California Avocado Society 1953-54 Yearbook 38: 87-95

DEVELOPMENT AND EVALUATION OF CLONAL ROOTSTOCKS IN THE AVOCADO -- PHASE TWO

H. L. Gillespie
Avocado nurseryman engaged in private research in the field covered by this article, as well as related horticultural studies.

In the past few years, considerable interest has been developing around the possibility of isolating, standardizing, and perpetuating a "predictable-performing" rootstock for the avocado. The need for this "standard" is self-evident in the production behaviour of even the best orchards in the avocado growing areas of the state. This rootstock would have to be one that would assure greater uniformity of tree size and overall average fruit production— and to be available at a cost that would be in line, on a production-potential basis, with presently available seedling-rooted nursery trees.

Abundant data prove the superiority of clonal rootstocks in ether tree crops; but the nursery trade still propagates the avocado on seedling roots, partly because their low cost meets the *public demand for low-priced trees* for orchard planting.

As a consequence, all cultivated avocado trees, with few exceptions, are a combination of two individual varieties. Of the *scion* variety we know: It produces a fruit we have found desirable, and through asexual reproduction processes (budding or grafting) it is reproduced virtually identical in all of its varietal characteristics. But, of the rootstocks we use to multiply these fruiting varieties we know specifically almost nothing. As each seed begins in a single cell, formed by the union of two gametes; and because of the hundreds, perhaps thousands, of character combinations possible in its genetic constitution, the resultant seedling becomes a new — an individual and an unknown — variety. Of such seedlings of uncertain parentage, we are accustomed to form rootstocks.

So far, very little is known about the interactions that result from indiscriminately combining these "sexual-variant" avocado seedlings with any of the "fruiting" varieties (by budding or grafting). That interaction does exist in these two-variety combinations is apparent even to the lay observer. The variation in tree size and productivity, so common to most of the commercially-grown varieties, cannot entirely be the result of environmental and cultural conditions.

According to evidence available relating to some other tree crops, uniform tree size and production are obtained only when the scion variety is grown upon rootstocks that are within themselves genetically identical. Also included in these data is evidence of a wide range in susceptibility to disease, tolerance of soil conditions, resistance to insects, quality of fruit, length of productive life, and other factors. It is a matter of record that the avocado industry is not the first to have suffered "economic pain" as a result of the character variance of seedling rootstocks.

Four Casper No. 2 root clones side grafted with Fuerte scions from the same tree. Seedling stocks cut back, but not yet removed.

The first large-scale development of any rootstock standards appears to have started in Europe, and to be primarily identified with the Mailing Research Station, England. This work resulted in the tabulation and vegetative reproduction of apple stocks having definite characteristics, and predictable growth and fruiting behaviour when combined with selected fruiting varieties. "Mailing" serial numbers were employed for identification of the various rootstocks.

The Massachusetts Experiment Station, in 1924, imported many of these clonal apple stocks for trial. They were budded to two American varieties of apple. In 1928, the resulting trees were planted in orchard form, with seedling-rooted and own-rooted trees also being planted for comparative purposes. The results, in the early 'forties, indicated the following.

Production was generally correlated with tree size. Two of the Mailing clonal rootstocks out-produced both the own-rooted and the seedling-rooted trees in both of the fruiting varieties. Two of the root clones appeared to induce early productivity in one of the fruiting varieties. Several of the root clones appeared to influence the shape of the fruits. One of the fruiting varieties was far more variable on seedling roots than on any of the clonal rootstocks. Other factors noted were in the adaptability of the several clonal rootstocks to soil moisture conditions. Several were tolerant to heavy watering; others were distressed in this condition. Some showed sensitivity to low moisture concentrations, while others appeared very tolerant of this condition.

In the citrus industry, clonal rootstocks are available as seedlings. This is the result of a phenomenon, peculiar to citrus, that initiates mitotic division of the cells of the nucellus. These cells develop into embryos formed entirely without contribution of male (pollen) cells. As these embryos are formed only from the tissue of the seed parent, they are genetically identical. They have neither lost nor gained characteristics.

In the years since this vegetative process was isolated, quite a "book"[1,2,3] has been compiled on various combinations of citrus rootstock and scion varieties. As is a matter of record, these seed-produced clonal rootstocks have made possible the production of "standardized" nursery trees, at prices well in line with the economics of citrus culture.

In the avocado, however, this "nucellar embryony", or the asexual reproduction through the seed, has not yet made an appearance[4]. Seed production of the avocado seems to depend entirely upon the successful fertilization of the female cell in the embryo sac. Cell division otherwise initiated and continued appears to result in the formation of seedless fruits, commonly known as "cukes". Attempts at artificial stimulation (2,4-D) resulted only in these seedless fruits[5].

In view" of the foregoing, it would appear that the door to avocado root-stock "standardization" through the seed is closed.

The Fuerte variety, by the end of the 'twenties, had resolved avocado growing into a "business", and had made a place for it in the economy of the state. It also brought with it production irregularities that indicated that this part of the state's economy might not be very stable. Research was started with a study of flower behavior and pollination requirements. Conclusions arrived at, from this and other functional research, indicated the need of more suitable varieties. Work on this started in 1941, with the initiation of an avocado breeding program.

In the interim, considerable progress had been made, in the citrus industry, from a study of bud mutations that had appeared as variant growth and fruit forms on otherwise normal trees of citrus varieties. Many off-type and unproductive trees frequently found in citrus orchards were ascertained to have been unintentionally propagated from limb or whole-tree "sports" of worthless types. The importance of sports, or bud mutations, to the citrus industry, with particular reference to the economics concerned, is well described by Shamel in Vol. 1 of *The Citrus Industry* (Chapter 10). That work is drawn upon for the following remarks.

Studies carried on in more than a hundred Washington Naval orange groves in southern California showed that about 25% of the trees were made up of striking entire-tree variations, with a minimum of less than 10% and a maximum of 75%,' in the different groves. Indicating that the percentage of variants was increasing with each propagation, the percentage of variants was smallest in the older orchards and larger in the younger orchards. In one of the best and most uniformly productive old Washington Navel orchards in the San Dimas district, about 3% to 5% of the trees were of a particularly unproductive and undesirable strain. These same trees, however, had an abnormally large proportion of vigorous-growing limbs—considered at the time as most desirable as nursery sources of budwood. Several years later, in a young orchard, it was found that approximately 90% of the trees showed the rank-growing, unproductive, and inferior fruiting characteristics observed previously in the few trees in the older orchard. Investigation into the history of the younger trees indicated without doubt that they had been propagated from buds taken from the vigorous, but unproductive, trees in the older orchard. Subsequent top-working of the undesirable trees in the young orchard brought them into normal production.

Eight Casper No. 2 root clones. Four at left have been side grafted with scions from the same tree. Four at right still have the sexual-variant tops intact.

In an eight year old Valencia orchard in the Corona district, where individual tree records were carried on for several years, many of the trees were found to have limb variations carrying corrugated fruits. A study of the orchard revealed that 12% of the total trees were entire-tree variants which had undoubtedly been propagated from similar limb variations.

A study of an eight year old Eureka lemon orchard, Corona area, found 3,200 out of 16,000 trees variantly substandard enough to warrant top-working to a superior strain. This was successfully done.

One of the most valuable Marsh grapefruit orchards in the Riverside district, when 12 years old, was found to contain 123 markedly undesirable tree variants out of 500 trees. These undesirable trees produced seedy, rough, corrugated, and decidedly inferior fruits—identical with those borne by limb variations in otherwise normal trees in the same orchard. The undesirable trees were successfully top-worked to a superior strain of the Marsh variety.

Although the definite cause or causes of bud mutation are unknown, the occurrence of such variations in citrus has been established beyond doubt. But also beyond doubt is this: Bud variation produces horticulturally inferior variants far more often than it

produces superior variants.

Several of the citrus bud-variations have proven superior to the variety giving rise to them, and to have considerable commercial value. The Thompson (Pink Marsh) grapefruit originated as a bud mutation on an otherwise normal tree of the Marsh variety of grapefruit. This pink-fleshed Thompson in turn gave rise, by bud mutation, to the Ruby and Webb (red-fleshed) varieties in the late 'twenties. These, and kindred local occurrences, helped in no small way to develop the "strain" hypotheses that seek to explain the occasional "outstandingly productive" individual Fuerte trees that have appeared since introduction of the variety.

Before any comparison is made between citrus and avocado, with regard to occurrence of mutations, it might be well to look at the evidence. In citrus, as noted above, bud variation has produced new *varieties,* capable of perpetuation through asexual processes. In the Fuerte, no recognizable changes have appeared in the fruits produced by allegedly superior "strains". The fruits from these "strain" selections appear *varietally* identical to those from trees with low yield. Significantly, this desirable productivity factor in the "strain" parent trees has not been perpetuated through propagation. There is reason to believe that evidences of "strain" are more likely the result of physiological or functional phenomena, brought about by the influence of climate, soil, and cultural care. There is reason to wonder, too, whether the prolific Fuerte tree may not be influenced by the genetic individuality of the sexual-variant seedling *rootstock.*

The occurrence of bud variation appears to the author to be tied to horticultural species, and varieties thereof, that are capable of producing adventitious growing points from somatic, or vegetative, cells. A few examples are citrus, apple, and rose. Its appearance in the avocado—at least with respect to the "strain" trees—seems to the author to be based upon evidence of empirical nature. Limited investigation (again, by the author) has failed to disclose supportable evidence. These remarks, it should be noted, refer to the question of "strains" in the Fuerte variety which make certain trees different from others. There have been instances of apparently true mutations in the Fuerte, though few in number and productive of fruit unlike the Fuerte.

Young avocado seedlings, stripped of growing points by cutting buds out of the leaf axils (and cutting well into the meristem), failed to produce adventitious growth. Two-year-old seedlings decapitated well below the stem-root transition zone have all failed to initiate a growing point, and all have died.

In 1946, individual tree production records were initiated in a grove of Fuertes owned by Mr. Kenneth Casper, Yorba Linda, Orange County[8]. This grove, located approximately one "bee-line" mile from the historic Whedon planting, was an ideal subject for analysis. The trees were of the same age, and of identical bud source. The rootstocks were all grown from seed obtained from a single Mexican-type seedling—a regular producer itself, and a good producer of the Fuerte variety when topworked to it. Soil, temperature, and water factors appears to be uniform for all the trees. The uniformity of these conditions, however, did not result in uniformity of fruiting behaviour or productive regularity. By 1949, it was evident from individual tree production records that five trees in the grove were outstanding producers. The four-year (1946-49) aggregate annual

average for these five trees was 522 pounds, in contrast to the 164.5 pounds averaged by the 119 trees in the study. With everything plausible taken into consideration, the responsibility for the variable tree behaviour was almost necessarily credited to the sexual-variant seedling rootstock.

Convictions (fortunately) usually require more than mere circumstantial evidence. To prove that the genetic-variant seedling rootstocks were responsible, "as charged", required asexual reproduction of the root tissue that supports these particular Fuerte trees. This re-grown root tissue then would have to be recombined with scion wood from these very trees, and a reasonably large number of the resultant trees would have to be grown to fruiting maturity. Only in this way could the question of productive responsibility be resolved.

Eight Casper A *o. 2 root clones. Four at left have been side grafted with scions from the same tree. Four at right have had the seedling tops grafted with scions from the same tree. Size of "sandwiched" trees directly reflects growth of the prior seedling tops.*

From the wealth of information available from root-relationship studies in other tree crops (completely lacking in the avocado, unfortunately), we concluded that the project was not impossible. With the cooperation of Mr. Casper, root-cuttings were taken from three of the outstanding Fuerte trees. These root-cuttings were whip-grafted onto the stubs of the tap roots of young avocado seedlings, these seedlings having their cotyledons attached and turgid. The root-grafted young trees were placed in a cable-heated hot bed, with the seedling section supported above the growing medium. Successful regeneration of two of these clones was accomplished in 1952. Observations follow, relative to one of these tissues designated "Casper No. 2".

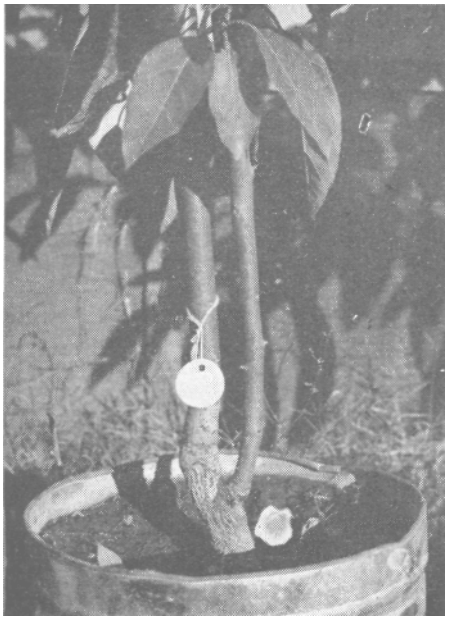
First: If a vigorous seedling was selected, and a successful union established, the Casper No. 2 root clone produced a fast-growing tree—probably as fast as this particular "top" would have grown on its own root in the same time.

Second: If an off-type or slow-growing seedling was used, and a successful union established, the resultant tree was not a fast grower. Growth was probably no faster than this particular "top" would have grown on its own root.

Third: When these clone-rooted seedlings were top-worked to Fuerte, using scions from the same tree providing the root cuttings, the growth rate appeared to reflect the prior behavior of the interstock, and not the known vigor of the root clone.

Fourth: When Fuerte scions, from the same tree as provided the root cuttings, were grafted into the re-grown tissue *below* the union of the seedling and root clone, the subsequent growth and vigor were expressive of the Casper No. 2 Fuerte tree.

Preliminary conclusions: 1) the vigor of the "juvenile" is retained in the root tissues of the particular seedlings that were used as the rootstocks for the outstanding Casper Fuertes. 2) These re-combined Fuerte trees exhibit a *growth uniformity* not heretofore observed in sexual-variant seedling-rooted nursery trees of like stock caliper.



Typical Casper No. 2 root clone and Fuerte scion union. Note regenerative-seedling and root-clone union above Fuerte union. Seedling stock and root section not yet removed.

As this is a matter concerned with the economics of avocado growing, let's look at some figures. They are taken from the Orange County Avocado production Cost Analysis for 1952 (twenty-third summary).

Orange County Averages for 1948-52—Analysis of 15 groves

Tree age, average	17 years
Trees per acre	70
Average yield per tree	53.5 lbs.
Average yield per acre	3,744 lbs

With which, compare—

Casper Grove Average for 1948-52 (included in above table)

Tree age, average	22 years
Trees per acre	56
Average yield per tree	176 lbs.
Average yield per acre	9,891 lbs.
Average yield for the five"top" trees in grove, per tree	*700 lbs.*

Hypothetically, a 56-tree-per-acre grove, consisting of "root and top tissue duplicated" trees, could produce an average yield per acre of 39,200 pounds. Land investment and cultural care costs would be the same as for the present low-yield groves. Nursery trees would cost more—perhaps five times as much, but greater yields would offset that cost quickly.

It would cost somewhat more to pick the hypothetical crop, no doubt; but with that kind of yield, would you mind?

GLOSSARY OF TERMS USED

Clone: A group of individuals resulting from asexual multiplication; examples: Fuerte, Nabal, Ganter, etc.

Variety: "In horticultural nomenclature as used with citrus and most fruit crops, a variety is a group of individuals, all possessing the same characters that have been propagated from one parent individual or branch of an individual by asexual methods, such as by budding or grafting."- *Webber.*

Gametes: A mature sex cell, or germ cell, capable of uniting with another of like origin to form a new plant or animal.

Mitotic: Descriptive of the four successive stages of the division-multiplication of a body cell in the formation of two new, or additional, cells.

Nucellus: The central and chief part of the body of an ovule, containing the embryo sac.

Meristem: Embryonic or undifferentiated tissue, the cells of which are capable of active division.

Strain: "The term 'strain' is here used to designate a group of individuals of a horticultural variety differing from all other individuals of the variety in one or more constant and recognizable characteristics and capable of perpetuation through bud

propagation."- *Shamel, as a footnote on page 920, The Citrus Industry, Vol. I.*

The word strain is commonly used to indicate comparatively slight differences within what is considered a single variety.

Somatic: Cells of the soma, or body. (Egg cells and sperm cells are gametes.)

REFERENCES CITED

1. Batchelor and Webber—Rootstocks: Character and Reaction. The Citrus Industry. Vol. II.
2. Batchelor and Rounds—Choice of Rootstocks. The Citrus Industry, Vol. II.
3. Bitters and Batchelor—Citrus Rootstock Problems. California Agriculture, Feb. 1952.
4. Schroeder—Multiple Embryos in the Avocado. California Avocado Society Yearbook. 1936: p. 51.
5. Stewart, Smoyer, and Puffer—Progress Report on the Effect of Plant Regulator Sprays on Avocados. California Avocado Society Yearbook, 1948: p. 113.
6. Hodgson—The University's Research Program on the Avocado. California Avocado Society Yearbook, 1937; pps. 228-234.
7. Shamel—Bud Selection in Avocado Varieties. California Avocado Society Yearbook, 1936: pps. 146-149.
8. Gillespie—Asexual Reproduction of Rootstock from High Producing Fuerte Avocado Trees. California Avocado Society Yearbook. 1952: p. 97.
9. Goebel—Organography of Plants, 1900. Authorized English Edition, Part 1; Clarendon Press, Oxford.
10. Stoutmeyer—Regeneration in Various Types of Apple Wood. Iowa Agric. Exp. Sta. Bul, 1937; pps. 308-352.