VARIATION IN CITRUS ORCHARDS*

THE variation in production and size of individual trees in citrus orchards is not a mere bogey of horticultural advisers but a fact demonstrated on each occasion of testing.

Thus Webber writing in 1925 of South African groves gives the following opinion: "Some groves, particularly those 10 years of age or older, are made up in considerable measure of trees of variable types, frequently almost or quite worthless as commercial sorts. This is particularly true of the early planted Navel groves. In many orchards of all ages there are frequently variations in size of trees and in yielding capacity, many trees existing that apparently year after year are regularly low yielders or 'boarder' trees."

Again Hodgson in urging Californian growers to take simple tree records, notes the presence in orchards of 3 types of tree, i.e., the entirely unprofitable, the self-supporting and the profitable tree. He gives analysed figures of cropping from three orchards as follows:

Orchard	1	2	3
Percentage of unprofitable trees	13·0	14·0	32·0
Percentage of self-supporting trees	19·0	32·5	42·0
Percentage of profitable trees	68·0	53·5	26·0
Total number of trees	5,736	800	1,525

It will be seen that even in an outstandingly profitable orchard such as number 1 segregation of trees into these classes shows that approximately one tree in three is either only self-supporting or is actually carried at a loss. He writes in 1923: "In the average orchard it is believed that the percentage of such trees is near 50".

Again there is the evidence of Batchelor and his co-workers to show the existence of variation. In preparation for fertilizer experiments they aimed at uniformity of material, treatment, and, as far as possible, soil. To ensure uniformity of material they adopted the following methods of propagation:

(1) The sweet orange (C. *sinensis*) stock, transplanted from seed bed to nursery in 1914, was culled and undersized trees amounting to about 15 per cent. eliminated. (2) At the time of budding in 1915 a further elimination of small sized trees was made. (3) On transplanting the budded trees in 1917 a third discarding of trees lacking vigour and size was carried out.

In budding only plump, well-formed buds from mature old wood were used, and only such twigs were used as showed blossoms formed or in process of formation on the tips of the current season's growth at the time the budwood was cut.

* From Imperial Bureau of Fruit Production, Technical Communication No. 3., November, 1932.

And yet yield and growth records taken during the twelve years of life, from planting up to the time of first application of fertilizer treatment, revealed the existence of considerable variation. Thus there was a difference of practically 109 per cent. between the lowest yielding plot, which produced an annual average of 79 lb. of oranges per tree for the 6-year period recorded, and the highest yielding plot which produced 179 lb. This variation was not considered strange by the authors in comparing areas of soil one-third mile apart. But, in addition, they also found variation in production of adjacent plots amounting to 30-40 per cent. They consider that the two groups of factors which are most persistently operative in causing the variation in production are (1) the inherent qualities of the trees due to character and vigour of rootstock and top, (2) the variability of the productivity of the soil. They comment as follows: "In spite of the variations in the growth and production of the trees of this field, it is believed that this planting is singularly uniform in comparison with the average citrus grove. This fact has been brought out in statistical studies of the variability of this and other orchards. In appearance the trees are strikingly similar."

In an earlier study on the nature and extent of the casual variability of yields of Navel and Valencia oranges and Eureka lemons under field conditions, and its bearing on the reliability of plot trials, Batchelor and Reed selected for their trials orchards uniform in treatment and appearance, and yet found considerable variability in productivity. The coefficient of variability for the yield of individual trees of these clonal varieties ranged from $29{\cdot}27 \pm 0{\cdot}69$ to $41{\cdot}23 \pm 1{\cdot}52$ per cent. Computations made on the yields of the orange trees for several consecutive years showed little annual fluctuation in their variability.

Many other workers have testified to the variations usual in citrus orchards. Thus Webber in 1920 noting the existence of variation in yield of trees of the same variety in the same orchard, planted at the same time and cultivated alike, considers it likely to be due primarily to one of the following factors:

1. Variations inherent in the buds due to different heritage.
2. Different kinds and characters of stock used.
3. The character of the union obtained in the budding and grafting.
4. Differences in environment.
5. Accident.

The influence of the stock on the variation observed is believed by him to be fundamental. He notes the normal practice to be as follows:

The stocks are grown from seed and the seedlings transplanted from seed-bed to nursery about 12 months after sowing, at which time they vary greatly in size, ranging from a few inches to 18 inches or more, this variation being always seen. In ordinary practice all of the seedlings are transplanted or at most only a few of the smaller ones are discarded.

After another 12 months those large enough are budded, there being considerable variation in the size of the seedlings, the smaller ones being budded later.

The budded trees then wait another two years and are transplanted to the grove. At this time they still vary greatly in size. Quite commonly only the largest trees in the nursery will be dug and sold, while the rest will be left for a future occasion.

The smallest trees for the smallest stocks will finally reach sufficient size and ultimately find their way into the grove. He considers that the question whether this small tree is as likely to produce a good productive orchard tree as the vigorous growing one selected previously remains unanswered.

Webber describes here an experiment in which he graded out seedlings of sour and sweet orange on transference to the nursery, discarding for inadequate size about 15 per cent. of the sweet and 25 per cent. of the sour stocks.

A further grading preceded budding, when those surviving the test were budded with 4,000 buds of Washington Navel and Valencia orange and Marsh grapefruit on the sweet stocks and 4,000 Eureka lemon buds on the sour stocks. All buds were specially selected from trees of known and uniform history for the previous five years. Throughout the rest of the time in the nursery the trees gave every appearance of good growth and comparative uniformity.

Two years later the trees were regraded, 15 per cent. being rejected as undersized, while the rest were planted out. Even so the author was able to grade those remaining into 3 lots, small, medium and large.

The Eureka lemons did not stand transplanting and the experiment with them lapsed.

Of the remainder, two and a half years after transplanting, the large intermediate and small trees were found to retain the same relative size that they had when dug in the nursery.

An examination of other possible causal factors led Webber to consider that the most important cause of difference lay in the stocks.

He says that among rootstocks in common use such as sour orange, sweet orange, grapefruit, lemon and trifoliate "It is known that there are hundreds of different variations, types or varieties within each".

In examination of a stock nursery of sour and sweet orange, Webber, Mertz and Thomas, were able to select out 16 different types of sour orange and 4 different types of sweet orange. Many more could have been selected. Two years after planting out these different types were found to be remarkably distinct from each other in character and size of growth, branching foliage and other important characters. An examination of stocks at Berkeley confirmed these findings, so that Webber remarks "There is no doubt that citrus nursery stocks as ordinarily grown are made up of a very large number of widely different types of different genetic constitution, exhibiting a wide range of characters". He recommends: "When any seedling is found to be a good stock type, it can be propagated by buds and each nurseryman can grow enough trees to supply the seeds required for his nursery use."

He then gives definite recommendations to those wishing to produce uniform citrus trees. As a preliminary necessity he urges that good stock varieties of sweet and sour orange should be found, named and grown regularly to furnish seed for the production of nursery trees. (This is actually practised in the Aegean Islands and near Milazzo in Sicily. In these localities extensive, isolated areas are devoted to the raising of sour orange seedlings for ultimate use as rootstocks in an endeavour to maintain the purity of selected strains and thereby secure some measure of uniformity.) The rest of his recommendations concern the elimination of variants by selection.

Twelve years' further work and observations in California have merely served to confirm his opinions and his recommendations remain materially unchanged. In *Hilgardia* for June, 1932, he writes: "in any lot of seedlings grown from seed of the same variety and from the same source, from 5-40 per cent. are highly variable types, which apparently differ in genetic constitution from the prevailing type and each other". The remainder are of the same general type, and are, he considers, probably of apogamic, i.e., asexual origin. He notes in great detail the evidence of variation and its causes and demonstrates the existence of very strong evidence that small seedlings and small budlings tend to produce small, low-yielding trees, and large seedlings and large budlings to produce comparatively large, high-yielding trees. The most important factor is the elimination of the variant seedlings, "that are found almost uniformly to produce weak and dwarfed orchard trees".

He considers the normal methods of elimination and makes a searching comparison from an economic standpoint of pre-budding and post-budding selection, which results much in favour of the former.

Dealing with sour and sweet orange stocks and the production of good uniform trees, he recommends the adoption of the following practice in the nursery:

Seed Bed Selection.—This should consist of a discarding of the smallest plants up to 25 per cent. of the total at the time of transplanting to the nursery, i.e., one year after sowing. This would eliminate a considerable proportion of the variant types.

Nursery Selection.—This should be made just prior to budding when the seedling has been 2 years in the nursery and has had plenty of time to show its top characters, and should consist of the elimination, irrespective of size, of any seedlings differing from the normal in any way and in addition of small seedlings up to a limit of 25-30 per cent. of the remainder "After this elimination," he writes," the entire remaining population may be budded and safely regarded as propagated on good, uniform, highly selected stocks. As the seedlings up to this time have little value, it does not entail very great financial loss."

Budling Selection.—Small and inferior budlings should be removed before digging for planting out. This elimination should be very slight, with good buds and selected stock probably not more than 1-5 per cent.

The problem of achieving uniformity in the citrus orchard has never lacked attention from Webber and it is interesting to note his experiences in South Africa.

In 1924-25 he conducted a special enquiry on the citrus industry of the Union, and as a result he recommended a serious trial of vegetative propagation methods for raising not only scions but also rootstocks.

In his report he notes the proved unsuitability for South Africa of the Seville orange as a rootstock and discusses the possibilities of the rough lemon.

He considers that the lesson to be derived from his own work and experience seems clear, namely that we cannot expect to obtain uniformly good lots of trees from ordinary, unselected seedling stocks, every individual of which differs in some character. Thus as a casual example, an examination of a group of 30 seedling rough lemon trees of about 7 years old showed 4 distinct types, strains or varieties, each of which he considers, would react somewhat differently as a stock.

In future he concludes: "We will have to select good stock strains that by experiment are known to produce uniform seedling progeny, or it will be necessary to find good stock strains and propagate budding stocks from these by some means such as layering or by cutting in order to ensure uniformity.

His search for good strains propagated asexually was successful. He found that in practically all cases layered trees seemed to possess rather an exceptional vigour and fruiting capacity. Fair comparisons were made between Valencias and Navels budded on rough lemons and others growing on their own roots from layers. In no case did the layered tree suffer by the comparison. He saw no evidence of the layered trees being rooted only on one side, nor did they appear more liable to uprooting by the wind, though he did note that they had no taproots. Sweet orange layers were indeed susceptible to collar rot, but no more so than sweet seedlings. Wherever plantings of layered trees were found they were clearly as good or better than any other trees in the grove.

The Washington Navel is in general a slow growing rather weak tree and one could understand the failure of such a variety on its own roots. Yet some forty ten-year old layered Washington Navel trees near Grahamstown were seen to be growing just as well as others budded on rough lemon. If then this does fairly well, sorts such as Valencia which are naturally vigorous, should do very well.

But the method of propagating by layering as practised in S. Africa, of which Webber gives an illustration, is not conducive to the production of a large number of rooted layers, only one tree being produced by each layered branch, nor had it been used for getting rootstocks. Despite this, Webber suggests that the use of layers or cuttings for propagating citrus stock might prove extremely valuable and he considers that experiments might profitably be made with the method of layering so successfully used for deciduous fruit trees (i.e., pinning down and covering the young shoot, which will then root at the nodes). A warning note is sounded by him in this connection. This method of propagation, however, would in general give no opportunity to effectively practise bud selection, and this is a serious drawback with unstable sporting varieties like the Washington Navel and the Valencia, and is somewhat risky with any citrus variety."

Webber was thinking here in terms of own rooted trees as a possible method of achieving uniformity. The more economic practice being, however to bud or graft varieties on to particular rootstocks, the actual problem is to achieve uniformity of production from this compound plant of rootstock and scion. Uniformity of soil and treatment are essential. As regards the rootstocks, standardization of seedling stocks by elimination of variants in the nursery has already been touched on and will be referred to again in the section on polyembryony, while details of propagation by layering cuttings, etc., will be found in the following pages together with notes on the work of bud selection of scion varieties.

POLYEMBRYONY

That variation exists within such groups as sour orange, sweet orange, trifoliate, pomelo, etc., has been shown by many workers.

Bonns, and Mertz, moreover, who made notes on the first five year's cropping of Valencia and Washington Navel orange and Eureka lemon on the above stocks, came to the following conclusions: " . . . It is evident however, that there is no ground at present for recommending any particular stock for increase yield or quality of fruit of either of the oranges or the lemon used in this experiment." They note considerable variation in cropping indeed, but this variation was not necessarily any greater between trees on different types of rootstock than between trees on the same type of rootstock. As they point out it was scarcely to be expected that evidence would be available in so short a time, but later trials, some of which are referred to in the previous section of this memorandum, show that variation still exists at a much later date than 5 years from planting, even when every effort has been made by careful selection to ensure uniformity.

Can approximate uniformity of rootstock be attained, and if so, what is the easiest method? If it can be attained without recourse to the usual methods of vegetative propagation, the problem will be much simplified. In the case of citrus the factor of polyembryony may offer a solution.

It has long been known that the seeds of certain species of citrus are polyembryonic. As early as 1719 Leeuwenhoek discovered two embryos in orange seed, but the real nature of the phenomenon as regards citrus remained unexplained until demonstrated by Strasburger in 1878.

Polyembryony, or the presence of more than one embryo within the seed may arise in various ways. In the orange it may be ascribed to a stimulus received by the cells of the nucellus, both above and below the embryo sac, even though separated from it by several cells. Strasburger found that no seeds develop without pollination and from this concluded that fertilization of the egg cell is necessary for the formation of the nucellus embryos. The experiments of Toxopeus bear this out. Toxopeus states, however, that strictly speaking his own experiments have proved only that pollination is essential and do not definitely decide the point whether actual fertilization is also necessary. The possibility remains that the stimulus may be given by the mere ingrowing of the pollen tube, though his cytological investigations strongly suggest the necessity for actual fertilization.

Neither spore nor egg cell being involved in this reproduction process, the formation of these additional embryos may in effect be regarded as a case of adventitious budding, analogous in its results to true vegetative

reproduction. It follows that on germination one seedling will be a true cross while presumably the remainder, numbering sometimes as many as ten, will differ in no way from the mother plant.

Frost found that citrus seeds varied greatly both in the number and in the size and shape of the adventitious embryos contained. He thinks there must be much competition both between themselves and between them and the fertilized embryo, and that the chances of survival to germination point must depend largely upon size, position, relative age and vigour of the two classes of embryo. In it he sees a form of developmental selection, natural selection acting within the soma of the parent (the evolutionary significance of this has been discussed by Buchholz in 1922) since genotypes inferior to the seed parent would tend to be severely handicapped by the competition of the vigorous apogamic embryos.

The economic significance of the phenomenon has not remained unobserved. Thus, if it is possible to distinguish the sexual seedling from the others in the seed bed, it would seem that nothing further should be needed to preserve a pure line of vegetatively reproduced descendants beyond the elimination of this seedling. Work by Webber, discussed by him in a paper at the Ninth International Horticultural Congress, seems to suggest that polyembryony can be turned to good account. Dealing with the variation seen in the seed bed and their persistence he writes: "The evidence seems thus to indicate that, if the variant types of seedlings (i.e., the generative offspring) were eliminated in citrus at the seed bed, the remaining seedlings, would show different sizes due to crowding and environment, but that the important inherent differences in the growth rate, if any exists, cannot be recognized at this time. It also seems to be the case that the remaining seedlings, after the variants are excluded, would show less genetic variation than would be expected in apple seedlings after excluding the marked variants, and should thus give a lower coefficient of correlation between size of nursery stock seedling and size of budded trees. "He notes incidentally that smaller correlations were obtained in his experiments than in the case of various repeated experiments with deciduous fruits.

Toxopeus, experimenting in Java with many kinds of citrus varieties, found the percentage of vegetative progeny arising from self and foreign fertilization in these varieties. Actually in the case of many hybrids the type of fertilization did not affect this percentage appreciably. Varieties examined included types of the following: C *nobilis*, C. *aurantium*, C. *grandis*, C. *aurantifolia*, C. *medica*. As regards the elimination of generative seedlings he found that there was no difficulty in picking out the offspring of complicated hybrids at a very early stage. Thus the generative offspring from such hybrids sown in December, 1928, were readily picked out in July, 1929. He states that, if negative selection of these off-type seedlings is made, 90-100 per cent. of the remaining plants will be of vegetative origin. This has also been found to be the case with two of the best stock varieties possessed by the Horticultural Extension Service in Java, namely Japanese citron (C. *nobilis* Lour. hybr.) and Citronella rough lemon. When on the other hand the mother parent is

not a hybrid and the percentage of vegetative seedlings is known by trial to be less than 90 per cent., the generative seedlings can be made distinguishable from the vegetative type by crossing with a very different father plant. For the pollen plant to be employed usefully, it should possess one or two characteristics, which in the resulting hybrid will dominate pronouncedly at an early age over those of the seed parent.

In a recent article Toxopeus describes the method of differentiating between the generative and vegetative seedlings of Japanese citron and rough lemon. As regards the latter he notes that the generative offspring amount to only 4 per cent. generally show very poor growth and are very easily distinguishable. The seeds produced 2 or 3 embryos which obstruct each other's growth very considerably. The weakest may be removed in the seed bed and the rest transplanted after developing their second leaves. In Japanese citron, where the percentage of generative offspring is much greater, 50-60, there is a greater danger of confusion, though to an observant eye the differences are obvious. To help those not conversant with the typical characters Toxopeus gives a comparative table of leaf and stem characteristics which mark the vegetative seedling, and the several characteristics which are likely to be seen in the generative seedling. Again, the generative plant is usually a weak grower.

As regards the hybrids themselves Toxopeus has found that only a very small proportion are compatible, i.e., some 28 out of 1,400. It is interesting to note that none of these 28 had pomelo as a parent. There is as yet, of course, no evidence as to the growth and cropping of citrus varieties worked on any of the compatible stocks, but at least there is hope that some, at any rate, will by field trials be proved valuable as stocks, in which case their reproduction by vegetative methods, including selection from polyembryonic seed beds, cuttings, layers, etc., might be a source of uniform rootstock material.

Webber in the current volume of the *Proceedings of the American Society for Horticultural Science* notes that apogamy, or the production of seedlings from unfertilized embryos, presents considerable difficulty to the breeder, but may be turned to good account by the nursery man, "who should thus apparently be able to obtain easily from any good stock type large batches of seedlings, which can be depended upon to be of nearly uniform genetic type and to react uniformly on the scion." Writing in 1931, he noted, however, that the percentage of apogamic embryos in certain citrus species and varieties used as stocks varies considerably. Obviously any stocks to be perpetuated by this vegetative seedling method, however desirable their other qualities, must possess the polyembryonic factor to a marked degree. The actual range of apogamy has been determined for certain varieties by different workers, and is given by Webber as follows:

Species	Range in Percentage of apogamic embryos
Sweet orange varieties, C. *sinensis*	40-95
Sour ,, ,, , C. *aurantium*	75-85
Grapefruit ,, , C. *grandis*	60-95
Mandarin orange ,, , C. *nobilis*	10-100
Lemon ,, , C. *Limonia*	10-96
Citron ,, , C. *medica*	40-50
Trifoliate orange ,, , *Poncirus trifoliata*	72

A factor of exceptional interest is the high percentage of apogamy shown by some FI hybrids of radically distinct species and the fact that such hybrids are often likely to be valuable as stocks. Thus progenies of the citronge, a hybrid of P. *trifoliata* (72 per cent. apogamic) and C *sinensis* (40-95 per cent. apogamic), have in Webber's experience always reproduced the citronge type, and may thus be considered as 100 per cent. apogamic. Among varieties which are not entirely apogamic he has found that there is a proportion of seedlings differing from the prevailing types. These variants are commonly, but not always, of comparatively small size and "almost invariably produce some degree of dwarfing in scions grown on them. The evidence available indicates that these variants apparently are seedlings produced from the normal (sexual embryos, probably mainly by self-fertilization. The roguing out of these variants from a batch of nursery seedlings before they are budded is the most important selection that can be made in the nursery. The seedlings remaining after such an elimination can be safely considered to be chiefly of apogamic origin."

Nevertheless, even among apogamic seedlings remarkable variations have been noted from time to time, the phenomenon being ascribed by Frost to chimeral conditions in the parent trees. Thus a pollen-sterile Navel orange has been known to produce apogamically, several fertile, non-Navel progeny. Again Webber's experience during the last twelve years shows that reproduction by vegetative means does not eliminate the necessity for selection in the nursery. He finds that "within comparatively homogeneous, apogamic seedling progenies of citrus the large seedlings in general produce larger budlings than the small seedlings of the same progeny and that this larger size is maintained in the orchard for at least several years. Trees on large seedling stocks during the first 8 and 10-year periods in the orchard have yielded over 19 per cent. more than comparative trees on small seedling stocks from the same apogamic progeny." He considers that though eventually the difference may disappear, it may be maintained for many years in long-lived, slow-growing perennials, such as citrus, and may be attributed to the hold-over influence of large size and vigour in the young seedlings or cuttings. Although the heritage of such plants is unlikely to be affected by the process of selection in the nursery bed, he considers such selection to be economically sound, and gives figures to support this view.

Toxopeus, summing up the possibilities offered by the polyembryony, says that it enables us in one way or another to raise in one year from a single tree a very much greater number of vegetative progeny than would be possible by methods of budding and grafting without spoiling the mother tree. Further, new varieties can be imported as seed, thus minimizing the risk of importing disease. If the seed is found satisfactory, then by observation of the number of embryos an estimate of the percentage of apogamic offspring can be made. If this is high, a single sowing will furnish a large percentage of replicas of the mother plants; if low, twinning will be necessary. (In twinning the seedling is split vertically in two, thus securing two plants instead of one.)