (dotted line with open circles)

The x-axis represents time after pretreatment in hours, ranging from 0 to 20. The y-axis represents growth in mM/L, ranging from 0 to 600. The graph indicates a linear relationship between concentration and growth rate.
In addition to testing dwarfing stocks for inhibitors, chemicals known to have anti-auxin properties were applied to standard trees in an attempt to produce the dwarf characteristics in apples without the process of graftage.

Seventy-five standard Golden Delicious trees, 2 year cut-backs, were lined out in the field in the spring of 1955 to be treated with several of the so-called anti-auxins, coumarin, nicotine sulfate, TIBA (2,3,5-triiodobenzoic acid), 2,4,6-T (2,4,6-trichlorophenoxyacetic acid), and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). All lateral branches were cut back to the trunk when the trees were planted.

The nicotine sulfate used was of technical (40%) grade, 2,4,5-T and TIBA were manufactured by Eastman Kodak Co. and the 2,4,6-T was presented by the Dow Chemical Co. The 2,4,6-T contained about 3% of material which was insoluble in alcohol but soluble in water. Coumarin and nicotine sulfate were dissolved directly in water, the coumarin with much difficulty. The 2,4,5-T, TIBA and 2,4,6-T were dissolved in as small a quantity of ethanol as was possible, an equimolar quantity of triethanolamine added, and the mixture poured into a large volume of distilled water. Triton B-1956 a non-ionic wetting agent was added at the rate of one or two drops per liter as
noted. The sprays in almost every case were applied in the evening.

Instead of one or two large doses of these materials many sprays of dilute concentration were applied all through the period of rapid growth. The concentration of materials was increased throughout the season; but whenever signs of burning of leaves or shoots were observed the subsequent spray was omitted or its concentration reduced.

Although several extra trees were planted in the field experiment, there was a considerable number which did not grow. In addition to this, about a dozen trees in the design were found near the end of July to be of another variety; the variety Grimes is often confused with Golden Delicious in the nursery, though they are easily distinguished later in the season. All blocks in the statistical design were broken and it was necessary to combine blocks for the analysis.

The following season, 1956, 120 very uniform Golden Delicious one year whips were put into 12-inch pots in the greenhouse. When growth began, 100 were selected for uniformity and placed in two 5 x 5, linear latin square designs on a greenhouse ground bench, two plants representing one experimental unit. Kentucky-31 Fescue grass seed was planted on the surface of the pots as a deterrent to effects of over-watering as is so often the case where small bare root plants are placed in large pots. The greenhouse trees were sprayed
as in the above experiment using a polyethylene hood to isolate the pairs of trees during treatment. The coumarin treatment was omitted.

The data taken at the end of the seasons involved three known indications of dwarfing, namely, change in branch angles, reduced growth, and precocious flowering. Branch angles were measured with a Lufkin adjustable protractor No. 890 with extensions added; and growth increase was calculated by measuring the length (L) of the shoot times the diameter at the mid-point squared ($D_m^2$). Dana (1952) used a similar factor $L \times D^2$ in which D represented the diameter at the base of the shoot. His correlation coefficient, $r = 0.94$, was satisfactory but the flaring of the base of the shoot, especially in certain of the inhibitor treatments, tended to make the accurate measure of D difficult. Twenty shoots ranging from 40 cm to 110 cm long were sampled from the greenhouse trees and correlations between the $L \times D_m^2$ factor and both wet weight and dry weight were calculated as $r = 0.996$. 
RESULTS

Growth Measurements

In 1957, trunk diameter measurements were made at weekly intervals on two-year old dwarf stocks in the nursery, and on twelve, eight-year old interstem trees in the dwarf orchard. Data taken over a period of 16 weeks for trunk diameter increase of dwarf trees are plotted in Figure 6. Each point represents the average of 5 trees, one of each replication of the 5 x 5, linear latin square. EM IX averaged more trunk diameter growth than the other stocks, although it is considerably shorter and more spreading than EM II and EM VII. The total trunk diameter growth increment for the 15 weeks (to Sept. 8) is summarized in Tables 1a and 1b and analyses of variance and multiple range test appear in Tables 1c and 1d, respectively. EM IX was significantly different only from EM VIII and Clark Dwarf. Clark Dwarf was significantly different from all but EM VIII. It is interesting to note in the graph, Figure 6, that Clark Dwarf had grown slowly for the first six weeks measured, then its growth paralleled that of EM VIII almost exactly.

Trunk diameter measurements were made on twelve, eight-year old Clark interstem dwarfs in the dwarf orchard. Four measurements were made, one about two inches above the
Figure 6. Diameter growth of trunks of dwarfing stock trees in the nursery
Table 1a. Total diameter growth of trunks of two-year old dwarf apple trees in the nursery over 15 weeks period (Measurement in thousandths of an inch)

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Table 1b. Summary by varieties

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Table 1c. Analysis of variance

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<th>Source of variation</th>
<th>Degrees of freedom</th>
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**Significant difference at the 0.01 probability level.
Table 1d. Multiple range test for comparison of variety means

|                | $\overline{X}_{EM 
IX}$ | $\overline{X}_{EM 
VII}$ | $\overline{X}_{EM 
II}$ | $\overline{X}_{EM 
VIII}$ |
|----------------|-----------------|-----------------|-----------------|-----------------|
| $\overline{X}_{Clark 
Dwarf}$ | 138.0**          | 112.8*          | 88.0*           | 44.4            |
|                | 83.9             | 83.2            | 80.7            | 76.9            |
|                | 118.9            | 116.9           | 107.9           |                 |
| $\overline{X}_{EM 
VIII}$   | 93.6*            | 68.4            | 43.6            |                 |
|                | 83.2             | 80.7            | 76.9            |                 |
|                | 116.9            |                 |                 |                 |
| $\overline{X}_{EM 
II}$    | 50.0             | 25.0            |                 |                 |
|                | 80.7             |                 |                 |                 |
| $\overline{X}_{EM 
VII}$   | 25.2             |                 |                 |                 |

**Significant difference at the 0.01 probability level.

*Significant difference at the 0.05 probability level.

interstem, one at the top of the interstem at the enlargement, one at the bottom of the interstem, and one below the interstem. Four of the trees had interstem pieces which were three inches in length, while eight had interstems which were eight inches long. Six of the dwarfs with long interstems bore little or no fruit during the season of measurement. All of the four trees with short interstems, and two with long interstems were heavily loaded. There seemed to be no consistent difference in trunk diameter growth when grouped according to
interstem length; however, when grouped according to crop size, trunk diameter increase differences were apparent.

No statistical analysis is shown for these data. However, 20 weeks growth measurements are plotted in the graphs, Figure 7 and Figure 8, for trees with little or no fruit and those with a heavy crop, respectively. Besides less total trunk increase in the trees with the heavy crop, it can be seen that growth had stopped by the first of September. Trunk diameter was still increasing as of 15 September in trees which bore little or no fruit.

There is evidence that the growth of the interstem varied throughout the season though it finally surpassed that of the standard woods both above and below the interstem. A distinct reduction in trunk diameter growth rate of the top of the interstem of trees heavily laden with fruit, Figure 7, was apparent in June; even some shrinkage was measured the week ending 23 June. This is about the time when apples not well set are dropped and the remaining fruits grow rapidly. In Figure 8 a reduction occurred at the same time, but it was much less distinct and no shrinkage occurred.

It was shown in this work that EM IX had the greatest trunk growth of the five stocks. EM VII and EM II were intermediate, while EM VIII and Clark Dwarf had least trunk growth. This was not correlated with tree size. EM VII and EM II were
Figure 7. Diameter growth of interstem dwarfed apple trunks of trees with heavy crop of fruit.
Figure 8. Diameter growth of interstem dwarfed apple trunks of trees with little or no fruit
obviously the larger trees, EM IX was intermediate and EM VIII and Clark Dwarf were smallest.

Clark Dwarf and EM VIII trunks grew at the same rate after July 7; Clark Dwarf trunk grew less before that time.

Comparison of trunks of Clark Dwarf interstem trees with and without fruit confirms the statement that fruiting is a dwarfing process, at least in regards to trunk diameter.

In those Clark Dwarf interstem trees bearing fruit, the rate of growth at the top of the interstem dropped off sharply during the three weeks ending 23 June. This was the period in which the fruit was attaining size. Neither the trunk above the interstem, the trunk below the interstem, nor the bottom of the interstem showed this change in growth rate. The tops of interstems of the trees which bore little or no fruit showed only a comparatively slight decrease in growth rate.

If this enlargement at the top of the interstem is an effect of girdling and contains an accumulation of food substances then the decrease in growth rate may have been caused by the competitive withdrawal of these materials by the rapidly growing fruits.

Anatomical Studies

Transverse sections of the xylem of the five dwarfing stocks EM II, EM VII, EM IX, Clark Dwarf, and EM VIII were
made with a sliding microtome in August, 1957. The fresh
sections were placed in a killing fluid for 24 hours, stained
and mounted in Canada balsam. Three 350μ diameter fields
were photographed from each plant; the fields were located
350-500μ from the cambium and were spaced at 120 degrees, or
about equally around the stem. The photomicrographs were en­
larged onto single weight papers and were cut into pieces
representing separately the vessels and rays of each group of
each field. Differences in the amount of xylem parenchyma and
tracheids were so obvious that they were not separated.

The combined cutouts of the vessels and of the rays were
weighed and calculated as percentages of the field from which
they were taken, and the three fields from each section were
averaged. The percentages are shown in Tables 2a and 3a for
vessel cross-sectional area and ray tissue, respectively.
Likewise Tables 2b and 3b present the summaries by varieties,
Tables 2c and 3c the analyses of variance, and Tables 2d and
3d the multiple range tests for comparison of variety means.

Clark Dwarf and EM VII had almost the same amount of
vessel area; EM IX somewhat more while EM II and EM VII were
considerably higher. EM IX, however, was not significantly
different at the 0.05 probability level from EM VIII, Clark
Dwarf or EM II, although the difference from EM II was very
close to 5 percent significance.
Table 2a. Percentage of vessel cross-sectional area in stems of dwarf apple stocks

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Table 2b. Summary by varieties

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Table 2c. Analysis of variance

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**Significant difference at the 0.01 probability level.
Table 2d. Multiple range test for comparison of variety means

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<td>6.65*</td>
<td>4.74</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>5.29</td>
<td>5.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.44</td>
<td>7.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$ EM IX</td>
<td>2.17</td>
<td>1.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$ EM VIII</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.

*Significant difference at the 0.05 probability level.

Table 3a. Percentage of ray tissue in stems of dwarf apple stocks

<table>
<thead>
<tr>
<th>Order</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>E: 28.62</td>
</tr>
<tr>
<td>2</td>
<td>D: 21.66</td>
</tr>
<tr>
<td>5</td>
<td>A: 20.29</td>
</tr>
<tr>
<td>Sum</td>
<td>116.59</td>
</tr>
</tbody>
</table>

Table 3b. Summary by varieties

<table>
<thead>
<tr>
<th>A: EM II</th>
<th>B: EM VII</th>
<th>C: Clark</th>
<th>D: EM IX</th>
<th>E: EM VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum</td>
<td>85.75</td>
<td>80.51</td>
<td>131.84</td>
<td>112.58</td>
</tr>
<tr>
<td>Mean</td>
<td>17.15</td>
<td>16.10</td>
<td>26.37</td>
<td>22.52</td>
</tr>
</tbody>
</table>
Table 3c. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24</td>
<td>680.5969</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>4</td>
<td>448.2717</td>
<td>112.0679**</td>
</tr>
<tr>
<td>Replication</td>
<td>4</td>
<td>102.8880</td>
<td>25.7220*</td>
</tr>
<tr>
<td>Orders</td>
<td>4</td>
<td>38.7969</td>
<td>9.6992</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>90.6403</td>
<td>7.5534</td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.
*Significant difference at the 0.05 probability level.

Table 3d. Multiple range test for comparison of variety means

<table>
<thead>
<tr>
<th></th>
<th>$\bar{x}_{\text{Clark Dwarf}}$</th>
<th>$\bar{x}_{\text{EM VIII}}$</th>
<th>$\bar{x}_{\text{EM IX}}$</th>
<th>$\bar{x}_{\text{EM II}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM VII</td>
<td>10.27**</td>
<td>9.51**</td>
<td>6.42**</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>4.60</td>
<td>4.56</td>
<td>4.42</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>6.52</td>
<td>6.41</td>
<td>6.23</td>
<td></td>
</tr>
<tr>
<td>EM II</td>
<td>9.22**</td>
<td>8.46**</td>
<td>5.37*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.56</td>
<td>4.42</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.41</td>
<td>6.23</td>
<td>5.91</td>
<td></td>
</tr>
<tr>
<td>EM IX</td>
<td>3.85</td>
<td>3.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM VIII</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.
*Significant difference at the 0.05 probability level.
Variation within fields was not large but that between sections was extremely great. Samples from more trees per replication would likely have confirmed the differences in the means.

In percentage of xylem ray tissue the results were in reverse order. EM VIII and Clark Dwarf were strikingly similar, EM IX slightly less, and EM II and VII had comparatively less ray tissue. The range test placed them into two groups, one containing EM VII and II and the other EM IX, VIII, and Clark Dwarf.

The large amount of xylem parenchyma characteristic of Clark Dwarf and EM VIII stems can be seen in the photomicrographs of Figures 9 and 10, respectively, and compared with that of EM IX, Figure 11. Photomicrographs of the other two East Malling stocks, EM II and VII are shown in Figures 12 and 13, respectively; they are not dissimilar to those of two-year old stems of Golden Delicious and Virginia Crab, Figures 14 and 15, respectively.

In addition, the number of vessels were counted, Table 4a, and summarized in Table 4b. EM IX had the least number per unit area, Clark Dwarf and EM VIII were about equal, and EM VII and II had a somewhat greater number. The analysis of variance, Table 4c, shows there were no significant differences at the 0.05 probability level.
Figure 9. Transverse section of Clark Dwarf stem. Note the small elliptical vessels, abundance of ray and other xylem parenchyma, and the scattered groups of tracheids.

Figure 10. Transverse section of EM VIII stem. Note the similarity to Figure 9. Some investigators believe that Clark Dwarf and EM VIII are identical.

Figure 11. Transverse section of EM IX stem. Note the slightly larger vessels as compared with EM VIII and Clark Dwarf above. There is less xylem parenchyma and more area of tracheids.
Figure 12. Transverse section of EM VII stem. Note the abundant vessel area as compared with EM VIII, Clark Dwarf, and EM IX in Figures 9, 10, and 11, respectively. There is less area of medullary rays, almost a complete absence of other parenchyma cells, and an abundance of tracheids.

Figure 13. Transverse section of EM II stem. Note the general similarity to EM VII above.

Figure 14. Transverse section of Golden Delicious stem. Note the many large round vessels.

Figure 15. Transverse section of Virginia Crab stem. This is a vigorous stock. Note the many large round vessels.
### Table 4a. Relative number of vessels in cross-section of stems of dwarf apple trees

(Each number represents the average of three 350μm diameter microscope fields)

<table>
<thead>
<tr>
<th>Order</th>
<th>Replications</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E: 19.2</td>
<td>B: 24.2</td>
<td>D: 15.3</td>
<td>A: 17.5</td>
<td>C: 16.2</td>
<td>92.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>D: 11.0</td>
<td>C: 21.0</td>
<td>B: 15.2</td>
<td>E: 16.5</td>
<td>A: 16.7</td>
<td>80.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C: 14.0</td>
<td>E: 14.3</td>
<td>A: 27.7</td>
<td>D: 15.2</td>
<td>B: 15.8</td>
<td>88.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B: 16.0</td>
<td>A: 19.7</td>
<td>E: 13.0</td>
<td>C: 18.5</td>
<td>D: 15.0</td>
<td>82.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A: 27.0</td>
<td>D: 17.0</td>
<td>C: 14.3</td>
<td>B: 24.3</td>
<td>E: 17.7</td>
<td>100.8</td>
<td></td>
</tr>
</tbody>
</table>

Sums 87.2 96.2 86.0 92.0 82.4 443.8

### Table 4b. Summary by varieties

A: EM II  B: EM VII  C: Clark  D: EM IX  E: EM VIII

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum</td>
<td>108.6</td>
<td>96.5</td>
<td>84.5</td>
<td>73.5</td>
<td>80.7</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>21.7</td>
<td>19.3</td>
<td>16.9</td>
<td>14.7</td>
<td>16.1</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4c. Analysis of variance

<table>
<thead>
<tr>
<th>Source of varieties</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24</td>
<td>440.08</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>4</td>
<td>168.86</td>
<td>42.22</td>
</tr>
<tr>
<td>Replication</td>
<td>4</td>
<td>69.30</td>
<td>17.32</td>
</tr>
<tr>
<td>Order</td>
<td>4</td>
<td>38.23</td>
<td>9.56</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>163.69</td>
<td>13.64</td>
</tr>
</tbody>
</table>
The results of this study show that the number of vessels, percentage of vessel cross-sectional area, and percentage of ray tissue in stems of five dwarf apple stocks are correlated with the size of the trees and, likewise, with their dwarfing propensities when used as interstems. EM VIII and Clark Dwarf are most dwarfing, have the fewest vessels, lowest percentage of vessel cross-sectional area, greatest amount of ray tissue and xylem parenchyma, and fewest tracheids. EM IX was intermediate in these quantities while EM VII and EM II were opposite.

It has been mentioned earlier that EM IX rootstock is considered to be the criterion of dwarfing in England. This has been true in America as well, but it was reported by Tukey and Brase (1943) that the effect of a stem piece of EM IX is much less, than when used as a rootstock.

The anatomical differences of vessel area point to possible restriction of the movement of water and minerals through the interstem piece of dwarfs. Restricted water supply to the top would limit terminal growth resulting in the accumulation of carbohydrates which would otherwise be utilized in growth; this would swing the balance to differentiation and early flowering.

Trunks of several Clark interstem dwarfs in the dwarf orchard were sectioned longitudinally to observe the morphology of the interstem. Both an eight inch long interstem and a
three inch one are pictured in Figures 16 and 17, respectively.

Enlargement of the Golden Delicious variety immediately above the top graft union is very apparent, and has been the factor suggesting to early investigators some impediment to downward translocation at the union. It has been compared to a girdle such as is pictured in Figure 18.

Another characteristic of the Clark Dwarf interstem is the heavy bark development. It is shown in Figures 16 and 17 to be three to five times as thick as that of the standard variety; it would be much thicker were it not for its constantly sloughing off. This separation of the bark was found to be greatest during the early part of July, but the separation of the large scales continues throughout the year.

The inward curving of the xylem at the unions in Figures 16 and 17 might be noted also. Although this is considered to be evidence of incompatibility by Chang (1938) there has been no evidence of weakness at the graft unions in trees in the orchard up to 19 years old.

Two-year old unions were sectioned longitudinally and sliced for microscopic examination. There was considerable vascular contortion and whorls of xylem in the Golden Delicious enlargement above the dwarf interstem union. Golden Delicious on Golden Delicious unions were smooth, as were Golden Delicious on Virginia Crab unions. The dye, Light
Figure 16. Longitudinal section through the trunk of a Clark Dwarf (eight inch) interstem tree. Especially noticeable is the characteristic enlargement of the Golden Delicious wood above the top union and the development of thick bark on the Clark Dwarf. The Xylem of the interstem tapers downward. Note also the slight incurving xylem at the lower union with Virginia Crab.
Figure 17. Longitudinal section through the trunk of a Clark Dwarf (three inch) interstem tree. Note incurving xylem at both unions. The outer layers of the heavy development of bark on the interstem was beginning to slough off; a few of the dry scales are visible on the outside of the upper union.
Figure 18. Longitudinal section through a one year old girdle of apple. Note that limb is larger above the girdle and that there is an enlargement immediately above the girdled area. Dwarfing interstems have been compared to such a girdle because of their resemblance and their similar effects of reduced growth and precocious flowering and fruiting.
Green S F, was injected into one plant each of Golden Delicious and Clark Dwarf interstem trees. The dye path was straight and clearly seen passing through the Golden Delicious interstem, but was much contorted and intermittently visible through the Clark Dwarf interstems. Such contortions suggest interference in upward translocation through the xylem and, according to Chang (1938), are symptoms of incompatibility.

In addition to a study of the morphology and anatomy of the union, the two year old dwarfing stocks were regularly observed in regards to the identity of the Clark Dwarf. Erase (1953) has pointed out its similarity to EM VIII. Many observers at this station have seen differences in the two stocks when grown in different locations or on different roots. The author has found no morphological differences between EM VIII and Clark Dwarf in two years of growth side by side on the same rootstock or own root. They grew equally poorly and both suffered losses during the first dry, open winter to which they were exposed; both survived -30°F during the second winter. Both EM VIII and Clark Dwarf have shown only slight susceptibility to fire blight. EM IX is especially susceptible to fire blight; EM VII and EM II are somewhat more resistant.
Experiments with Radioactive Phosphorus

Accumulation of $^{32}$P

Six grafted interstem trees were treated on 1 May, 12 noon with 0.17 mc - 10% $^{32}$P through cut roots according to the schedule following (Table 5); these six trees were in the early stages of leafing, only the first emerged leaves being fully expanded.

Table 5. Schedule of $^{32}$P treatment and harvest of the first six trees

<table>
<thead>
<tr>
<th>Type of interstem</th>
<th>Pair 1</th>
<th>Pair 2</th>
<th>Pair 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard, Dwarf</td>
<td>Standard, Dwarf</td>
<td>Dwarf, Dwarf</td>
</tr>
<tr>
<td>Time of treatment</td>
<td>1 May, 12 n</td>
<td>1 May, 12 n</td>
<td>1 May, 12 n</td>
</tr>
<tr>
<td>Time of harvest</td>
<td>2 May, 6 pm</td>
<td>7 May, 12 m</td>
<td>11 May, 12 n</td>
</tr>
<tr>
<td>Time lapsed</td>
<td>1 1/4 days</td>
<td>5 1/2 days</td>
<td>9 1/2 days</td>
</tr>
</tbody>
</table>

Immediately after treatment the radioactivity was followed by placing the probe of a side window survey counter on the trunk of the tree. The beta-radiation from $^{32}$P will penetrate only approximately 7 mm of tissue; accordingly, it was possible to differentiate movement straight up on one side of
the tree. In fact, as will be shown later, the radioactivity remains reasonably confined to this same side even after several days. The apparent affect of any of the graft unions was to spread this area slightly. Also in some cases, the position around the tree might be offset after crossing a union. It was also observed that the radioactivity traveled up the trunk considerably faster through the standard interstem tree than through the dwarf; the full milliliter of solution was taken up in both cases, but at a somewhat slower rate in the dwarf.

Harvest consisted of cutting the trunk into sections each of 5 cm length. Table 6 lists the data obtained from the first two trees, pair 1, harvested after 30 hours. It is

Table 6. Radioactivity assay of briquets from pair-1 trees harvested 30 hours after treatment of root

(5 cm sections in order down the trunk)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th>Standard interstem</th>
<th>Dwarf interstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Above interstem</td>
<td>22,800</td>
<td>5,100</td>
<td></td>
</tr>
<tr>
<td>2-Top of interstem</td>
<td>24,000</td>
<td>10,900</td>
<td></td>
</tr>
<tr>
<td>3-Bottom of interstem</td>
<td>21,900</td>
<td>7,000</td>
<td></td>
</tr>
<tr>
<td>4-Below interstem</td>
<td>21,300</td>
<td>6,400</td>
<td></td>
</tr>
</tbody>
</table>
of course not possible to distinguish between the radioactivity that was deposited on the way up the trunk and that which had been utilized by the leaves and had moved downward.

A tendency to accumulate radioactive phosphorus in the interstems of both the dwarf and the standard trees of pair 1 was apparent after 30 hours. The high activity at the top of the interstem might indicate impedance at the top union. The higher counts in the tree with a standard interstem may have been related to the more rapid absorption by this plant, although so much difference would not have been expected after 30 hours.

Radioactivity of sections of the trunk below and above the union of the seedling roots and Virginia Crab are included in Table 7, the assay of the pair-2 trees harvested 5 1/2 days after treatment. The sections were taken serially from top to bottom and are so listed in the table. Again there was a tendency for activity to be higher near the top of the dwarf interstems. Section 2 of the dwarf tree was high in radioactivity, also; this was wood opposite lateral branches, and it was found generally that the leaves and adjacent wood accumulated the greatest activity. As before it was found that the solution was taken up faster by the standard tree than by the dwarf. Differences between the data of Tables 2 and 3 are associated with the longer period between treatment and sampling—5 1/2 days and 30 hours.
Table 7. Radioactivity assay of briquets from pair-2 trees harvested 5 1/2 days after treatment of root (5 cm sections of trunk in order from top to bottom)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard interstem</td>
<td>Dwarf interstem</td>
<td></td>
</tr>
<tr>
<td>1-</td>
<td>14,800</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>15,800</td>
<td>18,700</td>
<td></td>
</tr>
<tr>
<td>3-Above interstem</td>
<td>11,300</td>
<td>20,000</td>
<td></td>
</tr>
<tr>
<td>4-Top of interstem</td>
<td>9,500</td>
<td>18,100</td>
<td></td>
</tr>
<tr>
<td>5-Bottom of interstem</td>
<td>10,800</td>
<td>13,500</td>
<td></td>
</tr>
<tr>
<td>6-Below interstem</td>
<td>11,800</td>
<td>14,000</td>
<td></td>
</tr>
<tr>
<td>7-Above bud (Va. Crab)</td>
<td>11,700</td>
<td>13,300</td>
<td></td>
</tr>
<tr>
<td>8-Below bud (West. Seed.)</td>
<td>12,000</td>
<td>14,600</td>
<td></td>
</tr>
</tbody>
</table>

Two dwarf interstem trees, pair 3, were treated and harvested after 9 1/2 days. The data, given in Table 8, show a much greater drop in activity than would be expected from radio decay alone. Probably the P³² was being transferred into the rapidly growing leaves. It was observed that the solution was taken up rapidly by the first plant, while the milliliter of solution was taken up by the second plant only after about an hour. The dwarf of the right hand column shows, as before,
Table 8. Radioactivity assay of briquets from pair-3 trees harvested 9 1/2 days after treatment of root
(Numbered parts are 5 cm trunk sections in order from top to bottom)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th>Dwarf interstem (A)</th>
<th>Dwarf interstem (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>4,900</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>2-Above interstem</td>
<td>4,500</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>3-Top of interstem</td>
<td>4,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Bottom of interstem</td>
<td>3,400</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>5-Below interstem</td>
<td>3,000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>6-</td>
<td>--</td>
<td>1,100</td>
<td></td>
</tr>
<tr>
<td>7-</td>
<td>1,800</td>
<td>1,100</td>
<td></td>
</tr>
<tr>
<td>8-Above bud union</td>
<td>1,400</td>
<td>4,800</td>
<td></td>
</tr>
<tr>
<td>9-Below bud union</td>
<td>1,100</td>
<td>6,300</td>
<td></td>
</tr>
</tbody>
</table>

an accumulation in the interstem, both top and bottom sections of which were lumped into one briquet.

The results from these three pairs of trees point to the probability of \( P^{32} \) accumulation in or above the top of the dwarf interstem; however, the differences were not great. The bark of the dwarf is much thicker than that of the variety, and this simple volume difference may explain this small
accumulation. There is also more living tissue in the xylem of the dwarf interstem, which would likewise tend to favor accumulation.

A second set of six trees was treated with 0.2 mc \( \text{P}^{32} \) each, through the root as before, and harvested according to the following schedule (Table 9).

Table 9. Schedule of \( \text{P}^{32} \) treatment and harvest of second six trees

<table>
<thead>
<tr>
<th>Type of interstem</th>
<th>Pair 4</th>
<th>Pair 5</th>
<th>Pair 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard, Dwarf</td>
<td>Standard, Dwarf</td>
<td>Standard, Dwarf</td>
</tr>
<tr>
<td>Time treated</td>
<td>23 May, 2 pm</td>
<td>22 May, 2 pm</td>
<td>21 May, 2 pm</td>
</tr>
<tr>
<td>Time harvested</td>
<td>26 May, 2 pm</td>
<td>29 May, 2 pm</td>
<td>2 June, 2 pm</td>
</tr>
<tr>
<td>Time lapsed</td>
<td>3 days</td>
<td>7 days</td>
<td>11 days</td>
</tr>
</tbody>
</table>

The second set of six trees was harvested as the first except that a 0.5 cm slice was taken from the top of each 5 cm (approximate) section of trunk, sanded smooth, and placed on Kodak Tri-X film for autoradiographs. A piece of black, 0.001 inch thick polyethylene was interspersed between the cross-sectional slices and the film, since it has been shown that phenolic compounds from some woods placed directly on film may result in false exposure (Yagoda, 1949). Exposure
was calculated from the piece with maximum radioactivity, and all of the films used for each pair of trees were exposed, developed, and printed uniformly.

Tree trunks are pictured in Figures 19, 20, and 21; the autoradiographs of cross sections are shown opposite the position from which the section was taken, except where otherwise indicated by arrows. Autoradiographs of the sections are reduced in size, the amount of the reduction being constant throughout. Kodak Velox printing papers were used because of their slow speed so as to obtain proportionately less error in timing the exposure. Exposure was 100 seconds; development in Kodak Dektol (D-72 type) was timed carefully at 90 seconds at a controlled temperature of 20° C. The radioactivity counts of the briquets from these trees are shown in Tables 10, 11, and 12.

The autoradiographs appear to indicate an accumulation of phosphorus in the dwarf interstem; this is likewise borne out by the briquet analyses. It can be plainly seen that even after 3 days the activity was localized on one side. The localization on one side tended to be less after 7 or 11 days (see Figures 20 and 21) but was still distinct.

Accumulation of activity in the xylem as well as the bark after 11 days is apparent in Figure 17. That the bark of dwarfs is considerably thicker is well known; furthermore, Beakbane et al. (1936, 1939, 1941) found more living tissue
Figure 19. Autoradiographs of pair-4 apple trunk cross-sections 3 days after treatment of root

Left: Standard interstem tree.
Right: Dwarf interstem tree. Note that cross-section at top of dwarf was taken from the main continuation of the trunk and shows very little activity; the activity was localized on one side, the side which fed the small limb arising on the left and not autoradiographed. The briquet samples assayed in Table 6 include a portion of this limb as well as the main trunk.
Table 10. Radioactivity assay of briquets from pair-4 trees harvested 3 days after treatment of root

(Numbered parts are 5 cm sections in order down the trunk)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard interstem</td>
</tr>
<tr>
<td>Leaves from branch on side opposite that treated</td>
<td>46,400</td>
</tr>
<tr>
<td>Green twigs (current) from branch on side treated</td>
<td>53,100</td>
</tr>
<tr>
<td>Leaves from branch on side treated</td>
<td>80,900</td>
</tr>
<tr>
<td>1-</td>
<td>19,300</td>
</tr>
<tr>
<td>2-Above interstem</td>
<td>16,100</td>
</tr>
<tr>
<td>3-Top of interstem</td>
<td>13,600</td>
</tr>
<tr>
<td>4-Bottom of interstem</td>
<td>8,000</td>
</tr>
<tr>
<td>5-Above bud union</td>
<td>9,300</td>
</tr>
<tr>
<td>6-Below bud union</td>
<td>12,100</td>
</tr>
</tbody>
</table>

in the dwarfs that she examined than in standard trees.

Since it has been shown that radioactive phosphorus tends to accumulate more in parenchymatous, meristematic, and actively growing tissues (Wiebe and Kramer, 1954, Lundegarth, 1950, Biddulph, 1951, Moore, 1949, Sisakyan and Voronkora, 1950,
Figure 20. Autoradiograph of pair-5 apple trunk cross-sections 7 days after treatment of root

Left: Standard interstem tree
Right: Dwarf interstem tree
Table 11. Radioactivity assay of briquets from pair-5 trees harvested 7 days after treatment of root
(Numbered parts are 5 cm sections down the trunk)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th>Standard interstem</th>
<th>Dwarf interstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green twigs and leaves</td>
<td>56,200</td>
<td>25,800</td>
<td></td>
</tr>
<tr>
<td>1-</td>
<td>27,200</td>
<td>12,800</td>
<td></td>
</tr>
<tr>
<td>2-Above interstem</td>
<td>23,400</td>
<td>12,400</td>
<td></td>
</tr>
<tr>
<td>3-Top of interstem</td>
<td>23,700</td>
<td>13,300</td>
<td></td>
</tr>
<tr>
<td>4-Bottom of interstem</td>
<td>29,200</td>
<td>11,400</td>
<td></td>
</tr>
<tr>
<td>5-Below interstem</td>
<td>31,400</td>
<td>9,600</td>
<td></td>
</tr>
<tr>
<td>6-</td>
<td>--</td>
<td>12,100</td>
<td></td>
</tr>
<tr>
<td>7-Above bud union</td>
<td>44,600</td>
<td>8,500</td>
<td></td>
</tr>
<tr>
<td>8-Below bud union</td>
<td>29,100</td>
<td>11,000</td>
<td></td>
</tr>
<tr>
<td>9-Below original ground line</td>
<td>26,900</td>
<td>10,500</td>
<td></td>
</tr>
</tbody>
</table>

Klechkovskiy, et al., 1951) it is not surprising that such accumulation was observed.

Differences in accumulation of radioactive phosphorus in the dwarf interstem as compared to the adjacent standard woods are small when measured by the briquet method. It is apparent from the autoradiographs that most of the activity is in the
Figure 21. Autoradiographs of pair-6 apple trunk cross-sections 11 days after treatment of root

Left: Standard interstem tree
Right: Dwarf interstem tree
Table 12. Radioactivity assay of briquets from pair-6 trees harvested 11 days after treatment of root
(5 cm sections in order down the trunk)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Dwarf</td>
<td></td>
</tr>
<tr>
<td></td>
<td>interstem</td>
<td>interstem</td>
<td></td>
</tr>
<tr>
<td>1-</td>
<td>--</td>
<td>19,800</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>3-Above interstem</td>
<td>10,500</td>
<td>31,700</td>
<td></td>
</tr>
<tr>
<td>4-Top of interstem</td>
<td>8,400</td>
<td>34,300</td>
<td></td>
</tr>
<tr>
<td>5-Bottom of interstem</td>
<td>9,400</td>
<td>29,500</td>
<td></td>
</tr>
<tr>
<td>6-Below interstem</td>
<td>12,300</td>
<td>22,600</td>
<td></td>
</tr>
<tr>
<td>7-</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>8-</td>
<td>20,400</td>
<td>24,200</td>
<td></td>
</tr>
<tr>
<td>9-</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>10-</td>
<td>--</td>
<td>20,500</td>
<td></td>
</tr>
<tr>
<td>11-</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>12-Above bud union</td>
<td>25,700</td>
<td>9,800</td>
<td></td>
</tr>
<tr>
<td>13-Below bud union</td>
<td>20,900</td>
<td>11,100</td>
<td></td>
</tr>
<tr>
<td>14-Below original ground line</td>
<td>22,500</td>
<td>20,000</td>
<td></td>
</tr>
</tbody>
</table>
bark; consequently, the wood in the briquet is chiefly effective as a diluent. The larger volume of wood in the dwarf interstem tends to level out differences in measuring of the briquets.

Measurement of radioactive phosphorus translocated downward from the leaves is confounded with that which was deposited in its upward course through the xylem. A seventh pair of trees was handled as before with the exception that they were treated through leaf petioles. Autoradiographs of trunk cross section are shown in Figure 22 and assay data in Table 13. Accumulation of phosphorus in the dwarf interstem bark was again observed but accumulation in the interstem xylem was not shown clearly.

In order to confirm the accumulation of phosphorus in the interstem xylem, a dwarf interstem tree was treated with p$^{32}$ through a cut root and harvested after a 21 day equilibration period. The interstem and portions of adjacent standard woods were removed in one piece and plunged into dry ice to prevent any further translocation or diffusion. They were then split longitudinally and planed smooth while still frozen to prepare them for autoradiography. The samples were kept frozen during the exposure period. The autoradiographs are presented in Figures 23 and 24. The upper parts of the dwarf interstem and the Golden Delicious scion were placed on the film as quarters of trunk section, but the lower sections are shown partly as
Figure 22. Autoradiographs of trunk cross-sections from pair-7 trees harvested 7 days after P\textsuperscript{32} treatment of leaf petiole

Left: Standard tree
Right: Dwarf tree
Table 13. Radioactivity assay of briquets from pair-7 trees harvested 7 days after P\textsuperscript{32} treatment of leaf petiole

(Numbered parts are 5 cm sections from top to bottom of trunk)

<table>
<thead>
<tr>
<th>Section</th>
<th>Cts/min</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard interstem</td>
<td>Dwarf interstem</td>
<td></td>
</tr>
<tr>
<td>Green parts of fork bearing treated lateral\textsuperscript{a}</td>
<td>82,500</td>
<td>56,100</td>
<td></td>
</tr>
<tr>
<td>Green parts of other fork</td>
<td>5,900</td>
<td>54,400</td>
<td></td>
</tr>
<tr>
<td>1-Section of treated fork opposite treated lateral</td>
<td>61,000</td>
<td>High\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>2-Section of treated fork</td>
<td>--</td>
<td>66,900</td>
<td></td>
</tr>
<tr>
<td>3-Section of treated fork</td>
<td>12,000</td>
<td>38,600</td>
<td></td>
</tr>
<tr>
<td>4-Section of treated fork</td>
<td>--</td>
<td>17,700</td>
<td></td>
</tr>
<tr>
<td>5-Above crotch</td>
<td>9,400</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>6-Above interstem</td>
<td>4,800</td>
<td>18,900</td>
<td></td>
</tr>
<tr>
<td>7-Top of interstem</td>
<td>4,100</td>
<td>32,000</td>
<td></td>
</tr>
<tr>
<td>8-Center of interstem</td>
<td>2,900</td>
<td>19,700</td>
<td></td>
</tr>
<tr>
<td>9-Bottom of interstem</td>
<td>4,600</td>
<td>17,300</td>
<td></td>
</tr>
<tr>
<td>10-Below interstem</td>
<td>4,900</td>
<td>10,800</td>
<td></td>
</tr>
<tr>
<td>11-</td>
<td>--</td>
<td>11,900</td>
<td></td>
</tr>
<tr>
<td>12-Above bud union</td>
<td>3,500</td>
<td>17,500</td>
<td></td>
</tr>
<tr>
<td>13-Below bud union</td>
<td>3,500</td>
<td>16,700</td>
<td></td>
</tr>
<tr>
<td>14-Below original ground line</td>
<td>5,400</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Does not include parts of treated lateral itself.

\textsuperscript{b}Activity too great to measure without dilution.
Figure 23. Autoradiograph of two longitudinal half sections of lower graft union of dwarf tree. Arrows point to union. The portion above the arrows is the bottom of the Clark Dwarf interstem, the portion below is Virginia Crab. Note the P$_{32}$ activity in the xylem of the dwarf wood, as contrasted to the crab.

Figure 24. Autoradiograph of two longitudinal quarter sections of upper graft union of dwarf tree. Arrows point to the union. The portion above the arrows is Golden Delicious variety, the portion below is the Clark Dwarf interstem. Again, note very little activity of P$_{32}$ in the standard xylem.
halves. Clark Dwarf interstems are comparatively brittle and were difficult to split accurately.

The work just presented shows that there is more accumulation of phosphorus in the Clark Dwarf interstem than in the standard scion and rootstock of the same tree. This is to be expected in view of the greater development of bark and greater volume of living cells in the xylem. $P^{32}$ has been shown by several investigators to accumulate most in meristematic areas and regions of high metabolic activity, therefore such accumulation points to the interstem as being of relatively high metabolic activity.

Dana (1952) has shown that growth habits of dwarfed plants indicate a difference in auxin relations. It is possible that the higher metabolic activity of the interstem results in more rapid destruction of auxin, or the differentiation of anti-auxins which may be related to dwarfing.

It is also of interest to note that treatment of a root or petiole with a $P^{32}$ solution resulted in uptake of the solution on one side of the plant. Even after 11 days the one sided pattern could be distinguished in autoradiographs of stem cross section.

**Downward translocation of $P^{32}$**

Early in the nineteenth century Thomas Andrew Knight (1822) compared the union between dwarfing stocks and standard
variety to a girdle, postulating that the union impeded phloem transport downward. Several investigators have tried in vain to prove such an impediment, assuming that the impediment would cause an accumulation of carbohydrates immediately above it. Biddulph and Marple (1944) stated that phosphorus moving by way of the phloem may diffuse into the xylem to be carried upward again by the transpiration stream. Thus, it is possible that accumulation of materials does not occur even above a girdle.

A one year old potted Golden Delicious tree was girdled two weeks prior to leaf petiole treatment with radioactive phosphorus. Eighteen hours after treatment the trunk was cut above and below the girdle, the bark separated from the xylem of the isolated section, and the whole plunged into dry ice and kept frozen during the subsequent operations. Autoradiographs made of the flattened bark and smoothed longitudinal half sections of the xylem are reproduced in Figure 25. A strong accumulation of radioactive phosphorus above the girdle is shown, both in the bark and in the wood. A considerable amount of callus had formed above the girdle in the two weeks prior to treatment and the accumulation may possibly be related to this increase in actively metabolizing tissue.

Seven dwarf and two standard trees were treated with $^{32}\text{P}$ through leaf petioles or green stems to determine if an
Figure 25. Autoradiographs of girdled tree 2 days after $\text{P}^{32}$ treatment

Left: Flattened bark from above and below the girdled area.  
Right: Longitudinal half sections of wood. 
Pieces were cut transversely at the girdle.  
Arrows on top piece show the position of the lower edges of the bark.
impediment to phloem transport could be detected. The radioactivity was followed after treatment and was found to descend the main stem below the place of intake for 5 to 7 cm in a period of one to five minutes. It then ascended to the topmost leaves. The amount of radioactivity transferred upward was considerably greater in the standard trees than in the dwarf interstem trees; more radioactivity was deposited along the stems of the dwarfs. After several hours a slow downward movement of radioactivity was detected. The trees were harvested at periods ranging from 14 hours to 3 days. The bark was separated in the vicinity of the interstem, pressed flat, quickly frozen in dry ice and kept frozen during autoradiography. In no case was there definite evidence of blocking in the phloem such as was indicated in the girdled tree. In all of the dwarf interstems, however, considerable contortion of the phloem pathways was indicated. In Figures 26 and 27, one of the autoradiographs is shown along with the piece of bark.

Radioactive phosphorus was applied by spraying on all of the leaves of one Clark Dwarf interstem tree. The trunk and lower branches were protected from the spray with aluminum foil and cotton. This tree was harvested in 5 hours and handled in the method described above. It was evident even in this short time that practically no impediment to phloem transport of phosphorus was present. In fact slightly higher
Figure 26. Autoradiograph of bark removed from the interstem and vicinity of a dwarf tree 21 hours after petiole treatment with radioactive phosphorus. Note that there is no evidence of buildup above the top union at the left of the picture.

Figure 27. Bark from which the autoradiograph above was produced. Note that the unions have considerably contorted contours.
radioactivity was present in the interstem than above it. It was assumed that the $^{32}\text{P}$ which was translocated out of the leaves was present in the bark in organic form. Barrier and Loomis (1957) found that 80% of the $^{32}\text{P}$ was in organic form after 24 hours in leaves.

Upward movement of $^{32}\text{P}$

The second set of six trees (3 pairs) was also used to estimate the rate of movement of the $^{32}\text{P}$ up the trunk after entering the cut root. Immediately after treatment a radioactive front of an arbitrary counting rate was chosen and followed up the trunk of the trees at several marked positions to determine the rate of upward movement. Time measures were in minutes. Tables 14a and 14b represent the results of the pair-4 trees, with sketches of the tree trunks to indicate their lengths, placement of interstem, and the marked positions. Interstem lengths were 5 cm. The radioactive front progressed upward at a slower rate through the dwarf interstem trunk than the standard interstem trunk.

The activities of the fronts chosen were close to the maximum which could be obtained at the top of the tree; generally those in the standard trees were higher.

The data show that the rate of movement through the interstem, or from position 2 to position 3, was less than
### Table 14a. Rate of movement of radioactive front\(^a\) up the trunk of a standard interstem apple tree of pair 4

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5 cm(^b)</td>
<td>1</td>
<td>7 min</td>
<td>1 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>15 3/4 cm</td>
<td>2</td>
<td>11 min</td>
<td>0.88 cm/min</td>
</tr>
<tr>
<td>1</td>
<td>4 cm</td>
<td>3</td>
<td>29 min</td>
<td></td>
</tr>
</tbody>
</table>

Average rate from position 1 to position 3 is 19 3/4 cm in 22 min or 0.90 cm/min

\(^a\)10,000 cts/min.

\(^b\)Length of interstem.

### Table 14b. Rate of movement of radioactive front\(^a\) up the trunk of a dwarf interstem apple tree of pair 4

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5 cm(^b)</td>
<td>1</td>
<td>7 min</td>
<td>1 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>12 cm</td>
<td>2</td>
<td>11 min</td>
<td>0.54 cm/min</td>
</tr>
<tr>
<td>1</td>
<td>4 cm</td>
<td>3</td>
<td>33 min</td>
<td></td>
</tr>
</tbody>
</table>

Average rate from position 1 to position 3 is 16 cm in 26 min or 0.62 cm/min

\(^a\)3,500 cts/min.

\(^b\)Length of interstem.
that below the interstem, from position 1 to position 2. Here and in the following experiments, the apparent rate of movement, that is, the rate of movement of a specific radioactive front, decreased as it progressed upward. It is supposed that this change can be attributed to absorption of the phosphorus$^{32}$ by the tissues of the stem. Stout and Hoagland (1939) found that when the phloem was left attached to the xylem of willow and geraniums, the phloem contained P$^{32}$ almost as soon as the xylem itself. More phosphorus would be absorbed from the slower column, resulting in a progressive reduction of activity; consequently, there would be an exaggeration of the differences in rate of movement. It is probable that more absorption takes place in the dwarf interstem, since it contains more living tissue (Beakbane et al., 1936, 1939, 1941).

In Tables 15a and 15b, are given the results of the pair-5 trees. The tendency for the apparent decreasing rate of movement of the front is shown again. Rate of movement of the radioactive front is considerably less in the dwarf interstem tree than in the standard interstem trees. The dwarf and standard interstem are about twice as long as in the previous experiment, 10 cm and 11 cm, respectively. Heights of the trunk are greater, also.

The third replication, pair 6 of this experiment, is summarized in Tables 16a and 16b with trees of considerably greater height. Rate of upward movement of the radioactive front is again greater in the standard interstem tree.
### Table 15a. Rate of movement of radioactive front\(^a\) up the trunk of a standard interstem apple tree of pair 5

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>11 cm(^b)</td>
<td>1</td>
<td>5 min</td>
<td>3.25 cm/min</td>
</tr>
<tr>
<td></td>
<td>16 cm</td>
<td>2</td>
<td>7 min</td>
<td>1.83 cm/min(^c)</td>
</tr>
<tr>
<td>3</td>
<td>5 1/2 cm</td>
<td>3</td>
<td>10 min</td>
<td>3.20 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>6 1/2 cm</td>
<td>4</td>
<td>15 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average rate from position 1 to position 4 was 28 cm in 10 min or 2.80 cm/min</td>
</tr>
</tbody>
</table>

\(^a\)15,000 cts/min.  
\(^b\)Length of interstem.  
\(^c\)Position 3 found to be spot where wood was knotted.

### Table 15b. Rate of movement of radioactive front\(^a\) up the trunk of a dwarf interstem apple tree of pair 5

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>10 cm(^b)</td>
<td>1</td>
<td>10 min</td>
<td>0.40 cm/min</td>
</tr>
<tr>
<td></td>
<td>15 cm</td>
<td>2</td>
<td>29 min</td>
<td>0.33 cm/min</td>
</tr>
<tr>
<td>3</td>
<td>10 1/2 cm</td>
<td>3</td>
<td>61 min</td>
<td>0.22 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>7 1/2 cm</td>
<td>4</td>
<td>128 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average rate from position 1 to position 4 was 33 cm in 118 min or 0.28 cm/min</td>
</tr>
</tbody>
</table>

\(^a\)3,500 cts/min.  
\(^b\)Length of interstem.
Table 16a. Rate of movement of radioactive front\(^a\) up the trunk of a standard interstem apple tree of pair 6

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 min</td>
<td>1</td>
<td>6 min</td>
<td>1.35 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>16 min</td>
<td>2</td>
<td>16 min</td>
<td>1.12 cm/min</td>
</tr>
<tr>
<td>3</td>
<td>29 min</td>
<td>3</td>
<td>29 min</td>
<td>0.34 cm/min</td>
</tr>
<tr>
<td>4</td>
<td>73 min</td>
<td>4</td>
<td>73 min</td>
<td>0.75 cm/min</td>
</tr>
<tr>
<td>5</td>
<td>93 min</td>
<td>5</td>
<td>93 min</td>
<td>0.75 cm/min</td>
</tr>
</tbody>
</table>

Average rate from position 1 to position 5 is 55 1/2 cm in 87 min or 0.64 cm/min

\(a\) 10,000 cts/min.  
\(b\) Length of interstem.

Table 16b. Rate of movement of radioactive front\(^a\) up the trunk of a dwarf interstem apple tree of pair 6

<table>
<thead>
<tr>
<th>Position</th>
<th>Distance between positions</th>
<th>Position</th>
<th>Time</th>
<th>Rate of movement between positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 min</td>
<td>1</td>
<td>13 min</td>
<td>0.23 cm/min</td>
</tr>
<tr>
<td>2</td>
<td>121 min</td>
<td>2</td>
<td>121 min</td>
<td>0.22 cm/min</td>
</tr>
<tr>
<td>3</td>
<td>161 min</td>
<td>3</td>
<td>161 min</td>
<td>0.07 cm/min</td>
</tr>
<tr>
<td>4</td>
<td>309 min</td>
<td>4</td>
<td>309 min</td>
<td>0.25 cm/min</td>
</tr>
<tr>
<td>5</td>
<td>362 min</td>
<td>5</td>
<td>362 min</td>
<td>0.25 cm/min</td>
</tr>
</tbody>
</table>

Average rate from position 1 to position 5 is 57 cm in 349 min or 0.16 cm/min

\(a\) 10,000 cts/min.  
\(b\) Length of interstem.
Beakbane (1947) postulated that, because of differences in size, number, and cross-sectional area of vessels which she found consistently in dwarfing stock, there might well be an impedance to water uptake. The present work also indicates that the water relations of interstem dwarfs may be different from standard trees. Because the roots were cut for treatment it cannot be estimated precisely what these differences may be but the indications in all three replications are that the transpiration stream moved considerably faster in standard than in dwarf trees of the same size. Although there is considerable variation some evidence is shown in the data to suggest that $\text{P}^{32}$ movement through a trunk containing a short dwarfing interstem is not as slow as movement through trunks containing longer interstems.

Growth Inhibitors of Apple Bark

Growth inhibitor analyses, using a straight growth wheat coleoptile assay, were made of the five dwarfing stocks EM IX, EM VIII, Clark Dwarf, EM VII, and EM II, sampled according to the following methods:

1. Small, 1/2 cm sections of the green tissue of the stems about 2 cm from the terminal buds were inserted directly into the growth solution at the time of adding of the coleoptiles.
2. A No. 1 cork borer was used to obtain bark punches (From wood of approximately 1 1/2 cm diameter) which were placed directly in the growth solution before adding the coleoptile sections.

3. Extracts were made of bark collected in the late summer, lyophilized, and ground to 20 mesh in a micro-Wiley mill. The solvents tried were ether, acetone, ethanol, and water, used at the rate of 1 ml solvent to 1 mg of bark. The extracts were placed directly into the test vial and the solvents evaporated by heat or suction. The use of small sections of green tissues resulted in no appreciable differences in coleoptile growth.

Two bark punches were dropped into each test vial with a sucrose-buffer solution containing IAA at a concentration of 0.1 mg/l. The vials were then allowed to stand at 23°C for about 8 hours until the coleoptiles were ready to be added. The data and analysis of variance for three replications are presented in Tables 17a and 17b, respectively. There appeared to be less inhibition from the presence of the EM IX bark than from the others, although the difference was not significant at the 0.05 probability level.

Several assay trials were made using one or three bark punches per vial, at growth solution concentrations of 0.5 mg/l and 1 mg/l, and with and without sucrose. In almost
Table 17a. Fresh bark inhibitor analyses
(Each number is the average of 25 coleoptiles and represents thousandths of an inch less growth than the control)

<table>
<thead>
<tr>
<th>Replication</th>
<th>EM IX</th>
<th>EM VIII</th>
<th>Clark</th>
<th>EM VII</th>
<th>EM II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td>107</td>
<td>95</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>98</td>
<td>95</td>
<td>96</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>74</td>
<td>66</td>
<td>87</td>
<td>72</td>
</tr>
<tr>
<td>Mean</td>
<td>65</td>
<td>93</td>
<td>85</td>
<td>87</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 17b. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>1,568</td>
<td>392</td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>1,772</td>
<td>886*</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>910</td>
<td>114</td>
</tr>
</tbody>
</table>

*Significant difference at the 0.05 probability level.
every case EM IX bark gave either most inhibition or least inhibition. There was no consistency as to what conditions were responsible for such a discrepancy. Golden Delicious bark punches from wood of the same diameter were assayed with results usually indicating more inhibition than dwarfing stocks.

Lyophilized bark was used to make extracts using ether, acetone, ethanol, and water as solvents. The use of water extracts gave results similar to those above. Ethanol extracts gave erratic results but generally stimulated coleoptile growth.

Extracts of apple bark made with acetone and ether as solvents were tried with fair results. One ml of the extract or a dilution of it was placed into each culture vial and evaporated to dryness at 70° C.; the growth solution was then added to the residue before inserting the coleoptile sections. It was found that all but the lower concentrations resulted in flaccid or waterlogged coleoptile sections. This type of action was also described by Barlow et al., (1955) in neutral extracts of Crab C stock. Where the ether was evaporated by suction, flaccidity was slightly less.

Residues from evaporated ether and acetone were tested. The acetone gave slight stimulation. The ether was erratically inhibitory. In subsequent tests the ether used was distilled
according to the method detailed by Reimers in Paech and Tracey (1955, pp. 565-625). It was found that distilled ether could only be stored for about three days before it became inhibitory, even when kept at 0°F. Another, more stable, less volatile, solvent would certainly be desirable for this work.¹

The data and analysis of variance from total ether extract assays for three replications are presented in Tables 18a and 18b.

In Table 18c, the multiple range test for comparison of treatment means², proposed by Newman (1939) and Keuls (1952) and further developed by Duncan (1955), shows differences significant only for EM II; EM II and EM VII were not different at the 0.05 probability level. However, it is of interest to note that the treatment means were in the reverse order of tree size as they were grown in the field. EM VIII and Clark Dwarf are short and spreading and about equally small, EM IX is spreading but somewhat larger, EM VII and EM II are upright.

¹Ether now produced by the Mallinckrodt Chemical Company contains 0.05 ppm of sodium diethyldithiocarbamate (C₄H₇)₂NCS₂Na as a preservative in the prevention of ether peroxides. If this additive is found not to interfere in coleoptile growth, use of Mallinckrodt's ether may obviate the necessity of ether distillation (Mallinckrodt Chemical Works, 1955).

²For each cell of the multiple range table, the first line is the difference between the treatment means, the second line—if it exists—is the difference calculated for significance for the 0.05 probability level, and the third line—if it exists—is the difference calculated for the 0.01 probability level.
Table 18a. Total ether extract bark inhibitors

(Each number is the average of 25 coleoptiles and represents thousandths of an inch less growth per coleoptile than the control)

<table>
<thead>
<tr>
<th>Replications</th>
<th>EM IX</th>
<th>EM VIII</th>
<th>Clark</th>
<th>EM VII</th>
<th>EM II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>67</td>
<td>61</td>
<td>43</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>75</td>
<td>61</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>90</td>
<td>106</td>
<td>84</td>
<td>35</td>
</tr>
<tr>
<td>Mean</td>
<td>71.7</td>
<td>77.3</td>
<td>76.0</td>
<td>58.0</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Table 18b. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>5,297</td>
<td>1,324.0**</td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>2,288</td>
<td>1,144.0**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>1,295</td>
<td>161.9</td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.
Table 18c. Multiple range test for comparison of variety means

<table>
<thead>
<tr>
<th></th>
<th>$\bar{x}_{EM VIII}$</th>
<th>$\bar{x}_{Clark Dwarf}$</th>
<th>$\bar{x}_{EM IX}$</th>
<th>$\bar{x}_{EM VII}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}_{EM II}$</td>
<td>50.3**</td>
<td>49.0**</td>
<td>44.7**</td>
<td>31.0**</td>
</tr>
<tr>
<td></td>
<td>22.4</td>
<td>22.2</td>
<td>21.5</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>31.7</td>
<td>31.2</td>
<td>30.3</td>
<td>28.8</td>
</tr>
<tr>
<td>$\bar{x}_{EM VII}$</td>
<td>19.3</td>
<td>18.0</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>$\bar{x}_{EM IX}$</td>
<td>5.6</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x}_{Clark Dwarf}$</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.

and considerably larger. Ether extracts of Golden Delicious bark were generally found to be about as inhibitory as EM IX.

It should be pointed out that the coleoptiles used were slightly over 1 cm in length at the time of insertion into the growth solution, i.e. 0.410 inch. Total length of the controls after 12 hours was about 0.79 inch, or a growth of 0.380 inch. About the greatest amount of inhibition possible from total ether extracts of apple bark was roughly one-third of the growth of the controls. When more bark inhibitor was supplied the coleoptiles were "waterlogged", indicating serious toxic effects.
Some separations of bark extracts were attempted by placing ether extracts on chromatographic paper and developing with isopropanol-ammonia-water mixture, 10-1-1. The ascending chromatograms were run to 10 inches at 23° C for 10 hours, dried, and cut into sections, which were analysed by placing strips of the paper directly into the growth solution. At least three areas of inhibition were found in each case, one each at the bottom and top, and one about one-third of the way up. Differentiation of the inhibitor spots was relatively poor and a spot that appeared to be a single inhibitor was spread over three inches of the ten inch chromatogram.¹ There was no effect of flaccidity of coleoptile sections when portions of paper chromatograms were assayed, even when the inhibition was almost complete. Evidently, this phenomenon is an action involving more than one substance.

Evidence is presented that there may be differences in the total amounts of inhibitors present in different varieties of apples. A trend is shown to more total inhibitors in ether extracts of the bark of small growing dwarf stocks than of the larger growing dwarf stocks. It is indicated that the assay method used may be unsatisfactory for mixtures.

¹According to Block et al., (1955) developing solvents which run slower may give better separation. It is suggested that a butanol-ammonia-water solvent be tried, although it has generally been found poor for the separation of indole compounds.
Treatment of Standard Trees with Antiauxins

Golden Delicious, two year old cut-back trees were sprayed with dilute "antiauxin" solutions through the growing season according to the following schedule (Table 19). Application was made in the evening.

Table 19. 1955 field spray schedule of antiauxin solutions on standard trees

<table>
<thead>
<tr>
<th>Date</th>
<th>Molar concentrations x 10^{-4}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coumarin 2,4,6-T 2,4,5-T TIBA nicotine sulfate</td>
</tr>
<tr>
<td>June 4,19,22, 25,July 3, 8,16,25,29</td>
<td>10 1 1 0.25 10</td>
</tr>
<tr>
<td>Aug. 4</td>
<td>20 2 2^a 0.5 20</td>
</tr>
<tr>
<td>Aug. 9,16</td>
<td>20 2 0 0.5 20</td>
</tr>
<tr>
<td>Aug. 23,Sept. 7</td>
<td>20 2 1 0.5 20</td>
</tr>
</tbody>
</table>

^aDamage noted from this treatment.

No leaf damage was noted from any of the sprays until 2,4,5-trichlorophenoxyacetic acid was applied at 0.0002 M. Not only did the edges of the leaves yellow and some fall, but also a spreading of the branches was apparent, and in some cases the angle of branches with trunk seemed to change.
In some instances knotty growths were formed at the bases of these branches.

Measurements proportional to the dry or wet weight increase of tops were made by the $L \times D_m^2$ factor. Tables 20a and 20b present the measurements of five replications and analysis of variance, respectively. The trees treated with TIBA and 2,4,5-T averaged only about half the growth of the control. But the variation was extremely high and these differences were not significant at the 0.05 probability level. The high variation in growth increase is attributed partly to the fact that newly transplanted nursery trees characteristically come into growth at various times after planting.

Table 20a. Proportional growth of antiauxin treated trees in the field. (Measured 1 Nov. 1955)

<table>
<thead>
<tr>
<th>Rep</th>
<th>Control</th>
<th>2,4,6-T Nicotine sulfate</th>
<th>Coumarin</th>
<th>TIBA</th>
<th>2,4,5-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>481</td>
<td>447</td>
<td>413</td>
<td>220</td>
<td>162</td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td>221</td>
<td>153</td>
<td>142</td>
<td>132</td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>183</td>
<td>108</td>
<td>124</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>114</td>
<td>150</td>
<td>106</td>
<td>145</td>
<td>107</td>
</tr>
<tr>
<td>5</td>
<td>238</td>
<td>79</td>
<td>248</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>Means</td>
<td>206</td>
<td>216</td>
<td>206</td>
<td>140</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 20b. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>Sums of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>5</td>
<td>64,638</td>
<td>12,928</td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>172,888</td>
<td>43,222**</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>105,464</td>
<td>5,273</td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.

The angles of branches with the trunk were measured, and the arithmetic mean calculated for each tree. The data for branch angle and analysis of variance are presented in Tables 21a and 21b.

No significant differences at the 5 percent level were shown either in growth increase or branch angle, even though both TIBA and 2,4,5-T averaged considerably less growth and the 2,4,5-T treatments caused a more spreading growth. Measurements of branch angles were made against the trunk to a specified distance away from the trunk. Many of these trees had branches which joined the trunk at a sharp angle but drooped or spread farther away from the trunk. No flowers were noted the following spring.

Spray treatments were continued on this same block of trees through the next summer according to the schedule in
Table 21a. Branch angles of antiauxin treated trees in the field (Measured 1 Nov. 1955)

<table>
<thead>
<tr>
<th>Rep</th>
<th>Control</th>
<th>2,4,6-T</th>
<th>Nicotine sulfate</th>
<th>Coumarin</th>
<th>TIBA</th>
<th>2,4,5-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.1</td>
<td>52.0</td>
<td>54.7</td>
<td>55.9</td>
<td>49.3</td>
<td>51.3</td>
</tr>
<tr>
<td>2</td>
<td>52.6</td>
<td>59.4</td>
<td>47.9</td>
<td>52.9</td>
<td>51.4</td>
<td>50.1</td>
</tr>
<tr>
<td>3</td>
<td>51.4</td>
<td>52.0</td>
<td>52.3</td>
<td>50.7</td>
<td>52.9</td>
<td>52.8</td>
</tr>
<tr>
<td>4</td>
<td>44.0</td>
<td>47.7</td>
<td>49.5</td>
<td>55.8</td>
<td>52.4</td>
<td>63.9</td>
</tr>
<tr>
<td>5</td>
<td>48.3</td>
<td>45.9</td>
<td>49.8</td>
<td>44.0</td>
<td>54.5</td>
<td>56.2</td>
</tr>
<tr>
<td>Mean</td>
<td>49.9</td>
<td>51.4</td>
<td>50.8</td>
<td>51.9</td>
<td>52.1</td>
<td>54.9</td>
</tr>
</tbody>
</table>

Table 21b. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>5</td>
<td>115.1</td>
<td>23.0</td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>32.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>351.6</td>
<td>17.6</td>
</tr>
</tbody>
</table>
Table 22. There were long intervals between spray treatments from July on because of extreme drought conditions. Coumarin was omitted. It was thought to be ineffective at the concentration used and it was not possible to increase the concentrations further. Audus (1948) pointed out in his work that the

Table 22. 1956 field spray schedule of antiauxin solutions on standard trees

<table>
<thead>
<tr>
<th>Date</th>
<th>Molar concentrations x 10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,4,6-T</td>
</tr>
<tr>
<td>May 28</td>
<td>10</td>
</tr>
<tr>
<td>June 4</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>July 26</td>
<td>10</td>
</tr>
<tr>
<td>Aug. 11</td>
<td>10</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
</tr>
</tbody>
</table>
limits of coumarin concentrations are set by the smaller effectiveness of coumarin on the one hand (no significant inhibition of root growth with 1 ppm) and its solubility on the other (saturated solution approximately 100 ppm).

Measurements were not made on the experiment in the fall but flowering was noted on three trees the following spring. One tree treated with 2,4,5-T held one normal appearing terminal flower truss which set fruit. Two trees of the TIBA treatment flowered with several terminal trusses, the flowers of each which opened at varying times within a period of three weeks. The flowers had different lengths of peduncles, some did not open completely, parts of the flowers were twisted, and one remained a solid green blob on a peduncle without opening. No fruit was set.

The experiment was repeated in the greenhouse with 100 potted, one year whip trees of unusual uniformity. All trees came into growth during the same week and grew vigorously. They were sprayed in groups of two and in place in the greenhouse according to the schedule in Table 23. The data and analyses of variance of Tables 24a, b and c indicate a significant growth inhibition due to treatment with TIBA. It was observed that a bulge was present at the base of many branches on trees treated with TIBA. The concentration of 2,4,6-T was increased to its maximum solubility without causing damage. 2,4,5-T caused obvious damage to the leaves; the trees
Table 23. 1956 greenhouse spray schedule of antiauxins on standard trees

<table>
<thead>
<tr>
<th>Date</th>
<th>Molar concentrations x $10^{-4}$</th>
<th>2,4,6-T</th>
<th>2,4,5-T</th>
<th>TIBA</th>
<th>Nicotine sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 21, 23, 26, 28, May 1, 3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>May 5&lt;sup&gt;a&lt;/sup&gt;, 8, 10</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>May 15, 17, 19, 23, 25, 28, 30, June 1</td>
<td>10.0</td>
<td>0.5</td>
<td>0.5</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>June 7, 10, 12</td>
<td>20.0</td>
<td>0.5</td>
<td>0.5</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>June 14, 17, 19</td>
<td>25.0</td>
<td>0.75</td>
<td>0.75</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>June 21</td>
<td>25.0</td>
<td>0.75</td>
<td>0.75</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>July 25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Aug. 20</td>
<td>20</td>
<td>0.75</td>
<td>0.75</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>2,4,5-T and TIBA sprays discontinued temporarily because of damage to trees.

appeared to have caught up on growth loss when the spray was discontinued. The nicotine sulfate was increased to the point where the operator was forced to wear a gas mask and protective clothing; nevertheless, there was no apparent inhibitory effect on the apple trees. This was in contrast to the work of Fisher (1955), who found nicotine sulfate to inhibit the growth of soybeans and to act as an antiauxin.
Table 24a. Proportional growth of antiauxin treated trees in the greenhouse (Measured 1 Nov. 1956)

<table>
<thead>
<tr>
<th>Order</th>
<th>Replication</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A: 743</td>
<td>B: 1,292</td>
<td>E: 1,094</td>
<td>D: 812</td>
<td>C: 1,652</td>
<td>5,593</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B: 926</td>
<td>A: 1,206</td>
<td>C: 1,011</td>
<td>E: 1,754</td>
<td>D: 558</td>
<td>5,455</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C: 1,165</td>
<td>E: 1,253</td>
<td>D: 887</td>
<td>B: 1,734</td>
<td>A: 880</td>
<td>5,919</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E: 2,012</td>
<td>D: 738</td>
<td>A: 1,361</td>
<td>C: 1,536</td>
<td>B: 1,136</td>
<td>6,783</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>D: 1,053</td>
<td>C: 1,102</td>
<td>B: 1,442</td>
<td>A: 1,453</td>
<td>E: 1,489</td>
<td>6,539</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sum 5,899</td>
<td>5,591</td>
<td>5,795</td>
<td>7,289</td>
<td>5,715</td>
<td>30,289</td>
<td></td>
</tr>
</tbody>
</table>

Square II

<table>
<thead>
<tr>
<th>Order</th>
<th>Replication</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E: 1,360</td>
<td>C: 893</td>
<td>D: 661</td>
<td>A: 2,210</td>
<td>B: 2,189</td>
<td>7,313</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B: 1,813</td>
<td>D: 1,074</td>
<td>E: 851</td>
<td>C: 1,970</td>
<td>A: 1,596</td>
<td>7,304</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C: 1,674</td>
<td>A: 1,465</td>
<td>B: 1,176</td>
<td>D: 850</td>
<td>E: 934</td>
<td>6,099</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A: 1,425</td>
<td>B: 1,130</td>
<td>C: 1,532</td>
<td>E: 2,038</td>
<td>D: 915</td>
<td>7,040</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>D: 425</td>
<td>E: 1,362</td>
<td>A: 1,984</td>
<td>B: 1,707</td>
<td>C: 1,409</td>
<td>6,887</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sum 6,697</td>
<td>5,924</td>
<td>6,204</td>
<td>8,775</td>
<td>7,043</td>
<td>34,643</td>
<td></td>
</tr>
</tbody>
</table>
Table 24b. Summary by antiauxins

<table>
<thead>
<tr>
<th></th>
<th>A: 2,4,5-T</th>
<th>B: Nicotine</th>
<th>C: Control</th>
<th>D: TIBA</th>
<th>E: 2,4,6-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1,432</td>
<td>1,454</td>
<td>1,394</td>
<td>797</td>
<td>1,415</td>
</tr>
</tbody>
</table>

\[ \text{LSD}_{0.05} = 343. \]
\[ \text{LSD}_{0.01} = 462. \]

Table 24c. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>3,161,391</td>
<td>790,348**</td>
</tr>
<tr>
<td>Replication within squares</td>
<td>8</td>
<td>1,391,147</td>
<td>173,893</td>
</tr>
<tr>
<td>Orders within squares</td>
<td>8</td>
<td>469,441</td>
<td>58,680</td>
</tr>
<tr>
<td>Squares</td>
<td>1</td>
<td>379,147</td>
<td>379,147*</td>
</tr>
<tr>
<td>Error</td>
<td>28</td>
<td>3,920,118</td>
<td>140,004</td>
</tr>
</tbody>
</table>

**Significant difference at the 0.01 probability level.
* Significant difference at the 0.05 probability level.
The data of Tables 25a and 25b indicate also a trend of the TIBA treatment to increase the average angle of branches. However, the analysis of variance in Table 25c shows that the difference was not significant at the 0.05 probability level.

Table 25a. Branch angles of antiauxin treated trees in the greenhouse (Measured 1 Nov. 1956)

<table>
<thead>
<tr>
<th>Order</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Square I</td>
</tr>
<tr>
<td>1</td>
<td>A: 35.6</td>
</tr>
<tr>
<td>2</td>
<td>B: 38.1</td>
</tr>
<tr>
<td>3</td>
<td>C: 30.4</td>
</tr>
<tr>
<td>4</td>
<td>E: 40.4</td>
</tr>
<tr>
<td>5</td>
<td>D: 47.6</td>
</tr>
<tr>
<td></td>
<td>Sum</td>
</tr>
</tbody>
</table>

|       | Square II  |   |   |   |   |      |
| 1     | E: 41.9    | C: 40.8 | D: 40.1 | A: 35.3 | B: 38.0 | 196.1 |
| 2     | B: 38.4    | D: 32.4 | E: 30.9 | C: 43.2 | A: 31.9 | 176.8 |
| 3     | C: 32.8    | A: 39.4 | B: 36.2 | D: 43.8 | E: 39.4 | 191.6 |
| 4     | A: 50.0    | B: 29.2 | C: 44.7 | E: 39.4 | D: 34.9 | 198.2 |
| 5     | D: 43.9    | E: 32.9 | A: 35.9 | B: 37.2 | C: 39.8 | 189.7 |
|       | Sum        | 207.0 | 174.7 | 187.8 | 198.9 | 184.0 | 952.4 |
Table 25b. Summary by antiauxins

<table>
<thead>
<tr>
<th>Source</th>
<th>A: 2,4,5-T</th>
<th>B: Nicotine</th>
<th>C: Control</th>
<th>D: TIBA</th>
<th>E: 2,4,6-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>38.8</td>
<td>38.4</td>
<td>38.9</td>
<td>43.0</td>
<td>37.2</td>
</tr>
</tbody>
</table>

Table 25c. Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>196.8</td>
<td>49.2</td>
</tr>
<tr>
<td>Replications within squares</td>
<td>8</td>
<td>219.3</td>
<td>27.4</td>
</tr>
<tr>
<td>Orders within squares</td>
<td>8</td>
<td>66.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Squares</td>
<td>1</td>
<td>68.2</td>
<td>68.2</td>
</tr>
<tr>
<td>Error</td>
<td>28</td>
<td>768.6</td>
<td>27.4</td>
</tr>
</tbody>
</table>

The apparent spread of these trees, however, was distinctly greater.

The greenhouse potted trees were placed out of doors from early autumn until frost. They were put into common storage until the following spring when they were brought into the
greenhouse to be observed for flowering. Some normal flower trusses were formed on terminals, but flowering showed no trend to favor any treatment.

The chemical production of the dwarfing characteristics of decreased growth, wider branch angle or spreading aspect, and precocious flowering has been demonstrated to be a possibility by the use of TIBA. However, decreased growth has been attended by definite injury to leaf and bud tissues and the precocious flowering resulted in deformed and apparently non-functional flowers.
DISCUSSION

Dwarf fruit trees have been prized for hundreds of years in European countries. Because of the high cost of fruit production on standard trees in America, considerable interest has been aroused in the use of dwarf trees in commercial orchards as well as in the backyard amateur garden. By combining a strong rootstock with a hardy interstem such as the Clark Dwarf, a small tree of early productivity and of high quality fruit may be produced. This study was designed to add to our knowledge of dwarfing and to determine the physiological basis of the dwarfing which follows the use of interstems.

Dwarfing may be artificially accomplished in many ways, including the tying of branches in horizontal positions, reverse bark grafting, upside down budding, tying a knot in the trunk, girdling the tree by cutting out a ring of bark, persistent pruning of tops and roots, and restricting minerals or water. Most of these methods result either in the ultimate destruction of the tree, or require constant care to maintain the dwarf condition. Relatively permanent and satisfactory dwarfing can be accomplished by graftage onto a stock known by experience to induce dwarfing when used with standard varieties.

Dwarfing by graftage may be induced also when a piece of a dwarfing stock is grafted between a vigorous root and a scion
of a vigorous variety. Thus, the dwarfing effect may be derived from this piece of interstem alone. Some of the suggested physiological causes of dwarfing by such interstems are interference with water and mineral transport upward to the tops, interference with phloem translocation either upward or downward, and the production of an antiauxin or growth inhibitor or the rapid destruction of auxin by the interstem.

Trunk diameters of six interstem trees each of trees bearing a heavy crop and of those with little or no fruit were measured weekly. Differential growth at four positions on the trunk were plotted. The trunk above and below the interstem and the bottom of the interstem grew at a reasonably steady rate on both groups; however, the growth rate of trunks of the trees bearing fruit leveled off toward the latter part of August in contrast to the trunk growth rate of trees with little or no fruit, which was just beginning to level off on September 15.

A striking difference in growth of the enlargement just above the top of the interstem was shown. Growth here stopped in the middle of June and some shrinkage occurred on plants with a heavy crop of fruit (Figure 7). If the enlargement at top of the interstem is due to an accumulation of food materials, this growth phenomenon may be related to the competitive withdrawal of food materials by the rapidly enlarging fruit. Growth measurements of trunk diameters of five dwarfing stocks
indicate that EM IX made the greatest diameter growth during the season. EM VII, EM II, EM VIII, and Clark Dwarf followed in that order. Growth rate of trunks of EM VIII and Clark Dwarf was almost identical after July 7. Before that time Clark Dwarf trunks grew at a somewhat slower rate.

Doubt about the distinctness of the Clark Dwarf variety from EM VIII has been expressed. The rootstocks EM VIII and Clark Dwarf have been grown in the same location on similar roots or own rooted and have been compared with each other and with three other dwarfing stocks in a number of tests. Where trunk diameter growth measurements were plotted through one growing season, Clark Dwarf was found to grow more slowly for the first few weeks and then to parallel the growth of EM VIII. Both EM VIII and Clark Dwarf have grown rather poorly and this small difference is interpreted as due to normal variation. In all other comparisons they have performed identically, about equal numbers succumbed to the first winter which was dry and relatively open, and all survived -30°F the following winter. Maney (1943) claimed more hardiness for Clark Dwarf; until a valid hardiness test is made, this claim must stand. Otherwise Clark Dwarf appears to be identical with Malling VIII.

An anatomical study was made to fill in gaps in our knowledge of dwarf stems. Beakbane (1941) described the stems of EM IX, EM II, and EM XII. Included here are those of EM IX, EM II, EM VII, EM VIII, and Clark Dwarf. Numbers of vessels
were not significantly different in any of the five stocks, but the means were in the order of EM II and EM VII-highest, EM VIII and Clark Dwarf-lowest, and EM IX-intermediate. This order is correlated with tree size in the nursery and the dwarfing capabilities of the stocks when used as interstems. Vessel cross-sectional area and amount of medullary ray tissues were measured by weighing the respective parts cut from photomicrographs. Vessel cross-sectional area differences were significant and in the order above. Amount of medullary ray tissue was in reverse order.

Actual volume of total xylem parenchyma was not measured but was obviously greatest in EM VIII and Clark Dwarf, considerably less in EM IX, and least in EM VII and EM II.

Beakbane and Thompson (1939) postulated that differences in vessel area might be effective in reducing the flow of water and nutrients to the tops of trees and therefore be the influential factor in dwarfing. Dwarfing by rootstocks was correlated with the vessel area of the root and other anatomical features of the roots which she measured.

Facts in regard to vessel area and anatomical structures of the stem presented in this work also fit in with our knowledge of interstem dwarfing. Although EM IX has the greater dwarfing propensities when used as a rootstock it has been reported by Tukey and Brase (1943) to produce interstem dwarfs which are larger and scarcely earlier maturing than
standard interstem trees. The other Malling stocks have apparently not been used as interstem grafts. Further evidence of the importance of reduced xylem transport is shown by the impediment indicated by the conduction of dyes through the interstem. Microscopic examination has suggested more contortion of xylem elements above the dwarf union. A reduced water supply to the top could be the major cause of interstem dwarfing. Harvey (1923) showed that terminal shoot growth of Grimes trees stopped before the water content of the top one-third of the shoot fell to 63 percent. A water content of 70 percent allowed rapid shoot growth.

Radioactive phosphorus, $^{32}\text{P}$, was introduced into dwarf and standard interstem apple trees for varying equilibration periods. Sections were cut for analysis, and an accumulation of radioactivity in the dwarf interstems was shown by counter measurements and by autoradiography. This accumulation may be related to the heavier bark formation in the interstem, to a greater volume of living cells in the dwarf wood than in the standard, and to a probably higher metabolic activity.

Translocation downward through the phloem was investigated by feeding radioactive phosphorus into the tops, either through cut petioles or cut green stems. The radioactivity traveled rapidly downward into a branch for about a decimeter, and then passed upward into the foliage above. After several hours a slow, general downward translocation of $^{32}\text{P}$ occurred.
It is assumed that this downward movement consisted of organic $P^{32}$ translocated in the phloem. Trees were sampled at times varying from 5 hours to 4 days after treatment and the bark autoradiographed. No evidence of accumulation above the graft unions could be found. The phosphorus evidently transversed the unions unimpeded.

Upward translocation in the phloem was studied by Dana (1952) who found that nitrogen supplied to the roots of nitrogen starved trees reached the tops slightly faster in own stems and self-interstem trees than in dwarf interstem trees. He interpreted this as evidence of a phloem transport impediment. Biddulph (1941), using $P^{32}$, discovered that where the plant was deficient in phosphorus, the result was less migration. He said that it appeared that phosphorus deficiency retarded migration of phosphate through such tissues, presumably until they were restored to some minimum phosphorus content. If this phenomenon applies to nitrogen, then the slight delay in reaching the tops might be attributed to "filling" the minimum nitrogen requirements of the trunk, which would be slightly more in the case of the interstem. The fact that nitrogen transport through bridged interstems was intermediate would support the initial interpretation, however. Loomis (1935) showed that upward translocation of nitrogen from the roots of woody plants occurs in the phloem. Dana's work was the first instance that impeded translocation in the phloem,
either upward or downward had been satisfactorily demonstrated as a factor in dwarfing.

Translocation upward in the transpiration stream was studied by introducing the radioactive tracer solution into cut roots and tracing it upward with a Geiger-Müller counter. The rate of upward movement of P$^{32}$ was found to be markedly slower in dwarf interstem trees than in standard interstem trees. Measurement of the rate of water movement has been shown to be confounded by the withdrawal of phosphorus from the ascending stream by cells adjacent to the xylem vessels and/or absorption on the walls of the xylem cells. These factors would operate to exaggerate differences in the measurement of the rate of water movement since the phosphorus would have more chance to move out of the slower moving transpiration stream. It was observed in six trials, however, that water was taken up notably faster by cut roots of equal size on standard interstem trees than on dwarf interstem trees.

Standard interstem trees were used as a comparison in this and the other P$^{32}$ experiments to duplicate the condition of two graft unions. Vyvyan (1955) has shown that some reduction in growth may be associated with the presence of a union. It is therefore probable that differences might have been even greater had the dwarf interstem trees been compared to single worked, standard trees.
Observed growth habit differences have suggested that hormonal relations may differ in interstem trees. An inhibitor study was undertaken first to determine if growth inhibitor concentrations were in any way correlated with dwarfing propensities of the dwarf interstems and secondly to see if some of the known dwarfing responses could be duplicated by chemical anti-auxins.

Only a beginning was made on a coleoptile inhibitor analysis; total inhibition by fresh bark samples and total inhibition of ether soluble samples of lyophilized bark were measured. Fresh bark of EM IX had the least inhibitive effect on coleoptiles, though none of the differences were significant. Tests of total ether extracts of lyophilized bark samples of EM VIII and Clark Dwarf gave the most inhibition, followed by EM IX and EM VII; EM II gave least inhibition. Amount of inhibition was in inverse order of the size of trees as they were grown in the field and in direct order of their dwarfing propensities when used as interstems.

Inhibitor concentrations were limited to a narrow range in the analysis of a total ether extract; this was attributed to the presence of toxic substances causing the coleoptiles to be flaccid or curled and therefore unmeasurable. Preliminary chromatographic separations were carried out, using an isopropanol-ammonia-water mixture as developer, a mixture recommended for indole compounds. In each of the apple woods
tested there were three areas of inhibition. The assay of inhibitor concentration was not limited when the substances were separated in this manner; almost total inhibition of coleoptile growth could be attained without toxic phenomena.

The accumulation of P³² in dwarf interstems indicates a greater metabolic activity which might act to destroy auxin faster, or to produce more antiauxin. More work needs to be done along this line before conclusions can be drawn regarding the relationship between growth inhibitors and dwarfing.

Standard two year old apple trees were sprayed with 2,4,5-T, 2,4,6-T, nicotine sulfate, coumarin, and TIBA chemicals which have been shown to have antiauxin properties under certain conditions. It was attempted to apply dilute concentrations often, rather than a concentrated solution once or twice. Nevertheless, where dwarfing responses were noted, they were accompanied by symptoms of toxicity. Of the chemicals tried, only TIBA gave significant differences in total growth. The spreading growth habit was apparent and two such trees bore abnormal flowers which did not set fruit. This experiment demonstrates the possibility that dwarfing may be associated with auxin inhibitors.

Both Colby (1935) and Rao and Berry (1940) base their conclusions on the cause of dwarfing by EM IX rootstock on the reduced water content caused by cyclic suberization of the roots. This implies that EM IX, though dwarfing as a root-
stock, would not necessarily induce the same degree of dwarfing when used as an interstem, which is indeed the case. An interference with downward translocation by compatible dwarf unions has never been demonstrated. Impeded upward translocation of nitrogen (Dana, 1952) would have the effect of slowing terminal growth, the consequence of which would be carbohydrate buildup. Restriction of water supply also would reduce terminal growth. These combined effects would be similar to a girdle with the exception of the girdle's limited downward translocation to the roots. A greater concentration of carbohydrate should be apparent throughout the tree rather than confined to the tops as in girdled trees. Other investigators have found this to be true.

Growth inhibitors extracted from lyophilized bark of stems of dwarfing stock were found to be correlated with the dwarfing propensities of the stocks when used as interstems. They may be the cause of dwarfing or only the result of greater differentiation in the dwarfs, or such inhibitors may be a linking reaction between the carbohydrate buildup and the induction of flowering.
SUMMARY

Trunk diameters of five dwarfing stock varieties were measured weekly through one growing season. EM IX had greatest diameter growth, followed by EM VII, EM II, EM VIII, and Clark Dwarf. Growth rates of EM VIII paralleled that of Clark Dwarf trunks after July 7. Clark Dwarf trunks grew slower before July 7.

EM VIII and Clark Dwarf were compared. They have been found nearly identical, but the claim that Clark Dwarf is more hardy is not disputed since conclusive hardiness tests were not made.

Weekly trunk diameter measurements of Clark Dwarf interstem trees, with and without fruit, were plotted through one growing season. The trunk above and below the interstem and the bottom of the interstem grew at steady rates. The enlargement at the top of the interstem ceased growth and shrunk during a two week period in June on plants bearing a heavy crop. It was suggested that this resulted from the competitive withdrawal of food materials by the rapidly enlarging fruit during this critical period. Subsequent growth of the top of the interstem was such that it surpassed the growth of the other parts of the trunk.

Cessation of diameter growth of trunks occurred in late August on fruiting trees. Trunks of trees without fruit were still growing in late September.
Anatomical studies of stem cross-sections of five dwarf apple stocks show the number of vessels and vessel cross-sectional areas to be negatively correlated with tree size as grown in the nursery and with the dwarfing propensities of the stems when used as inter-pieces. Medullary ray tissue and xylem parenchyma were in inverse order. Treatment of cut roots with a dye indicated a possible impediment to the transpiration stream in a Clark Dwarf interstem as compared with a standard interstem.

Radioactive phosphorus was applied to dwarf and standard interstem trees. When $^{32}$P was introduced via cut roots, upward conduction through the stem was slower in trees with dwarf interstems than those with standard interstems. $^{32}$P was applied to the tops, and the bark of the stem was autoradiographed after varying periods of time. No buildup of radioactivity could be detected above the dwarf unions.

$^{32}$P accumulated in both the bark and the wood of the Clark Dwarf interstems as compared to other parts of the trunk. This accumulation is attributed to the greater bark development and larger volume of living tissue of the Clark Dwarf wood.

A study was made of auxin inhibitors in dwarfing stocks. Growth inhibitor assays of the bark of five dwarf apple stocks were made with a straight growth, coleoptile method. There were no significant differences in the test of fresh bark;
however, EM IX gave the least inhibition. Tests of ether extracts of lyophilized bark indicated that EM VIII and Clark Dwarf were most inhibitory, EM IX intermediate and EM II and EM VII least. This order is correlated with the size differences of the trees in the field as well as their interstem dwarfing propensities.

Anti-auxin chemicals were applied to standard trees in the nursery and greenhouse. TIBA sprayed on nursery trees induced dwarfing responses, as indicated by increased spread of branches, reduced growth, and precocious flowering; however, the flowers were abnormal and did not set fruit. Toxicity symptoms accompanied the dwarf responses.

Interstem dwarfing is explained on the basis of reduced water conduction through the dwarf interstem xylem and reduced nitrogen translocation through the phloem, resulting in reduced shoot growth. Such slowing or cessation of shoot growth would result in a carbohydrate buildup and the attending induction of flowers. Fruiting is itself a dwarfing process, which effect added to the continuing effects of the interstem would tend to perpetuate the dwarfing response. More work should be done however in considering the role of anti-auxin in dwarfing of apple. It is proposed that anti-auxins may be related to a linking reaction between carbohydrate accumulation and floral induction.


Banta, Eldon S. Dwarf apple orchards are "growing up". Am. Fruit Grower 75(3):20-21, 40-41. 1955b.


Physiological observations upon the effects of partial decortication, or ringing the stems or branches of fruit trees. Trans. Hort. Soc. London 4:159-162. 1824.


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