NUCLEAR EMBRYONY—A MEANS OF FREEING CITRUS CLONES OF VIRUSES[1]

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INTRODUCTION

Most varieties of *Citrus*, *Fortunella*, and *Poncirus* produce two very distinct types of embryos: (*a*) gametic embryos, which are produced sexually from egg cells fertilized by sperm cells; and (*b*) nucellar embryos, which are produced asexually from somatic cells of the nucellus of the seed parent. These nucellar embryos grow into the embryo sac and lie alongside the normal embryo. Upon germination the nucellar embryos give rise to seedlings which are called "nucellar seedlings."

Since nucellar embryos are produced asexually from seed-parent cells without meiotic divisions, seedlings derived from these embryos inherit from the seed parent alone and are therefore identical with the seed parent in genetic constitution, except for occasional differences due to mutation or somatic variation. Nucellar seedlings have one feature, however, which distinguishes them from the mother plant and makes them of value in propagating desirable citrus varieties. In almost all cases, nucellar progenies are more vigorous and grow faster than the parent stock.

NUCELLAR EMBRYONY AS A POSSIBLE MEANS OF ELIMINATING VIRUS INFECTION IN CITRUS CLONES

Many theories have been advanced to explain (*a*) the gradual senescence or degeneration of citrus clones that are vegetatively propagated continuously over a long period of time and (*b*) the comparatively high vigor of nucellar seedlings grown from the old weakened clones (*1, 16, 17*). Some think that possibly one of the reasons for increased vigor in nucellar strains is that viruses are "screened out" of the old-line tissues during the development of the nucellar embryo. While it is true that many varieties of citrus carry one or more viruses and that virus infection is a common cause of deterioration of many citrus clones, it is not known what role virus infection plays in the regularly occurring senescence of citrus clones that are repeatedly propagated by budding, grafting, and other comparable means.

For purposes of this discussion it will be sufficient to point out cases where nucellar embryony has been studied as a means of eliminating viruses from citrus clones. We shall discuss nucellar embryony from the standpoint of a control measure for citrus virus diseases and forego discussion of the relationship of virus infection to clonal senescence and nucellar reinvigoration in citrus.

Psorosis. In a general article published in 1948, Fawcett (*12*) pointed out that as early as 1934 he had demonstrated that the psorosis virus was not transmitted through seeds in any appreciable amount. In many of his published articles, Fawcett indicated that the virus of psorosis failed to pass from psorosis-infected seed-parent trees of

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Valencia sweet orange, *Citrus sinensis* (Linn.) Osbeck, into their nucellar progeny. In later tests Fawcett (*10*) examined more than 3,000 seedlings grown from seed from diseased Valencia orange trees and found only one Valencia seedling with symptoms suggestive of psorosis. Wallace[3] grew almost 3,000 seedlings from seeds of psorosis-infected trees of Eureka lemon, *C. limon* (Linn.) Burm. Of this total number of seedlings only two developed psorosis symptoms. Thus it can be concluded that psorosis is only rarely transmitted through seeds and that this is not an important means of spreading the virus.

One notable case further demonstrates the infrequency of seed transmission of psorosis. In Ventura County, California, a block of sweet orange trees has been maintained as a commercial seed source for sweet orange rootstocks. For many years all of these trees have shown severe scaly-bark symptoms of psorosis. Thousands of seeds have been harvested from these trees and sold to nurserymen to be grown as seedlings for rootstocks. Of the many thousands of citrus trees propagated on the seedling progeny from these infected seed parents, only rare instances of psorosis which appear to be the result of seed transmission have been encountered.

Tristeza. Considerable attention has been given to the study of seed transmission of tristeza virus. Evidence presented by Bennett and Costa (*2*) indicates that tristeza is not transmitted through the seeds of sweet orange and sour orange, *Citrus aurantium* Linn. In tests involving more than 1,400 plants, they found that seedlings grown from diseased sweet orange and sour orange trees did not develop symptoms of tristeza when the sweet orange seedlings were grafted onto the sour orange seedlings. Oberholzer (*22*), in South Africa, found that buds from seedlings grown from tristeza-infected Valencia orange and Triumph grapefruit, *C. paradisi* Macf., developed normally when budded into sour orange and lemon rootstocks, whereas buds from the infected orchard trees developed into twigs with symptoms of tristeza when propagated on these rootstocks.

McClean (*19*) reports that not one case of seed transmission of tristeza has been observed among many thousands of acid-lime, *Citrus aurantifolia* (Christm.) Swing., seedlings raised from seed of infected parents for use as test plants in transmission studies with the tristeza virus in South Africa. The same was true with many seedlings of grapefruit; sweet lime, *C. limettioides* Tanaka; and citron, *C. medica* Linn. Among many hundreds of seedlings of sweet orange; mandarin, *C. reticulata* Blanco; Rough lemon, *C. jambhiri* Lushington; and tangelo, *C. reticulata* X *C. paradisi*, grown from seeds of diseased parents and then tested for tristeza virus by graft inoculation to seedlings of West Indian lime, no seed transmission was detected.

Exocortis. Bitters *et al.* (*3*) in 1954 reported that old-line Frost Eureka lemons propagated on trifoliate orange, *Poncirus trifoliata* (Linn.) Raf., and Morton citrange, *P. trifoliata* X *C. sinensis*, rootstocks developed severe symptoms of exocortis on both rootstocks, whereas the same kinds of rootstocks under the nucellar-line Frost Eureka lemons developed no exocortis. Weathers *et al.* (*30*) in 1955 reported that old lines of Allen Eureka lemon were carrying exocortis virus but that nucellar progeny of these trees were apparently free of the virus. The authors have also noted that nucellar seedlings from seeds of old-line Sawyer Eureka lemon infected with exocortis do not produce symptoms of exocortis when propagated on *P. trifoliata*.

In other studies related to seed transmission of exocortis, the authors have examined several hundred 4- to 10-year-old nucellar-line lemon and sweet orange trees growing on *Poncirus trifoliata* and trifoliate orange hybrids. None of the trees examined exhibited any exocortis symptoms traceable to seed transmission, even though many of the seed parents of the nucellar progeny are known carriers of the exocortis virus.

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Fraser and Levitt (*15*) reported that in tests involving more than 1,200 *Poncirus trifoliata* seedlings grown from seed of exocortis-infected parents, no symptoms of exocortis have developed in the *P. trifoliata* stocks after more than eight years of growth.

Rangpur Lime Disease. Rangpur lime disease was first described in 1952 in Texas (*23*) on trees of Red Blush grapefruit and of Valencia orange budded on Rangpur lime, *Citrus limonia* Osbeck, rootstock. Rangpur lime disease was found by the junior author near Indio, California, in 1952. Moreira (*21*) later described the disease in Brazil and showed it to be a bud-transmitted disease which he considered to be identical with exocortis. The disease was later reported to be present in Florida (*28*).

Transmission of Rangpur lime disease is readily effected by grafting buds or scions from diseased trees onto healthy trees. There is no evidence that transmission of the virus is accomplished by any other method or that there is natural spread of the disease. Moreira (*21*) made the observation that nucellar clones of Bahianinha sweet orange from seeds of infected parent stock failed to cause Rangpur lime disease when propagated on Rangpur lime. Infected parent stock caused Rangpur lime disease symptoms on Rangpur lime and exocortis symptoms on trifoliate orange.

Cachexia. Cachexia was first observed and described by Childs (*7*), in Florida, as a disease of Orlando tangelo trees. Childs (*8*) later reported the transmissibility of cachexia and noted the marked similarity between cachexia on Orlando tangelo and xyloporosis on sweet lime. Subsequently, the disease was reported from Texas (*24*), and it has recently been found in mandarin and tangelo varieties in California and Arizona.

Seed transmission of cachexia was first studied by Childs (*9*). He reports that his studies indicate that cachexia is not transmitted through Orlando tangelo seeds, or is transmissible only in rare cases. Cachexia symptoms did not develop in 50 Orlando tangelo seedlings grown from cachexia-infected parent trees. However, in a test with more than 250 Orlando tangelo seedlings from a commercial source, 3 seedlings developed mild symptoms suggestive of cachexia. Olson and Shull (*25*) found that Orlando tangelo seedlings grafted with buds of one-year-old Red Blush seedlings did not develop symptoms of cachexia, whereas buds from many old-line Red Blush grapefruit trees caused symptoms of cachexia on Orlando tangelo. Whether or not the Red Blush seedlings came from seed of diseased parent trees was not indicated.

Studies by the authors indicate that cachexia is not transmitted through seeds of Wekiwa tangelo. Some 50 seedlings were grown from Wekiwa tangelo trees showing severe symptoms of cachexia. Buds were taken from some of the seedlings and propagated on Orlando tangelo seedlings. After more than three years, neither the Wekiwa seedlings nor the grafted Orlando seedlings have developed symptoms of cachexia.

Xyloporosis. This disease was first reported in Palestine as a disease of sweet lime rootstock (*27*). The disease was subsequently reported from South America (*20*), Florida (*8*), and Texas (*26*).

Xyloporosis has not been reported to be spread by vectors or by any mechanical means. The disease has been reported to be transmitted by budding and grafting and perpetuated in clonal progeny of diseased trees (*9*). The causal agent of xyloporosis of sweet lime in Florida appears to be transmitted through the seed. Childs (*9*) reported that 20 of 30 commercial sweet lime seedlings developed symptoms of xyloporosis, which he considered to be the same virus disease as cachexia, within four years after planting in nursery rows. Further controlled experiments need to be made to establish clearly whether or not the xyloporosis virus is seed transmitted.

Vein Enation. Vein enation was reported first from California (*29*) and later from South Africa (*18*). The virus is bud-transmissible and in South Africa it has been shown to be transmitted by aphids. Vein-enation virus appears not to be transmitted through the seeds of lemon. To determine the possible seed transmissibility of vein enation, the authors examined several hundred Rubidoux Eureka lemon seedlings grown from a vein-enation-infected parent tree and found no symptoms to suggest that vein enation had passed through the seed.

Stubborn Disease. This is a transmissible disease of navel oranges and grapefruit trees. Stubborn has been reported as being widespread in California and Arizona (*11*, *13*, *14*) and the Mediterranean area (*6*). Little is known regarding the spread of the virus of this disease or its transmission by seed. An insect vector has not been reported. Barring the possibility of an efficient insect vector of stubborn, it is hoped that this disease can be eliminated from future plantings by the use of nucellar lines of desirable varieties.

Growth-retarding Virus of Eureka Lemons. Calavan *et al.* (*5*) reported that inoculation of nucellar-line Eureka lemon trees by means of tissue transplants from old-line Eureka lemons caused a significant retardation of growth, in comparison with noninoculated controls. Growth retardation occurred in inoculated young-line Eureka trees on grapefruit, sour orange, and sweet orange rootstocks. They concluded that a transmissible factor (or factors), probably of virus nature, is at least partly responsible for the poor vigor of old-line Eureka lemon trees. The apparent absence of this factor (or factors) from nucellar seedlings indicates that it is not transmitted through seeds.

DISCUSSION

It is now clearly evident that the geographic distribution of most of the virus diseases affecting citrus is traceable to distribution in infected propagative stock. Most citrus viruses become so well distributed in diseased plants that cuttings, scions, buds, or other propagative tissues taken from them usually carry the viruses that are in the source tree.

Once a citrus tree becomes infected with a virus, there is no practicable means of eliminating the virus from that particular tree and thereby effecting a cure. Control of citrus virus diseases thus becomes either a procedure of prevention or the selection and use of varieties of rootstocks and scions that are tolerant or resistant to the viruses.

There is no question but that all citrus virus diseases have been and are still being increased and distributed by indiscriminate propagation of infected material. On the basis of the knowledge of the means by which the citrus viruses are spread and increased, elimination of viruses from propagative material in most instances must precede measures applied in the orchard for effective control of the disease. Since they are known to be carried in nursery stock, a logical starting point, in the case of viruses that are not insect transmitted, is to make use of methods to eliminate the viruses from scion and budwood sources. Growers and nurserymen then should use only certified virus-free sources for propagating their stocks.

Citrus clones completely free of all viruses are difficult to achieve for a number of reasons: The complete host range of many of the citrus viruses has not yet been determined. Suitable index hosts have not been determined for all of the citrus viruses. Certain viruses appear to be transmitted to some extent through the seed of some citrus varieties. New virus diseases are being discovered. Some citrus clones appear to be universally infected with viruses, particularly with some of the latent viruses, and are in danger of being abandoned unless the viruses can be removed. It is for the last-mentioned reason that considerable interest is being shown in the use of nucellar seedlings as a means of rejuvenating old clones and freeing them from virus infection.

Most citrus species produce nucellar seedlings, which, because of their asexual origin, conform closely to the parent tree but are generally more vigorous than the parent tree. It has been pointed out in this review that, with the possible exception of xyloporosis in sweet lime, most viruses found in citrus are apparently not transmitted through the seed in any significant amounts. However, some objections have been raised against the use of nucellar citrus on the grounds of thorniness, slowness in coming into bearing, poorer fruit quality, and the uncertainty of longevity of vigor. Work in California indicates that most of the objections to nucellar lines are not too serious and that most of the undesirable characters of nucellar clones can be overcome by selective propagations with buds and scions from older and nearly thornless wood. From the many thousands of acres of nucellar citrus, principally lemons, now in production in California, it appears that nucellar clones will produce vigorous trees with high yields of good-quality fruit.

While it has been shown that, with some exceptions, the use of nucellar seedlings can be an effective method of freeing citrus clones of virus infection, reservations should be employed before making definite conclusions regarding nucellar embryony as a method for controlling all virus diseases of citrus. Tests for seed transmission of a particular virus in most cases have been limited to tests with one or two varieties of citrus. Whether or not a virus is eliminated from the tissue during the development of the embryo would seem to be more a property of the host plant than of the virus.

Thus it cannot be assumed that because a virus is eliminated from nucellar seedlings of one variety such will be the case with other varieties. Some evidence for this concept is provided by Childs (*9*), who showed that xyloporosis of sweet lime is apparently transmitted through the seed of sweet lime, whereas cachexia, which he considers to be the same virus as xyloporosis, is apparently not transmitted through the seed of Orlando tangelo. Furthermore, even though some viruses can be eliminated from nucellar progeny of certain varieties, other disease factors may be transmitted through seed. Weathers *et al.* (*30*) reported that old-line Eureka lemons carry either a virus or an incompatibility factor capable of moving through a sweet orange interstock and causing a severe disorder on Troyer citrange and *Poncirus trifoliata*. It has been demonstrated that this as yet unidentified factor or agent is also present in nucellar strains of Eureka lemons. According to Calavan (*4*), wood pocket of lemons, which appears to be a chimeral disorder, is often perpetuated in seedlings from diseased trees. Thus, in light of the observations reported above, the general assumption that nucellar seedlings, as such, are disease-free should be viewed with some doubt.

Nevertheless, the use of nucellar seedlings as stocks makes possible the propagation of a clone in its most vigorous and disease-free condition. If the only available material of a variety is infected with virus, then passage of the variety through the nucellar seedling stage to eliminate the virus and rebudding in a few years from nearly thornless wood, offers a very practical and invaluable method of rejuvenating the variety. The value of this method cannot be overestimated. Without it many of our most desirable clones might otherwise be lost.

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