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Tristeza

Reaction of Some New Citrus Hybrids and Citrus Introductions as Rootstocks to Inoculations with Tristeza Virus in California

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THE SEARCH for new citrus rootstocks at the Citrus Research Center is a continuing project (2). An integral part of it involves testing rootstocks for tolerance to tristeza virus (1, 3). Similar experiments have been conducted elsewhere (7, 8). This testing is considered of primary importance to the overall rootstock program as it serves as the basis of determining which new rootstocks to evaluate in long-term horticultural trials. In California nearly 400 citrus species, varieties, hybrids, and relatives have been tested and evaluated in this phase of the program. Many have proved to be susceptible and others, while tolerant, have susceptibility to other diseases or other undesirable horticultural characteristics—making them unacceptable as rootstocks. Results of testing some new hybrids (4, 5, 6) and recently introduced cultivars are presented in this paper.

Procedures

The experiments were conducted at the University of California South Coast Station, a coastal environment, characterized by natural infestation with both tristeza and vein-enation viruses. One hundred seed of each accession considered for testing were planted either in a greenhouse or in a lathhouse seedbed with bottom heat. Because assigned planting space was limited some restriction had to be placed on the number of accessions to be planted. Any variable lots of seedlings, those lacking in vigor, those which were chlorotic, and some of those representing excessive duplication of F_1's, were discarded. Since it takes 3 years under California field conditions to produce a budling ready to plant in the field, the first seed plantings were made in 1961 for the first field planting in 1964.

From 1961 to 1965, 106 seed lots were discarded for the above-stated reasons. Many of these were F_1's of Rangpur lime × trifoliate orange or of *Citrus sunki* × Swingle trifoliate orange.

Seedbed seedlings were carefully culled and, with few exceptions, 35 seedlings of each accession were lined out in the nursery. These were reduced by selection to 25 just prior to budding. The soil at South Coast Field Station is somewhat calcareous in nature, and some of the seedlings of the trifoliate orange hybrids showed excessive chlorosis in the nursery, even after treatment with iron chelates. The chlorosis effect, however, did not carry over to the budded trees. Because of poor nursery response 35 accessions were eliminated from the nursery, prior to budding.

All the seedling lots were budded to Valencia orange scions. The bud source used for the 1964 and 1966 plantings was an Olinda Nucellar Valencia obtained from outside the tristeza–vein-enation area and was a clonal line similar to one from the Citrus Variety Improvement Program (CVIP) (10). The bud sources for the 1968 and 1969 plantings were Campbell and Cutter nucellar Valencias and were obtained from the CVIP accessions. These bud sources are considered to be free of known citrus viruses, except possibly the pathogen of mild stubborn disease, and were grown outside the area of natural infection by tristeza and vein-enation viruses. No other virus has been found in these plots. Occasional questionable stubborn trees have appeared, but they do not seem to be related to bud source.

Trees in the 1964 planting were inoculated in the nursery with 3 tristeza virus sources, several weeks prior to balling and planting in the field plots. Trees in the 1966 and 1968 plantings were inoculated when they became established, approximately 3 months after planting in the field. The sources of inoculum were constant for all plantings. They were originally obtained from Dr. J. M. Wallace of the Citrus Research Center and consisted of 3 tristeza virus strains which were increased and maintained in West Indian Lime seedlings in the greenhouse. These source trees have been repeatedly indexed throughout the course of these experiments to be sure that no other virus contamination had occurred.

Inoculation consisted of placing 3 buds, 1 from each strain source, into the budling trunk near the scaffold branches. Care was exercised to make certain all 3 buds "took," and any bud failures were replaced with exactly the same bud source. Shoots of any inoculation buds that grew were removed immediately. Every inoculated tree had exactly the same sources of tristeza inoculum for all planting dates.

The trees are planted in an area that has never been cropped to citrus before, and there have been no problems with *Phytophthora* spp. or citrus nematodes. The planting plan consists of 3 trees per plot, with a healthy check tree between 2 inoculated trees. All rootstocks were completely randomized within a replication and there are 5 replications. For each planting date a Valencia orange on Bittersweet orange serves as a basis of comparison for susceptibility. There are 18 rootstock selections in the 1964 plantings. Observations are confined to visual symptoms of the disease in the top, tree size, and stem pitting in the bud-union area. Reported observations and conclusions were made as of July 1969.

The check trees were periodically indexed to determine the rate of natural spread of tristeza and vein-enation viruses and also to ascertain whether any exocortis virus was a factor since many of the rootstocks involve trifoliate orange hybrids. The rate of natural spread of tristeza virus has been slow. In the 1964 planting it amounted to about 2 per cent after 3 years but increased to 11 per cent in 4 years. In the 1966 plantings it was also 2 per cent in 2 years but jumped to 9 per cent in 3 years. The rate was slightly faster than in an adjacent rootstock planting in which natural spread reached 98 per cent after 9 years, but might be related to the high incidence of infection as inoculated trees.

All sources showed a rapid spread of vein-enation virus, which reached 100 per cent infection within 3 years of planting for the 1964 and 1966 plantings. Probably none of the effects observed in any of the rootstock combinations can be attributed to vein-enation virus. Vein-enation virus in California has never been observed to cause stem pitting, decline symptoms in the tops, or stunting of trees. In no case were any definite woody gall formations found on any of the rootstocks. No exocortis has been detected as of July 1969.

Results

A summary of the observations and conclusions for the 1964 planting may be found in Table 1. Field codes are used in some instances to distinguish hybrids of similar sources of origin used as rootstocks. Top symptoms generally appeared around 15–16 months after inoculation and coincided with the second flush of growth of the second growing season. Rootstocks were classified into 4 categories: tolerant in which there were no effects of the inoculations, or only slight effects; less tolerant in which there was obviously a slight effect of the inoculation, but the rootstock-scion combination could still probably be commercially usable; susceptible in which the rootstock-scion combinations were markedly affected but generally there were no collapsed trees; and very susceptible in which the rootstock-scion combinations were markedly affected and frequently there were collapsed trees.

The time of symptom appearance and rate of symptom development on the Bittersweet orange served as criteria for judging the other rootstocks. At the end of 5 years, only 1 of the check trees on Bittersweet showed symptoms whereas all the inoculated trees were seriously affected—in fact, 5 were dead. CRC No. 1449 Citremon was undoubtedly seriously affected by the virus, but other factors must also have been involved. Nearly half the trees showed an orange brown discoloration at the union, suggestive of an incompatability, and the check trees were as much affected as the inoculated trees, something which happened in no other rootstock-scion combination. The rootstock itself was frequently discolored and distorted. Similar observations have been made in other rootstock plantings by the author. Incompatability is rather common within trifoliate orange hybrids.

The Uvalde citrange must be considered susceptible; although top symptoms were light, stem pitting was rather severe. The trifoliate orange and its hybrids are generally susceptible to stem pitting and most of the hybrids are not tolerant to tristeza. The Cunningham citrange showed little or no effect of the inoculation and must be considered tolerant. It is quite dwarfing in its growth habit which might be an important factor to consider in orchard plantings. There are 2 hybrids of King tangor × Batangas mandarin and of these, H-1 must be considered susceptible on the basis of moderate top symptoms and severe stunting. On the other hand, H-7 showed only slight effects of the inoculation and was classed as less tolerant. Both sweet orange cultivars, Argentina and Sanguine Grosse Ronde, are classed as less

TABLE 1. Reaction of 18 rootstock combinations with Valencia orange scions to inoculation with tristeza virus in the 1965 plantings as of July 1969

Code[a]		Top symptoms[b]	Rootstock pitting[b]	Stunting[b]	Tentative rating[c]	Comments
C-2	CRC No. 1449 Citremon	S	0	0	Sc+	Discoloration at bud union
C-4	Uvalde citrange	0–L	M	0	Sc	
C-10	Cunningham citrange	0	0	L	T	
D-1	Bittersweet orange	S	0	S	Sc+	
H-1	King tangor × Batangas mandarin	M	0	S	Sc	
H-7	King tangor × Batangas mandarin	0–L	0–L	L–M	T—	
J-2	Sanguine Grosse Ronde sweet orange	0	0	S	T—	
J-3	Argentina sweet orange	0	0–L	M	T—	
M-1	India lemon	0	L–M	L–M	Sc	
M-2	India lemon	0	M–S	0	Sc	
M-3	Rough lemon 'A'	0	L	0–L	T	
M-4	Rough lemon 'B' (Estes)	0	L–M	0	T—	
N-6	Yuzu	0	L–M	M	Sc	
N-7	Yuzu	L	M–S	M–S	Sc	
N-9	Laranja Cravo mandarin	L	0	L	T—	
N-10	Volkamer lemon	0–L	0–L	L–M	T—	
N-12	Webber's weeping Philippine hybrid	0–L	0–L	L	T—	
N-15	Alemow	L	S	S	Sc+	

a. Field code used to identify species and source.
b. 0, none; L, light; M, moderate; S, severe.
c. T, tolerant; T—, less tolerant; Sc, susceptible; Sc+, very susceptible.

tolerant. Although several of the Argentina sweets showed stem pitting, the trees in general are not so severely stunted as those of Sanguine Grosse Ronde, and Argentina is the best of these 2 rootstocks. Sweet orange cultivars as rootstocks have been shown to react to tristeza inoculations (3, 11). Trees on the 2 India lemon selections M-1 and M-2, although expressing no top symptoms and being the largest and healthiest trees in the 1964 plantings, showed severe stem pitting, and M-1 also showed some stunting. Both are classed as susceptible. Rough Lemon B (Estes) was not so tolerant as rough lemon A, based on the fact that it showed more stem pitting. Both sources of Yuzu, N-6 and N-7, are susceptible although N-7 was the poorer, showing more stem pitting and more stunting. This result is in agreement with other observations by the author in other rootstock experiments and with other sources of Yuzu. It is difficult to understand how Yuzu can be used as a rootstock in Japan in the presence of tristeza. Laranjo cravo mandarin, Volkamer lemon, and Webber's Philippine hybrid are all classed as less tolerant. The alemow was very susceptible, based on severe stem pitting and severe stunting, but showed little in the way of a top symptom.

Of the 18 rootstocks only 2 were classed as tolerant, 7 as less tolerant, 6 as susceptible, and 3 as very susceptible. Of 263 trees examined none showed pitting in the scion. Of the 18 rootstocks examined, 12 showed pitting. Thirty-four per cent of the inoculated trees, alemow and Uvalde citrange, were 100 per cent pitted and both Yuzu selections ranked next.

Results of observations on the 1966 plantings and conclusions may be found in Table 2. Again the Bittersweet orange was used for comparison; after 3 years all the inoculated trees on Bittersweet orange showed severe symptoms and 4 of these were dead. One of the check trees showed moderate symptoms. One hundred per cent of the trees of Valencia orange scion with a Rubidoux trifoliate orange interstock on sour orange understock were seriously affected, but none of the check trees were. No pitting was found. This clearly reconfirms the observation (3) that the use of a tolerant interstock does not filter out the effect of the virus when a susceptible rootstock is used. Toxopeus (13) clearly pointed this out and there appear to be no exceptions.

The Fairhope trifoliate orange was rated as susceptible on the basis of light top symptoms, light to moderate pitting, and light stunting. Two-thirds of the trees showed stem pitting and associated with the pitting was a peculiar bumpy characteristic of the stock. The extent of pitting agrees with observations at Baldwin Park (3) and also observations by the author in other rootstock trials. There are many strains of trifoliate orange and many strains of tristeza virus. It appears obvious that certain strains of tristeza virus can cause deleterious effects in certain strains of trifoliate orange and each one must be tested separately. CRC No. 2866 citrange (No. 1416) showed light to moderate symptoms and moderate stunting and must be classed as susceptible as are many other citrange strains, such as Savage and Morton. Of 4 new citrange hybrids under test, C-32 gave the best performance and was classed as tolerant. However C-33 and C-35 were classed as less tolerant and C-34 was classed as susceptible. Of the two shekwasha × Swingle trifoliate orange hybrids, C-12 showed definite indications of incompatibility. Six of the trees showed a brownish discoloration at

TABLE 2. REACTION OF 47 ROOTSTOCK COMBINATIONS WITH VALENCIA ORANGE SCIONS TO INOCULATION WITH TRISTEZA VIRUS IN THE 1966 PLANTINGS AS OF JULY 1969

Code[a]		Top symptoms[b]	Rootstock pitting[b]	Stunting[b]	Tentative rating[c]	Comments
D-1	Bittersweet orange	S	0	S	Sc+	
S-1	Valencia orange/trifoliate orange/Keen sour orange	S	0	S	Sc+	Interstocked
B-1	Fairhope trifoliate orange	L	L–M	L	Sc	Peculiar bumpy bark
C-3	CRC No. 2866 Citrange (No. 1416)	L–M	0	M	Sc	
C-32	Ruby sweet orange × Webber-Fawcett trifoliate orange	0	0	0–L	T	
C-33	Ruby sweet orange × Webber-Fawcett trifoliate orange	L	0	L	T—	
C-34	Ruby sweet orange × Webber-Fawcett trifoliate orange	L–M	0	L	Sc	
C-35	Ruby sweet orange × Webber-Fawcett trifoliate orange	L	0	L	T—	
C-12	Shekwasha × Swingle trifoliate orange	L	0	0	T?	Incompatibility?
C-13	Shekwasha × Swingle trifoliate orange	0	0	0	T	
H-14	Shekwasha × Koethen sweet orange	L	0	L	T—	
H-16	Shekwasha × Koethen sweet orange	L	0	L	T—	
H-17	Shekwasha × Koethen sweet orange	L	L	L	Sc	
H-18	Shekwasha × Koethen sweet orange	0–L	0	0	T	
H-20	Shekwasha × rough lemon	0	0	0	T	
C-22	Citrus sunki × Swingle trifoliate orange	L	0	0	T—	
C-23	Citrus sunki × Swingle trifoliate orange	L	0	L	T—	
C-24	Citrus sunki × Swingle trifoliate orange	L–M	0	L	Sc	
C-25	Citrus sunki × Swingle trifoliate orange	L–M	M–S	L	Sc+	Discoloration at bud union
C-26	Citrus sunki × Swingle trifoliate orange	L	0	L	T—	
C-27	Citrus sunki × Swingle trifoliate orange	M–S	0	M–S	Sc+	
C-28	Citrus sunki × Swingle trifoliate orange	L–M	0	L	Sc	
C-29	Citrus sunki × Swingle trifoliate orange	0	0	0	T	
C-30	Citrus sunki × Swingle trifoliate orange	0	0	0	T	Discoloration at bud union
C-31	Citrus sunki × Swingle trifoliate orange	S	0	S	Sc+	
H-9	Clementine mandarin × Koethen sweet orange	0–L	L	L	Sc	
K-1	Shangyuan	L	L–M	L	Sc	
N-3	Faustrime	M	S	0	Sc	Gum in pits
N-4	Chinese box orange	S	0	S	Sc+	Discoloration at bud union

(TABLE 2—Continued)

TABLE 2—Continued

Code[a]		Top symptoms[b]	Rootstock pitting[b]	Stunting[b]	Tentative rating[c]	Comments
N-5	Eremolemon	L	S	L	Sc+	Ropy trunk
N-8	Mauritius papeda	L-S	S	S	Sc+	Ropy trunk
N-14	Melanesian papeda	L-M	M-S	L	Sc+	
G-2	Tachibana	L-M	L	L	Sc	
G-3	Girimikan	L	L	L	Sc	
N-17	Kikudaidai	L	L	L	Sc	
N-21	Kinukawa mikan	S	0	S	Sc+	Bud-union problems?
N-30	Ujukitsu	M	0	M	Sc	
N-31	Hanaju	L	L	0	T—	
N-32	Tosu	L	0	M	Sc	
N-33	Funadoko-mikan	L-M	0	S	Sc	
N-34	Kinkoje	L	0	0	T—	
N-35	Shunkokan	0	0	0	T	
N-36	Sudachi	L-M	L	M	Sc	Ropy trunk
N-37	Otachibana	S	0	S	Sc+	
N-38	Yama-mikan	L-M	0	L	Sc	
N-39	Rokugatsu-mikan	S	0	S	Sc+	
N-40	Keraji	L-M	0	M	Sc	

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the union and 1 of these had bud-union crease and heavy deposits of gum at the union. Only 1 tree showed severe top symptoms, and it had no stem pitting and no discoloration at the union. No stunting was evident. C-13, however, showed absolutely no symptoms and must be classed as tolerant. There are 4 hybrids of shekwasha × Koethen sweet orange. Of these, H-14 and H-16 showed only light top symptoms and light stunting and are classed as less tolerant; H-18 was classed as tolerant. H-17, on the other hand, showed light stem pitting, light top symptoms, and light stunting, and must be considered susceptible. Shekwasha × rough lemon, H-20, showed no reaction whatsoever to the virus and must be considered a tolerant rootstock.

It is somewhat unfortunate that severe cullage in both the seedbed and nursery minimized the populations available for study within a given cross. Ten F_1 hybrids of *C. sunki* × trifoliate orange are, however, represented in this planting and clearly demonstrate the heterozygous nature of resistance to tristeza virus and of other factors. C-25, while it showed a severe reaction to tristeza virus, showed in 5 trees a discoloration at the bud union suggestive of an incompatibility; C-30, which showed no reaction at all to tristeza virus, showed discoloration at the union in 4 trees, making any further consideration of it as a rootstock unwise. This, again, points out the problems of incompatibility within trifoliate orange hybrids. C-29 was classed as tolerant; C-22, C-23, and C-26, as less tolerant; C-24 and C-28 as susceptible; and C-25, C-27, and C-31 as very susceptible.

In a somewhat miscellaneous group, the shangyuan clone was susceptible as other genotypes of it also have been. The faustrime, a trigeneric hybrid of *Fortunella* × *Microcitrus* × *Citrus* was very susceptible and generally displayed gum deposits in the pits. The Chinese box orange was very susceptible, as has been reported by other workers (9), and showed some discoloration at the bud union. The Eremolemon, a possible hybrid of *Eremocitrus glauca* × Meyer lemon was also extremely susceptible with some of the stocks having such severe pitting as to exhibit a ropy trunk character. The stocks of the inoculated trees on *Citrus hystrix* were 100 per cent pitted and so severely that many exhibited ropy trunk. None of the check trees showed pitting. The stocks of *Citrus macroptera* were 70 per cent pitted, but again none of the checks showed pitting. Both are classed as very susceptible.

Of the 15 Japanese varieties under test in these plantings, only 1, the shunkokan, was tolerant; 2 were less tolerant, 9 were susceptible, and 3 were very susceptible. Stocks of sudachi showed some ropy trunk character and trees on kinukawa mikan, some bud-union problems—a tight bark and bud-union creasing which was not associated with stem pitting, but at least partly with the severity of top symptoms, as 3 of the inoculated trees were dead and others were in the last stages of collapse.

Kinkoje was classed as less tolerant. This reaction is rather interesting, since seedlings of kinkoje express a strong reaction to tristeza virus, and the variety itself is at least distantly related to sour orange.

Hanaju is genetically very close to yuzu and, although tentatively classed now as less tolerant, it showed top symptoms and stem pitting almost exactly as yuzu does, which lends further credence to the susceptibility of yuzu to tristeza virus. Tanaka (12) mentions the failure of satsuma orange grafted on *C. tachibana* in Japan and this probably was also due to tristeza virus according to my trials. Whereas he indicated favorable results on *C. neo-aurantium* and *C. intermedia,* the results in this planting are so far very discouraging.

Of the 47 rootstocks in the 1966 plantings, 8 are classed as tolerant, but 2 of these may have bud-union problems. Nine rootstocks are classed as less tolerant, 17 as susceptible, and 13 as very susceptible. At least 5 of the rootstocks showed some type of bud-union incompatibility. Of a total of 672 trees examined for stem pitting, 97 showed pitting. Eighteen per cent of the inoculated trees were pitted and only 8 per cent of the check trees. Of all rootstocks, 19 showed some pitting symptoms; 28 did not. Most severe pitting was found on faustrime, *Eremocitrus glauca* hybrid, and *Citrus hystrix*. These were followed by C-25 (*C. sunki* × Swingle trifoliate orange), *C. macroptera*, shangyuan, Fairhope trifoliate orange, and *C. hanaju*. No pitting was found on any of the scions.

Observations on these plantings will be continued to allow ample time for full symptom development in susceptible combinations. Comparisons between the check trees and the inoculated trees will be less valid and more difficult as the check trees become infected. The low rate of natural spread of tristeza in this area is helpful in this regard. Probably none of the affected combinations will improve with time; in fact, symptoms will most likely become more severe. It is also possible that some of the tolerant and less tolerant combinations will decline further and necessitate removal from those categories. The reaction of some combinations might also be different if other strains of tristeza virus were introduced.

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