Relation of Cristacortis Virus to Other Citrus Viruses

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CRISTACORTIS WAS first reported in 1964 and shown to be a graft-transmissible disease (6). From further experiments, it was concluded that cristacortis was caused either by a yet unreported virus, by a new strain of a known virus, or by a complex of known viruses (7). These conclusions as well as the results on which they were based were confirmed later (4).

The experiments to be described in this paper were made principally to relate more precisely cristacortis virus to known viruses of citrus or differentiate it from them. The results indicate that cristacortis is probably caused by a new virus.

Typical symptoms of cristacortis have been observed by the authors in Corsica, Sicily, Sardinia, Spain, Morocco, and Algeria (6, 7). Further reports on the occurrence of the disease in Mediterranean countries have appeared (2, 3, 4, 5, 8). Symptoms have been found in orange trees more then 100 years old in Corsica.

Procedures and Results

SUSCEPTIBLE CULTIVARS.—Three different types of trees were graft-inoculated with a field source of cristacortis virus: 6-year-old trees, 1-year-old seedlings, and budlings on sour orange roots. The inoculum, 3–5 bark patches, was grafted into the main limbs of the scion or into the stem of the seedling. Many different cultivars of those inoculated have thus far developed cristacortis symptoms (Table 1). Orlando tangelo plants showed symptoms in the shortest time, often within 12 months after inoculation of either trees, seedlings, or budlings. Sour orange seedlings did not show symptoms within 15 months. Symptoms developed sooner in budlings of mandarin and sweet orange than in seedlings.

Even though several cultivars developed symptoms within a year, not all plants of a given cultivar did so in less than 3 years (Table 1). Not only the scion portion of the budlings developed symptoms but the stock also often did. The sour orange rootstock in 7 of 18 plants in one experiment developed symptoms within 30 months after inoculation and an additional 5 developed symptoms after 45 months. In another experiment, the rootstock in 3 of the 9 Orlando tangelo budlings developed symptoms within 40 months, and an additional 3 did so after 51 months. The Orlando tangelo scion portion of 8 of these seedlings showed symptoms within 14 months, and the remaining 1 within 32 months.

The cultivars that have thus far failed to develop symptoms within specified periods after graft inoculation are: budlings of bergamot orange, chinois, combava, and Nagami kumquat within 41 months; budlings of Etrog citron within 66 months; seedlings of Troyer citrange, Eureka lemon, and Mexican lime within 66 months; and trees of Eureka lemon within 66 months.

In addition to the data listed in Table 1, the following species or varieties have shown symptoms in the field: Ovale Calabraise sweet orange in Corsica, Tardive de Ciac-

TABLE 1. CITRUS CULTIVARS DEVELOPING SYMPTOMS OF CRISTACORTIS AT VARIOUS TIMES AFTER INOCULATION WITH SEVERAL SOURCES

Species or variety	Type of plants inoculated	Source of inoculum	Months between inoculation and appearance of first symptoms	Months between inoculation and last observation
Citrus pectinifera	B[a]	I[b]	38 (1/4)[c]	41 (1/4)[c]
Clementine	B	I	38 (1/4)	41 (1/4)
Clementine	T	II	63 (1/1)	
Grapefruit, Marsh	T	II	41 (2/2)	
Marsh	S	I	28 (1/2)	36 (2/2)
Ruby	T	II	28 (1/2)	41 (2/2)
Thompson	T	II	26 (1/1)	
Mandarin, Carvalhal	T	II	37 (1/1)	
Carvalhal	B	I	11 (2/4)	41 (3/4)
Commune	S	I	41 (2/2)	
Commune	B	V	10 (6/8)	16 (7/8)
King of Siam	B	I	20 (1/4)	41 (1/4)
Trabut	S	I	28 (1/2)	36 (2/2)
Wilking	T	II	26 (2/2)	
Cleopatra	S	I	28 (1/2)	36 (2/2)
Sweet orange, Cadenera	T	I	37 (1/2)	41 (2/2)
Cadenera	B	I	11 (4/4)	
Caipira	S	I	45 (1/4)	45 (1/4)
Double Fine	T	II	28 (1/2)	37 (2/2)
Double Fine	B	I	11 (1/4)	38 (3/4)
Golden Buckeye	S	I	26 (1/2)	41 (2/2)
Grosse Sanguine	T	II	28 (1/2)	37 (2/2)
Hamlin	T	II	37 (1/2)	41 (2/2)
Moro	T	II	28 (2/2)	
Pera	S	I	26 (1/2)	41 (2/2)
Petite Jaffa	S	I	69 (2/2)	
Sanguine d'Espagne	T	II	28 (1/1)	
Sanguine de porto-Vecchio	B	I	11 (2/3)	43 (3/3)
Tardive Italienne	T	I	26 (1/1)	
Tarrochino	B	I	28 (1/6)	41 (1/6)
Tarocco	B	I	12 (5/12)	42 (6/12)
Tarocco	B	I	11 (1/6)	41 (1/6)
Tarocco	B	I	12 (9/40)	39 (11/40)
Valencia late	T	II	26 (2/2)	
Valencia late	S	I	26 (1/2)	36 (2/2)
Vernia	T	II	26 (2/2)	
Washington Navel	T	II	37 (1/2)	41 (2/2)

(TABLE 1.—Continued)

TABLE 1—Continued

Species or variety	Type of plants inoculated	Source of inoculum	Months between inoculation and appearance of first symptoms	Months between inoculation and last observation
Trifoliate orange	S	I	16 (1/1)	
Rough lemon	S	I	30 (2/2)	
Owari satsuma	S	I	26 (1/2)	28 (2/2)
Siamelo	B	I	38 (2/4)	41 (2/4)
Sour Orange	S	I	15 (2/5)	31 (5/5)
Sour Orange	S	III	16 (1/4)	25 (4/4)
Sour Orange	S	IV	26 (1/4)	29 (1/4)
Sweet lime Millsweet	S	I	40 (1/2)	66 (1/2)
Tangelo, Orlando	T	I	13 (1/6)	41 (6/6)
Orlando	S	V	10 (2/24)	19 (10/24)
Orlando	S	I	21 (2/4)	45 (4/4)
Orlando	B	I	14 (8/9)	32 (9/9)
Sampson	B	I	38 (2/4)	44 (4/4)
Sunshine	B	I	11 (1/4)	38 (4/4)
Watt	B	I	38 (3/4)	44 (3/4)
Webber	S	V	11 (1/4)	77 (1/4)
Tangor, Temple	B	I	16 (1/3)	40 (2/3)

a. T, tree; S, seedling; B, budling (see text).
b. I, Tarocco orange tree C-4; II, Tarocco orange tree V-31; III, Clementine tree Z-28; IV, orange tree P-2; V, Orlando tangelo tree 21.
c. Number of plants showing symptoms over total number inoculated.

culi mandarin in Spain (1), and Navelate and Navelina sweet orange in Morocco. Other susceptible species or varieties have been reported in Sicily (3, 4) and in Sardinia (5, 8).

TIME FOR VIRUS TO PASS FROM SCION TO STOCK.—Tarocco sweet orange buds from a nucellar tree were grafted to 1-year-old sour orange seedlings, and bark pieces from a cristacortis-affected Tarocco orange tree (C 4-8) were then grafted both below and above the bud. The bark pieces serving as inoculum were removed 2, 4, 8, or 14 days, respectively, after inoculation. Five test plants were used for each time period and an additional 5 were used as controls in which the inoculum was allowed to remain. The tree used as a source of inoculum is known to be infected not only with cristacortis virus but also with exocortis and concave-gum viruses. Presence of cristacortis and concave-gum viruses was detected by symptoms that developed in the inoculated test plants. Presence of exocortis virus was checked twice by the Etrog citron test, with bark inoculum being taken from the inoculated plants 24 and 37 months, respectively, after inoculation.

The data (Table 2) indicate that 2 days suffice for cristacortis virus to pass from the bark inoculum to the test plant. Psorosis virus also passed to 1 of 5 plants within 2 days. Exocortis virus, on the contrary, did not pass from the graft within 2 days, but only within 4 days. This failure made it possible to obtain a source of cristacortis virus free of exocortis

TABLE 2. TIME REQUIRED FOR CRISTACORTIS VIRUS TO PASS A GRAFT UNION

Time between inoculation and removal of bark inoculum (days)	Number of plants showing symptoms within 54 months after inoculation		Number of plants with exocortis symptoms 37 months after inoculation
	Cristacortis virus	Concave-gum virus (leaf symptoms)	
2	2	2	0
4	1	1	1
8	2	2	0
14	3	3	3
No removal of inoculum	4	4	5

virus, but unfortunately still contaminated with concave-gum virus.

RATE OF TRANSLOCATION OF CRISTACORTIS.—Sour orange seedlings were inoculated by grafting with bark from a cristacortis source tree (Tarocco orange C 4-8), which also carries concave-gum and exocortis viruses. Subsequently, bark patches taken from the inoculated seedlings at different distances above and below the graft were grafted to other sour orange seedlings serving as indicator plants. Symptoms in the indicator seedlings showed that cristacortis virus was present 5 cm below and 5 cm above the point of the initial grafts 10 days after inoculation, and 20 cm above the graft 16 days after inoculation. Removing a ring of bark either above the point of inoculation or below—immediately after inoculation—did not prevent the passage of virus through the girdle within 16 days following inoculation. However, no attempts were made to scrape the girdle periodically in order to prevent healing.

METHODS OF TRANSMISSION.—Cristacortis virus was transmitted by grafting when either bark tissue, root tissue, or leaf tissue was used as inoculum.

No evidence of seed transmission has been noticed so far in an experiment in which 157 sour orange seedlings produced from seed of a cristacortis-affected tree have been under observation for 3 years. Finally, there is no field indication in Corsica that the virus is spread by a vector. No transmissions were obtained in preliminary experiments with aphids, *Aphis spiraecola* Patch and *Toxoptera aurantii* B. de F.

EXPERIMENTS TO TEST FOR AN IDENTITY WITH, OR RELATION TO, OTHER CITRUS VIRUSES. *Tristeza.*—Six Mexican lime seedlings inoculated 6 years ago have failed to show stem pitting and vein clearing, whereas 6 Orlando tangelo seedlings inoculated at the same time with the same source of virus have developed typical cristacortis stem pitting. The results confirm our previous conclusions (7) that, on the

basis of symptoms and host susceptibility, cristacortis virus is not related to tristeza virus.

Exocortis.—Thus far, practically all sources of cristacortis virus have been found to contain both exocortis and concave-gum viruses. As mentioned above, however, a source of cristacortis virus free of exocortis virus was obtained experimentally. Therefore, it was possible to determine that the symptoms of cristacortis are independent of the presence or absence of exocortis virus. Thus cristacortis and exocortis viruses are unrelated.

Cachexia.—Although Orlando tangelo is a good indicator for both cristacortis and cachexia viruses, the symptoms produced by the two in Orlando tangelo seedlings are clearly different (7). Additional evidence for a difference was obtained in an experiment in which 3 1-year-old Orlando tangelo seedlings were inoculated with a pure strain of cachexia virus (Calavan, code 114). Distinctive symptoms of cachexia were present in the trees 6 years later. At that time, each of the 7-year-old seedlings was inoculated with 5 bark patches from a cristacortis-affected Orlando tangelo tree (Orlando tangelo n°2). Typical cristacortis symptoms appeared 13 months later. Thus, the presence of cachexia virus in these seedlings has neither prevented the appearance of cristacortis symptoms nor delayed them.

In a more recent experiment with Orlando tangelo seedlings on sour orange stocks, 9 were inoculated with cachexia virus. All 9 showed symptoms of cachexia 51 months later but no symptoms of cristacortis or concave gum. A second set of 9 seedlings was inoculated with cristacortis virus; all of them showed cristacortis symptoms 14 months later but no symptoms of cachexia; 5 of the 9 also showed symptoms of concave gum after 48 months. A third set of 9 plants was inoculated with both cachexia and cristacortis viruses. None of them showed symptoms of cachexia within 29 months or of concave gum within 32 months; 6 showed cristacortis symptoms within 15 months, and 2 more within 25 months.

The results of these experiments bring considerable evidence for the belief that cristacortis and cachexia viruses are not related.

Concave gum.—All Corsican sources of cristacortis virus also contain a strain of psorosis virus since young-leaf symptoms of psorosis have always been obtained in susceptible species inoculated with these sources. From the bark symptoms that have recently appeared in the source trees, as well as in certain inoculated Orlando tangelo trees, it has become evident that the form of psorosis virus involved is that of concave gum.

In one experiment, a source of concave-gum virus (code 158-62, also containing a mild strain of exocortis virus) was used to inoculate 8 Tarocco orange trees on sour orange roots. Six identical trees were inoculated with a source of cristacortis virus (Tarocco C 4-8,

also containing concave-gum and exocortis viruses). Within 54 months, the 8 plants inoculated with the concave-gum virus source all showed young-leaf symptoms but no bark symptoms. Of course, no cristacortis symptoms have appeared. Within 29 months, 4 of the 6 plants inoculated with cristacortis virus had shown cristacortis stem pitting; all had shown concave-gum leaf symptoms but not the bark symptoms.

The results suggest either that certain concave-gum virus strains contain a stem-pitting component or, more likely, that a stem-pitting factor—the cristacortis virus—is also present in certain sources. Differences in host ranges and symptomatology (7) also suggest that cristacortis and concave-gum viruses are not related.

MIXTURES OF CONCAVE-GUM, EXOCORTIS, AND CACHEXIA VIRUSES.—Since the Corsican sources of cristacortis virus also contain concave-gum and exocortis viruses, there was a possibility that cristacortis results from a synergistic reaction of concave-gum, exocortis, and cachexia viruses. An experiment was performed to test this possibility. Sets of Orlando tangelo budlings on sour orange rootstock, each containing 9 budlings, were inoculated with cachexia virus alone, cristacortis virus alone, and simultaneously with cachexia, exocortis, and concave-gum viruses. All 9 of the budlings in the first group showed cachexia symptoms in 51 months but no symptoms of cristacortis or concave gum within the same time. All 9 of the budlings of the second group developed cristacortis symptoms in 14 months; 5 of the 9 developed concave-gum symptoms in 48 months; none of the 9 showed symptoms of cachexia in 48 months. The only symptoms to appear in the third group were those of concave gum in 1 of the 9 plants within 56 months. It seems clear from the evidence that cristacortis is not caused by a mixture of concave-gum, exocortis, and cachexia viruses.

Discussion and Conclusions

The results so far obtained with cristacortis virus indicate that it is different from tristeza, exocortis, cachexia, and concave-gum viruses and that cristacortis is not caused by a mixture of the last three. The techniques used to obtain these results are based, of course, on the effects these viruses produce in certain hosts. They give no information about the intrinsic nature of the pathogens.

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