

Long-term Cross Protection of Severe Stem Pitting *Citrus tristeza virus* in Peru

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ABSTRACT Economic disasters caused by the stem pitting *Citrus tristeza virus* (SP-CTV) led the authors to investigate for the recovery of the sweet orange industry in Peru. 1) By a search for and identification of highly productive symptomless carriers of the SP-CTV in the Washington navel orange and in the Key (Mexican) lime, *Citrus aurantiifolia* (Christm.) Swing. 2) By cross protection to obtain symptomless individuals of diverse cultivars of oranges, grapefruit and hybrids that are susceptible to SP-CTV. Evidence is presented that compares average fruit weight and sizes in adequately cross protected cultivars nine years after inoculation, and in their unprotected controls. Remarkable protection was obtained for all sweet orange and grapefruit varieties. The 37A, 37B and 37C selections that had been obtained after passage through *Passiflora* spp. showed outstanding and consistent cross protective capability. Similar cross protective capability was shown by L-1, L-2, R-2 and *C. aurantiifolia* cv. Topara selections of spontaneous origin that had been identified under open field conditions. Maps of commercial plantations up to 5 yr of age are shown comparing the incidence of SP-CTV in unprotected and in adequately cross-protected orchards. Adequate cross protection that was obtained for the Lane Late navel orange on UCLA rough lemon rootstock remained stable when such budwood was used for propagation on rootstocks other than UCLA rough lemon. Surprisingly, adequate cross protection that was obtained for the Star Ruby grapefruit on UCLA rough lemon was highly specific to this rootstock and broke down when such budwood was used for propagation on rootstocks other than UCLA rough lemon.

Bederski et al. (1) reported that during the 1970's and early 1980's the commercial production of oranges, specifically the Washington navel, was virtually terminated in the coastal citrus growing regions of Peru. This was due to extremely severe stem pitting isolates of the *Citrus tristeza virus* (SP-CTV) which affected scions regardless of rootstock. These severe and destructive isolates were introduced into Peru in the 1950's with the known importations of Satsuma mandarin budwood from Japan. A search was initiated in the mid 1980's for productive surviving trees of the popular Washington navel orange. Thirty promising budlines of Washington navel were identified and extensively tested. Five of them were finally selected as protective navel orange sources. In addition, the search also identified two other protective sources. One was a highly productive Mexican (Key) lime tree that was growing under the cool

Mediterranean climatic conditions of coastal Peru. This tree was fruitful with large fruit and showed no stem pitting. The other sources were imported scion budsticks of Duncan grapefruit and Madam Vinous sweet orange containing attenuated cross-protective isolates of codes 37 and 40 derived by vector passage of CTV through *Passiflora* spp. and introduced from California in 1990. This paper continues reporting the results of these cross protection isolates 4 yr later.

Background of the protective isolates. Studies continued on 11 protective sources coming from 1) the "L" and "R" selections collected from surviving navel oranges in Peru; 2) a small fruited lime source "*Citrus aurantiifolia* Topara" coming from a lime tree found in Peru without stem pitting and without severe decline; 3) Codes 37 and 40 which were derived from passage of severe SP-CTV through *Passiflora* at

Riverside, California and brought to Peru. Details of the background and derivation of these sources are given in Bederski et al. (1).

1) *The L and R protective sources:* Beginning in 1984, approximately 10,000 Washington navel orange trees in different orchards were surveyed by the senior author for their performance against the prevailing severe SP-CTV present in all orchards in coastal Peru (7) the coastal regions of Peru (“L “ and “R” are code names for orchard owners). The L1, L2, R1 and R2 were selections of four of the best trees and were the end product of a severe selection process by the senior author where he collected budwood from superior surviving trees from orchards in the Cañete and Chincha valleys, south of Lima. Budwood from 10 of the best surviving trees was collected in each orchard. A total of 6,000 rough lemon seedlings were grown at the Topara nursery and budded with material from three selected orchards (200 seedlings x 10 of the best surviving trees x three source orchards = 6,000 trees). However, trees grown from one of the selected orchards showed severe decline and this selection was dropped, leaving only the L and R selections. After observing the 4,000 trees of the L and R budded trees over a period of years, the very best trees from the L and R selections were selected for further tests and labeled L1, L2, R1 and R2. An additional Washington Navel selection of unknown origin was later found in the Supe valley as spontaneous symptomless carrier of SP-CTV and labeled S.

2) *The small fruited lime protective source:* This came from one of two neglected lime trees found outside of a private home close to the ocean in a cool area favorable for CTV infection for limes. One tree showed severe stem pitting and small fruit and the other tree was vigorous with larger fruit. Budwood of both trees were collected and brought to the Topara

nursery and propagated. The good tree was named *C. aurantifolia* cv Topara. Six trees of this selection were budded on rough lemon seedlings in 1988 and nursery increase trees propagated from this source were planted in the Topara nursery trial block and distributed to growers.

3) *Codes 37 and 40 sources.* A detailed history of research and performance of these code sources derived from passage of severe SP-CTV through *Passiflora* by *Aphis gossypii* is given by Roistacher and Bar-Joseph (6) and Roistacher et al.(10). These two cross protection isolates were obtained by a passage of a severe stem pitting CTV through *Passiflora* sp. *Aphis gossypii* was the vector for transmission from a sweet orange holding plant of SY-563 into *Passiflora caerulea* and then vector transmitted out of *P. caerulea* into seedlings of Mexican lime. The origin isolates Code 37 and 40 was a Brazil navel from the UCR variety collection coded as SY-563 (CRC-957). It was originally imported as USDA Plant Introduction (P.I.) 37757 in 1914 and was almost certainly free of tristeza when introduced at this early date. However, when indexed, budwood from the field tree of this Brazil navel was found positive for both seedling yellows and stem pitting CTV. After passage of this isolate through *P. caerulea* and then into a series of indicators, a single positive Mexican lime plant showing only mild CTV symptoms was designated as Code 37 in September 1982. A bud-inoculation from this Mexican lime to a sweet orange holding plant was designated as Code 37A (6). Code 40A was an independent source derived by vector transmission in the same way from the Brazil navel SY-563 holding plant to *P. caerulea* and vector transmitting from CTV-positive *P. caerulea* to a Mexican lime seedling in November, 1982. It was then sub-inoculated to a sweet orange holding plant in March, 1983 and designated as

Code-40A. In December, 1989, budsticks of Codes 37A, 37B, 37C and code 40A were taken to Klaus Bederski's Topara nursery in Peru and grafted on field grown rough lemon seedlings (four per coded source). The objective was to test the long term stability of these attenuated protective isolates against the severe challenge of the local Peruvian stem pitting CTV. Despite the risk of having exotic California CTV strains at the Topara nursery and in Peru, the perceived benefits of stopping the destruction of citrus by the severe CTV stem pitting isolates currently in Peru was felt to justify any risks involved (1, 7, 10).

MATERIALS AND METHODS

Cross protection research requires seeds, cultivar budwood and sources of cross protective inoculum. Seeds were obtained from the Mother Block seedling trees that existed at the Topara nursery. These trees originated from Willits & Newcomb (W & N) nursery budwood that was imported in the late 1970's. Commercial cultivar budwood was obtained from W & N nursery in California and from AVASA in Spain in 1997. Cross protective inoculum was obtained from sources that existed at the Topara Nursery at that time (8). The initial choice of rootstock for cross-protection work was UCLA rough lemon. Seeds were sown in 1997. The resulting seedlings were transplanted into 8 X 14 PE containers and grown in open field nursery rows until they were ready for budding. They were inoculated (blind-budded) with cross-protective sources immediately prior to their budding with commercial cultivars in 1998. Controls were established by budding unprotected seedlings to the same commercial cultivars (Fig.1).

In 1999, four trees of each combination of commercial cultivars and cross-protective SP-CTV sources and their

corresponding controls were planted in the open field under heavy SP-CTV inoculum pressure and with the endemic presence of *Toxoptera citricida*. All trees were evaluated once each year at the end of winter to record overall growth, leaf color and stem pitting symptoms (Fig. 2).

Beginning in 2004, the evaluations also included fruit size and weight. Variance analysis was initiated in 2007.

Additional choices of rootstock: Cleopatra mandarin and Swingle citrumelo 4475. Container field grown seedlings of Cleopatra mandarin and of Citrumelo 4475 were budded in 2003 with budwood taken from the best looking cross-protected trees on rough lemon rootstock that were already producing fruit at that time. These new trees were planted in the open field in 2004 and evaluated thereafter to compare their SP-CTV symptoms and productive efficiency against the results that were being observed on rough lemon rootstock.

RESULTS

Cross protection for susceptible cultivars on UCLA rough lemon. Initial results of protection by these *Passiflora*-attenuated CTV strains and by strains of spontaneous origin in Peru have been published (1). After 19 yr in the field, the CTV protective isolates were still doing well under the severe SP-CTV inoculum pressure at the Topara nursery. A slide show illustrating cross protection in Peru reviewing the history and development of protective isolates can be seen in the EcoPort slide show #142 (9).

Nine years of evaluation after inoculation has shown that all SP-CTV susceptible cultivars can be protected by one or more sources. Successful cross protective sources for sweet orange and grapefruit cultivars are highlighted in Fig. 3.

PROTECTION VARIETIES	PROTECTION										
	L-1	L-2	R-1	R-2	W. N Supe	M. Vinous 37A	C. aurantifolia Topara	Duncan 37B	Duncan 37C	Duncan 40A	UNPROTECTED
ORANGE											
Navelina	X			X	X	X	X				X
Fukumoto	X		X	X	X	X					X
Cara Cara		X	X		X	X	X				X
Navelate	X			X	X	X	X				X
Lane late	X		X	X	X	X					X
GRAPEFRUIT											
Star Ruby							X	X	X	X	X
Flame							X	X	X	X	X
Marsh							X	X	X	X	X
Oro Blanco							X	X	X	X	X

Fig. 1. Commercial citrus cultivars and sources for cross-protection against stem pitting *Citrus tristeza virus* in Peru.

Rating	Stem pits	Color and feeling	Cross section
0	none		
1	Isolated, superficial		
2	Semi-dense, superficial		
3	Dense, superficial		
4	Isolated, deep		
5	Dense, deep		
A		Normal green, moist	
B		Pale, dry	
a			Normal green
b			Pale,
c			Brownish stains, necrotic points.

Fig. 2. Tristeza-induced stem pitting and color evaluation criteria for selection of cross protecting strains in Peru.

PROTECTION \ VARIETIES	L-1	L-2	R-1	R-2	W. N Supe	Madam Vinous 37A	<i>C. aurantifolia</i> Topara	Duncan 37B	Duncan 37C	Duncan 40A	UNPROTECTED
Navelina sw.or.	216			318	263	329	282				172
Fukumoto navel	207		237	277	274	341					226
Cara Cara navel		328	253		264	248	270				205
Navelate	215			218	210	256	254				207
Lane late navel	358		289	324	292	273					254
Star Ruby gft.							X	X	X	X	X
Flame gft.							418	303	318	177	276
Marsh gft.							X	X	X	X	X
Oro Blanco							532	548		448	272

Fig. 3. The average weight per fruit for the citrus crop of 2007, 9 yr after inoculation with cross protecting *Citrus tristeza isolates*. The two most successful sources for each variety under Peruvian coastal conditions are highlighted. (gft=grapefruit).

Successful cross protective sources for navel sweet oranges and for grapefruit cultivars are defined as those which show only mild stem pitting while allowing their

host trees to be productive and to produce fruit of commercial diameter and weight (Figs. 4, 5, and 6).

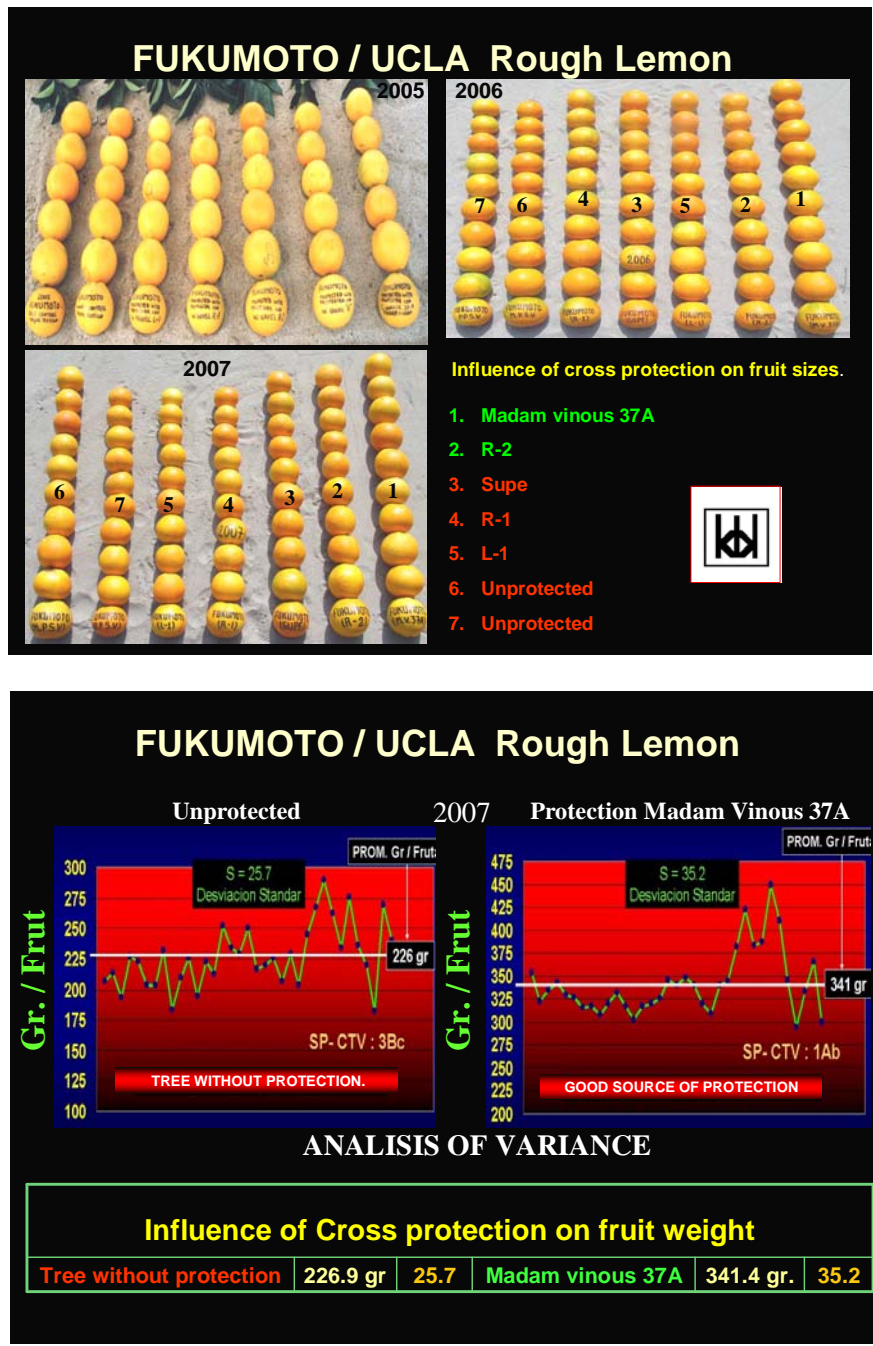


Fig. 4. Results of the effects tristeza cross protection fruit weight of Fukumoto navel on UCLA rough lemon.

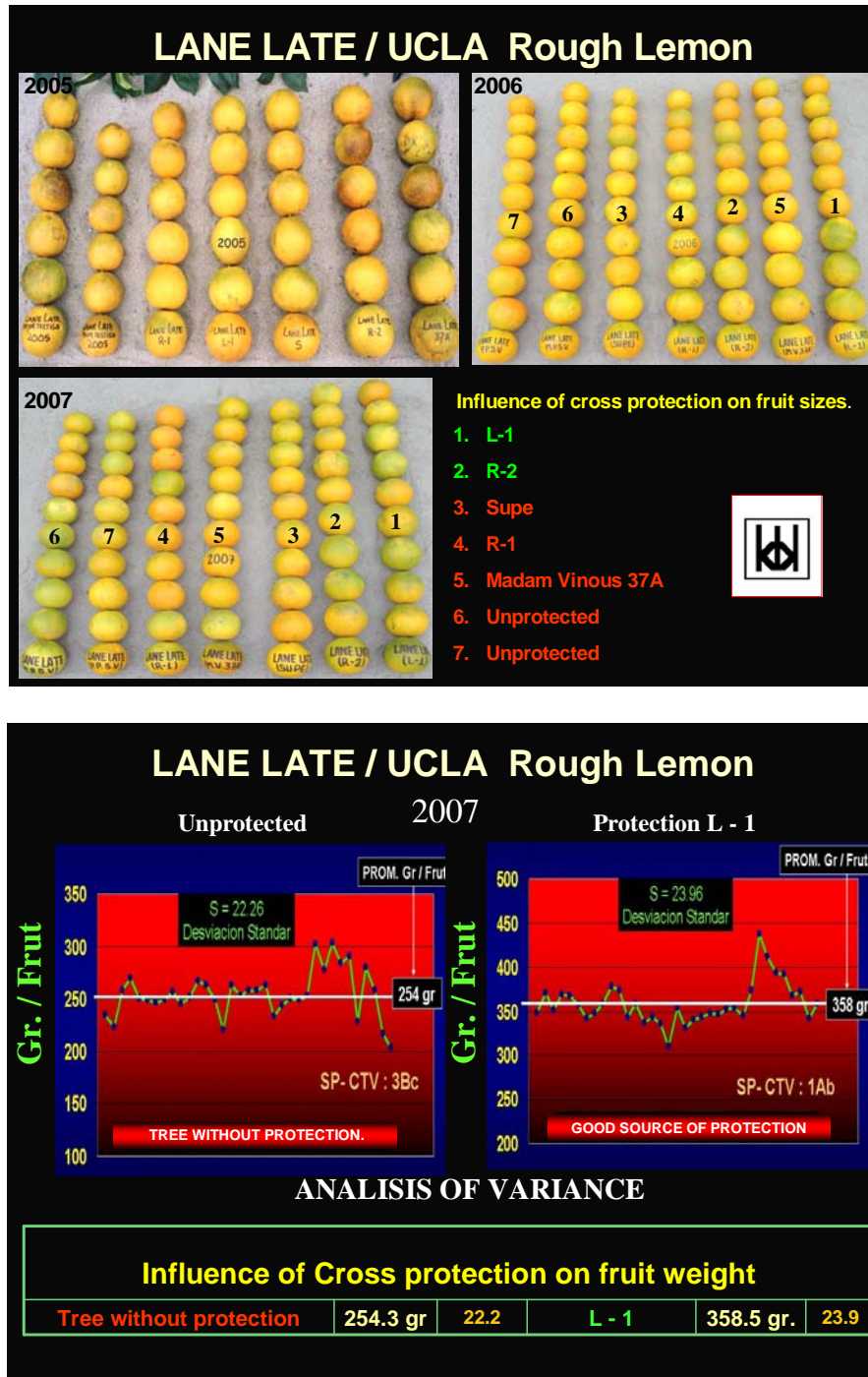


Fig. 5. Results of the effects tristeza cross protection fruit weight of Lane Late navel on UCLA rough lemon.

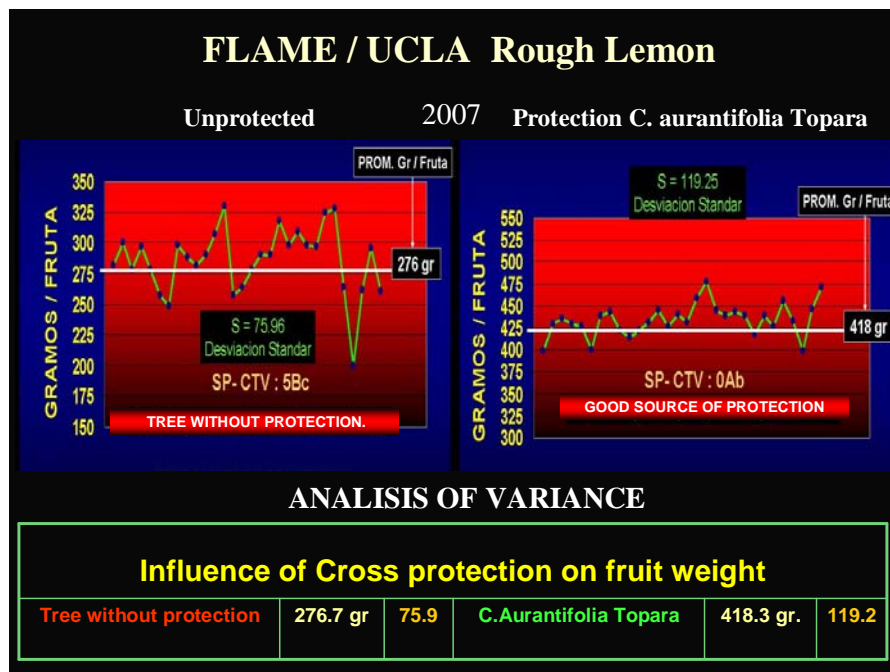
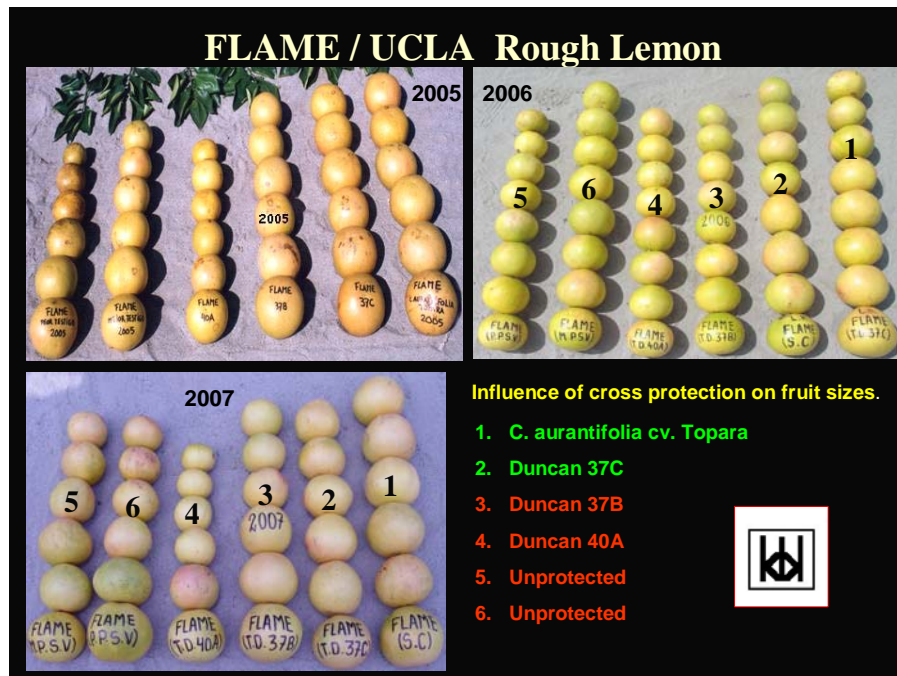


Fig. 6. Results of the effects tristeza cross protection fruit weight of Lane Late navel on UCLA rough lemon.

Fruits of Fukumoto navel, Lane Late navel and Flame grapefruit are shown in the top portion of Figs. 4, 5, and 6, respectively. The pictures were taken over a period of 3 yr and in all cases demonstrate that the two best sources of protection remained invariable over time. The results of analysis of variance in fruit weights of Fukumoto navel, Lane Late navel and Flame grapefruit are shown in the lower portion of Figs. 4, 5, and 6, respectively. It indicates consistently that the average weight of fruit in unprotected trees is much lower than in adequately protected trees and explains why unprotected orchards have a very high proportion of fruit that can not be marketed because of their small caliper and weight.

Influence of rootstocks on the stability of cross protected cultivars. The best cross protected trees for each cultivar on UCLA rough lemon rootstock were

chosen as ‘foundation trees’ to provide budwood for further propagation on rough lemon or on other rootstocks. In the case of oranges, such budwood had not caused changes of economic importance when it was budded on Cleopatra mandarin, citrumelo 4475 or other rootstock choices. Fig. 7a, shows a profitable 5-yr-old orchard of Lane Late navel on citrumelo 4475 rootstock which was cross protected with the L-1 source. The orchard map showed only 2.13% of severely stem-pitted trees. These trees are highlighted in red and were replaced. Fig. 7b, for simple illustration purposes, shows an equally 5-yr-old but unprotected Navelate orchard on citrumelo 4475 rootstock. Severe SP-CTV was rapidly spreading in this orchard. The map shows a total 8.1% of trees that have already been replaced (highlighted in black) or newly stem-pitted trees in need for elimination (highlighted in red).

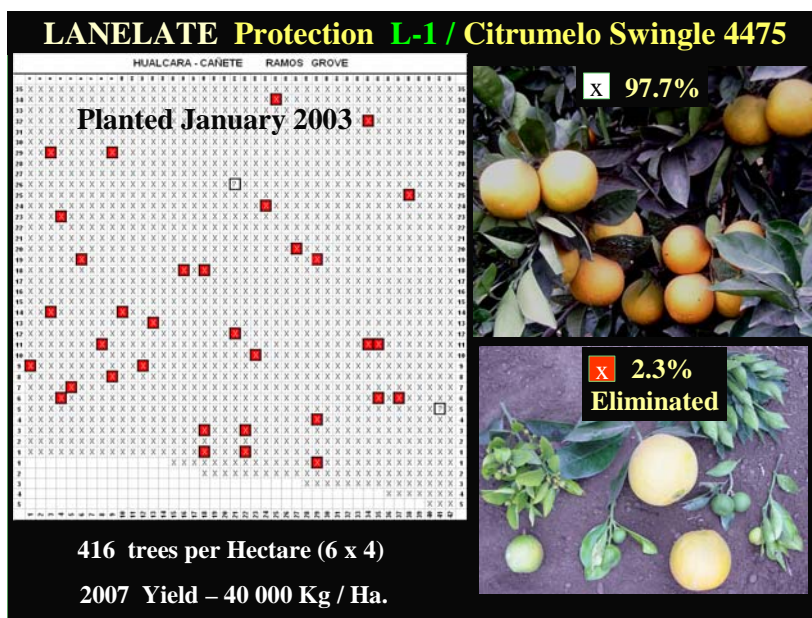


Fig 7a. Stem-pitting *Citrus tristeza virus* survey of a 5-yr-old Lane Late on Citrumelo 4475 grove protected with source L-1 derived from Lane Late on UCLA rough lemon. Trees blocked in red showed stem pitting and were replaced.

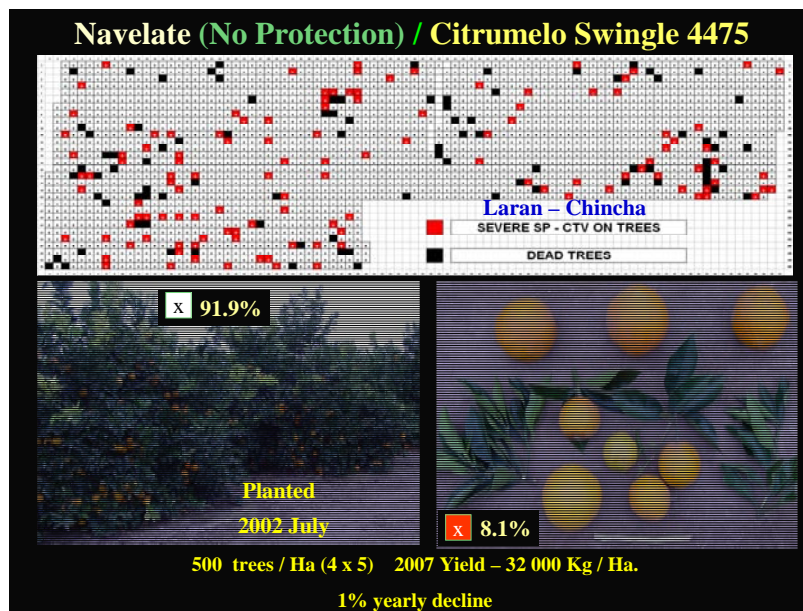


Fig 7b. A 5-yr-old but unprotected Navelate orchard on Citrumelo 4475 rootstock with a severe SP-CTV problem. The map shows a total 8.1% of trees that had already been replaced (blocked in black) or newly stem-pitted trees in need for elimination (blocked in red).

In the case of grapefruit, such budwood breaks down and causes changes of economic importance when it is used on other rootstock choices. None of the cross protective sources for SP-CTV which were successful on UCLA rough lemon rootstock repeated this success when the budwood taken from the same foundation trees were used to grow trees on rootstocks **other** than rough lemon. Fig. 8 shows severely pitted trunks of Star Ruby grapefruit on one year old nursery trees of various rootstocks.

DISCUSSION AND CONCLUSIONS

Cross protection of citrus against severe stem pitting strains of CTV is currently the only way for preserving a citrus industry which has been ravaged, to the point of destruction, by the combined

effect of the presence of severe CTV strains and the efficiency of the primary aphid vector for tristeza, *T. citricida*. The classical studies on the preservation of the Brazilian citrus industry through tristeza cross protection was reported and reviewed by Müller and Costa (3, 4) and Costa and Müller (2).

With the second wave of destruction of Peruvian citrus industry due to exceptionally severe SP-CTV strains brought into the country by importation of Satsuma mandarins on trifoliolate rootstock from Japan, the senior author followed the example of these pioneers in an intensive search for trees of navel oranges which appeared to survive the severe stem pitting, stunting, unproductive trees with small fruit. The successful results of these selections were reported (1). In addition, the senior author located a lime tree in front of a

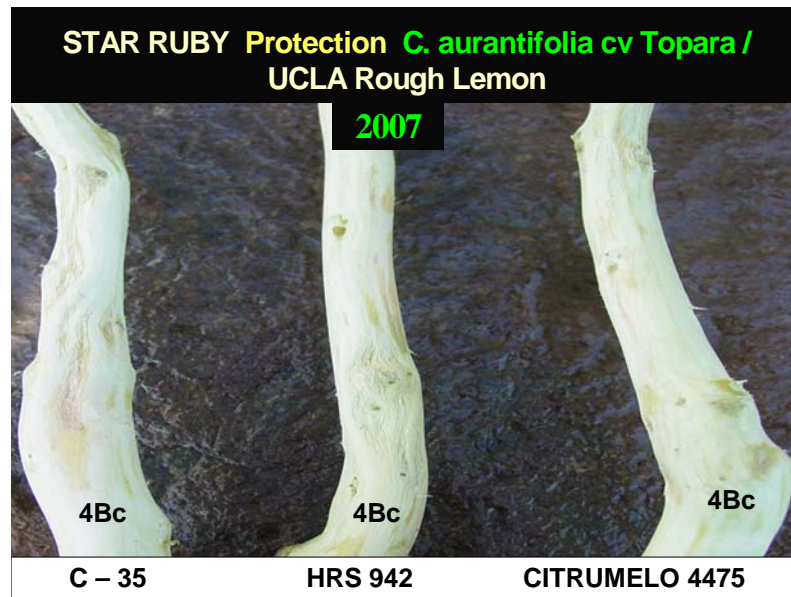


Fig 8. Severe stem pitting of 1-yr-old nursery Star Ruby grapefruit budded on rootstocks other than UCLA rough lemon.

private home which was not in decline whereas its sister tree was in severe decline. Both of these trees were infected with CTV. However, when put under test, the tree without apparent symptoms of stem pitting or small fruit proved to have excellent protective abilities. Protection was not only successful for the small fruited lime, but also for navels, grapefruit and the grapefruit hybrid Oroblanco.

A third source of protection was brought into the country from the Rubidoux quarantine greenhouse at the University of California at Riverside, California. These protective isolates were derived by passage of severe SP-CTV through *Passiflora* spp. and were shown to have excellent protective abilities when tested under greenhouse conditions (6, 7). The results of this protection under field conditions and heavy inoculum pressure in Peru were given by Bederski et al. (1).

This paper brings to date the various experiments previously reported on the continued success of all three sources of protective isolates against the severe SP-

CTV existing in Peru and gives promise to the revival of Peruvian citrus industry.

It should be kept in mind that in 1987, when many groves were visited in the region north and south of the Topara nursery, not a single young or old grove could be found that was not in severe decline with severe stem pitting found on most trees in every grove of navel oranges. Where grapefruit trees were observed they were debilitated, stunted and severely stem pitted (7, 8, 9). The presence of small fruited lime trees was virtually non-existent. Tangelo and Valencia orange trees were found severely stem pitted and stunted. In contrast, as reported by Bederski et al. (1) navels, limes and grapefruit could now be grown successfully in this region once devoid of these orchards due to the severity and intensity of SP-CTV.

In this continued study nine years after inoculation with protective CTV sources, we report continued protection at the Topara nursery by selected and tested sources. The navel protective source L-1 was superior for protecting the Lane Late

navel (Figs. 3 and 5), the navel protective source L-2 was superior for protecting the Cara Cara navel (Fig. 3), and the navel protective source R-2 was superior in protection of the Navelina, Fukumoto and Lane Late navels (Figs. 3, 4 and 5).

The lime selection *C. aurantiifolia* cv Topara continued to be highly successful for protecting the small fruited lime trees in commercial orchards and a new industry of growing limes has developed. Of interest, this *C. aurantiifolia* cv Topara protective selection was also successful for protecting the Cara Cara and Navelina navels and also the Flame grapefruit and was outstanding in its protection of the Oroblanco hybrid grapefruit 9 yr after inoculation (Fig. 3).

The *Passiflora* protective source Madam Vinous code 37A was the outstanding protective source for the Navelate, Fukumoto and Navelina navels (Figs. 3 and 4). *Passiflora* protective source code Duncan 37B was the leading protective source for the Oroblanco hybrid grapefruit (Fig. 3). *Passiflora* protective source Duncan code 37C was second to the *C. aurantiifolia* Topara in protection of Flame grapefruit. (Figs. 3 and 6).

The results herein obtained, infer that the experiments have to be long lasting to achieve the “finest tuning”, represented by

the best relationship of virus and citrus tissue and in the last analysis expresses itself in maximum performance and ideal balance of CTV, canopy and rootstock. Furthermore, it is unique that the *C. aurantiifolia* Topara affords protection to a wide range of citrus making it a “universal” protective isolate.

Field trials of a block of 1,549 5-yr-old Lane Late navels on citrumelo-4475 rootstock protected with L1 showed only 2.13% stem pitting and the removal of only 33 trees (Fig 7).

Seven isolates of CTV from the Topara citrus nursery were collected and established as *in planta* cultures in Madam Vinous sweet orange at the Exotic Plant Quarantine greenhouse at Beltsville, MD. Which included sources of the L-1, L-2, R-1, *C. aurantiifolia* cv Topara and other sources. These isolates were tested by the multiple molecular markers and the strain group specific probes to determine the relatedness of the isolates. Further studies on sweet orange and grapefruit are under way (5).

In conclusion, the remarkable protection afforded by these three sources of tristeza cross protection continues in Peru 20 yr after the search for improvements was initiated and 10 yr after inoculation for cross-protection was done.

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