

Some Factors Affecting the Rooting of Citrus Species¹

R. P. ARMOUR²

INTRODUCTION

The propagation of citrus species by cuttings is not new. Citron has been propagated by cuttings for centuries and Garey (8) spoke of the easy rooting of lemon and citron cuttings as being well known in 1882. As early as 1924 Swingle, Robinson and May (32) had demonstrated the use of a solar propagating frame for rooting citrus and other subtropical plants. A year later Mowry (21) reported the successful propagation of the "Rough" lemon by means of leaf cuttings, and by 1926 Halma (9) had presented a general treatise on the subject, based on his experiments with three of the main commercial species of citrus, i. e., lemon, orange and grapefruit.

Commercial propagation by cuttage of the most important varieties of citrus, however, met with limited success, and Halma, as quoted by Webber (37), doubted if any method, other than budding on seedling rootstocks, would be utilized commercially in the near future, at least in California. Comparative studies on the initial and subsequent size of citrus cuttings and buddlings were also made by Halma (10) in 1940. He concluded that the rootstock was not a factor in the decreasing correlation between initial and subsequent tree size in the grove.

In the field of research, however, the importance of the technique was early recognized (32). In 1924 Malloch (19) pointed out the significance of asexual propagation as an aid to the breeding of fruit tree rootstocks, particularly in maintaining uniformity in the stock itself. Others (24, 30) have stressed the desirability of studies on fruit tree rootstocks which would be commercially valuable when propagated by vegetative means.

-
1. The work here reported is taken from the thesis submitted to the Graduate Council of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science.
 2. Assistant Director, Escuela Agrícola Panamericana, Honduras.

The so-called "miniature" citrus trees used in the ornamental horticulture industry are, at present, extremely popular and they, too, are produced from cuttings of selected citrus species.

For the reasons listed above, a survey of the techniques involved in the propagation of citrus by cuttings acquires new emphasis. The purpose of the present investigation is to examine several basic factors which, according to existing knowledge, have most influence on the successful rooting of citrus cuttings and to study the effect of these factors on five commercial citrus varieties which are considered among the more difficult species to root.

REVIEW OF LITERATURE

The literature specifically dealing with the rooting of citrus cuttings is not extensive and, as has been indicated, is largely confined to experimental work. With the exception of the lemon and citron the genus *Citrus*, as a whole, is considered as rather difficult to root. Several of the commercial varieties have not infrequently been used as difficult subjects when testing propagating techniques and, more specifically, the reactions obtained from the root-inducing growth regulators.

References in the literature to the basic principles of propagation by cuttings are, of course, extremely numerous and these have been reviewed to supplement the citrus literature in each area of basic study as outlined below.

Reasons for Use of Cuttings

Hartmann and Kester (11) have summarized the reasons for the use of asexual propagation as follows:

- 1) Perpetuation of a clone.
- 2) Impossibility of seed propagation.
- 3) Ease of propagation.

In the purely research field most of the motives for propagating by cuttings, rather than by the easier budding operation in citrus, would fall within reasons one and two.

Malloch (19) and Swingle *et al.* (32) have pointed out the importance of uniform stocks when testing fruit varieties. Citrus hybrids may also have merit as stocks but produce few seeds. Such hybrids, being heterozygous, if they produce seeds may segregate in succeeding generations and the particular genes giving the desired character of resistance may be lost. Hybrid vigor is also maintained when propagation is by vegetative means.

Opitz (24) reported that cuttings of the Troyer citrange make an adequate substitute for seedlings in the absence of sufficient seed of this hybrid.

Sinha and Vyvyan (30) have stressed the desirability of further study on rootstocks which would be commercially valuable if they could be readily propagated by vegetative means.

Johnston *et al.* (16) have indicated that propagation by cuttings is the preferred means where the plants are to be sold in containers, and this applies to the so-called "miniature" ornamental species mentioned earlier.

Growth Regulators and Methods of Application

In 1939 Thimann and Delisle (34) discussed the vegetative propagation of difficultly rooted plants. Citrus species were not included in the plants tested (*Pinus* spp., *Tsuga* spp., *Picea* spp., *Taxus baccata*, *Quercus* spp., and *Acer* spp.), but the response of cuttings of these species to auxin treatment was observed. Indoleacetic acid (IAA) was used and, although faster rooting with more roots per plant was obtained in those cuttings which rooted, the species which failed to root entirely as controls also failed to root under IAA treatment. The authors considered that the age of the tree from which the cuttings came, and not auxin treatment, was the most important factor in the rooting of these difficult species.

During the same year Hitchcock and Zimmerman (13) made a study of the comparative activity of several root-inducing substances and methods for the treatment of cuttings. They concluded that relatively dilute solutions and concentrated solutions produced the same results when 24-hour soaking periods were allowed for the dilute solutions and a quick dip method employed with the concentrated material. Potassium salts were more effective than acids and, of all acids tested, indolebutyric acid (IBA) gave superior results in most cases.

The reviews by Pearse (25) indicate that several workers, who published their results during the years 1938-40, made common use of IAA at various concentrations and periods of treatment. Significant increases in the rooting of lemon, lime, sour and sweet orange, grapefruit and citrange were obtained by such treatments.

In 1943 Cooper (4) experimented with the concentrated-solution-dip (quick-dip) method of treating cuttings with growth-regulator substances. He used tropical plantation-crop species as his subjects, namely derris (*Derris elliptica* L.), cinchona (*Cinchona ledgeriana* L.), and cacao (*Theobroma cacao* L.). Indolebutyric acid (IBA) was used at concentrations of 0 to 10.0 mg./ml. in 50 per cent alcohol. The results, based on the average number of roots per cutting, showed conclusively that a 5-second dip at a high concentration was superior to a 24-hour soaking period in a water solution at a low concentration. Among other advantages of the quick-dip method, according to this author, were (a) speed and (b) fewer utensils required.

Since 1952 most workers (9, 15, 25) who have dealt specifically with the propagation of citrus species from semi-hardwood cuttings have either reported the superiority of IBA or have used it exclusively.

Fungicides

The initial healing of the wound (3, 11) is an essential phase in the successful rooting of any cutting. Newton (23) has discussed at length the effects of the application of fungicides to wounded plant tissue. Working with cuttings of cypress (*Cupressus lawsoniana erecta viridis* Cy.) he demonstrated that dusting the cut bases with Arasan and Spergon gave favorable results and increased rooting. He pointed out that applications of fungicides containing copper or mercury are frequently injurious to wounded plant tissues. Applications of fungicide were made with and without 10 ppm. NAA and this had no added beneficial effect on the rooting of cypress. The possible synergistic action of the fungicide was discussed.

Doran (5) has also reported on the beneficial effects of treating cuttings of woody plants with both a root-inducing substance and a fungicide. Using Phygon XL in a dilution of 0.3 to 0.6 gm. per liter of water, plus IBA at the rate of 0.3 to 0.8 per cent in water, he obtained faster and more rooting with woody cuttings of *Franklinia* sp., *Rhododendron* sp., and *Ilex* sp.

Singh *et al.* (29) have proved the value of the fungicide Seradix A in treatments for the rooting of sweet lime cuttings in India. Cuttings were made at two distinct seasons, September and January-February. Seradix A proved to be of particular value in the case of cuttings taken in late winter.

In all of the experiments reported above, the fungicide was applied directly to the basal cut just before insertion into the propagation medium

Bottom Heat

Provision of bottom heat to the rooting medium, preferably in excess of that in the atmosphere, has long been considered one of the basic aids to root development, particularly with species known to root only with difficulty (11, 31).

It has been pointed out that the ideal rooting environment for the cutting, i. e., with the medium warmer than the air temperature, is more difficult to achieve under mist than in the usual cutting bench. Slow or poor rooting under mist has often been attributed to the low temperature of the rooting medium (27).

Of those workers who have reported specifically on the rooting of citrus cuttings with the aid of bottom heat, Halma (9) considered

a temperature of 76-80° F. ideal for the medium. Others (16) considered a minimum of 75° F. essential and pointed out that the upper limit was not known. The early work of Swingle *et al.* (32) indicated that a soil temperature of 80-90° F. was beneficial for the rooting of citrus species, and more recently (1956) it has been reported that a minimum night temperature of 90° F. in the medium, which rose to 105° F. at mid-day, gave best results when used with intermittent mist for the rooting of leaf-bud cuttings of a sour orange clone (6).

Preparation of Cuttings

Despite the rather limited scope of the work done on the rooting of citrus cuttings, considerable attention has been focused on the type of material used and, frequently, on specific information concerning the position and angle of the basal cut in relation to the stem.

In the experiments carried out in the 1920's terminal cuttings of semi-mature wood and 5 to 6 inches in length were most frequently used (9, 32). Mowry (21), however, used mature leaf cuttings of lemon with petiole attached. The age of the leaf was not stated.

Experiments carried out in Trinidad in 1932 (14) confirmed the semi-hardwood stem cutting as being the most promising among a total of six types tested: root, layered stem, marcotted stem, ringed-hardwood stem, semi-hardwood stem and leaf cuttings. Citron, lime, sour orange, and mandarin were used as the test materials in this case.

The favorable rooting response of leaf-bud cuttings was reported for citrus species in 1939 (36). This method was recommended for use where the amount of propagation material was limited; otherwise the shoot cutting develops into a young plant in a much shorter time period. As mentioned earlier the leaf-bud technique has also been used more recently (6) to multiply clonal material of a sour orange rootstock which was in short supply.

Girdling to induce carbohydrate accumulation in the cuttings of sweet lime was also used by Jauhari and Rahman (15) in India in 1958. Ringing was done 1/4-inch below a pre-selected point at which each cutting was to be taken and 2 weeks before removal from the parent tree.

Hitchcock and Zimmerman (12), in discussing rooting response in relation to age of tissue at the base of greenwood cuttings, concluded that rooting varied both with type of cutting and with species of plant. As a general rule, however, when the cut was made at the base of the current season's growth, rooting was more successful.

The relationship of the basal cut to buds on the stem has not been considered of great importance by most writers (7, 14, 16). Swingle *et al.* (32) considered that the cut is best made just below a node, but Chadwick (3) concluded that a cut either at the node, or

1/2-inch below, would give equally satisfactory results in most cases. Hunter (14) reported that there was no advantage to leaving a heel on citrus cuttings. A few workers (16) have agreed that a cut made at a 90° angle to the stem is preferable to a cut at an acute angle, irrespective of where the cut is made in relation to the node.

Wounding of the base of the cutting and its effect on rooting have been studied simultaneously with the selection of the type of cutting as discussed above. Notching of the base of the cutting increased the number of roots obtained but did not increase the weight of roots per unit area of leaf on the cutting. Other workers (22) have found that crushing the basal end of the cutting, combined with growth-regulator treatments (IBA), increased the percentage of rooting as well as the number of roots.

Relative Ease of Rooting of Several Citrus Species

Many reports of the rooting of citrus species are found in the literature of propagation (25, 27), but for the most part they deal with single species. Consequently they provide no basis for a critical comparison of rooting performance of one species with another.

Specific evaluations of the ease of rooting of certain species have been made by some workers, however (9, 16, 28). In summary, these authors agree that ease of rooting is in descending order as follows: citron, lemon, lime, sweet orange, grapefruit, trifoliolate orange, citrange, sour orange, mandarin, and kumquat. There is nothing in the literature to indicate that the two commercial species of kumquat have been successfully rooted at any time. Johnston *et al.* (16) have contributed most to this listing in their study of citrus propagation published in 1959.

In 1951 Mes (20) made a critical study of citrus species which are difficult to root. In an experiment using sour orange and citron cuttings she removed cylinders of bark from the base of the cuttings and, by grafting, interchanged those of the sour orange onto the citron cuttings and vice versa. By this means she established that the ease of rooting could also be transposed in this way and concluded that capacity for rooting was inherent in the bark from which the roots originate.

Some years later Ryan *et al.* (28) successfully carried out a similar experiment transposing grafted cylinders on difficult and easy-to-root species. In this case the kumquat (*Fortunella margarita* Swing.) and the sour orange were chosen as species being difficult to root and these were grafted on twigs of sweet orange and rough lemon, respectively, which had been chosen for their comparative ease of rooting. Here again the authors concluded that the capacity for rooting is determined by the stem area from which the roots originate.

The occurrence and inheritance of preformed root primordia in the citron have been studied by Carpenter (2), who has shown that they are formed shortly after differentiation of the primary stem and have their origin usually near the pith. Differentiation of the vascular structure of the primordium occurs within the medullary ray as the stem grows in thickness.

MATERIALS AND METHODS

Selection and Preparation of Plant Materials

Based on information obtained from the literature the following five varieties of commercial citrus species were chosen as possessing fair extremely poor rooting potential in the order listed:

<i>Date Inserted</i>	<i>Scientific Name</i>	<i>Common Name</i>
Dec. 3	1. <i>Citrus sinensis</i> Osbeck.	"Hamlin" orange
Nov. 29	2. <i>C. paradisi</i> Macf.	"Ruby" grapefruit
Nov. 22	3. <i>C. reshni</i> Tan.	"Cleopatra" tangerine
Nov. 29	4. <i>C. unshiu</i> Marc.	"Owari" satsuma
Dec. 5	5. <i>Fortunella margarita</i> Swing.	"Nagami" kumquat

Only mature, bearing trees were used as a source of propagation material and the trees selected for each species were of one age and from one uniform block.

Terminal cuttings 7 to 9 inches long of semi-mature wood and bearing 8 to 12 leaves were selected from within a band 4 to 8 feet high on the south side only of the chosen trees. The only exception to this rule was in the case of the "Owari" satsuma where the trees were a maximum of 7 feet in height.

Immediately after removal from the tree the cuttings were placed in plastic bags for transportation from grove to greenhouse. The period between cutting from the parent tree and insertion in the rooting medium varied from 4 to 12 hours, but turgor was maintained by successive sprinklings with water during the interim.

Final preparation of the cuttings comprised shortening to uniform length for each variety, i. e., 6 to 7 inches for all varieties with the exception of the kumquat cuttings which were only 5 to 6 inches long. This cut was made with a sharp knife immediately below an appropriate node and at a 90° angle to the stem. The lower 3 to 4 leaves were then trimmed off close to the stem, thus leaving each cutting with 5 to 7 leaves. A total of 480 cuttings of each species was prepared in this way.

Growth-regulator and fungicide treatments took place a few minutes after preparation and immediately prior to insertion in the medium. Growth-regulator application was by means of the quick-dip method, i. e., 1-second immersion to a depth of 1 to 1 1/2 inches. Fungicide treatments were applied after excess hormone solution had

evaporated and consisted of covering the basal cut with a coating of the fungicide in the dry, powder form.

The four levels of growth regulator concentration, viz. 0, 2,000, 3,000 and 4,500 ppm., of indolebutyric acid were obtained by appropriate dilution of the concentrate with 50 per cent ethyl alcohol in water. A fresh portion of the diluted solution was used for each batch of 480 cuttings, thus avoiding contamination and affording the optimum degree of uniformity in the solution used for each lot of cuttings. When not in use the stock solutions were kept in a household refrigerator at 41° F.

The fungicide was prepared by mixing 50 per cent Captan (N-Trichloro-methylmercapto-4-cyclohexene-1, 2-Dicarboximide) wettable powder with 20 per cent PCNB (Pentachloronitrobenzene) in equal proportions.

The cuttings were then inserted in the bench to a depth of 1 1/2 to 2 inches.

Propagation Bench and Misting Equipment

The propagation bench was located in a standard greenhouse with ridge ventilation and supplemental steam heat. The thermostat was set for a minimum night temperature of 65° F.

The experiment utilized 24 feet of a standard propagation bench, 36 inches wide by 8 inches deep, mounted on concrete columns 2 feet above ground level. The construction of this particular bench comprised sides of smooth 1/4-inch transite with a base of corrugated transite to permit free drainage. The bench was divided into three equal 8-foot lengths to enable three distinct temperatures of bottom heat to be utilized, and insulation of one treatment from the other was accomplished by dividers of 1/2-inch-thick transite.

A 2-inch layer of vermiculite (N° 3) was first placed directly on top of the corrugated transite in the bottom of the bench to provide insulation from underneath and to enable the heating cables to function efficiently. In each of sections I and II of the bench a 60-foot length of General Electric HS 11040 lead-sheathed heating cable was then laid directly on top of the packed vermiculite in the form of loops spaced approximately 7 inches apart, so that the 60 feet of cable were evenly distributed within the 3- by 8-foot area.

The rooting medium comprising 50 per cent peat (Premium sphagnum peatmoss) and 50 per cent perlite (Perl-lome grade of horticultural perlite) was then mixed and added to all three sections of the propagating bench to within 1/2 inch of the top, i. e., to an approximate depth of 5 1/2 inches.

Thermostat controls (General Electric Mod. HSC3.) were attached to the heating cables in sections I and II and set at 80° F.

and 90° F. respectively. The cables were then connected to a standard power outlet and temperatures adjusted to desired minimums over a five-day period.

Finally a light shade, comprising fibre-glass cloth tacked to a wooden frame, was suspended 4 feet above the bench in order to stabilize mid-day temperatures during periods of intense sunlight and to prevent excessively high temperatures in the medium. This covering reduced light intensity by 30 per cent at mid-day.

Intermittent mist was supplied during daylight hours under a cycle of 3 seconds on and 57 seconds off. Automatic control was provided by means of an Intermatic Time Clock Mod. N° TS 60SP. Six Thompson baffle spray nozzles N° 215 were located at 39-inch intervals down the center of the bench and approximately 12 inches above the cuttings. Water pressure was 40 to 60 psi. and gallonage delivered by each nozzle was rated at 0.3 gallon per minute at 50 psi. Since the valve was only open for 3 minutes during each hour, 0.9 gallon per hour per nozzle was applied.

Experimental Design

A split-split-plot design was used as described by LeClerc *et al.* (18). Since this design affords less precise comparison of the whole plot treatments than of the sub-plots, the species constituted the whole plots, as the rooting response between species is known to vary considerably. Treatments with and without fungicide comprised the sub-plots, thus leaving the four levels of growth-regulator concentration to occupy the sub-sub-plots where they would receive a more precise comparison in the statistical analysis.

A randomized, complete block design was used for the whole plots and the sub- and sub- sub- plots were likewise randomized in each case. The four replications were also randomized within each experiment, i. e., for each temperature setting of the medium. Randomization was carried out by use of a set of Random Sample numbers (18). The experimental unit was 5 cuttings in each case. Since each block comprised 5 species with 8 treatments for each, the total number of cuttings per replicate was $5 \times 5 \times 8$ or 200 cuttings. Four replications of each gave a total of 800 cuttings per experiment for a grand total of 2,400 cuttings over the 3 temperature ranges. By reference to the specifications for bench area involved it will be seen that the space allocated was approximately 24 square inches per 5-cutting unit, although this varied somewhat with overall size and leafiness of each species.

Scoring

Rooting was determined after 12 weeks and the results scored according to the system of ranks as described by Preston *et al.* (26). In this case the following weighting was given:

- 5 Good rooting
- 4 Medium rooting
- 3 Poor rooting
- 2 Callused but no rooting
- 1 Dead

Mean values for degree of rooting in any one treatment were then obtained by dividing the sum of the scores for each of the cuttings by the total number of cuttings. These values were then analyzed statistically.

Statistical Analysis

The data from each of the three experiments were analyzed statistically to show first and second degree interactions, using the method outlined by LeClerc *et al.* (18).

RESULTS AND DISCUSSION

General

The percentage of cuttings rooted per variety in each of the three experiments is given in Tables 1, 2 and 3.

When compared with some of the figures for per cent rooting of sweet orange and grapefruit given in the literature the results of the experiments must be considered, in general, to be low. Only Cleopatra tangerine gave results approaching what would be considered economically feasible in a commercial operation. Means did not exceed 60 per cent although the highs for individual plots reached 80 per cent.

Heavy dropping of leaves occurred on many cuttings and appeared to be a major contributing factor in the lack of rooting. With the exception of Cleopatra tangerine, this mainly took place 7 to 14 days after insertion. Leaf fall was slower in the unheated bed but number of leaves held at the end of the 12-week period was approximately the same for all three experiments.

Van Overbeek (35) has pointed out that the retention of leaves is of prime importance in the successful rooting of most cuttings and especially of the more difficult-to-root species. Leaf drop in the present experiment, therefore, is of particular significance in these species and treatments where rooting performance was poor.

TABLE 1.—Per Cent Rooting Obtained with Five Citrus Species and Eight Treatments; Experiment I, 90 F. Bottom Heat.

TREATMENT SPP	Cleopatra Tangerine		Hamlin Orange		Ruby Grapefruit		Owari Satsuma		Nagami Kumquat	
	Av.	High*	Av.	High	Av.	High	Av.	High	Av.	High
1. 0 ppm. IBA	45	60	10	20	10	40	0	0	5	20
2. 2000 ppm. IBA	50	80	10	20	0	0	0	0	5	20
3. 3000 ppm. IBA	60	80	0	0	10	20	0	0	15	40
4. 4500 ppm. IBA	30	40	5	20	35	60	5	20	5	20
5. 0 ppm. IBA + Fungicide	5	20	0	0	5	20	0	0	5	20
6. 2000 ppm. IBA + Fungicide	20	40	15	20	0	0	0	0	15	20
7. 3000 ppm. IBA + Fungicide	25	60	15	40	5	20	10	40	5	20
8. 4500 ppm. IBA + Fungicide	25	60	5	20	5	20	0	0	5	20

* Highest individual plot.

TABLE 2.—Per Cent Rooting Obtained with Five Citrus Species and Eight Treatments; Experiment II, 80° F. Bottom Heat.

TREATMENT SPP	Cleopatra Tangerine		Hamlin Orange		Ruby Grapefruit		Owari Satsuma		Nagami Kumquat	
	Av.	High*	Av.	High	Av.	High	Av.	High	Av.	High
1. 0 ppm. IBA	5	20	15	20	15	40	0		5	20
2. 2000 ppm. IBA	35	60	20	60	15	40	0		0	
3. 3000 ppm. IBA	55	80	15	40	10	40	10	20	5	20
4. 4500 ppm. IBA	50	80	20	40	20	20	5	20	0	
5. 0 ppm. IBA + Fungicide	20	40	0		5	20	0		0	
6. 2000 ppm. IBA + Fungicide	10	40	15	40	10	20	0		0	
7. 3000 ppm. IBA + Fungicide	15	40	15	20	30	60	0		0	
8. 4500 ppm. IBA + Fungicide	35	80	20	40	5	20	5	20	5	20

* Highest individual plot.

TABLE 3.—Per Cent Rooting Obtained with Five Citrus Species and Tilt Treatments; Experiment III,
No Bottom Heat.

TREATMENT SPP	Cleopatra Tangerine		Hamin Orange		Ruby Grapefruit		Owari Satsuma		Nagami Kumquat	
	Av.	High*	Av.	High	Av.	High	Av.	High	Av.	High
1. 0 ppm. IBA	5	20	5	20	15	40	0	0	0	0
2. 2000 ppm. IBA	0		10	20	15	20	0	0	0	0
3. 3000 ppm. IBA	15	20	0		15	40	0	0	0	0
4. 4500 ppm. IBA	15	40	15	40	10	20	10	20	0	0
5. 0 ppm. IBA + Fungicide	0		5	20	0		0	0	0	0
6. 2000 ppm. IBA + Fungicide	5	20	20	40	15	20	5	20	0	0
7. 3000 ppm. IBA + Fungicide	5	20	15	20	20	40	5	20	0	0
8. 4500 ppm. IBA + Fungicide	5	20	15	20	35	60	0	0	0	0

* Highest individual plot.

Although bottom heat would seem to be a contributing factor in the drying out of the beds during the night hours (when intermittent mist was not supplied), an even greater drying effect on the foliage was noted during nights when supplementary steam heat was in operation to maintain a minimum air temperature of 65° F. This would indicate that intermittent mist during the full 24-hour period may be necessary to maintain leaf turgidity when the greenhouse is being steam heated, and desirable where the higher ranges of bottom heat are utilized.

A spot check on per cent moisture in the beds, taken twice with a 12-hour interval to point up any day-to-night alteration, showed no appreciable difference between beds and only a slight change, within a range of 2.60 per cent to 5.35 per cent in day-to-night variation. It would not appear, therefore, that such slight changes would have a very strong influence on leaf turgidity, and reduced humidity in the night air, when steam heat was in operation, was perhaps the greatest contributing factor.

The drying influence of high winds has been mentioned by Joiner and Sheehan (17), and it was noted during this experiment that such winds can also cause severe deflection of the mist off the bed, particularly at the extremities where overlap is not sufficiently large. This wind factor necessitated the use of missing plot technique to obtain data for one plot in the extreme northeast corner of experiment II. Lack of significance between blocks (Table 4), however, shows that on the whole the influence of wind on the three experimental beds was not great. Where wind is a problem the use of a 3-foot-high baffle on the windward side of the bed is recommended. This not only reduces air currents but helps to deflect the spray back onto the bed.

The weighting (1 to 5) for the system of ranks (26) used to determine degree of rooting in response to treatments is shown in Figure 1.

IBA Concentration

Reference to the Analysis of Variance (Table 4) shows that there was a significant difference between treatments, at the 4 levels employed, in each of the three experiments. This difference was significant at the 1 per cent level in experiments II and III and at the 5 per cent level only in experiment I (90° F. bottom heat).

The Duncan Range Test for IBA levels, Table 5, enables us to study these differences more closely. This table shows that in experiments I and II the 3,000 ppm. concentration gave optimum rooting results and was followed by 4,500 ppm., 2,000 ppm. and 0 ppm. (control) in descending order of influence. Furthermore it can be noted from this table that although all three IBA treatment were significantly different from the control in the experiment having 90° F. bottom heat, these treatments were not significantly different from each other.

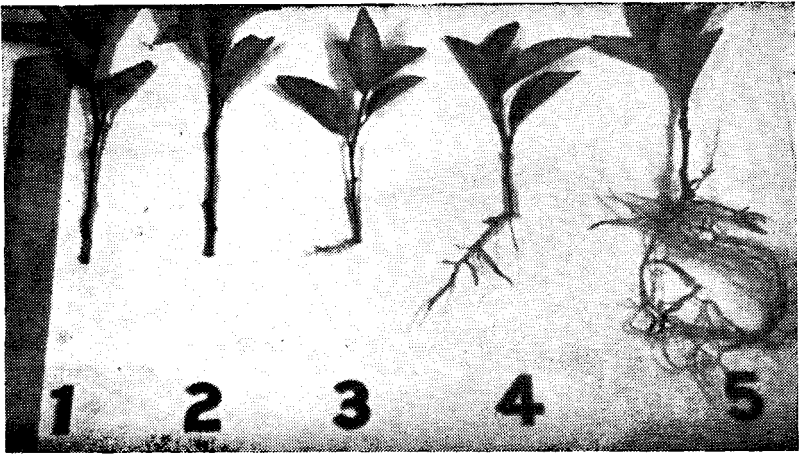


Figure 1. Showing system of ranks (after Preston [26]) used to determine degree of rooting response to treatments (Cleopatra tangerine). 1, Dead or necrotic. 2, Callused but no rooting. 3, Poor rooting. 4, Medium rooting. 5, Good rooting.

In experiment II, 80° F. bottom heat, however, each treatment level gave results significantly different from its neighbors even at the 1 per cent level. Indeed, all treatments here were rather evenly distributed, with a factor of 1.0 between successive levels.

TABLE 4.—Analysis of Variance, F Values for Effect of Several Factors on Rooting of Five Species of Citrus.

SOURCE OF VARIATION	F Value		
	Exp. I 90° F.	Exp. II 80° F.	Exp. III Control
Blocks	2.39	2.26	0.93
Species	6.38**	29.00**	4.63*
Fungicide	0.01	4.16	9.33**
Species x Fungicide	7.37**	1.67	1.94
IBA Concentration	2.81*	5.49**	6.35**
Species x IBA Concentration	1.26	2.09*	0.64
Fungicide x IBA Concentration	0.28	1.51	1.23
Species x Fungicide x IBA Concentration	0.59	2.95**	1.06

* Significant at the 5% level.

** Significant at the 1% level.

In the case of experiment III, no bottom heat, the sequence of levels is altered slightly and, as indicated by Table I, the highest concentration of IBA (4,500 ppm.) gave best results. Once again all three levels of concentration were significantly different from the control but concentrations of 2,000 and 3,000 ppm. did not give results significantly different from each other.

From the above it would appear that where bottom heat is applied, an adequate response can be obtained from IBA at 3,000 ppm. concentration, and inhibition occurs after this point. Where no bottom heat is applied we may expect greater response at the 4,500 ppm. level, however. Figure 5 indicates that, for the majority of the species tested, the trend is still upwards on the graph even for this level, and the zone of inhibition had not been reached at the highest concentration used.

Fungicide

Response to fungicidal treatment was only obtained in experiment III (no bottom heat). Here it was extremely significant at the 1 per cent level. Degree of rooting for any one species was not significantly high enough in this experiment to allow conclusions to be drawn, but there was an indication that, while controlling disease organisms, the fungicide inhibited somewhat the rate of healing and callus formation.

In the case of the two experiments where bottom heat was applied, the fungicide was not effective in preventing necrosis in those cuttings

where the wound did not heal. Indeed, with some species and at the highest temperature there appeared to be a phytotoxic reaction to the fungicide. This is discussed at greater length in the appropriate paragraph under first degree interactions.

TABLE 5.—Duncan Range Test for Significance Between IBA Levels.

		<i>IBA LEVELS</i>			
EXPERIMENT		<u>C₃</u> 3,000 ppm.	<u>C₂</u> 4,500 ppm.	<u>C₁</u> 2,000 ppm.	<u>C₀</u> 0 ppm.
I	90° F.	18.25a ⁽¹⁾	18.20a	18.15a	16.10b
II	80° F.	20.35a	19.35b	18.45c	17.35d
		<u>C₃</u>	<u>C₂</u>	<u>C₁</u>	<u>C₀</u>
III	No Bottom Heat	20.25a	19.30c	19.25c	17.45b

(1) Each value is the mean of ratings for 5 species and 4 replications.

Means in the same line followed by like letters are not significantly different; means not followed by like letters differ statistically from each other at the one per cent level.

Temperature

Due to the impracticality of setting up randomized blocks for bottom heat with the equipment available, three separate experiments were run, i. e., one at each of the two temperature levels and the third as a control. As a result no statistical comparisons can be made between the data obtained from these individual experiments. Since all treatments and other external environmental factors, however, were common to all three experiments, some broad comparisons can be drawn from the data.

Figure 2 shows graphically the average temperature readings for all three propagation beds, taken at 2 1/2-inch depth in the medium, and also includes the air temperature readings over the same period for comparison.

As may be expected, the air temperature fluctuated more than any of the media temperatures.

Also the unheated bed (experiment III) experienced greater fluctuation than the heated beds supplied with thermostat control for

the 90° and 80° F. minima respectively. Minimum air temperature for the greenhouse was set for 65° F. and supplemental steam heat was required for approximately two-thirds of the 84 nights of the experimental period. This supplemental steam heat, however, in pipes running 2 feet below the propagation bench, was sufficient to raise the temperature in the two heated beds above the normal night minimum settings.

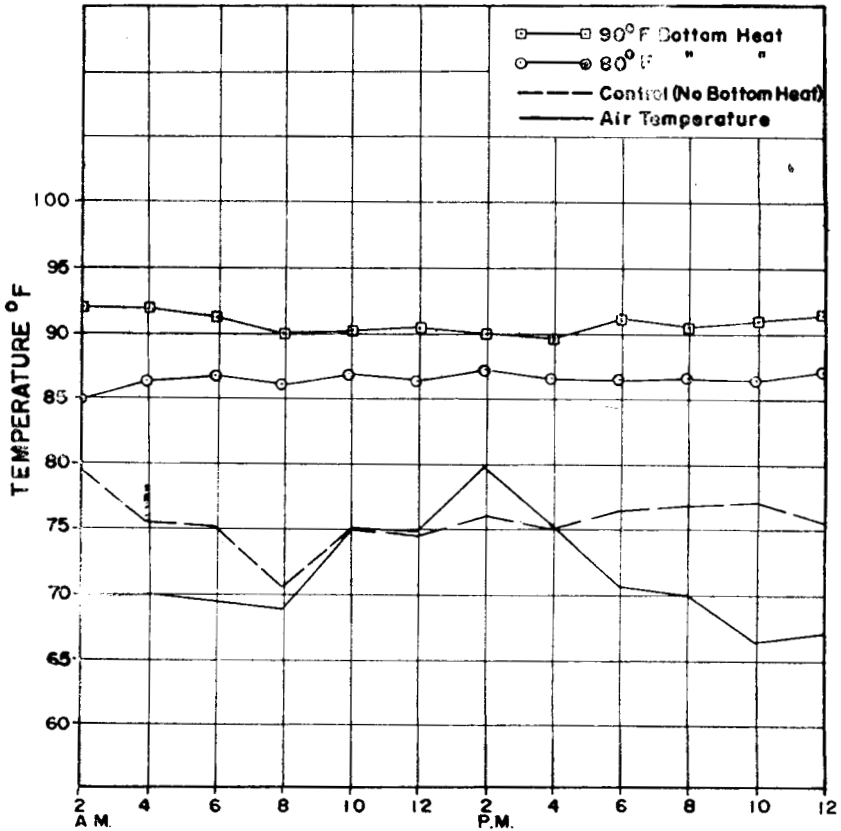


Figure 2. Average temperature readings, at a depth of 2 1/2 inches in the media of the three experimental beds, compared with that of air temperature in the greenhouse over a 24-hr. period.

Although it was not the purpose of these experiments to determine rate of rooting at the three temperature settings, it was, nevertheless, evident from the data obtained that rooting response was directly correlated to temperature, with the fastest rooting taking place at the highest temperature (29 days for Cleopatra tangerine).

It was also observed, from data on the degree of rooting obtained, that the difference between 90° and 80° F. temperatures was slight, possibly no more than one to two weeks depending on species, whereas the rooting response in the control bed (no bottom heat) came two to three months later for most species and not at all in the case of the kumquat.

It was also noted that a few Owari satsuma cuttings rooted successfully in 3 months, in the heated beds, as opposed to a 4-months minimum as reported in the literature (16).

Best overall results, based on degree of rooting, were obtained in the bed at 80° F. and this would indicate that there is no need to go higher except in cases where extremely difficult-to-root species show a response at the 90° F. level.

Interactions

As shown by table 4 three of the four interactions were significant in one experiment each and these are discussed below.

1) Species x Fungicide: Significant difference was shown between species at the 1 per cent level for the 90° F. experiment only.

The combination of high temperature, high moisture and fungicide, resulting in increased chemical reaction, was detrimental to cuttings of Cleopatra tangerine. Although no specific lesions which could be attributed to phytotoxicity of the treatment were noted, it seems possible that some damage or weakening of tissues was involved, resulting in advanced necrosis in most cases.

Hamlin orange and Ruby grapefruit showed no appreciable response to the treatment with fungicide. Some protection appeared to result from the use of fungicide in the case of Owari satsuma and this attained significant proportions in the case of the Nagami kumquat. Although overall results in the experiment with no bottom heat (experiment III) did not show statistical significance for this interaction, a study of the data shows a considerable increase in rooting for Cleopatra tangerine and Hamlin orange where fungicide was used. The other three species tested, however, showed no such increase from the fungicidal dip treatment, and the data for treated and untreated plots were almost identical in each case.

2) Species x IBA Level: This response was significant, at the 5 per cent level, only in experiment II. It is graphically illustrated by Figure 3, reference to which points up the following:

a) Greatest rooting response of Cleopatra tangerine to IBA is obtained between 2,000 and 3,000 ppm. concentration.

b) The response to IBA concentration has still not reached a peak for Hamlin orange at the highest concentration used, i. e., 4,500 ppm.

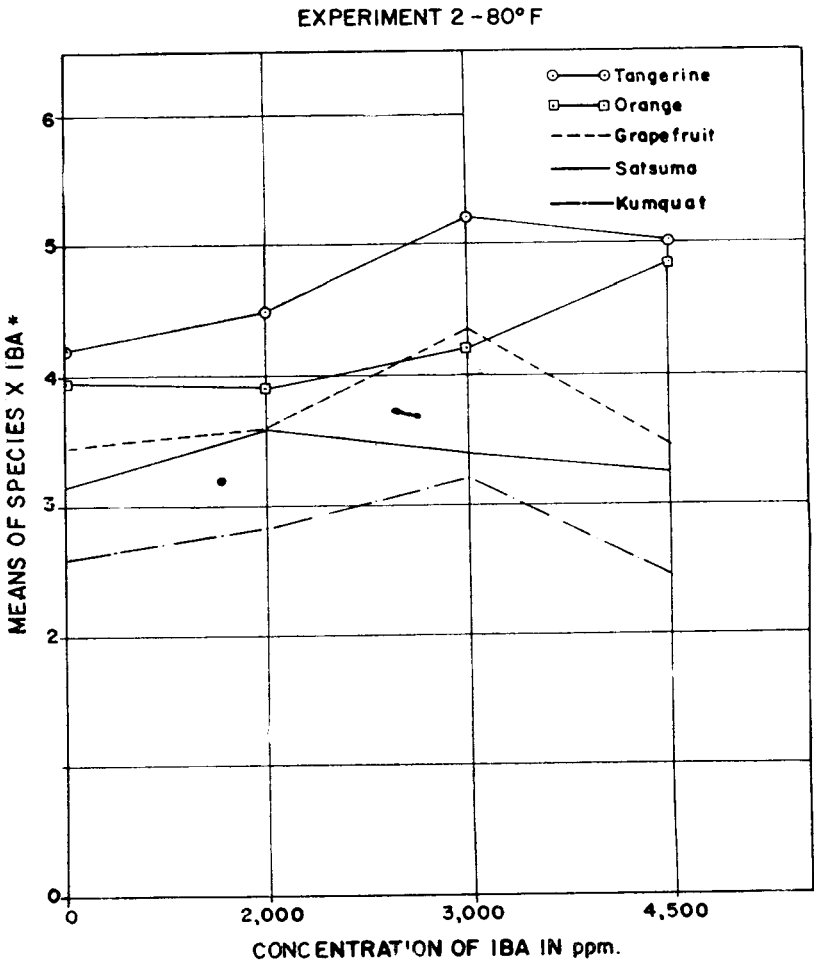


Figure 3. Graph showing response to interaction of species x IBA levels with bottom heat (Exp. II) at 80° F.

* Data derived from degree of rooting by system of ranks.

c) The response of Ruby grapefruit to IBA level is not marked at any point over the range.

d) A distinct apex in the response of Owari satsuma is observed at 3,000 ppm.

e) For the Nagami kumquat the same pattern is followed as in (d) although it is much less marked.

Reference to Table 4 shows that no significant difference was obtained for this interaction in the case of experiments I and III. However, graphs compiled from the means for this interaction, Figures 4 and 5, show overall interesting trends which are worthy of study.

Figure 4, for data pertaining to experiment at 90° F., shows a fairly wide separation in the reaction of the five species to the four levels of IBA concentration employed. Response was most significant in the case of Cleopatra tangerine. Four of the five species, the exception being Ruby grapefruit, show a peak of response at or before 3,000 ppm. concentration is reached. In the case of grapefruit the trend was still upward at the highest concentration, i. e., 4,500 ppm., but a valley occurred at the 2,000 ppm. level.

Figure 5, corresponding to the data for experiment III, with no bottom heat, shows considerable uniformity in the five species as indicated by the closeness of the lines on the graph. Further, since all lines run almost parallel within one unit on the X-axis it is obvious that overall response to IBA levels for all species was not great. Since all lines show an upward trend, even at the 4,500 concentration, this would suggest that the upper perimeter of response to IBA level, in the unheated bed, was not reached.

3) Species x Fungicide x IBA Level: Since 40 means are involved in this comparison, all of them falling within the comparatively narrow range of 3.20 to 1.10, it was to be expected that significant differences were not maintained over the range. Analysis by means of the Duncan Range Test indicated that significant variance occurred in the following areas:

a) Highest rooting performance was attained by Cleopatra tangerine with no fungicide treatment and 3,000 ppm. IBA. (Significant at the 1 per cent level).

b) Lowest recorded response was for Nagami kumquat, treated with fungicide and with 0 ppm. IBA.

EXPERIMENT I - 90° F

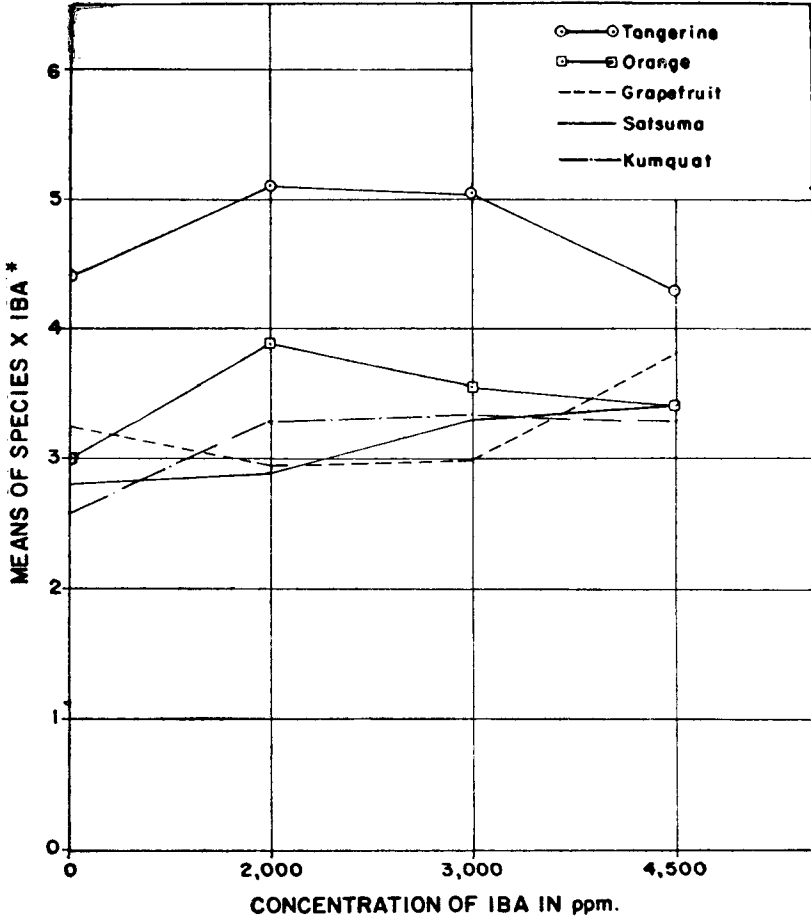


Figure 4. Graph showing response to interaction of species x IBA levels with bottom heat (Exp. I) at 90° F.

* Data derived from degree of rooting by system of ranks.

EXPERIMENT 3 - NO BOTTOM HEAT

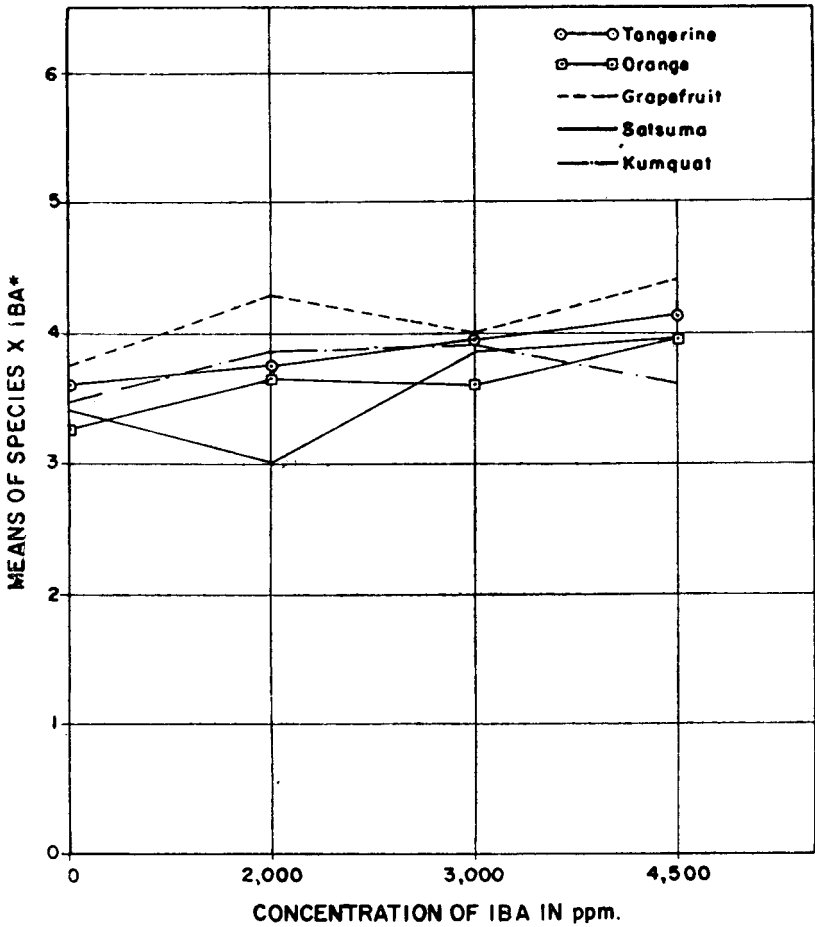


Figure 5. Graph showing response to interaction of species x IBA levels with no bottom heat (Exp. III).

* Data derived from degree of rooting by system of ranks.

c) Greatest significance occurred between species and IBA levels and these data may be summarized as follows:

<i>Species</i>	<i>High</i>	<i>Low</i>	<i>Significance Level</i>
Cleopatra tangerine	3,000 ppm. NF	0 ppm. NF	1 per cent
Hamlin orange	4,500 ppm. NF	0 ppm. F	5 per cent
Ruby grapefruit	3,000 ppm. F	0 ppm. F	1 per cent
Owari satsuma	2,000 ppm. F	0 ppm. F	not significant
Nagami kumquat	3,000 ppm. NF	0 ppm. F	not significant

NF — No Fungicide

F — Fungicide.

Season of the Year

The overall rooting response of the cuttings, taken during the last two weeks of November and the first week in December, would indicate that this is perhaps not the most favorable season for taking these cuttings.

Cleopatra tangerine, Hamlin orange, and Ruby grapefruit all flowered profusely in the cutting beds during the last three weeks of the experimental period. This drain of food reserves away from purely vegetative growth was detrimental to further root and shoot development during the period. Sykes and Williams (33) cited nutritional status as being one of the most important factors affecting rooting.

It also proved to be extremely difficult to find sufficient plant material in a uniform semi-mature state at the time the cuttings were prepared. Even though this must be attributed, in part, to the severe freeze damage during the previous December to most of the groves being sampled, this scarcity of suitable material was also noted in trees which had not been subject to severe frost damage. This was particularly evident in the case of the kumquat, where a heavy fruit crop had still to be harvested, and in Owari satsuma, where harvesting had just been completed.

In view of the above it would seem that the June-July cutting period, gives superior results both from the aspect of material selection and rooting response.

Position of Cut

The basal cut, just below a node and at right angles to the stem, gave uniform healing without excessive callusing. Argle (1) has pointed out that callus formation and root formation are distinct processes although both are affected by the same environmental factors. Callus-pad formation is also said to have a direct relationship to the air

moisture ratio of the medium. A small callus is thought to indicate too high a ratio, i. e., the medium too dry. However, the well branched roots with numerous root hairs (Figure 1) are reported by the same author as indicating that moisture is optimum for root development.

All roots formed on or close to the callus and there was no root development away from the location of the basal cut.

Relative Ease of Rooting of Species

Significant differences in the ease of rooting among the five species used in the experiment were evident throughout the Analysis of Variance table, and greatest differences in the two-way and three-way interactions were, as has been discussed previously, largely attributable to this factor.

Table 6, shows the results of the Duncan Range Test for significance between species, and it is interesting to note that in experiment II, using 80° F. bottom heat, the distribution of species is as listed in the review of literature (page 27). At 90° F. only Nagami kumquat and Owari satsuma, at the lower end of the range, are transposed, although the data are not significantly different at this point and do not permit of statistical separation.

In experiment III, with no bottom heat, it will be seen from Table 6 that the rooting of the Ruby grapefruit was relatively improved under these conditions. Cleopatra tangerine did less well than in the two other experiments and significant difference between that species

TABLE 6.—Duncan Range Test for Significance Between Species.

SPECIES		I	II	III	V	IV
<i>Experiment</i>						
I	90° F.	18.25a ⁽¹⁾	13.90d*	13.00c*	12.55bc	12.40bc
		I	II	III	IV	V
II	80° F.	19.35a	16.80d	14.85cd	13.40c	11.1b
		III	I	IV	V	II
III	No Bottom Heat	16.70a	15.40c	14.90c	14.80c	14.45b

(1) Each value is the mean of ratings for 8 treatments and 4 replications.

Means in the same line followed by like letters are not significantly different; means not followed by like letters differ statistically from each other at the one per cent level with the exception of two starred items (*), where difference is at the 5 per cent level only.

and the two which are considered most difficult to root, i. e., Nagami kumquat and Owari satsuma, was not established. Hamlin orange rooted very poorly under conditions of no bottom heat, and losses, due to necrosis at the basal end of the cutting, were heavy.

It is interesting to note that there was no rooting of Nagami kumquat in the unheated bed although callus formation and general condition of most of the cuttings of this species was good, even at the end of the experiment.

Hitchcock and Zimmerman (12) have stressed the fact that different species of the same genus and different varieties of the same species may show distinctly different response in rooting. It was to be expected, therefore, that strong significant differences would be obtained in the present experiment. It is also evident that these results may only apply to the specific varieties used in the experiment.

SUMMARY AND CONCLUSIONS

1. Three split-split-plot experiments were carried out with varying temperatures of the media. Eight treatments were used to study the effects of IBA levels and fungicide application on five citrus species in each experiment. Results, taken after a 12-week period, were interpreted by Analysis of Variance and Duncan Range Test techniques.

2. Excessive leaf drop was noted for all species except Cleopatra tangerine during the second week of the experiment. This may have been due to drying out of the foliage during the night hours when supplemental steam heating of the greenhouse was in operation.

Electric heating cables in the beds having bottom heat treatment did not appear to lower per cent moisture appreciably in the media of those beds and apparently did not contribute to drying out of the foliage. The use of intermittent mist during the entire 24-hour period is recommended, especially where it is necessary to counteract the influence of steam heat.

3. Strong winds can influence the proper distribution of mist over the beds. The use of baffles on the windward side of the beds is recommended.

4. Significant increases in rooting were noted at the 4 levels of IBA concentration used. Greatest significance occurred at 80° F. bottom heat and in the bed at ambient temperature (approximately 75° F.). High temperature in the medium appeared to substitute for higher IBA levels and, vice versa, best rooting results were obtained in the unheated bed at the highest IBA concentration. Where bottom heat is applied, an adequate response to IBA can be obtained at the 3,000 ppm. level and inhibition may occur above this point. Where no bottom heat is used, 4,500 ppm. IBA does not seem to constitute the upper perimeter of response for the five citrus species tested.

5. Response to fungicide was significant in one experiment only (no bottom heat), and even here a possible inhibition to callus formation was suspected. Under the conditions of these experiments it would seem that the use of a Captan-PCNB dip is not to be recommended. The need for a suitable fungicide for use at the higher temperatures of the medium would seem worthy of further investigation.

6. Temperature of the medium played an important role in rooting response. Fastest rooting took place at the highest temperature and difficult-to-root species showed greatest response at 90° F. Greatest difference for overall degree of rooting, for the five species tested, was noted between the heated beds and those with no bottom heat. Differences between 80° and 90° F. bottom heat were slight.

7. Citrus species vary in their response to fungicide treatment. Under conditions of bottom heat the results were distinctly negative for Cleopatra tangerine and favorable results were noted only in the case of Nagami kumquat. All other species tested showed insignificant to slightly negative results. Under conditions of no bottom heat much less necrosis was noted in cutting of Cleopatra tangerine and Hamlin orange under fungicide treatment.

8. Response of the species tested to IBA concentration varied with the temperature of the medium. The apex of response occurred at 80° F. bottom heat, within a 2,000 to 4,500 ppm. IBA range, for all species except orange.

9. Greatest second degree interaction response between the three variables in any experiment, viz., species, fungicide and IBA levels, occurred in the experiment with 80° F. bottom heat. In the majority of cases highest response was obtained from Cleopatra tangerine with no fungicide and at 3,000 ppm. IBA. Lowest response was for Nagami kumquat with fungicide application and no IBA treatment.

10. Difficulty in obtaining suitable vegetative cutting material in late November and early December, and the subsequent performance of this material in the propagation beds, indicated that this season of the year was not optimum under the conditions of these experiments.

11. In general the rating, as to ease of rooting, for the five species tested was similar to that reported by previous workers. The relative position in this rating was altered somewhat under the influence of bottom heat.

The author wishes to express his profound gratitude to Dr. H. S. Wolfe, Professor of Fruit Crops, University of Florida, for his kind interest, thoughtful counsel, and constant encouragement throughout this investigation and preparation of the manuscript.

LITERATURE CITED

1. ARGLES, G. K. 1959. Root formation and root development in stem cuttings; a re-examination of certain fundamental aspects. *Ann. Appl. Biol.* 47:626-7
2. CARPENTER, J. B. 1961. Occurrence and inheritance of pre-formed root primordia in stems of citron. (*Citrus medica L.*) *Proc. Amer. Soc. Hort. Sci.* 77:211.18.
3. CHADWICK, L. C. 1930. The influence of the position of the basal cut on the rooting and arrangement of roots on deciduous softwood cuttings. *Proc. Amer. Soc. Hort. Sci.* 27:287-94.
4. COOPER, W. C. 1943. The concentrated-solution-dip method of treating cuttings with growth substances. *Proc. Amer. Soc. Hort. Sci.* 44:533-41.
5. DORAN, W. L. 1952. Effects of treating cuttings of woody plants with both a root-inducing substance and a fungicide. *Proc. Amer. Soc. Hort. Sci.* 60:847-91.
6. FORD, H. W. 1957. A method of propagating rootstock clones by leaf bud cuttings. *Proc. Amer. Soc. Hort. Sci.* 69:204-7.
7. GARDNER, R. J. 1958. Propagation of woody plants by cuttings. *Jour. Royal Hort. Soc.* 83:335-43.
8. GAREY T. A. 1882. Orange culture in California. p. 185. Pacific Rural Press, San Francisco, Cal.
9. HALMA, F. F. 1926. Propagating citrus by cuttings. *Cal. Citrog.* 11:225.
10. —————. 1940. Comparative study of initial and subsequent size of citrus cuttings and budlings. *Proc. Amer. Soc. Hort. Sci.* 38:336-8.
11. HARTMAN, H. T., and D. E. KESTER. 1960. Plant propagation: principles and practices. Chap. 10. Prentice-Hall, Inc., N. J.
12. HITCHCOCK, A. E., and P. W. ZIMMERMAN. 1932. Relation of rooting response to age of tissue at the base of greenwood cuttings. *Contrib. Boyce Thompson Inst.* 4:85-98.
13. ————— and —————. 1939. Comparative activity of root-inducing substances and methods for treating cuttings. *Contrib. Boyce Thompson Inst.* 10 (4):461-480.
14. HUNTER, R. E. 1932. The vegetative propagation of citrus. *Trop. Agric. (Trin.)* 9 (5): 135-40.
15. JAUHARI, O. S., and S. F. RAHMAN. 1958. Effect of hormones on rooting in cuttings of sweet lime (*Citrus limetoides Tanaka*). *Sci. and Cult.* 23:615-16. (in *Hort. Abstr.* 29 (1), N^o 789, 1959).
16. JOHNSTON, J. C., K. W. OPITZ and E. F. FROLICH. 1959. Citrus propagation. *Cal. Agric. Exp. Stat. Circ.* 475. pp. 16-19.
17. JOINER, J. N., and T. J. SHEEHAN. 1961. Mist propagation. *Fla. Agr. Ext. Serv. Circ.* 127A.
18. LeCLERG, E. W., W. H. LEONARD and A. G. CLARK, 1962. Field plot technique. 2nd. ed. Chap. 13 and p. 369. Burgess Publishing Co., Minneapolis, Minn.
19. MALLOCH, W. S. 1924. Asexual propagation as an aid to the breeding of rootstocks. *Jour. Agr. Res.* 29:515-21.
20. MES, M. G. 1951. Cuttings difficult to root. *Brooklyn Bot. Gard. Rec.* 7:95-7. (in *Hort. Abstr.* 22 (1), N^o 872. 1952).

21. MOWRY, H. 1925. Studies with citrus propagation. Fla. Agr. Exp. Stat. Ann. Rept. 1925, R69-70.
22. NAUNDORF, G. and J. M. TERREROS. 1952. Contribución al enraizamiento de estacas de *Citrus limon* (Rooting lemon cuttings) Not. Agron. Palmira. 5:43-7 (in Hort. Abstr. 23 (2), N° 2148. 1953).
23. NEWTON, W. 1952. Effects of the application of fungicides to wounded plant tissue. Sci. Agr. 32:659-62.
24. OPITZ, K. W. 1951. Troyer citrange rootstock propagation. Cal. Citrog. 36:454-56.
25. PEARSE, H. L. 1948. Growth substances and their practical importance in agriculture. Commonwealth Bureau of Hort. and Plantation Crops. Tech. Comm. N° 20, pp. 115-16.
26. PRESTON, W. H. Jr., J. P. SHANKS and P. W. CORNELL. 1953. Influence of mineral nutrition on production, rooting and survival of cuttings of azalea. Proc. Amer. Soc. Hort. Sci. 61:497-507.
27. ROWE-DUTTON, PATRICIA. 1960. Mist propagation of cuttings. Commonwealth Bureau of Hort. and Plantation Crops. Digest N° 2. East Malling, Maidstone, Kent, England.
28. RYAN, G. F., E. F. FROLICH and T. P. KINSELLA. 1958. Some factors influencing rooting of grafted cuttings. Proc. Amer. Soc. Hort. Sci. 72:454-61.
29. SINGH, K. K., J. C. BAKHSHI and S. SINGH. 1961. Effect of callousing and Seradix treatments on rooting of sweet lime cuttings. Indian Jour. Hort. 17:193-7.
30. SINHA, ADYA C., and M. C. VYVYAN. 1942. Studies on the vegetative propagation of fruit tree rootstocks. II. By hardwood cuttings. Jour. Pom. 20:127-35.
31. STOUTEMYER, V. T., O. K. BRITT and J. R. GOODWIN. 1961. The influence of chemical treatments, understock and environment on growth phase changes and propagation of *Hedera canariensis*. Proc. Amer. Soc. Hort. Sci. 77:552-57.
32. SWINGLE, W. T., T. R. ROBINSON and E. MAY Jr. 1924. The solar propagating frame for rooting citrus and other subtropical plants. U. S. Dept. Agr. Circ. 310. pp. 1-13.
33. SYKES, J. T. and I. H. WILLIAMS. 1959. Factors affecting regeneration from cuttings using mist technique. Ann. Appl. Biol. 47:631-4.
34. THIMANN, K. V., and A. L. DELISLE. 1939. The vegetative propagation of difficult plants. Arnold Arb. Jour. 20:116-36.
35. VAN OVERBEEK, J., A. GORDON and L. E. GREGORY. 1946. An analysis of the function of the leaf in the process of root formation in cuttings. Amer. Jour. Bot. 33:100-7.
36. WATKINS, J. V., and G. H. BLACKMON. 1939. Leafbud cuttings for multiplying tropical shrubs. Proc. Amer. Soc. Hort. Sci. 37:1109-11.
37. WEBBER, H. J. 1948. Nursery Methods, Chap. I (pp. 53-57) in The Citrus Industry, Vol. II. L. D. Batchelor and H. J. WEBBER, eds., Univ. Cal. Press, Berkeley and Los Angeles, Cal.