Citrus Rootstock Improvement

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By their very nature, rootstock experiments take a long time. I feel most rootstock workers would like a 20 year period to draw their conclusions and make their recommendations to the industry. A citrus tree takes a long time in coming into full bearing but once in full bearing may have a long productive life. A rootstock is a very important part of any orchard and is not changed overnight like a cultural practice, a fertilizer program, or an irrigation schedule. Therefore, it is important to choose the right rootstock. What rootstock to use requires the right answer. The principal problem confronting the rootstock worker is that the answers he obtains from his plots today are the answers to questions the plot was designed to answer 20 years previously. These answers may not fit today's needs, and under today's conditions the answers we need tomorrow are still 20 years away. It is urgent, therefore, something be done to close this gap from 20 years to 15, or even 10 or less. If reliable information upon which dependable recommendations can be made to the industry can be obtained in a shorter interval than previously, then we owe this responsibility to the industry. To some extent we need to anticipate what a few of these problems might be and have some answers waiting.

I am happy to say progress is being made with *Citrus* rootstocks and even more progress lies ahead. This has come about through new ideas, new tools, new techniques, new equipment, and new facilities. It has come about through better collaboration and cooperation with those in associated fields of interest such as the nematologist, the plant pathologist, the nutritionist, the plant breeder, the plant physiologist, the antomist, and others. Each can and has contributed something. In a sense, the rootstock researcher must be the key to the rootstock puzzle. It is up to him to bring together the knowledge contributed separately or cooperatively by the other investigators and try to interpret meaningfully the overall rootstock picture. For example, what good is gummosis resistant if the rootstock is susceptible to tristeza? What good is tristeza resistant if the stock is susceptible to burrowing nematode? What good is resistance to the burrowing nematode if the tree is too susceptible to frost? The nematologist may not be interested in cold hardiness and the plant pathologist may not be interested in fruit quality, but the rootstock man is. In a sense, the rootstock man must be the hub around which the total rootstock program revolves. He must not be subservient to the other groups, and they must not be a service group for him. He must either work cooperatively with his associates or borrow techniques they have perfected and which are useful to his cause. Both may be to his advantage and to the advantage of rootstock progress. Progress is being made at Riverside as well as elsewhere, I'm sure, and I'd like to cite a few examples to illustrate some new horizons which I think have been attained.

One of the most important steps is the availability of clean bud lines for rootstock test purposes. Many a rootstock trial has been a survival test to disease, rather than a matter of measuring true rootstock increments. Clean bud lines may arise from several sources. They may arise from nucellar lines in those varieties in which this is possible, and they of course in due time can be recontaminated by mechanical means, by propagation, or by insect vectors. It is important to know at all times if and what viruses are present. In many instances, nucellar lines are not available and virus-free old lines are a necessity. These may be ascertained by virus indexing procedures which have been perfected by the plant pathologists. At Riverside we have a Citrus Variety Improvement Committee, which consists basically of plant pathologists, horticulturists, and plant breeders. The sole purpose of this committee is to provide to the industry and research personnel thru a budwood certification program virus-free budwood which is true to type. It is not only important to provide it initially, but it is equally important that it continues to be maintained as such through budwood foundation blocks and a budwood certification program which verifies the freedom from recognized viruses. Certified rootstocks must be recognized as well. The effectiveness of this program is based on the use of indicator plants, plants which, when inoculated to a particular virus to which it is extremely sensitive, express key symptoms of the disease in a very short period of time. I cite, for example, the use of the 'West Indian' lime as an indicator plant for tristeza, the 'Etrog' citron as an indicator plant for exocortis, and *Citrus excelsa* as an indicator plant for tatter-leaf. As horticulturists, we can assist the plant pathologist by supplying new plant material to be tested. With these techniques, it is possible to determine in months information which would take years to obtain under field conditions. We use these techniques to follow virus progression in our rootstock blocks in the field, although virus-free bud lines may have been used initially. Other pathological problems will be referred to later.

Great progress has also been made through increases in the gene bank of material available for rootstock testing. This has resulted from increased importations and exchange of genetic material, increased interest in the *Citrus* relatives, and increased efforts by the plant breeders. During the 1940's, citrus variety collections had somewhat stagnated. The inroads of tristeza, if it did nothing else, stimulated interest in rootstock and variety collections and really awakened interest in this field. Since the 1940's, there has been a tremendous exchange of citrus material between many of the citrus producing countries of the world, and varieties and species that were once extremely localized are now worldwide in their distribution. The researcher can try not only selections from his own native country but from many other countries as well.

Citrus relatives are relatively unexplored as rootstocks. Within the subfamily *Aurantioideae* there are 33 genera, of which *Citrus* is one. This means 32 other genera and their species are potential rootstocks. The success of the trifoliate orange, *Poncirus trifoliata*, and one of its hybrids, the 'Troyer' citrange, are well established. Precursory investigations indicate several of the relatives may also have a potential rootstock value. I cannot agree with some of the conclusions drawn by other investigators about non-congeniality of some of these combinations. Perhaps some of the reactions were based on diseased material or under improper ecological conditions. The fact that citrus may not do well with 1 species of a genus does not mean it won't do well with another. Interesting results have been obtained with species within some of the genera of *Severinia, Atalantia, Citropsis, Hesperethusa, Microcitrus, Clymenia* and *Pleiospermium*, to mention a few. It should also be pointed out that *Citrus* hybridizes readily with some of these other genera, as for example the hybrids, the citranges, the limequats, the faustrimes, etc. At Riverside, we have not succeeded in creating new bigeneric hybrids other than with *Poncirus*, although we have tried, but are hopefully looking forward to following such crosses and testing their progeny.

The citrus breeder has also become aware of the importance of breeding citrus for other purposes than edible fruits. Heretofore, if existing hybrids had no commercial value and served no useful purpose, they were as a last resort, tried as a rootstock. I am happy to say that the plant breeders at the Citrus Research Center at Riverside and the USDA Date and Citrus Field Station at Indio have made crosses with specific rootstock purposes in mind. At Indio, accent is primarily on rootstocks tolerant to salinity and to *Phytophthora*. At Riverside, nematode resistance has been a primary factor. In some instances they have taken the best rootstock performance of some species and crossed with the best rootstock performance of other species in hopes of improving the beneficial characters of both in the hybrids. Examples of such crosses are *Poncirus trifoliata* crossed with rough lemon, rangpur lime, cleopatra mandarin, *Citrus macrophylla*, and others. Some of the first generation seedlings have come into bearing and progeny plants are in various stages of testing.

Progress has also been made in the selection of *Phytophthora* resistant stocks. Under field conditions, it may take years for an epidemic to develop. Under laboratory conditions, considerable information may be developed in a rather short period of time. Two techniques developed by pathologists have proved very useful. One consists of developing a heavy aqueous spore suspension of the *Phytophthora* species in question. Immersing the roots of test species in the spore suspension for a given period of time and estimating the percentage of rotten feeder roots provides a fairly good index of susceptibility. Sometimes the technique is modified to a soil type pest hole, but again the degree of survival is determined. The second technique consists of inserting mycelium of the test organism under the bark of a host plant. The surface of the bark may be surface sterilized, a plug of bark removed with a cork borer, and a small bit of mycelium inserted underneath. The organism is permitted to incubate for a given period of about 60 days. The lesion area to which it has spread can then be calibrated and the susceptibility determined in this way. Both tests are somewhat indicative, in a short period of time, of the relative tolerance of the host plant. The soil pest hole may be too severe, and others argue that the bark itself may contain the resistance factor and inserting the organism underneath it doesn't answer the complete picture. Resistance must be structural, or chemical, or both, and an insight into the actual nature of resistance would be most helpful. We must know the why's and the wherefore's of these complex problems.

The *Citrus* replant problem is a good example of excellent cooperation between departments and individuals. Workers at Riverside were among the first to notice that a citrus tree planted where a citrus tree previously grew never grew as well as the original rootstock. Investigation of this problem revealed a number of causes, and the complete picture is finally emerging. The work of Dr. Baines has indicated the citrus nematode as one of the causal factors. However, the work of Dr. Martin indicated the reduction in growth could be just as great in the absence of the *Citrus* nematode. Martin suggested the possible role that other soil organisms might play and suggested *Fusarium* spp. and *Thialeviopsis basicola* as possible suspects. Further search into this problem by Tsao and others has demonstrated that the presence of *T. basicola* in soil does retard growth of citrus seedlings and that an extract made from it also retarded growth. I had the pleasure of working with Dr. Baines and checking the resistance of a large number of *Citrus* species and *Citrus* relatives to the *Citrus* nematode. Resistance within *Citrus* species does not exist, tolerance is only relative and true resistance is to be found only in the *Citrus* relatives. Apparently, it is a dominant character in the genus *Poncirus* and is inherited to a degree in the bigeneric cross with *Citrus*. Kirkpatrick, Van Gundy, and Bitters, examining the effect of bark extracts from resistant genera, found the extracts contained a nematocidal compound effective at almost the same concentration as chemical compounds on the commercial market. This compound (or compounds) needs to be isolated and identified so that it might serve as the marker in resistant hybrids. Kirkpatrick and Van Gundy also investigated the anatomical resistance of the 'Troyer' citrange and its 2 parents, *Poncirus trifoliata* and *Citrus sinensis*. They found resistance was directly related to the host plant's ability to form a wound periderm in the area of infection by the gravoid female. The resistant plants reacted rapidly to the invasion of the nematode and formed the wound periderm rapidly enough to slough off the nematode before it had caused serious damage. In the susceptible hosts there is a rudimentary wound periderm laid down which is inadequate to stop the penetration of the nematode. One can look at the infected roots of the trifoliate orange or the 'Troyer' citrange and quite clearly see the little pock marks where the tissue and the nematode have been sloughed off. This is making progress. In the replant program I also worked closely with Dr. Martin. Our studies in the absence of the citrus nematode showed also that there was only a relative resistance of citrus species to this problem and, again, only good replant growth came when citrus was followed by other genera. Martin and I conducted a rootstock replant experiment in which 6 different rootstocks were used. Each was replanted following itself and reciprocally with the others. Each rootstock had the greatest depressed growth following itself and, aside from this, the 'Troyer' citrange had the greatest overall depressing effect. As a result of these experiments, Martin and I suggested rootstock rotation might be a good practice. While soil fumigation can prevent this problem, it would be better to have a built-in resistance. It has been our experience that, following soil fumigation, the microflora of the soil may be upset, as the incidence of gummosis usually increases tremendously.

It has been observed through the years that rootstocks have an outstanding effect on the nutrition of the scion. Rootstocks have the ability to differentially absorb cations and anions from the soil water solution. In some cases this may be to excess or deficiency to the ultimate detriment of the plant. In others it is within the optimum range of the needs of the plant and favorable for ideal plant growth. Not a great deal is known about nutritional status as affected by rootstocks and particularly with rootstocks which may be termed experimental. It is known, however, that a rootstock such as *Citrus macrophylla* can partially exclude the uptake of boron and maintain it at an optimum level in leaves of the scion; whereas, the same scion on other stocks may be showing 2 or 3 times the boron concentration and showing severe leaf symptoms. A stock such as Yuzu can absorb greater amounts of iron from the soil but, on the other hand, it is very sensitive to sodium uptake. What mechanisms are involved are unknown but at present it is important to know that they are functioning. The old techniques of long drawn out chemical tissue analysis would greatly retard such investigations. Progress was made possible with the introduction of the flame photometer to the laboratory and today these have improved to atomic absorption and infrared spectrophotometers which do in minutes what the old laboratory manual techniques did in days.

Of specific interest will be to fully evaluate the approach initiated in Israel to the physiological and chemical selections of rootstocks. Feeling that rootstocks of the future will come from a planned rootstock breeding and selection program they are aiming at screening methods which will make such a program effective. Such a program would be based on biochemical and physiological tests for the rapid evaluation of thousands of potential rootstocks. In this approach they have demonstrated, for example, that the flavonones, which are phenolic compounds found in citrus tissue, act as growth inhibitors. Varieties whose tissues contain large amounts of these compounds are associated as being weak vegetative growers and may make poor rootstocks. An index of the growth rate of seedlings in the field, based on the total phenolic content of the bark of rootstock seedlings, correlates quite well. They hope to develop indicators for growth rate and duration of the adult phase, as they term it, as well as the juvenile stage and age for coming into bearing. Ultimately, they hope to be able to predict growth vigor, life span, compatibility, fruit quality, level of yield, degree of resistance to diseases and pests by using such techniques. This work has continued with studies of perioxidase activity, effects of salinization upon nucleic acids and ribosomes, ion exchange in citrus roots, etc., and these approaches must and will be watched with great interest.

Tissue culture offers a new approach to old but interesting problems. It has been effectively demonstrated with many plants that each plant cell possesses totipotentiality and by proper techniques it may be possible to reconstitute an entire new plant from a single cell. The plant tissue culture techniques may be capable of producing virus-free bud lines, either from single cell isolates of virus-free citrus plant tissues or the culture of apical meristems which may not as yet be virus infected. This has not as yet been accomplished with *Citrus* except thru micrografting the shoot apex unto a healthy seedling. Organ culture is much simpler. The effects of toxins and metabolic by-products also can be studied as they diffuse across the media from an infected transplant to a healthy transplant. The approach grafting of tissues from 2 distinct citrus species or varieties can be tried by using adjacent transplants and observing what type of tissue union takes place. Perhaps such studies would provide a short term index for predicting compatibilities or incompatibilities between 2 individuals. Also, some *Citrus* species and varieties, as well as certain *Citrus* relatives, are characterized by having very small or only partially developed seeds. Under normal conditions, they are weak and a high percentage are lost in germination. Using the tissue culture technique has been useful in this regard. It is also possible to culture citrus roots in either liquid or solid media, and this offers an opportunity to fully study root growth under completely controlled conditions. I am happy to announce that the tissue culture technique has just proved effective in producing nucellar embryos in what are normally considered as monoembryonic lines. With this tool clonal lines of rootstocks, either monoembryonic or polyembryonic, can be readily maintained.

One of the most practical, yet one of the most elusive of citrus rootstock effects would be that of dwarfing. Citrus growers and others associated with the harvesting operations are well aware of the fact that citrus trees just grow too large. Anyone growing mature citrus trees is very cognizant of the limitations they place on the cultural operations and removal of the fruit from the tree. With recognition of growing labor problems and the pending need for mechanical harvesting interest in growing smaller, more compact but productive trees becomes more acute. The success of dwarf trees in the apple, peach and pear industries only serve as a constant reminder of why not with citrus. While tree sizes vary between stock and scion combinations, none of the healthy trees appear to show much in the way of dwarfing tendencies. Sub-standard in size, yes, but not dwarfing. I say this because in the course of my investigations I have tested over 400 rootstocks in many combinations and none have demonstrated true dwarfing. Substandard trees are reduced in size by 25 to 30 percent. Dwarfed trees should be reduced in size by 50 and preferably 75 per cent. A truly dwarfed tree should be no more than 8 feet in height at full maturity. This would enable all the fruit to be picked from the ground without the aid of picking ladders.

Some combinations are dwarfed because of genetic dwarfing others are the result of viral dwarfing. Within citrus, there appears to be no genetic dwarfing but substandard sized trees are common. Even a natural dwarf like the chinotto does not produce dwarf tree but rather substandard trees with the standard scions. In addition, orange trees budded on it are very susceptible to tristeza. A good example of viral dwarfing is examplified by trees on Palestine sweet lime. Orange and grapefruit trees budded on it were 1/4 natural size but were infected with cachexia. They are also susceptible to tristeza. This possibility of using viral dwarfing in commercial orchards has long been recommended by Dr. Cohen of Fort Pierce. Examples of genetic dwarfs were observed when other genera were used as rootstocks. Grapefruit trees on trifoliate orange in an old rootstock block were stunted to between 8 and 10 feet in height at 33 years of age. No evidence of exocortis was present and unfortunately these trees were pulled before the etrog citron test was available. This is not to say that all grapefruit trees on trifoliate orange are dwarfed since some of the largest grapefruit trees I have observed have been on trifoliate root. Whether some genetic factor was involved in the particular case cited or whether viral dwarfing was involved is unknown. Grapefruit trees on *Severinia buxifolia*, the Chinese box orange, at 30 years of age were about 8 feet high and yielding 200-250 lbs of fruit. The trees were healthy but today could not be used in the presence of tristeza. The 'Thomasville' citrangequat also exhibits some dwarfing characters. From these experiences it would appear that dwarfing is more apt to be found using some genus other than citrus or by using bigeneric or trigeneric hybrids in which citrus is 1 of the parents. The use of citrus relatives as interstocks must not be overlooked. Many of these have been tried with rather discouraging results. One of these, however, is encouraging, an interstock of *Clymenia polyandra* on 'Troyer' citrange with a navel top. This combination is about 50 percent of normal size and yet appears healthy in all respects. In the past, rootstock plantings have not been designed with any accent on dwarfing aspects in mind. The efforts of Dr. R. L. Phillips from Lake Alfred are perhaps the first directed specifically to this phase of investigation. There is no question in my mind that progress will be made in this field in the future and that dwarf citrus rootstocks are just around the corner.

I think it has been clearly pointed out that we are making some advances with *Citrus* rootstocks. Other advances are being made in the knowledge gained in the sphere of still different rootstock effects. I may mention cold hardiness, and drought resistance, as a few. The citrus tree is being considered more and more in its entirety. It is the complete response of the rootstock and the scion to its total environment which is the final consideration. Of these, the rootstock influence has been 1 of the hardest to evaluate. There has always been a large number of rootstocks to evaluate, and it appears that breeding programs will bring more. Greater rootstock progress is being made now than ever before. Continued citrus rootstock improvement may be expected.

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