# THE INFLUENCE OF SECONDARY CHEMICAL TREATMENT UPON THE ROOTING RESPONSE OF FILBERT AND DOUCINE APPLE HARDWOOD CUTTINGS.

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# THE INFLUENCE OF SECONDARY CHEMICAL TREATMENT UPON THE ROOTING RESPONSE OF FILBERT AND DOUCINE APPLE HARDWOOD CUTTINGS.

## INTRODUCTIONS

The occurrence of growth stimulating substances in nature has been an extensive field of scientific investigation since the latter part of the 19th century. In studying the phototropic response of plant coleoptiles to unilateral illumination, Darwin concluded that the cause for the bending response was the transfer of some growth-accelerating influence from the terminal portion toward the base. Identification and quantitative determination of growth materials have been made since by measuring the degree of plant curvature induced through chemical stimulation.

The culture of excised root tips in liquid nutrient mediums has given definite knowledge of plant growth requirements. Nutrient medium have been prepared which will support growth of excised plant tissues almost indefinitely; apparently, the equivalent of all essential substances naturally required for growth in the normal plant are available in the solution. It is only within the past 10 years that growth-promoting substances have been found of value in commercial propagation of horticultural plants. Such materials as indoleacetic acid, naphthalene acetic acid, and indolebutyric

acid, vitamin $B_1$, amino acids, inorganic and organic salts, and other materials have been tested on widely varying plant species.

Organic acids are commercially prepared in dust, liquid, and paste form for use by plant propagators. Through extensive experimental work the most efficient materials have been selected and the optimum concentrations determined for various species of plants.

Since many plants strike root readily from cuttings, while others root with difficulty if at all, it is concluded that there must be inherent differences between the various species in relation to the supply of growth promoting substances naturally present in the tissues. According to Went, Bonner, and Warner (33, 3), Pearse (24), Doak (11), and White (36), one explanation for the inability of many plants to root from cuttings is an inherent deficiency within the plant itself of certain growth substances essential for root development. Experimental tests have shown that these deficient materials may be supplied by treating the cuttings with dust, solution, or lanolin preparations of growth substances at the time cuttings are placed in the propagation bed. Further work by the same workers has shown that the lack of thiamin (vitamin $B_1$) and amino acids in the tissues of certain plants has proven to be the limiting factor in root development. Cuttings of some species produce

root primordia (root initials) in the propagation bed, but unless thiamin is subsequently supplied, roots will not develop. The application of vitamin $B_1$ as a secondary treatment 2 to 4 or 5 weeks following an indolebutyric acid treatment has resulted in marked increase in rooting on many species. To be effective, this subsequent or secondary treatment must be applied at the appropriate time when the root initials are formed. Vitamin $B_1$ is of no value if applied as an initial treatment when the cuttings are first prepared.

Filbert and doucine apple stocks have never been successfully propagated from cuttings. As far as the writer is aware, very little experimental work has been done in the chemical treatment of hardwood cuttings of these two species. Present methods of obtaining doucine stock by seedlings and filbert plants by layerage are slow and comparatively inefficient methods of propagation. Any method whereby their propagation could be hastened would be of practical value and interest.

The basis for the present study is the work of Hitchcock and Zimmerman (21), Tincker (31), Brase (8), Pearse (23, 24), Poesch (25), Went, Bonner, and Warner (33), Doak (11), and others who have proven the effectiveness of primary and secondary hormone and vitamin treatments in the rooting of cuttings. The objective of the present work is to determine if the rooting of filbert and doucine apple

hardwood cuttings can be induced through primary treatment with indolebutyric acid plus the secondary application of various nutrient and growth stimulating solutions after callusing has occurred as subsequent treatments.

# HISTORICAL REVIEW OF PREVIOUS INVESTIGATIONS

## Early Studies of Growth Promoting Substances in Plants

The occurrence of growth substances in plants was first noted by botanists through studies on phototropisms. Unilateral stimulation of certain plant organs by light caused positive phototropic response. Darwin (10) in experimenting with the coleoptile of Phalaris canariensis found that unilateral illumination caused strong positive phototropic curvature. If the terminal portion of the coleoptile was protected from the light stimulus very little curvature resulted, while if the lower portion were covered and the tip exposed to illumination, there was a positive curvature. If the tip was removed, the coleoptile did not react phototropically. Darwin concluded that lateral light caused some influence to be transmitted from the upper portion to the lower part of the coleoptile, resulting in the bending response.

Fitting (14) in studying the Avena coleoptile, found that lateral incisions below the tip in no way hindered the phototropic response as compared to unwounded coleoptiles. The curvature response is due to more rapid cellular growth and activity on the shaded side of the coleoptile than on the illuminated side.

Boysen-Jensen (6) conducted more extensive studies similar to those of Fitting. Transverse incisions were made in coleoptiles and their phototropic response determined. He concluded that the growth promoting influence is conducted downward upon the shaded side and is not obstructed by lateral incisions if the wounded surfaces are in close contact. He further found that even though the coleoptile tip were severed and separated from the base by a drop of gelatin, the downward movement of the growth substance was not obstructed. However, a thin piece of mica inserted in a lateral incision did prevent the transfer of the growth substance.

Paal (22) found that if the coleoptile tip were severed and replaced on only one side of the stump, the greatest growth occurred on the side under the tip and the coleoptile showed distinct curvature. Stark (28) found that curvature of the coleoptile resulted from unilateral application of agar blocks containing sap from coleoptile tips. Went (32) showed that growth substance from various plant parts would diffuse into agar blocks and that these impregnated blocks when applied to the coleoptile stump caused curvature. This method was then used as a semi-quantitative test in identification of growth substances. Growth-promoting materials were present in such minute quantities that chemical analysis was not satisfactory.

Further studies have shown growth promoting materials to be widely distributed in plant and animal organisms.

Rhizopus and Aspergillus form growth substances in culture; many bacteria produce growth stimulating materials; human urine is the richest source of auxentriolic acid (Auxin a) and 3-indoleacetic acid (Heteroauxin); human saliva also contains these substances; both Auxin a and Auxin b (auxenolonic acid) have been obtained from maize oil and malt; yeast is also a common source of heteroauxin.

Growth stimulating substances have been found in foliage of plants, shoots, flower stalks, growing regions of grass stems, root tips, pollen, fruits, and seeds (7). Rapidly growing meristematic portions of plants contain higher concentrations of growth substances than older differentiated tissue.

## Plant Hormones in Relation to Adventitious Growths and Root Formation.

From both plant and animal materials a wide variety of growth substances have been isolated and produced in chemically pure state. The use of hormones and other growth promoting substances in treating horticultural plants began with the work of Hitchcock and Zimmerman in 1935 (18). They used the following materials: alpha naphthalene acetic acid, indolebutyric acid, indoleacetic acid, indolepropionic acid, 3-indolyl valeric acid, and phenylacetic acid.

Both lanolin paste preparations and water solutions were used. These materials when applied to the rapidly growing region of aerial roots of a tropical grape (Vitis sp.) caused new branch roots to appear in 3 to 5 days. Hitchcock (17) applied lanolin paste impregnated with indole-3-n-propionic acid to the stems of growing tomatoes and other plants. He also applied the material by hypodermic injections of aqueous solutions and by dipping basal portions of cuttings in aqueous solutions. These treatments induced bending, swelling, epinasty, and adventitious aerial parts. Concentration of the chemical markedly affected the degree of epinastic response. Zimmerman and Wilcoxon (38) used the above methods in applying 16 different growth substances to tomatoes and other plants and concluded that alpha-naphthalene acetic acid and indolebutyric acids are the most effective root forming substances. The effectiveness of treatment is determined by speed of rooting, number of roots produced, and size of roots. They also found (19) that the methyl, ethyl, and butyl esters of growth substances were effective in producing epinasty of plants and in some cases superior to the corresponding acids.

Cooper (9) applied beta-indolylacetic acid in lanolin paste to the side near the top of leafless Eureka lemon cuttings and obtained significant rooting. Untreated leafless cuttings did not root. Tincker (31) used beta-indolylacetic acid and a-naphthalene acetic acid as aqueous solution treatments on Escallonia Donard Seedling, Buddleia

alternifolia, Viburnam Carlesii and other plants. More roots were formed and root development was more rapid on the treated cuttings as compared to controls.

Studies were then conducted (20) to determine the comparative effectiveness of the acids, esters, and salts of growth substances. When applied unilaterally to stems of plants in lanolin preparations, these materials produced the typical epinastic response. The esters and salts were effective growth stimulators and in some cases superior to the standard acid forms.

Brase (8) used aqueous solutions of indolebutyric acid in the treatment of softwood cuttings of Prunus cerasus, Prunus tomentosa, Pyrus Malus, Pyrus communis x P. serotina (Kieffer). Treatment time varied from 30 minutes to 48 hours and hormone concentration varied from 10 to 66 milligrams per liter. His results though not definitely positive furnished a lead in the propagation of deciduous fruits by cuttings. Brase did obtain 55 percent rooting in one series of Prunus tomemtosa cuttings using a lanolin preparation of indolebutyric acid. Pearse and Garner (23) conducted limited experiments, using alpha-naphthalene acetic acid (30 to 40 mg. per liter) in treating deciduous fruit tree cuttings. They report 100 percent rooting of plum and pear cuttings when treated for 12 hours. None of the controls rooted. The same treatment also speeded up the rooting of black currant and fig cuttings.

Poesch (25) used the three most effective growth promoting materials, indoleacetic acid, indolebutyric acid, and naphthalene acetic acids in tests on 110 woody ornamental plant species. Of this number 57 showed significant rooting response from treatment. The basal part of the cuttings was dipped three-fourths inch into aqueous solutions of the materials for intervals of 6 to 24 hours. Concentrations varied from 5 to 100 mg. per liter. Indolebutyric acid constantly proved superior. Poesch states that 10 to 50 mg. per liter for 6 to 24 hours is the optimum treatment for the more succulent plants.

Snow (27) obtained 65 percent rooting of aspen cuttings taken in March by treating them for 27 hours with indolebutyric acid (10 mg. per liter). He obtained better results by removing two slices of bark from opposite sides of the basal portion. This technique of slicing the basal region increased the exposure of cambium area and the potential rooting surface.

Stuart (30), working with American holly, Ilex opaca, which is ordinarily difficult to propagate from cuttings, rooted 100 percent in 30 days by treating with 0.01 percent indolebutyric acid solution for 18 hours. The concentrations used varied from 0.001 to 0.02 percent and the treatment time from 6 to 18 hours. He found that 0.02 percent for 18 hours injured the cuttings. Stuart and Marth then began studies on the effect of wounding the base of holly cuttings

before treatment. Two thin slices of bark were removed from each side of the basal portion, exposing the wood. They also tried splitting the bases of cuttings upward about one-fourth inch. Cuttings which were wounded by either method produced more vigorous root systems than non-wounded cuttings. The use of indolebutyric acid as a deferred treatment was also found beneficial. A series of American holly cuttings were placed in the rooting bed untreated. After 76 days those which had not rooted were removed and treated with 0.01 percent indolebutyric acid for 18 hours. Of those cuttings which had been sliced, this deferred treatment resulted in 92 percent rooting 21 days later. Only 50 percent of the unwounded cuttings rooted following this treatment. Roots were produced at the base on unwounded cuttings while on wounded stems nearly all roots were formed at the margins of the slice.

Biale and Halma (1) treated Eureka lemon and other citrus cuttings with indole-3-acetic acid and noticed an increase in the number of roots as a result of the treatment. They found that the time of year when cuttings are taken and the physiological condition of the trees are very important factors in the rooting response.

Pearse (24) treated thousands of softwood cuttings of pear, quince, apple, plum, and cherry with both alpha-naphthalene acetic acid and indolebutyric acid at concentrations of 5 to 80 milligrams per liter for 24-hour

treatment periods. In some of the tests, he obtained 60 percent rooting of pear cuttings, 90 percent rooting of quince, and 100 percent rooting of apple, plum, and cherry. The optimum concentration of acid varied from 15 to 40 mg. per liter. The condition of the wood when cuttings are taken is of major importance. Pearse took cuttings in May, June, and July. Some species rooted best when in active growth; others just after growth had stopped. The physiological condition and source of origin of the cuttings is of utmost importance. He believes that softwood cuttings of the majority of even the most difficult varieties will root up to 60 percent or over if taken at the right season and given suitable treatment with indolebutyric acid or alpha naphthalene acetic acid.

Fisher (13) conducted similar studies using such plants as Grimes Golden apple, Multiflora rose, Mariana plum, Mazzard cherry, seedling peach, grape, quince, French crab, and others. He found that cuttings from some of these plants responded well to treatment with various organic acids. Fisher was able to obtain rooting on cuttings from juvenile wood when the mature wood failed to root. He also noted that cuttings which normally root without treatment, root quicker if organic acids are used.

Liquid and Dust Preparations of Growth Substances Compared.

Hitchcock and Zimmerman (21) conducted extensive experiments on 64 species of plants, using both the acids and salts of indoleactic, indolebutyric, and naphthalene acetic acids. Application was made in both liquid and powder forms. The use of a concentrated dip solution (1 to 20 mg. per cc.) in which cuttings were momentarily dipped in the liquid, proved more effective for such species as apple (Grimes Golden), hemlock, rhodendron, and many evergreens. Cuttings from many species considered hard to root such as rhodendron, English carnation, certain evergreens, and lilacs rooted well with proper indolebutyric acid treatment. Thirteen varieties of French lilacs, treated with 40 to 60 mg. per liter of indolebutyric acid, resulted in 50 to 100 percent in 25 to 28 days when treated with lanolin preparations of indolebutyric acid (12 mg. per gram). However, the cuttings rooted only when taken in May. Talc carrier was used in powder preparations and the concentration varied from 1 to 40 mg. of hormone per gram of talc. Cuttings were dipped in water and then stirred in the powder to obtain a uniform coating on the base of the cutting.

Indolbutyric acid in this series of tests again proved superior to all other growth substances used. Rooting depends upon species, age, and vigor of shoot, time of

year when taken, kind and concentration of substances, method of application, rooting medium, and propagation temperatures.

Grace (15) compared the effect of indolebutyric acid upon Lonicera tartarica, Spiraea Vanhouttei, and Cornus alba, when applied as a liquid and as dust. He found that the treatment had a significant effect upon the number and length of roots. The dust treatment gave 62 percent rooting and the solution 42 percent; also, there was greater leaf development following dust treatment.

Stoutemyer (29) in treating hardwood locust, Robinia pseudo-acacia, with various hormone solutions found that concentrations strong enough to produce rooting injured the cuttings. If allowed to callus prior to treatment, no injury occurred. Treatment with indoleacetic acid (100 mg. per liter for 24 to 27 hours) resulted in 91.5 percent rooted cuttings. Stoutemyer found that rapid callusing of cuttings resulted in more root formation than when cuttings were slowly callused. Griffith (16) treated Sitka spruce and Douglas fir cuttings with the various hormone materials. He took cuttings at various intervals from fall to late spring and found that material taken from December to March gave the best results. Sitka spruce rooted best when treated with 25 mg. per liter of indolebutyric acid for 24 hours and Douglas fir responded best from 50 mg. per liter of indolebutyric acid for 24 hours.

## Secondary Chemical Treatment of Cuttings.

Since 1937, a second major phase in the chemical stimulation of rooting began; namely, secondary treatment of cuttings subsequent to the primary treatment. Went, Bonner, and Warner (33) found that thiamin (vitamin $B_1$) is a hormone of plant growth. Normally, the minute amount of thiamin required is supplied by other parts of the plant. Without thiamin no root growth is possible and under some conditions it may be the limiting factor of growth. Extensive laboratory experiments have been conducted by various workers in which excised root tips, plant stems, etc., are artificially cultured in synthetic mediums of known composition to determine the essential nutrient materials and growth stimulating substances. The same workers also treated pea stems for 20 hours with auxin and then placed them in a 2 percent sugar solution. At different times following the primary treatment, varying concentrations of thiamin were added to the solution. The presence of thiamin markedly increased the number and size of visible roots formed, except in the highest concentration. A histological examination of the pea stems 5 days after the auxin treatment showed many root primordia developed. The number which developed into roots depended upon the amount of available thiamin present. $B_1$ treatment prior to auxin treatment was without effect

upon root formation. Leafy lemon cuttings given secondary thiamin treatment 7 days following the primary treatment with indoleacetic acid rooted within 13 days and twice as many cuttings rooted on the thiamin-treated as on the controls. More and longer roots developed. From a series of 200 Camellia cuttings treated with indoleacetic acid (200 mg. per liter), 30 failed to root. Twelve of these cuttings were then treated with vitamin $B_1$ for 24 hours and all rooted vigorously 5 days later. The conclusion is that vitamin $B_1$, if applied at the appropriate time after roots have been initiated by auxin, greatly increases root development. Pearse (24) in his hormone treatment of softwood cuttings from deciduous fruit trees, used vitamin $B_1$ (1 mg. per liter) secondary treatment 10 to 12 days after treatment with indolebutyric acid. The thiamin did not increase rooting on any of the varieties tested, but the cuttings receiving the secondary treatment grew more vigorously after removal from propagation beds than those given only the primary indolebutyric acid treatment. Bonner (3) in culturing isolated root tips of tomato, pea, and corn in vitro found vitamin $B_1$ to be an important constituent for root growth. A concentration of 0.002 gamma per cc. of $B_1$ crystals had a marked stimulating effect. As early as 1922, Robbins (26) found beneficial effects from autolyzed yeast in the culture medium of corn root tips. Subsequent work has shown the $B_1$ constituent

of yeast to be partly responsible for the beneficial effect obtained.

Bonner (4) noted that thiamin was produced in the leaves of normal plants and in the seed of seedlings. Root tips artificially cultured until the inherent vitamin $B_1$ was exhausted would not continue growth till additional $B_1$ was supplied. Bonner (5) believes that the difference in ability of plants to root at various times of year is determined by the seasonal amounts of $B_1$ and auxin inherently present in the tissue.

Doak (11) in his root tip tests substantiates the conclusions of Went regarding the importance of $B_1$ as a growth factor. Doak used a mixture of nine amino acids as a secondary treatment for cuttings of Rhodendron Maddeni var. Jenkinsii following primary treatment with naphthaleneacetic acid. The rooting response to this treatment indicated that one or more of the amino acids used had an effect on rooting. Also, Went predicted that the rooting of some cuttings may be limited by a lack of amino acids.

## Preparation of Synthetic Nutrient Solutions.

P. R. White has conducted extensive laboratory tests in the development of yeast extract and synthetic culture mediums. In 1934 he (34) developed a nutrient solution

which produced rapid and sustained growth of excised tomato root tips for over a year through 52 passages. The medium contained 2 percent by weight of sucrose, 0.01 percent by weight of dried brewer's yeast, and the following nutrient salts: calcium nitrate, magnesium sulfate, potassium nitrate, potassium chloride, potassium acid phosphate, and ferric sulfate.

In 1937 White (35) again used tomato root tips in preparing a nutrient medium containing five nutrient salts, eleven accessory salts, iron, sucrose, and yeast extract. One-hundred mg. of yeast per liter of nutrient solution was found to be close to the optimum for excised tomato roots. This yeast extract medium was found by other workers as well as White to be the best nutrient solution yet developed and is used as a standard of comparison for other growth promoting mediums. In the analysis of yeast extract to determine the growth stimulating ingredients, White found that it readily separated into two fractions, one soluble in 85 percent alcohol and insoluble in absolute alcohol, and the other soluble only in 100 percent alcohol. Free amino acids were found quite abundant in the absolute-alcohol fraction and vitamin $B_1$ proved to be the most important constituent of the 85 percent alcohol soluble fraction. By testing a large number of amino acids, 9 were selected which if supplied in proper

amount would nearly replace the 85 percent-alcohol-soluble yeast fraction.

Further tests (36) were made using excised tomato root tips in various modifications of the standard nutrient solution. To the standard solution containing accessory salts, standard salts, sugar, and iron were made the following additions: amino acids only, vitamin $B_1$ only, and amino acids plus $B_1$. Yeast extract was added for the control. Growth response from the vitamin $B_1$ addition alone was inferior to the medium containing yeast extract. Where both $B_1$ and the amino acids were added, root growth was superior and was maintained at a level only somewhat inferior to that with yeast extract. Vitamin $B_1$ was here proven of basic importance. However, its growth promoting effect was only detectable in the presence of the accessory salts which were also found essential.

In 1938 White (37) developed a synthetic nutrient solution which he considered optimal for root growth. This medium contains 9 standard and accessory salts, 9 amino acids, vitamin $B_1$, and sucrose. It proved to be almost if not quite the equal of the standard yeast extract medium which apparently contained all necessary nutrient substances naturally supplied by the plant.

Bonner and Addicott (2) in pea root tip tests using the basic nutrient medium with and without $B_1$ and amino

acids found that roots growing in basic medium containing $B_1$ only developed normally. The medium containing $B_1$ plus the amino acid mixture produced striking results superior to that obtained from nutrient solutions containing either the $B_1$ or amino acids alone.

Thus within the last 8 years the use of chemicals as growth stimulators in plant propagation has developed. Hormones, vitamins, amino-acids, and nutrient salts are now used in commercial propagation practice and the entire subject is one of much interest to many workers.

# MATERIALS AND METHODS

## Solutions

Indolebutyric acid was used as the standard primary treatment for all tests and applied in three concentrations, 20, 40, and 80 milligrams per liter. The following solutions were used for secondary treatments: vitamin $B_1$, White's synthetic nutrient medium (37), White's yeast extract solution (35), vitamin B complex (Lilly), and indolebutyric acid. Treatment was effected by submerging the basal portion (1 to 2 inches) of the cuttings in aqueous solutions of the above materials for a standard treatment time of 24 hours. Secondary treatments were given 3 to 4 weeks following primary treatment after the cuttings had become well callused and the root primordia were presumably formed.

All solutions were prepared in the Horticulture Department laboratory and the stock materials stored in the cold storage room at 32 degrees Fahrenheit. Indolebutyric acid crystals were dissolved in sufficient 95 percent ethyl alcohol to give a stock solution of 2 milligrams per cubic centimeter. Yeast extract was prepared by boiling 10 grams of Magic Yeast in 500 cc. of water

for 30 minutes. The solution was then centrifuged and the clear liquid decanted. This stock solution was equivalent to 20 mg. of yeast per cc. Vitamin $B_1$ solution was prepared each time immediately previous to use. The separate solutions required for White's yeast extract and also the synthetic nutrient medium were prepared once for the entire project and stored at 32 degrees Fahrenheit. Weights of materials were accurately determined using a quantitative balance.

## Preparation of Cuttings

Wiss hand shears were used in making the cuttings, which varied in length from 5 to 6 inches, depending upon spacing of the nodes. The basal cut was made just below the node on all cuttings, unless otherwise stated. Filbert cuttings usually included three nodes while the doucine cuttings bore an indefinite number. Unless otherwise stated, all cuttings were sliced by removing two half-inch strips of bark from opposite sides of the base of each cutting. This increased the amount of cambium surface exposed and resulted in better callusing.

## Number of Cuttings Per Test

Sixty cuttings of each type of wood were used for each secondary solution. Since the indolebutyric acid primary treatment was used in three concentrations (20, 40, and 80 mg. per liter), 20 cuttings were used for each concentration and the entire group then given the same secondary treatment.

## Types of Cuttings

The following types of filbert cuttings were used: terminal, median, and basal portions of one-year-old suckers 4 to 5 feet long; terminal and median portions of one-year-old and two-year-old limb wood; cuttings in which the basal wound severed the junction of one-year-old and two-year-old wood. Both sliced and simple cuttings were used. Material was taken in January while buds were still dormant, and also in February and March when the buds were swelling and the leaves emerging. Barcelona variety was used in most of the work, although some Nottingham material was treated also.

The Doucine apple cuttings were prepared from one-year-old suckers. Cuttings used were classified according to diameter as follows: 0 to one-eighth inch,

one-eighth to three-sixteenths inch, and one-fourth to five-sixteenths inch. Some were grouped according to position on the stem. Types of cuttings included simple (not sliced), sliced, and heal cuttings of lateral spurs. All apple wood was dormant at the time of treatment.

## Greenhouse Equipment

Ample greenhouse space was devoted to the work. The propagation bed measured 25 feet long, 4½ feet wide, and 12 inches deep. The rooting medium consisted entirely of washed mason sand placed in the bed to a depth of 8 inches. Bottom heat was supplied by an electrical heating element thermostatically controlled. The temperature variation of the bed from 72 to 80 degrees F. was caused by fluctuation in outdoor temperatures and intensity of the sunlight.

Some of the Doucine apple cuttings were placed in an outdoor coldframe which consisted of a wooden frame 3 feet by 5 feet with a fine gravel bottom. It was filled to a depth of 8 inches with washed mason sand and covered with a white-washed glass sash which was lifted during warm days to permit ventilation. No artificial heat was supplied and the temperature ranged from 50 to 75 degrees F. The bed was sprinkled occasionally with water to maintain adequate moisture supply.

The rooting medium of sand was considered preferable to sand and peat mixture because it is more sterile, more easily penetrable by air, and introduces practically no variable factors.

## Insect Injury on Cuttings in the Greenhouse

The early work of this project was severely damaged by insect injury to the cuttings. Small dipterous larvae, of the family Sciaridae, heavily infested the propagation bed 4 weeks after the experimental work was started. Feeding of countless numbers of larvae upon the tender, basal, callus tissue of the cuttings resulted in a disintegration and rotting of the basal region. Cuttings attacked were practically destroyed within 7 days after the initial infestation.

E. O. Essig (12) states of the family Sciaridae, "The larvae live largely on decayed vegetable and organic materials but many infest the roots and tubers of plants and do some damage, particularly in the warm humus soils of greenhouses". The adult is 2 mm. long.

This pest was found difficult to control in the greenhouse. Since "nicofume" fumigation proved ineffective, the entire propagation bed was emptied and refilled with clean sand. The cuttings were thoroughly fumigated with

hydrocyanic acid gas and replaced in the bed. A large part of the work was repeated with subsequent material prepared one month later. A second infestation, though less severe than the former, appeared again after the bed was cleaned. Apparently the larvae preferred doucine cuttings to the filbert wood, since the latter if segregated from the apple was only lightly infested.

This trouble necessitated final observations of the injured cuttings one week following the secondary treatment. Some conclusions were made then, but most of the work was repeated and final conclusions based on subsequent material. Cuttings placed in the outdoor coldframe were uninjured, although a few larvae were present.

## EXPERIMENT I.

## VITAMIN $B_1$ SECONDARY TREATMENT

### Filbert Cuttings

Nottingham variety. Over 500 Nottingham cuttings taken January 3 while completely dormant were given indolebutyric acid treatment, followed by a secondary vitamin $B_1$ treatment 3 weeks later. The following types of cuttings were used: heal cuttings of one-year-old wood, simple and sliced cuttings from one-year-old wood, cuttings from the terminal portion of one-year-old wood, sliced cuttings from two-year-old wood, and cuttings in which the basal wound severs the junction of the one and two-year-old wood. Sixty cuttings were used for each type of wood. Each group was divided into three series of 20 and the three standard concentrations of indolebutyric acid (20, 40, and 80 mg. per liter) applied. Vitamin $B_1$ was used at the rate of 5 mg. per liter and the cuttings were immersed for the standard 24-hour period.

At the time of the secondary treatment after the cuttings had been in the propagation bed 3 weeks, callus tissue was well developed. However, insect injury prevented final observations. Cuttings prepared from one-year-old wood were better callused.

Barcelona variety. A series of 180 Barcelona cuttings taken January 5 was treated with indolebutyric acid, placed in the greenhouse propagation bed, and retreated with vitamin $B_1$ 4 weeks later. Cuttings were taken from one-year-old suckers 4 to 5 feet high. These were prepared from the terminal, median, and basal portions of the suckers and were segregated into groups of 60. The three standard concentrations of indolebutyric acid (20, 40, and 80 mg. per liter) were used. Due to insect injury, final observations were made one week following the secondary treatment. The callus tissue at this time on practically all of the cuttings was dead. Since callus growth was alive and active when the cuttings were removed from the sand for the second treatment, it is probable that the removal and replacement of the cuttings in the rooting medium is the main cause for the severe callus injury. Cuttings not removed from the rooting medium showed little injury on the callus tissue. No indication of root development was found.

A third group of 40 Barcelona cuttings was prepared from suckers one-eighth to one-fourth inch in diameter. These were taken February 24 after the buds had swollen prior to leafing. Indolebutyric acid treatment (20 and 40 mg. per liter) was applied and vitamin $B_1$ (5 mg. per liter)

given after the cuttings had been in the propagation bed 25 days. Twenty percent of the total were well callused when the $B_1$ treatment was given. None of the cuttings rooted and by April 25, all were dead. Apparently better callus formation develops on the cuttings prepared from dormant wood than on the more actively growing material.

There are some indications that indolebutyric acid stimulates callus formation. Twenty cuttings of a group of 40 were treated with 40 mg. indolebutyric acid and the remainder dipped in water for 24 hours. Twenty-five days later, 95 percent of the hormone-treated group were well callused and only 25 percent of the untreated callused.

To avoid any mechanical injury to the cuttings by removal from the rooting medium for secondary treatment, 3 series of 20 cuttings each were taken March 1 and given the standard indolebutyric acid treatments (20, 40, and 80 mg. per liter), and placed in the propagation bed. Once every three or four days thereafter, the cuttings were sprinkled with 1 liter of $B_1$ solution (5 mg. per liter). This experiment was continued for 7 weeks during which time a total of 11 liters of $B_1$ solution was used. All cuttings were dead by April 25 and no roots had developed.

Hitchcock and Zimmerman (21) found that such species as apple, hemlock, rhodendron, and many evergreens rooted

better if dipped in a concentrated indolebutyric acid solution containing from 1 to 20 mg. of hormone per cc. of alcohol. This test was made on 60 cuttings prepared in groups of 20 from the terminal, median, and basal portion of one-year-old suckers. Indolebutyric acid was dissolved in 95 percent ethyl alcohol to give a concentration of 15 mg. per cc. Cuttings were dipped one inch in the solution for 10 seconds, then removed and placed in the propagation bed. Upon examination 25 days later, 7 of the 60 were callused and the remainder dead. This hormone treatment seemed to prevent normal callusing.

An additional series of 400 Barcelona filbert cuttings was prepared March 27 from one-year-old suckers. The stems were actively growing and had produced from one-half to one inch of new terminal growth. Half of the cuttings were taken from tips bearing the terminal growing part and the remainder were prepared from the stem immediately below the tip. Indolebutyric acid was used at 20 and 40 mg. per liter and the cuttings placed in the greenhouse propagation bed.

No secondary treatments were given as nearly all cuttings were dead 4 weeks later. There was no callus formation when examination was made. Evidently filbert wood, when actively growing, is unsatisfactory for cutting material.

## Doucine Apple Cuttings

The doucine apple material for this project was supplied by Simpson Nursery Company of Troutdale, Oregon. One-year-old suckers 2 to 4 feet long were used. Suckers were grouped according to length: 2, 3, and 4 foot sizes.

For the first test, cuttings were divided into two groups, those from the lower portion (three-sixteenths inch in diameter) and those from the upper portion of the suckers (two-sixteenths in diameter). A group of 280 cuttings was prepared and the indolebutyric acid treatment given in the three regular concentrations (20, 40, and 80 mg. per liter). Vitamin $B_1$ treatment at the rate of 1 mg. per liter was given in aqueous solution 2 weeks later. At this time, all cuttings were heavily callused. However, due to insect injury, final observations were not made. Within certain limits, the larger the diameter of the cutting, the greater the amount of callusing.

A second series of 200 doucine cuttings was made from dormant suckers taken February 20. Secondary treatment with vitamin $B_1$ (5 mg. per liter) was given 3 weeks later at which time injury by the Sciarid larvae resulted in destruction of 60 percent of the cuttings. The cuttings were then planted in outdoor soil under field conditions. Final examination was made April 25, at which time all were dead.

To avoid insect damage, a third series of 160 douoine cuttings was taken February 20 and placed in the outdoor coldframe. They were tied in bunches of 20, placed bottom-side-up, and completely covered with sand. Excellent callusing developed five weeks later when the $B_1$ solution (5 mg. per liter) was applied. Cuttings were returned to the coldframe, placed upright, and inserted 4 inches into the sand. Cuttings were watered occasionally to maintain adequate moisture supply. Final examination was made April 25, at which time two series of 20 each had rooted 30 and 35 percent respectively. (Refer to table 4; also, see Fig. 5 and 6). Control series not given indolebutyric acid treatment callused only very lightly, while on treated cuttings the callus extended up the side of the slice.

## EXPERIMENT II

## REPEATED INDOLEBUTYRIC ACID TREATMENT

To determine further if the failure of filbert and doucine stock to root from cuttings is due to a deficiency of inherent plant hormone substances, repeated treatment with indolebutyric acid at weekly intervals was tried. Concentrations varied from 10 to 80 mg. per liter using various ages of wood and types of cuttings. Cuttings were treated for 24-hour periods during which time they were placed in a cool basement room with subdued light.

### Filbert Cuttings

Nottingham variety. Forty heal cuttings from one-year-old wood were treated three times with indolebutyric acid at 7-day intervals. Half of the group were treated with 20 mg. per liter and the remainder with a 40 mg. solution.

Barcelona variety. A series of 180 cuttings was prepared January 13 from one-year-old suckers. The group included 60 cuttings from each of the stem regions, terminal, median, and basal. Three indolebutyric acid concentrations (10, 20, and 40 mg. per liter) were used on each type of cutting and repeated at weekly intervals for five weeks.

The above experiment was repeated using 70 heal cuttings prepared from two-year-old wood. Five hormone treatments were given at weekly intervals, using the same three concentrations.

Observations on all filbert cuttings given repeated indolebutyric acid treatment showed progressive callus injury with each succeeding treatment. Callus tissue after three treatments was severely injured and by the time of the fifth treatment, all cuttings were dead. There was no apparent difference in callus activity from the various concentrations used. The injury is apparently mechanical from removing and replacing the cuttings in the rooting medium; also, the hormone solution probably causes some injury.

## Doucine Apple Cuttings

A series of 300 doucine apple cuttings measuring three-sixteenths to one-fourth inch in diameter was used in experiments with repeated indolebutyric acid treatments. Sixty cuttings were given two indolebutyric acid treatments, 120 cuttings were given three indolebutyric acid treatments, and 120 cuttings were treated four times. Indolebutyric acid was used at concentrations of 10, 20, and 30 mg. per liter.

The reaction of doucine apple cuttings to repeated hormone treatments is similar to that of filbert. However, callus formation on the apple stock is tougher and more vigorous in growth and not as easily injured by removal from the propagation bed as that of filbert. Three treatments caused considerable injury and the application of four or more acid treatments is of no value. The injury is again caused mainly by the removal and replacement of cuttings in the sand rooting medium.

## EXPERIMENT III

### SECONDARY TREATMENT WITH WHITE'S YEAST EXTRACT SOLUTION

The yeast extract solution developed by P. R. White (34) is generally accepted by physiologists as the superior nutrient medium for plant tissue cultures. Materials and concentrations of this solution as given by White are as follows:

Table I.
P. R. White's Yeast Extract Medium

| | Amount per liter of stock solution. |
|---|---|
| **Solution # 1** | |
| Calcium nitrate | 14.0 grams |
| Potassium chloride | 6.7 grams |
| Potassium nitrate | 8.1 grams |
| Potassium acid phosphate | 1.22 grams |
| Dissolve the above salts in 800 cc. of water | |
| Magnesium sulfate | 7.4 grams |
| Dissolve separately in 200 cc. of water and then pour slowly into first solution | |
| **Solution # 2** | |
| Zinc chloride | 30.0 millegrams |
| Sodium silicate | 27.0 millegrams |
| Aluminum sulfate | 107.0 millegrams |
| Potassium iodide | 15.0 millegrams |
| Boric acid | 32.0 millegrams |
| Sodium chloride | 56.0 millegrams |
| Manganese sulfate | 44.0 millegrams |
| Nickel chloride | 4.0 millegrams |
| Lithium chloride | 3.9 millegrams |
| Cobalt chloride | 4.0 millegrams |
| Copper sulfate | 1.0 millegrams |
| Dissolve the above salts separately in 1000 cc. of water | |

Table # 2 - continued

| | Amount per liter of stock solution |
|---|---|
| Ferric sulfate<br>Prepare separately each time solution is to be used. | 2.50 millegrams |
| Sucrose<br>Prepare separately each time solution is to be used. | 20.0 millegrams |
| Yeast (Brewer's) | 100.0 millegrams |

To prepare one liter of P. R. White's Yeast extract solution, use 10 cc. of solution # 1 plus 10 cc of solution # 2, and add 2.5 mg. of ferric sulfate, 20 mg. of sucrose and sufficient yeast extract to give the equivalent of 100.0 mg. per liter and make up to volume.

This solution contains all important nutrient materials which have been proven necessary by the excised root tip cultures. The use of this medium as a secondary treatment for hardwood cuttings should theoretically supply whatever growth stimulating substances are deficient in the tissues.

## Filbert Cuttings

Nottingham variety. A group of 120 cuttings was treated with indolebutyric acid (20, 40, and 80 mg. per liter) January 1 and placed in the greenhouse propagation bed till February 6 when they were removed and the yeast

extract secondary treatment given. At this time the callus tissue, which was apparently well formed prior to February 6, had disintegrated. Insect injury was in part responsible. Cuttings of this group were heal cuttings prepared from one-year-old and two-year-old wood.

Barcelona variety. A series of 180 cuttings was prepared from one-year-old suckers in three series; 60 cuttings from the terminal, 60 from the median, and 60 from the basal portion of the suckers. The three standard concentrations of indolebutyric acid were used. In addition, it was used at 120 mg. per liter on one series of cuttings without injury from the chemical. The cuttings were placed in the propagation bed for one month and the yeast extract treatment then given. Of the cuttings prepared from the median and basal part of suckers, 50 percent were well callused at the time of the secondary treatment. These cuttings were all sliced and callus formation thereby more extensive than on non-wounded material. The concentration of indolebutyric acid used (20 to 80 mg. per liter) apparently has little effect upon the amount of callus formation, but when no primary hormone treatment is given, less callus tissue develops. Callus tissue on cuttings prepared from the terminal portion of suckers was dead at the time of secondary treatment.

Sixty heal cuttings, prepared from two-year-old wood,

were given the yeast extract secondary treatment one month following the indolebutyric acid application. Of this number 31 were well callused at the time of secondary treatment. Insect injury on these and the above group of cuttings prevented final observations.

A third group of 60 cuttings was taken February 24 after the buds had swollen. These were prepared from the middle part of one-year-old suckers and varied in diameter from one-eighth to one-fourth inch. Yeast extract solution was applied 4 weeks after the primary treatment, at which time cuttings were well callused. None of the cuttings had rooted by April 25.

To avoid any mechanical injury to the cuttings by removal from the sand for secondary treatment, 60 cuttings were treated with indolebutyric acid and placed in greenhouse propagation bed and the yeast extract solution sprinkled on the sand. The concentration of materials as given by White was modified for this experiment. Yeast extract was used at the rate of 400 mg. per liter instead of 100 mg. as previously used. All other materials were used at the standard concentrations (See table I.). One liter of extract was applied each 3 or 4 days to maintain optimum moisture content. No other water was used apart from the initial water content of the sand and the only

moisture surrounding the cuttings was that of the yeast extract solution. The treatment was continued for 8 weeks. Although cuttings remained alive throughout the experiment as evidenced by the single green leaf allowed to remain on each cutting , no roots were produced and callus tissue was largely disintegrated at the end of the experiment.

## Doucine Apple Stock

A series of 480 cuttings prepared from one-year-old doucine suckers taken January 12 was used in this test. Diameter of the cuttings in different series varied from one-eighth to five-sixteenths inch. Indolebutyric acid primary treatment was applied at the rate of 10, 20, 40, and 80 mg. per liter and the cuttings dipped one to two inches in the solution for the usual 24-hour period.

The yeast extract treatment was not applied, due to severe insect injury to callus tissue. From 50 to 100 percent of the cuttings in this group were completely destroyed. Apparently, the larvae are attracted more to doucine wood than filbert.

A second series of 120 doucine cuttings was prepared February 20 to duplicate the above group. The cuttings were placed in the propagation bed after indolebutyric acid primary treat, and removed three weeks later for secondary

treatment with yeast extract. They were then placed in fresh-spaded sandy loam soil outside. However, insect injury was again considerable. Final examination was made 5 weeks later, at which time none of the cuttings were alive.

To eliminate the insect injury, a third series of 220 cuttings was placed in an outdoor coldframe. Primary treatment was given February 20 and the cuttings inverted and covered with sand in the frame. Leaf buds were thus kept dormant and callusing developed normally. Cuttings were given secondary yeast extract treatment five weeks later and were then placed upright in the coldframe and inserted to a depth of 4 inches in the sand. Cuttings of this group were classified according to diameter and varied from one-eighth to five-sixteenths inch. Those five-sixteenths inch in diameter developed 2 to 3 times as much callus tissue as those one-eighth inch in diameter.

Final examination was made April 25. In one series (table 4) 20 percent of the cuttings rooted. On many of the cuttings which did not root, callus tissue had rotted, while on others of the same series, it was still active.

## EXPERIMENT IV

## SECONDARY TREATMENT WITH P. R. WHITE'S SYNTHETIC NUTRIENT SOLUTION

The use of P. R. White's synthetic nutrient solution (37) in root tip cultures proved to be almost if not quite the equal of the standard yeast extract medium and apparently contained all the materials naturally supplied by the plant for root growth. It was therefore considered feasible to use this solution also as a secondary treatment for apple and filbert hardwood cuttings. The materials of White's synthetic nutrient solution are as follows:

### Table # 2

### White's Synthetic Nutrient Solution

| Chemical | Milligrams per liter |
|---|---|
| Calcium nitrate | 70.0 Milligrams |
| Potassium nitrate | 80.0 " |
| Potassium chloride | 65.0 " |
| Potassium acid phosphate | 12.55 " |
| Potassium iodide | 0.75 " |
| Manganese sulfate | 4.40 " |
| Ferric sulfate | 2.50 " |
| Zinc sulfate | 1.50 " |
| Boric acid | 1.60 " |
| dl-phenylalanine | 1.50 " |
| dl-valine | 0.15 " |
| dl-lysine | 1.50 " |
| dl-serine | 0.05 " |
| dl-isoleucine | 0.0015 " |
| d-glutamic acid | 5.00 " |
| l-histidine | 1.50 " |
| l-proline | 0.50 " |
| l-leucine | 0.015 " |
| Vitamin $B_1$ | 1.00 " |
| Sucrose | 20.00 Grams |

## Filbert Cuttings

Nottingham variety. Eighty heal cuttings from two-year-old wood (one-eighth to three-sixteenths inch in diameter) were divided into groups of 20 and treated with 10, 20, 40, and 80 mg. per liter of indolebutyric acid. These were placed in the greenhouse propagation bed and secondary treatment with the synthetic nutrient solution given 4 weeks later.

Barcelona variety. A series of 200 cuttings prepared from one-year-old suckers was used in the second test. Cuttings were segregated into three groups, those from terminal part of the stem, those from the median, and those from the basal portion. Sixty cuttings were taken for each group and indolebutyric acid used at the three concentrations of 20, 40, and 80 mg. per liter. Insect infestation in the propagation bed necessitated final examination on both the Barcelona and Nottingham cuttings one week following the secondary treatment. However, at this time the callus tissue of nearly all cuttings, which was healthy and vigorous one week previous, was dead and had no apparent potentiality for root development. Control cuttings which were not removed for the secondary treatment bore normal active callus. Hence, retreatment of cuttings

by removing them from the propagation bed is detrimental to potential rooting ability.

A second group of 60 cuttings prepared from the median portion of one-year-old suckers was treated February 24, 20 cuttings being used for each concentration of indolebutyric acid. After 3 weeks in the propagation bed, secondary treatment with synthetic nutrient solution was given and the cuttings replaced. Insect injury was not significant. No roots had developed by April 25 (8 weeks after primary treatment) when final observations were made. Cuttings were still alive as evidenced by the single green leaf on each cutting.

## Doucine Apple Cuttings

A group of 320 cuttings varying from one-eighth to five-sixteenths inch in diameter was treated with indolebutyric acid (three concentrations) and placed in the greenhouse propagation bed January 12. All cuttings were removed and retreated 19 days later with White's synthetic nutrient solution. At this time many cuttings had developed short roots not over three-fourths inch long. The primary indolebutyric acid treatment on this group was identical with that of all other tests and yet appreciable rooting did not occur elsewhere. The table following indicates the extent of rooting.

Table # 3

Rooting of Dousine Cuttings in Propagation Bed

| Number of cuttings | Type of wood | Diameter of wood (Inches) | Days after primary treatment | Conc. of I.B.* Soln. (mg. per liter) | Total number roots (0-¾ inch) |
|---|---|---|---|---|---|
| 20 | sliced | 3/16-¼ | 22 | 20 | 6 |
| 20 | " | " | 22 | 40 | 5 |
| 20 | " | " | 22 | 80 | 0 |
| 20 | " | ¼-5/16 | 22 | 80 | 1 |
| 20 | " | " | 22 | 40 | 1 |
| 20 | " | " | 22 | 80 | 1 |
| 20 | " | 3/16-¼ | 22 | 10 | 5 |
| 20 | " | " | 19 | 20 | 5 |
| 20 | " | " | 19 | 40 | 1 |
| 20 | " | " | 19 | 10 | 11 |
| 20 | " | " | 19 | 20 | 4 |
| 20 | " | " | 19 | 40 | 0 |
| 20 | simple | ⅛ | 19 | 10 | 0 |
| 20 | " | " | 19 | 20 | 1 |
| 20 | " | " | 19 | 40 | 0 |

* Indolebutyric acid

Insect injury in the propagation bed prevented further observation on this group of cuttings.

A second series of 100 dousine cuttings was treated with indolebutyric acid and placed in the propagation bed. However, larval injury occuring 3 weeks later destroyed the series.

A third series of 200 cuttings was given the primary treatment and callused in the outdoor coldframe as previously described. Cuttings were grouped according to diameter

of the wood into three classes; one-eighth inch, three-sixteenth inch, and one-fourth inch. Five weeks later the synthetic nutrient solution was applied and the cuttings returned to the cold frame. Final examination was made April 25. Refer to table # 4 for rooting response. Also see Fig. 7. Apparently with proper treatment and propagation conditions significant rooting response could be obtained.

## EXPERIMENT V

## SECONDARY TREATMENT WITH VITAMIN B COMPLEX

Vitamin B complex (Lilly) is a commercial preparation sold in capsul form and contains the following materials:

| | Conc. per pulvule |
|---|---|
| Vitamin $B_1$ | 1.00 milligram |
| Riboflavin | 0.323 milligram |
| Vitamin $B_6$ HCl | 200 gamma |
| Pantothenic acid | 250 gamma |
| Nicotinic acid | 2.00 milligram |

Plus other vitamin B factors from liver-stomach concentrate.

Vitamin B complex was used as a secondary treatment by dissolving one pulvule in each liter of water. Cuttings to be treated were then submerged one to two inches in the solution for the standard 24-hour period.

### Filbert Cuttings

A series of 620 cuttings prepared January 11 was used in the first test with vitamin B complex. Indolebutyric acid was used in the three concentrations 20, 40, and 80 mg. per liter and the B complex solution prepared by dissolving one pulvule in each liter of solution. Cuttings used in this test include the following types: heals from one-year-old, two-year-old, and three-year-old wood; simple cuttings bearing the terminal bud, and sliced cuttings from one-year-old wood; cuttings from the terminal,

median, and basal portion of suckers. Vitamin B complex was applied to this group of cuttings 3 weeks following primary treatment. Insect injury necessitated final observations one week following the secondary treatment. Callus tissue on most of the cuttings at this time was dead. Injury caused by removal of cuttings from the propagation bed for secondary treatment is largely responsible.

A second series of 60 sliced cuttings was prepared from one-year-old suckers and treated with the standard indolebutyric acid February 24 and placed in the propagation bed. Thirty-seven of the 60 cuttings were well callused by March 18 when the secondary treatment was given. Final observations were made April 25. At this time cuttings were still alive, but no roots were formed.

To eliminate the injury otherwise caused by removing cuttings from the sand for secondary treatment, 60 cuttings were placed in the propagation bed after indolebutyric acid treatment and watered with vitamin B complex. One liter of solution was sprinkled on the cuttings once every 3 or 4 days for a period of 8 weeks. No water was added to the bed. Final examination was made April 25, but no roots were produced on any of the material. Some of the cuttings were still alive as evidence by the single green leaf allowed to remain per cutting (See figure 8).

## Doucine Apple Cuttings

A group of 380 doucine apple cuttings was used in the first test with vitamin B complex. Indolebutyric acid was used at 10, 20, 40, and 80 mg. per liter and the B complex (one pulvule per liter) was applied three weeks later. Cuttings were placed in the propagation bed. Both simple, sliced, and heal cuttings were prepared from one-year-old suckers varying from one-eighth to five-sixteenths inch in diameter. Due to severe insect injury, final observations were made one week following the secondary treatment. From the 380 cuttings, 11 had rooted. This cannot be considered a true test of the treatments due to insect injury.

A series of 240 cuttings including controls was treated with indolebutyric acid (20, 40, and 80 mg. per liter) and callused in an outdoor coldframe for 5 weeks prior to secondary treatment with vitamin B complex. Cuttings were prepared from one-year-old suckers and classified as small (one-eighth inch in diameter), medium (three-sixteenths inch in diameter), and large (one-fourth inch in diameter). Callus formation on cuttings three-sixteenths inch in diameter is much less than on cuttings one-fourth inch in diameter.

Final examination of these cuttings made 8 weeks after the primary treatment gave results as indicated in table 4. Ten percent of the cuttings in two series were rooted.

Doucine cuttings produce a typically weak and unbalanced root system. Two or three large fleshy roots usually appear instead of a strong system of many well balanced and well spaced roots.

Table # 4

Doucine Apple Cuttings Treated With Various Primary and Secondary Solutions

| Number Cuttings Per Test | Primary Treatment (mg. I.B.)* | Secondary Treatment | Diameter of wood (Inches) | Number Cuttings Rooted | Total Number Roots | Number cuttings strong rooted | Number cuttings weak rooted | Average Length of roots (Inches) |
|---|---|---|---|---|---|---|---|---|
| 20 | 20 | Vitamin $B_1$, 5 mg. | 3/16 | 7 | 44 | 2 | 5 | 3/8 |
| 20 | 40 | Vitamin $B_1$, 5 mg. | 3/16 | 0 | 0 | - | - | - |
| 20 | 80 | Vitamin $B_1$, 5 mg. | 3/16 | 0 | 0 | - | - | - |
| 20 | 20 | Vitamin $B_1$, 5 mg. | 3/16 | 1 | 7 | 0 | 1 | 3/8 |
| 20 | 80 | Vitamin $B_1$, 5 mg. | 3/16 | 0 | 0 | - | - | - |
| 20 | 20 | Vitamin $B_1$, 5 mg. | 1/8 | 6 | 33 | 1 | 5 | 1/2 |
| 20 | 80 | Vitamin $B_1$, 5 mg. | 1/8 | 0 | 0 | - | - | - |
| 20 | 40 | Vitamin $B_1$, 5 mg. | 1/4 | 0 | 0 | - | - | - |
| 20 | 20 | Yeast Extract | 3/16 | 5 | 12 | 0 | 5 | 1/4 |
| 20 | 40 | Yeast Extract | 3/16 | 0 | 0 | - | - | - |
| 20 | 80 | Yeast Extract | 3/16 | 1 | 9 | 1 | 0 | 5/8 |
| 20 | 20 | Yeast Extract | 3/16 | 1 | 1 | 0 | 1 | 3/4 |
| 20 | 40 | Yeast Extract | 3/16 | 1 | 10 | 1 | 0 | 5/8 |
| 20 | 80 | Yeast Extract | 3/16 | 0 | 0 | - | - | - |
| 20 | 20 | Yeast Extract | 1/8 | 4 | 21 | 1 | 3 | 1/4 |
| 20 | 40 | Yeast Extract | 1/8 | 3 | 13 | 1 | 2 | 5/8 |
| 20 | 80 | Yeast Extract | 1/8 | 0 | 0 | - | - | - |
| 20 | 40 | Yeast Extract | 1/4 | 0 | 0 | - | - | - |
| 20 | 80 | Yeast Extract | 1/8 | 0 | 0 | - | - | - |
| 20 | 40 | Yeast Extract | 1/4 | 0 | 0 | - | - | - |
| 20 | 40 | None | 1/4 | 0 | 0 | - | - | - |
| 20 | 20 | Synthetic Nutrient | 3/16 | 2 | 8 | 1 | 1 | 3/8 |
| 20 | 40 | Synthetic Nutrient | 3/16 | 0 | 0 | - | - | - |
| 20 | 80 | Synthetic Nutrient | 3/16 | 0 | 0 | - | - | - |
| 20 | 40 | Synthetic Nutrient | 3/16 | 0 | 0 | - | - | - |
| 20 | 40 | Synthetic Nutrient | 3/16 | 0 | 0 | - | - | - |
| 20 | 80 | Synthetic Nutrient | 3/16 | 0 | 0 | - | - | - |
| 20 | 40 | Synthetic Nutrient | 1/8 | 3 | 18 | 2 | 1 | 3/8 |
| 20 | 80 | Synthetic Nutrient | 1/8 | 0 | 0 | - | - | - |
| 20 | 40 | Synthetic Nutrient | 1/4 | 0 | 0 | - | - | - |
| 20 | 40 | Synthetic Nutrient | 1/4 | 1 | 3 | 0 | 1 | 1/4 |
| 20 | 20 | Vitamin B Complex | 3/16 | 2 | 6 | 0 | 2 | 1/2 |
| 20 | 40 | Vitamin B Complex | 3/16 | 0 | 0 | - | - | - |
| 20 | 80 | Vitamin B Complex | 3/16 | 0 | 0 | - | - | - |
| 20 | 20 | Vitamin B Complex | 3/16 | 1 | 4 | 0 | 1 | 1 |
| 20 | 40 | Vitamin B Complex | 3/16 | 0 | 0 | - | - | - |
| 20 | None | None | 3/16 | 0 | 0 | - | - | - |
| 20 | Water | Vitamin B Complex | 3/16 | 1 | 1 | 0 | 1 | 1 |
| 20 | 40 | None | 3/16 | 0 | 0 | - | - | - |
| 20 | Water | None | 3/16 | 0 | 0 | - | - | - |
| 20 | 20 | Vitamin B Complex | 1/8 | 2 | 6 | 0 | 2 | 1/8 |
| 20 | 40 | Vitamin B Complex | 1/8 | 0 | 0 | - | - | - |
| 20 | 40 | Vitamin B Complex | 1/4 | 0 | 0 | - | - | - |

* Milligrams indolebutyric acid per liter.

## EXPERIMENT VI

### SECONDARY TREATMENT OF MANETTI ROSE CUTTINGS

A limited experiment was conducted using the various nutrient solutions as secondary treatments on Manetti rose cuttings. Cuttings from wood three-sixteenths to five-sixteenths inch in diameter were prepared in the usual manner and placed in the greenhouse propagation bed. Indolebutyric acid was used at 20 mg. per liter and each of the secondary nutrient solutions applied at the same concentrations as for the apple and filbert cuttings. Fifteen cuttings were used per test.

Results are tabulated in the following table:

Table # 5

Rooting of Manetti Rose Cuttings

| Treatment | Number cuttings per test | Number weak rooted | Number strong rooted | Number roots per cutting - average |
|---|---|---|---|---|
| 10 mg. I.B.* plus 5 mg. $B_1$** | 15 | 8 | 7 | 8.13 |
| Water primary no secondary | 15 | 4 | 10 | 5.53 |
| 10 mg. I.B. no secondary | 15 | 4 | 11 | 11.80 |
| 10 mg. I.B. water secondary | 15 | 9 | 6 | 8.86 |
| 10 mg. I.B. yeast extract sec. | 15 | 6 | 9 | 8.80 |
| 10 mg. I.B. synthetic nutrient | 15 | 7 | 5 | 5.73 |
| 10 mg. I.B. vitamin B complex | 15 | 10 | 4 | 6.13 |

* Milligrams indolebutyric acid per liter.
** Milligrams vitamin $B_1$ per liter.

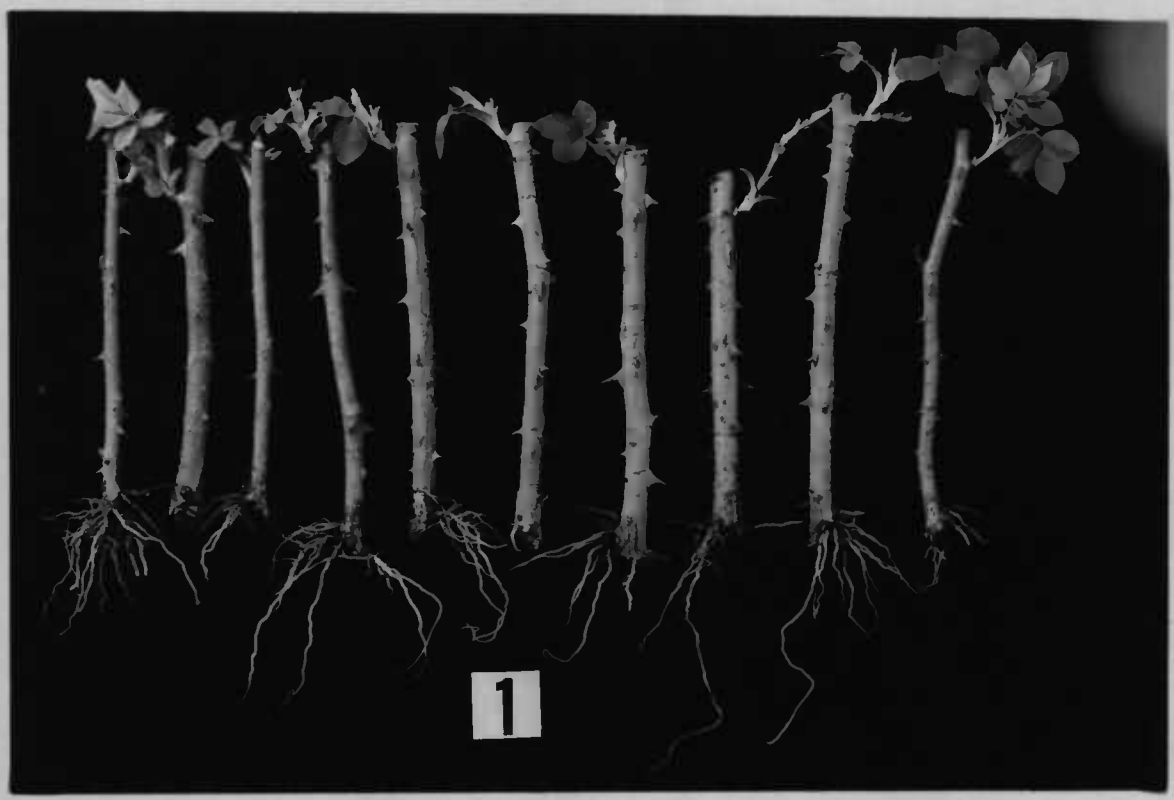
From the foregoing table, it is evident that there is no significant difference in rooting response from the various secondary treatments. Cuttings treated with indolebutyric acid without secondary treatment rooted better than any of the cuttings given additional secondary treatment. Twice the number of roots per cutting were produced on cuttings treated only with indolebutyric acid as compared to those receiving no chemical treatment. There is a significant increase in the number of roots produced by cuttings receiving only the indolebutyric acid treatment as compared to those receiving any of the secondary treatments in addition to the primary treatments.

Figure 1.

Rose cuttings treated with 10 mg. indolebutyric acid March 5. Vitamin $B_1$ (5 mg. per liter) secondary treatment given March 18. Photograph taken April 10.

Figure 2.

Rose cuttings not given primary or secondary treatment (control series). Photograph taken after cuttings had been in propagation bed 36 days.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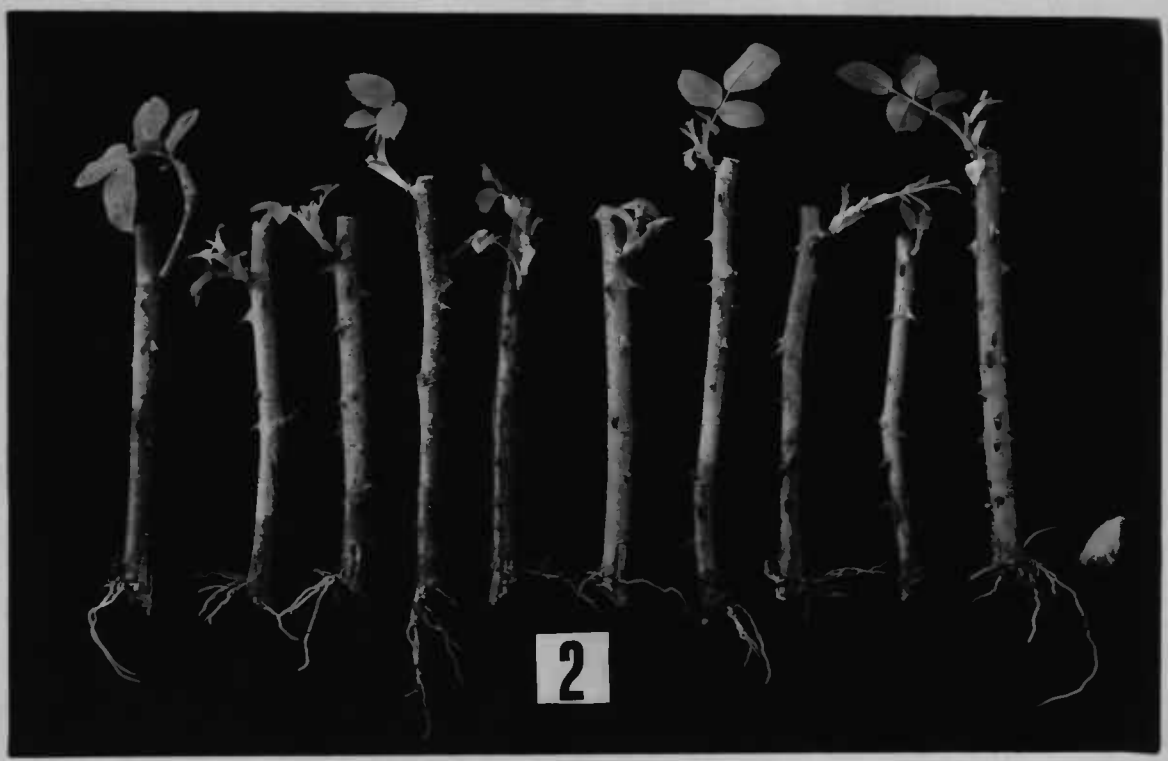
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Figure 3.

Rose cuttings treated with 10 mg. indolebutyric acid. No secondary treatment given. Photograph taken 36 days after primary treatment.

Figure 4.

Rose cuttings treated with 10 mg. indolebutyric acid March 5. Vitamin B complex secondary treatment applied March 18. Photograph taken April 10.


3


4

Figure 5.

Doucine apple cuttings (one-eighth inch diameter) treated with indolebutyric acid (20 mg. per liter) February 20; vitamin $B_1$ (5 mg. per liter) applied March 25; photograph taken April 30.

Figure 6.

Doucine apple cuttings (three-sixteenths inch diameter) treated with indolebutyric acid (20 mg. per liter) February 20; vitamin $B_1$ (5 mg. per liter) applied March 25; photograph taken April 30.

Note unusually well-developed root system on one of the cuttings.

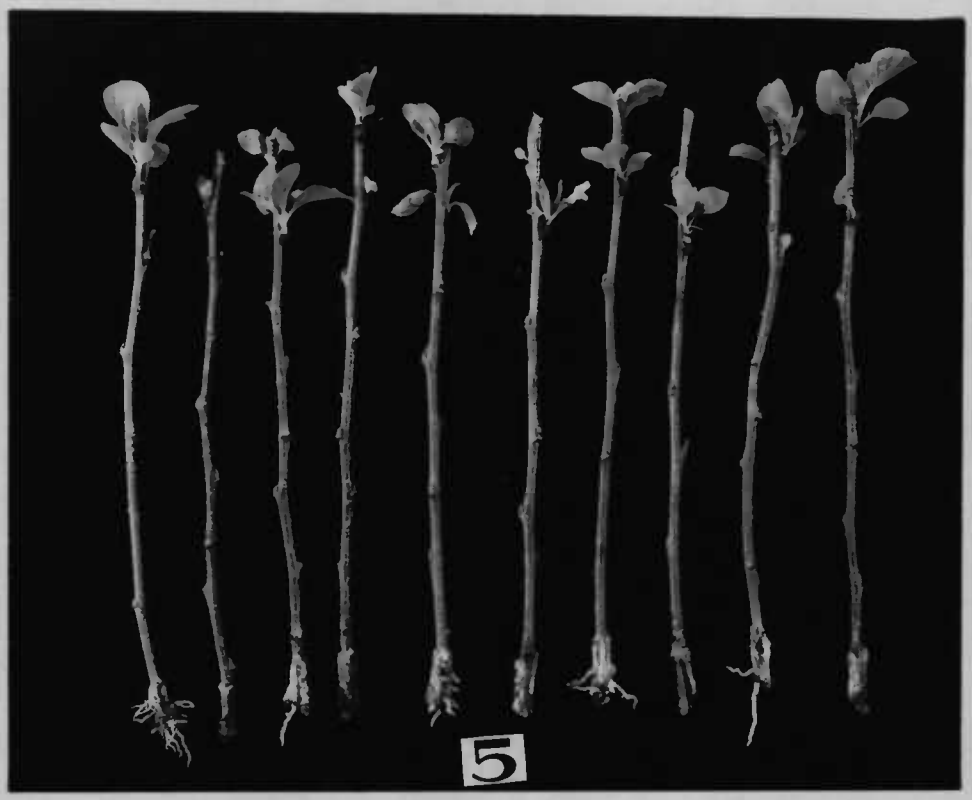
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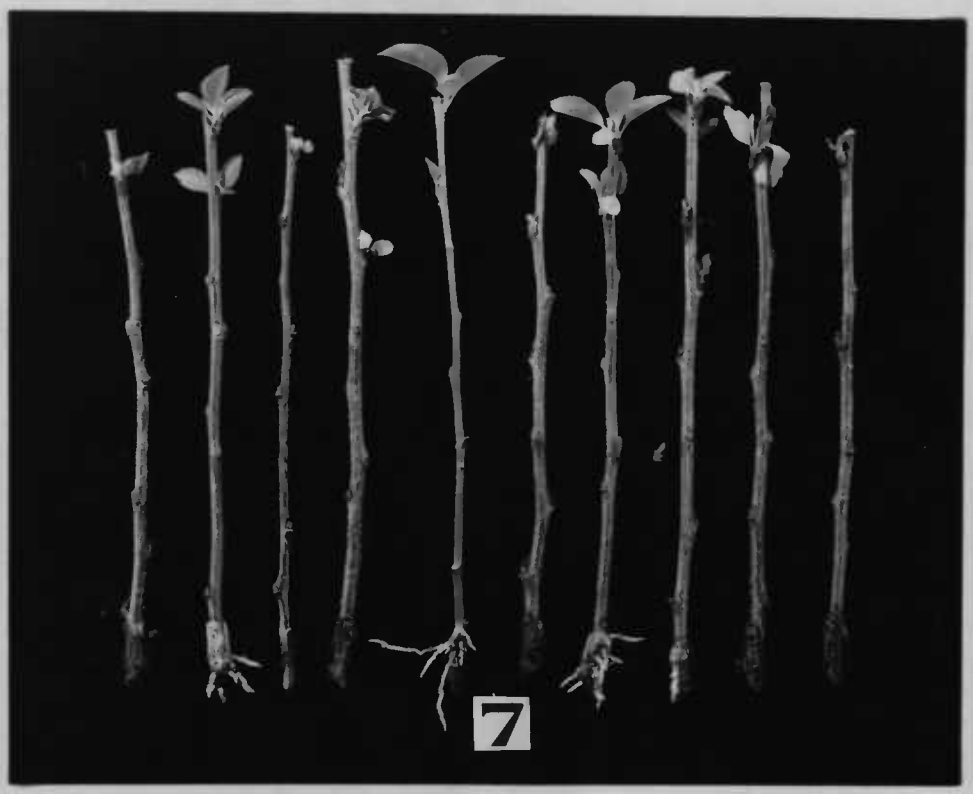
Figure 7.

Doucine apple cuttings (one-eighth inch diameter) treated with indolebutyric acid (40 mg. per liter) February 20; secondary treatment with P. R. White's synthetic nutrient medium gaven March 25; photograph taken April 30.

Figure 8.

Filbert cuttings from one-year-old suckers (one-eighth to one-fourth inch diameter) watered with vitamin B complex solution for 8 weeks. Shows typicak callouse development on filbert cuttings.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# SUMMARY

A study has been made of the rooting response of filbert, doucine apple, and Manetti rose cuttings to treatment with various growth substances, applied as supplementary treatments to indolebutyric acid. Substances used include vitamin $B_1$, P. R. White's yeast extract solution, P. R. White's synthetic nutrient medium, vitamin B complex, and indolebutyric acid. Various types of cuttings and different ages and sizes of wood were used.

Filbert cuttings were not found to respond to indolebutyric acid alone or followed by any of the secondary treatments.

Doucine apple cuttings were found to respond to indolebutyric acid treatment, but secondary treatments appeared to be of no benefit. The data of these experiments indicate that it should be possible to obtain vigorous rooting of doucine apple cuttings if given proper conditions of chemical treatment, cutting material, propagation mediums, and temperatures.

The removal of cuttings from the propagation bed for secondary treatment is apparently detrimental to the life of the cuttings and to ultimate root formation. This is especially true of filbert cuttings.

The use of various secondary treatments on Manetti rose cuttings subsequent to indolebutyric acid treatment gave no significant results.

It was found that feeding of the Sciarid fly larvae upon the callus tissue caused severe injury to the basal portion of the cuttings. The doucine apple wood was injured more than the filbert.

# DISCUSSION

More than 2600 filbert and 2500 doucine apple cuttings were used in these experiments, but in no case was there any apparent value from secondary treatment. In fact, when final examinations were made, the healthiest cuttings with the most active callus were those receiving only the primary indolebutyric acid treatment. With the filbert cuttings, good callus was found in most cases, but no roots were produced in any of the treated or untreated lots, even after a period of two months in the propagation bed. With the doucine apple cuttings, appreciable rooting occurred in a few of the test series which were given the primary indolebutyric acid treatment. However, cuttings given secondary treatment did not produce root systems superior to those receiving only the primary treatment.

Manetti rose, a plant known to respond to growth materials, was used to obtain comparative data for the various secondary treatments. Rooting was improved by the indolebutyric acid as compared to the untreated controls. However, there was no significant variation in rooting response from the various secondary treatments and all cuttings receiving secondary treatment were inferior to the series treated with indolebutyric acid only.

In practically all cases, considerable to severe injury resulted from secondary treatments. The injury to

filbert cuttings from secondary treatment is caused mainly by mechanical bruising of the callus tissue when removing and replacing the cuttings in the rooting medium. Filbert callus is very tender and delicate and even slight injury apparently causes the complete disintegration of the basal growth which is followed by death of the cutting. Any type of secondary treatment for filbert cuttings must be applied without disturbing the cuttings in the propagation bed.

Doucine cuttings produce far more vigorous and resistant basal callus growth than filbert and are not seriously injured by removal from the rooting medium for retreatment if the operation is done carefully. Doucine cuttings callused exceptionally well and displayed every preliminary appearance of root formation. Some growth factor or physiological condition is evidently lacking which, if it could be supplied or corrected, would probably result in satisfactory rooting.

Various factors make the present work an inconclusive test. The Sciarid fly larvae severely injured much of the material in the greenhouse, thus introducing an important variable. This was largely overcome by duplicate tests made later. Also intensity of sunlight during warm spring days increased greenhouse atmospheric temperatures from the normal 68 degrees to 85 degrees F. Temperatures of the

outdoor coldframe were also unfavorable for propagation work. The sand temperature varied from 52 to 72 degrees F. and the air temperatures usually somewhat higher. This, however, may emphasize the importance of the limited rooting response which was obtained.

Further experiments are necessary to obtain conclusive data both on the filbert and doucine cuttings. The studies of watering filbert cuttings with nutrient solutions should be continued. The same work should be done using doucine apple cuttings. Tests should be conducted to determine the relationship between time of application of the secondary treatment and ultimate rooting response. The various primary and secondary treatments should be repeated on both filbert and doucine cuttings taken in June while in active growth and also in July immediately following formation of terminal buds.

It would be of value to use other species in further tests of this type. Such species as Manetti rose, American holly, and lilac might give valuable results.

This study indicates the difficulty of rooting filbert from cuttings. However, it is quite probable that doucine apple cuttings in proper physiological condition can be rooted if chemically treated and placed in a propagation bed equipped with bottom heat (70 to 75 degrees F.)

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