

THE VEGETATIVE PROPAGATION OF CITRUS*

RESULTS OF PRELIMINARY EXPERIMENTS

INTRODUCTION

IN a previous note a description was given of the Solar Propagator and its application to the rooting of "semi-hardwood" cuttings of Citrus. In the present communication a description is given of all the methods of vegetative propagation which have been applied experimentally to various Citrus species. The ultimate aim in view has been the production of uniform plants suitable for use as rootstocks. Propagation has been achieved by means of roots, stems, and leaves. In stem propagation both old stems, "hardwood" propagation and young stems, "softwood" or "semi-hardwood" propagation, have proved successful with many species. For reasons which are given below, the further investigation of hardwood propagation was soon discontinued and all effort concentrated upon the more promising softwood type. The results obtained by various methods of hardwood propagation are described below in some detail for purposes of record and in the hope that they may be of some use to other investigators in the field of vegetative propagation.

MATERIALS AND METHODS

The following Citrus species and varieties have been used to a greater or lesser extent in the experiments on propagation:

Citron: *Citrus medica* L.

Ponderosa lemon: *C. medica* L. var. *Limoniu* Osb.

Rough lemon: *C. medica* L. var. *Limoniu* Osb.

Trinidad lime: This variety appears to have affinities with the Citron.

Rangpur lime: *C. medica* var. *acida*. *C. aurantifolia*, Swingle.

Kusai lime: *C. medica* var. *acida*, *C. aurantifolia*, Swingle.

West Indian lime: *C. medica* var. *acida* *C. aurantifolia*, Swingle.

T. I. lime: *C. medica* var. *acida*. *C. aurantifolia*, Swingle. This is a hybrid lime raised by the Trinidad Department of Agriculture.

Sour orange: *C. aurantium* L. var. *amara*.

Grapefruit: *C. decumana* L. *C. maxima* (Burm.) Merril.

Shaddock: *C. decumana* L. *C. maxima* (Burm.) Merril. The Shaddock has also been separated as *C. grandis* Osbeck.

C. nobilis: A loose skinned type with acid lime-like fruits introduced from Burma.

C. hystrix D. C.

C. excelsa, Wester: Provisional identification. Introduced from Burma.

In the earlier experiments, which are mainly with hardwood propagation, the numbers of branches and cuttings used in each experiment was of necessity rather small, owing to lack of experimental material. The plants furnishing the material were grown from seed and at first were not of sufficient size to yield large numbers of cuttings. In many cases the

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material had to be collected from several plants which introduced an error due to seedling variation. This error was reduced as far as possible by selecting plants of similar growth characteristics.

The physiological age of material used in the experiments was judged approximately by the position it occupied on the parent plant, and from its appearance, used in conjunction with a knowledge of the growth habit of citrus. The branch extension of mature citrus trees takes place by means of successive "flushes" or "cycles of growth". At certain times of the year there are main growth flushes when most of the branches extend in length. At almost any time, however, a few branches may be found in the activity elongating condition. During the dormant period when the branch is not elongating, very little secondary increase in stem diameter takes place along the length of the ultimate flush, i.e., the last length of stem to be produced. Marked thickening only begins when the terminal bud becomes active and adds a new length of branch. Secondary thickening is to a certain extent cyclic but succeeding cycles tend to merge into one another producing a steady increase in stem diameter as the particular branch ages, and the end of the branch grows further away from the original point observed. Secondary thickening of the branch is correlated externally with changes in the appearance of the stem surface. The epidermis becomes ruptured giving the stem a russeted appearance. The russetting first appears on small areas just above those buds which are situated towards the base of the particular flush and it gradually appears just above all the buds of the flush, spreading basifugally. Later the russetting appears as lines and streaks roughly parallel to the buds and finally becomes general all over the stem. In some species the russetting is delayed, as, for instance, in *C. excelsa*, where very little may be apparent except just above the buds, even on growth flushes of the third order in age. The growth of young citrus plants is less periodic than in older ones, the main seedling axis tending to grow steadily and continuously for about two years. In this respect, however, there is a certain amount of variation between the different species.

Information as to the rooting responses of various species has been sought by the following methods:

1. *Root Cuttings*.—Root cuttings have been taken from trees varying in age from two to five years. The cuttings were made to a length of about six inches and were taken from roots running horizontally at a depth of about eight inches. The root pieces were set in sand in the Solar Propagator and were placed in a horizontal position covered by sand to a depth of from one to two inches.

2. *Layering*.—For layering, branches were bent downwards and covered about two inches deep with sand and grit. In some cases the branches were left for a time after bending downwards until young shoots had developed to a length of about two inches, at which time the sand and grit were applied just leaving the tips of the young shoots uncovered. The layers were kept moist by watering daily.

3. *Wiring or Strangulation*.—Branches propagated by this method were surrounded by a couple of turns of soft brass wire drawn up fairly tightly and applied just below the region of the stem from which it was intended that the roots should develop. The stems were bound up at the region of the wire with damp moss which was kept moist by watering.

4. *Marcottage*.—This method with minor differences is variously known as Air Layering, Circumposition and Gootee. It is an ancient method of propagation which may have originated in the East and is mainly applied to woody plants which are not easily propagated by cuttings. In

the experiments a ring of bark about half-an-inch wide was removed from the stems to be propagated. The exposed wood was lightly scraped to make sure that all soft bark-tissues were removed and the ring-barked region bound up with damp moss and watered as in strangulation.

5. *Ringed-Hardwood Cuttings*.—In experiments with this type of cuttings the stems were ring-barked as in marcottage, but the ring was left bare and not bound up with moss or any other medium. When the upper cut made in ring-barking had healed and formed a visible callus tissue, the stem was clipped off and set as a cutting. Care was taken not to injure the healed surface as this becomes the base of the cutting when set in the rooting bed. For making these cuttings well matured stems were used such as had become russeted as explained above. They were cut to a length of about 12 inches. Five to seven leaves were left at the top, the rest being removed when the cuttings were set.

6. *Hardwood Cuttings*.—The material used in making cuttings of this type was similar to that used for ringed hardwood cuttings, differing only in that as the cuttings were made and set directly in the rooting bed, they were unhealed at the basal ends.

7. *Semi-hardwood or Softwood Cuttings*.—The term semi-hardwood applied to this type of cutting was apparently coined by Swingle to distinguish it from the horticulturists' common use of the term softwood, which is generally applied to a very sappy type of cutting. The cuttings consisted of leafy twigs of the last flush or growth cycle. After the growth in length of each flush ceases, the leaves, which are yellowish in the juvenile condition, begin to develop their full green colouration. As the leaves become dark-green the woody part of the stem begins to harden and the stems seem somewhat to dry. As far as possible twigs were selected for semi-hardwood cuttings, the leaves of which had just developed the full green colour. The cuttings were made to a length of from six to ten inches, all leaves being removed with the exception of five or six at the upper end. They were clipped from the parent tree with sharp secateurs and were set immediately in the Solar Propagator. The cuttings were made as far as possible on dull or rainy days when there was less chance of their wilting during transport to the Propagator.

From the results of the early experiments the use of "heel" cuttings was advocated. However, experimental evidence which has since accumulated indicates that there is on the whole no special advantage in making cuttings with a "heel" of older tissue.

8. *Leaf Cuttings*.—Experiments have been carried out with two types of leaf cuttings. In the first type the cuttings consisted of the leaf only, the leaves being removed by cutting through the leaf stalks close to the stem; whilst in the second type the leaf was removed together with a slip of stem tissue which included the bud, the cut being made in exactly the same way as in the removal of a bud for shield budding. Leaf cuttings were made with the same precautions, and received the same treatment as semi-hardwood cuttings.

Propagators.—A description of the Solar Propagator has previously appeared. This is a device for obtaining a certain amount of bottom heat for the rooting bed by utilizing the radiant energy of the sun. The rooting chamber has a close-fitting lid and thereby the atmosphere inside is kept saturated with moisture. In the Solar Propagator the semi-hardwood, the leaf, and the root cuttings were set. Watering was carried out once daily, in the early morning, and the Propagator was thereafter not opened again during the day, as with the leafy cuttings of the softwood type every precaution has to be taken to prevent the leaves from drying.

The hardwood cuttings were set in ten-inch pots in a tank in the greenhouse. The tank was covered with light wooden frames upon which was nailed a single thickness of Victoria lawn. The frames have since been glazed, which keeps the inside atmosphere at a higher degree of moisture saturation than formerly. However, it worked quite satisfactorily with the lawn covering, provided the sides and bottom of the tank were sprayed over with water twice daily to keep up the humidity.

Rooting Bed.—Well washed sand has been used for the rooting bed and two types have been tested in the experiments. The first was a coarse sea beach sand which contained a high proportion of broken shell fragments. The second was a fine quartz sand of which there are several deposits in Trinidad.

RESULTS OF ROOTING EXPERIMENTS

Root Cuttings

Soon after they were set, the root pieces began to produce callus tissue at both cut ends, but chiefly at the proximal end, that is, the end of the cutting which was towards the centre of the plant when the cuttings was still part of the parent tree. Later, buds appeared upon the callus at the proximal end and occasionally from other parts of the cuttings. The buds developed into leafy shoots and when these had remained in the Propagator for a sufficient length of time, roots were formed from the bases of the shoots. It will be noted that some species root from the shoots with far greater facility than do others. The West Indian lime roots much more easily than *C. excelsa*. The cuttings had been set for approximately nine months, at the end of which time over 50 per cent. of the cuttings in each species had rotted without producing any shoots. Root pieces of *C. nobilis* set at the same time all rotted without producing shoots.

Laboratory experiments of a preliminary nature indicate that root cuttings are very sensitive to over-watering, which causes decay before a callus tissue can develop. The use of a coarse sand for the rooting bed, the very careful control of watering and the use of a considerable degree of bottom heat would probably give better results for callusing and shoot production. These difficulties rule out the root cutting method as a practical measure. There is in addition the unwillingness of Citrus species to produce roots from the shoots except in those species most easily propagated by any of the methods used.

The bases of the shoots produced from root cuttings are perfectly etiolated, having pushed up through the rooting bed, but root production does not appear to be stimulated except in the readily rooting species. With a number of plants, etiolating the stem greatly facilitates root production, but with many Citrus species it appears to have little effect. These observations are in accord with some preliminary tests which were made with etiolated stem cuttings.

Layering

The success of layering as a horticultural practice is generally considered to be due in large measure to the production of etiolated shoots. In experiments with sour orange and grapefruit, the buds on the layered branches which were covered with sand and grit, failed to develop. From this it appears that the buds of Citrus are very susceptible to pressure and will not grow out if covered by even a shallow layer of sand. In some experiments, the buds were allowed to grow out to a length of about two inches before the sand and grit were applied. The shoots were then covered, leaving the tips free. This is a modification of the layering

method which is successfully applied to the propagation of apple and quince stocks. Although the shoots are not etiolated they root satisfactorily if covered while still young and tender. In the Citrus experiments the method proved quite unsuccessful and no rooting occurred. The two species used, sour orange and grapefruit, are not particularly easy to propagate and possibly greater success might have attended the use of the more easily propagated species.

Wiring

Some success has attended the use of this method. Kusai lime and grapefruit have been rooted, but a few tests with sour orange were quite unsuccessful. The method requires a considerable time for root production and as more rapid ways of rooting Citrus were found, the experiments were soon discontinued. Roots are only formed after the increase in diameter of the woody cylinder of the stem has constricted the bark tissues, by pushing them outwards against the coil of wire. The constriction prevents the downward translocation of food substances in the bark and as these accumulate above the wire, greatly increased stem growth takes place at this point. If the region above the wire is kept moist with damp moss, damp coconut fibre, or earth, roots are thrown out.

In this manner branches of Kusai lime wrapped up with damp moss were well rooted at the end of three months and grapefruits after five months.

Marcottage

This is probably the most certain method of propagating woody plants. By this method most species of Citrus can be rooted. In the early experiments the region of the stem which had been ring-barked was covered with grit and sand or bound up with coconut fibre, and watered every day. With both these treatments a very large callus tissue developed from the upper edge of the ring-bark but no roots were formed. Paring the callus, a procedure which assists rooting in some plants, had no beneficial effect. Finally, success was achieved by using damp moss with which to bind up the ring-bark. The moss "ball" so formed was surrounded on the outside with a sheet of rubber cut from old automobile tyre, tubes and tied on somewhat funnel-wise. Water was given every day and it was found that the rubber efficiently prevented the moss from drying out in between waterings. It thus appeared that neither the sand and grit nor the coconut fibre maintained sufficiently wet conditions for root formation. Experiments have shown that the position of the ring-bark in relation to the buds on the stem, whether nodal or internodal, is quite immaterial. The roots spring from the region of the callus tissue and not from any pre-formed root initials as in some plants.

The exact age of the stem has apparently little effect upon rooting, for, in different experiments branches varying in age from mature current flushes, i.e., the last growth cycle, having stems of approximately quarter inch diameter, and other branches of several cycles of growth having stems of about one inch diameter were rooted with equal facility. Below is a table giving approximately the number of days which elapsed from ring-barking to the first appearance of roots for several different species:—

Citron	20-25 days.
West Indian lime	20-30 days.
Kusai lime	20-30 days.
<i>C. excelsa</i>	25-40 days.
Sour orange	30-50 days.
Grapefruit	30-50 days.
<i>C. nobilis</i>	130 days. Result of one test only.

Ringed Hardwood Cuttings

The special feature of this type of propagation is that the cutting is set with a pre-formed basal callus. The bark tissue is already healed when the cutting is removed from the parent tree. This method was developed when early experiments with ordinary hardwood cuttings failed, owing to decay setting in at the basal ends which failed to callus when the cuttings were set. In most of these experiments the cuttings were ring-barked and left on the tree for 20 days before being clipped off and set. A few experiments on the effect of removal or non-removal of the leaves at the time of setting were inconclusive; there were, however, indications that if all the leaves were left on the cutting, then greater care had to be taken to keep the air in the Propagator thoroughly moisture laden. If the air at any time became too dry the leaves lost water and thereby tended to dry out the stems. Cuttings bearing from five to six leaves which were successfully rooted, developed a large number of roots, the roots were thicker and bore more secondaries than those cuttings which were completely defoliated. Some species, and in these experiments especially West Indian lime, tended to produce leafy shoots before any roots had formed. Having no water-absorbing organs, the shoots wilted rapidly and the cuttings died. In experiments with cuttings ring-barked for 20 days and then set, the approximate number of days from setting to first appearance of roots is given for a few species in the table below:

Citron	15-20 days.
Ponderosa lemon	25-40 days.
Rangpur lime	25-40 days.
Sour orange	60-80 days.

It is probable, however, that the 20 days given was not the optimum period for all species. In one experiment with Rangpur lime the period was varied from five days to 30 days with five-day intervals. The cuttings were set for 30 days and then dug up and examined, with the following results:

Days. Ring-Barked.	Number of Cuttings.	Cuttings Rooted 30 Days Set.
0 Control	5	0
5	5	1
10	5	2
15	5	5
20	5	1
25	5	1
30	5	1

For the Rangpur lime the optimum period for ring-barking was clearly in the neighbourhood of 15 days.

Hardwood Cuttings

Citrus cuttings of this type are not easy to root and on the whole little success has attended the use of this method. Failure is usually due to the cuttings decaying from base upwards. Only in the easily propagated species was a reasonable percentage of rooted cuttings obtained. Laboratory experiments upon the callusing of hardwood cuttings have shown that no callus forms if the cut end is continuously covered by a film of water. In practice this means that hardwood cuttings must only be watered very sparingly in the early stages of propagation. Later, when the basal ends have callused, watering may be increased to stimulate root production.

Semi-Hardwood Cuttings

Cuttings of this type have been rooted fairly successfully in the Solar Propagator. Compared with hardwood cuttings, semi-hardwood cuttings callus much more readily and few cuttings have been lost owing to decay setting in at the basal ends. The different species all have their own characteristic speed of root formation. Approximate periods from setting to root formation are given below. The first figure refers to the shortest time in which the cuttings of a given batch begin to form roots. At the end of the period given by the second figure all cuttings in the batch have usually produced roots. In the slower rooting species the lack of uniformity is much more marked and even at the end of several months there may be some cuttings still not rooted. When this occurs the second figure is omitted.

Species	Days to Root Formation
Citron	10-15
Trinidad lime	10-15
Rangpur lime	15-35
Kusai lime	15-35
West Indian lime	15-35
<i>C. excelsa</i>	25-
<i>C. hystrix</i>	30-
Sour orange	30-
Shaddock	35-
<i>C. nobilis</i>	250-

Leaf Cuttings

The first type of leaf cutting, consisting of leaf only, has not been found very successful. The time taken to root is longer than in the case with semi-hardwood cuttings, and after rooting a considerable time elapses before any shoot is formed. These eventually appear as tiny shoot-buds upon the callus developed from the cut end of the leaf stalk. The second method in which a portion of stem tissue is removed along with the leaf, has been very successful. These have rooted in just about the same number of days as the corresponding semi-hardwood cuttings. When the leaf is well established, the bud breaks and makes a good strong shoot. The method has not long been tried but appears promising. Many more cuttings can be taken from one tree and they occupy less space in the Propagator and are more easily handled than is the case with semi-hardwood cuttings.

DISCUSSION OF RESULTS

The hardwood methods of propagation, comprising wiring, marcottage, hardwood cuttings and ringed-hardwood cuttings, all suffer from one serious defect from the point of view of root-stocks, which is that the type of plant produced is not suitable for use as stock for budding. The plant produced consists of a hard stem, the bark of which does not "slip" easily for budding, whilst the shoots which are produced in profusion at the summit of the cuttings are too thin and sappy to bud for some time. The most promising of the hardwood method is the ringed-hardwood cuttings. This involves less time and labour than wiring or marcottage. Ordinary hardwood cuttings which require less labour are considered to be too uncertain owing to the liability to decay. One method of using the ringed-hardwood cuttings is to bud the stem at the same time that the ring-barking is done. When the cutting is set it is cut at the ring-bark and also just above the bud which by this time should have taken, but should not have grown out.

The cutting is then set and when rooted the scion bud will grow out along with others on the stock. The latter must be removed as they appear. This has proved successful using Citron as stock and T. I lime as scion. There was, however, a certain mortality of buds while the cuttings were set and before they had rooted.

The semi-hardwood or softwood methods of propagation on the whole appear the most promising. These cuttings grow away with a stem quite suitable for budding. The production of side shoots which have to be removed is probably not much more marked than in seedling stocks. Economically, the chief drawback to semi-hardwood propagation is the necessity for some fairly efficient type of Propagator, inside which the cuttings can be maintained in an atmosphere saturated with moisture until rooting takes place. The cuttings must only be made on dull rainy days or at early morning or in the evening, otherwise the leaves wilt before the cuttings reach the Propagator. Watering should be done once a day and that in the early morning so as to avoid having to open the Propagator during the heat of the day.

Reference has been made to "easy" and "difficult" species from the point of view of propagation. A rough classification of the species used in the experiments is as follows:

Easily propagated: Citrons, lemons, limes.

Not easily propagated: Oranges, grapefruit.

Only propagated with difficulty: *Citrus nobilis*.

The successful establishment of rooted cuttings may be said to take place in three phases. In the first phase the walls of these cells which lie close to the cut surface but which have not actually been themselves injured, become suberized. Fatty substances are apparently released from these cells which oxidize upon the surface of the cell walls to form a varnish-like layer of suberin. This suberin deposit protects the cells from invasion by micro-organisms which cause decay. Suberization takes place best in an atmosphere saturated with moisture and under conditions of ample aeration. It is prevented if the cut surface is kept covered with a film of liquid water, for under these conditions the fatty substances leach away from the cell walls and no suberin deposit results. The second phase is characterized by the formation of callus tissue which arises as a result of cell division and multiplication which takes place beneath the suberized layer. The callus tissue forms a second, and still more efficient barrier against the entry of decay organisms. The third phase is marked by the differentiation of roots from the wood cambium and their growth outwards piercing the callus tissue.

The results so far obtained indicate that callus formation, and therefore in all probability suberization first of all, takes place far more readily in semi-hardwood than in hardwood Citrus cuttings. In semi-hardwood cuttings callusing will take place under wetter conditions than are suitable for hardwood. There is some evidence also which indicates that the quantity of callus tissue formed is greater when the rooting bed is relatively dry. Rooting, on the other hand, is favoured by wetter conditions. The conditions to aim for in Citrus propagation are therefore to keep the rooting bed on the dry side at the start to encourage suberization and callusing.

Later on the watering should be increased to check excessive callus formation and to stimulate rooting. Semi-hardwood propagation is more successful than hardwood because far less care is required in controlling the preliminary watering. The success of ringed hardwood cuttings is due to their starting with a ready formed basal callus.

Some of the factors which have an effect upon the speed of rooting in semi-hardwood cuttings are receiving special attention. One of the most important of these is undoubtedly the age of the material. The most recent experiments seem to indicate that cuttings should be made from material, the leaves of which have not yet developed their full dark-green colour. The stems of such material are quite soft and fairly "sappy". Another factor which seems to be of importance, though results have so far been rather conflicting, is the size of the sand particles in the rooting bed. The indications are that if the sand can be kept sufficiently wet the size and type of sand grain is quite immaterial. If, however, water supply is cut down, then in the coarser grades of sand a large development of callus takes place and rooting is retarded. A treatment, which may prove of use in hastening rooting, in the soaking of the cuttings previous to setting in dilute solutions of potassium permanganate. Some encouraging results have been obtained, but require confirmation on a large scale.

SUMMARY

1. The results obtained from preliminary experiments on the Vegetative Propagation of various Citrus species are described.

2. The methods of Propagation which were used comprised: Root cuttings, layering, wiring, marcottage, ringed-hardwood cuttings, hardwood cuttings, semi-hardwood cuttings, and leaf cuttings.

3. It has been found that the relative speed of root formation in stem propagation is a specific attribute. The relative rates of root formation of various species remain approximately the same whatever the method of propagation used.

4. Semi-hardwood cuttings of Citrus are propagated with much more certainty than are hardwood, this being due to the inability of hardwood cuttings to callus at the basal ends except under very carefully controlled moisture conditions of the rooting bed. Semi-hardwood cuttings callus satisfactorily under far wetter conditions than do hardwood, and therefore demand less care in watering.