Cristacortis, a Virus Disease Inducing Stem Pitting on Sour Orange and Other Citrus Species

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In 1963, small depressions were noted on the trunks of three-year-old Tarocco sweet orange trees [*Citrus sinensis* (L.) Osb.] grafted on sour orange (*C. aurantium* L.) rootstocks, at the Citrus Experiment Station, San Giuliano, Corsica. At first sight, the symptoms resembled those of concave gum. However, they occurred not only on the orange part of the tree, but also on the sour orange rootstock. In addition, when the bark was removed from the concavities, deep vertical pits were found in the wood with corresponding pegs on the cambial side of the bark. As reported by Vogel and Bové (3), this so-called Tarocco stem pitting

could be bud-transmitted, which indicated the virus nature of the disease. However, they were unable to decide whether the causal agent represented a new strain of a known virus, a not yet reported virus, or a complex of viruses.

This communication reports the recent progress made in the study of the disease to which the name *cristacortis* (from the latin *cortex* = bark, and *crista* = peg) has been given.

SYMPTOMS.—The symptoms of cristacortis on the sour orange rootstock of a 3-year-old Tarocco orange tree before and after removal

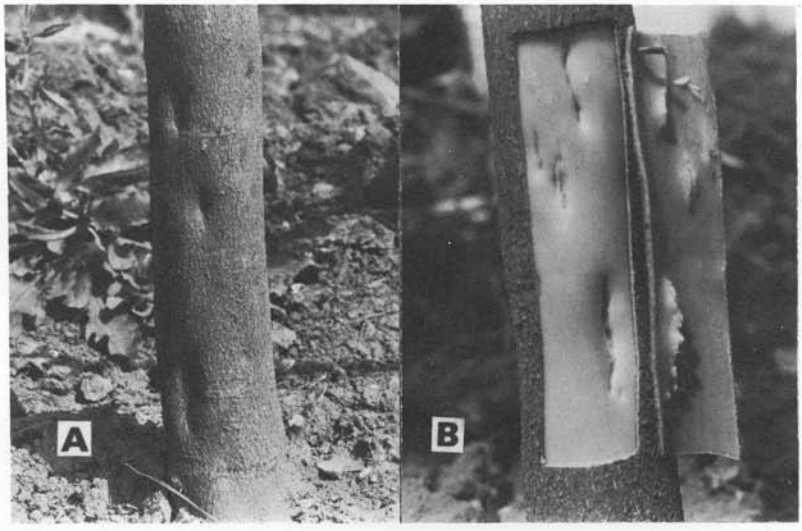

FIGURE 1. *Symptoms of cristacortis on sour orange rootstock of a three-year-old Tarocco orange tree with a trunk diameter of approximately 5 cm. A. With bark. B. With bark removed.*

of the bark are shown in Figure 1. The depressions usually appear first on the trunk of susceptible citrus varieties on sour orange rootstock; later, they can be found on the main limbs, the secondary branches, and even on young pencil-sized shoots (3). Symptoms on the trunk are not restricted to certain areas, such as the bud-union or the soil line, but may occur anywhere from the ground up to the limbs.

After removal of the bark, it is apparent that the depressions in the trunk result from pits in the wood that are penetrated by pegs from the cambial side of the bark. The pegs often present several peaks (Fig. 1,B). Gum-like material is always found in the bottom of the pits and within the pegs, but only in a few instances was gum observed in the

bark surrounding the pegs. A cross section of a branch or trunk through a pit, shows a line of discolored material extending from the bottom of the pit towards the center of the branch (Fig. 2,B). Pits in the wood and pegs in the bark have also been frequently observed on the roots, both large and small.

FIELD OBSERVATIONS.—Cristacortis was first observed at the Citrus Experiment Station in Corsica. The disease affected Tarocco orange trees

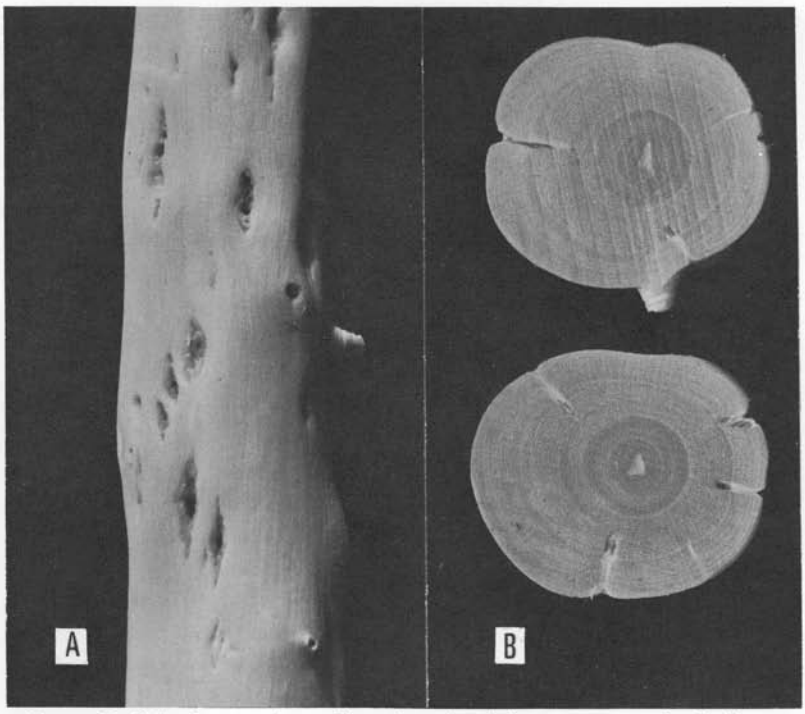

FIGURE 2. *Orlando tangelo seedlings inoculated four years previously from an affected Tarocco orange tree. A. Stem with bark removed to show pits. B. Cross sections of stem; diameter, 3 cm.*

grafted on sour orange rootstocks introduced from Morocco, and symptoms were found on both the scion and the rootstock. Of 160 trees planted in 1961, 53 (33 per cent) were affected in 1964, 71 (44 per cent) in 1965, and 77 (48 per cent) in 1966. Of 73 trees planted in 1962, 3 (4 per cent) showed symptoms in 1964, 19 (26 per cent) in 1965, and 24 (32 per cent) in 1966.

Budwood from Morocco was used in almost all Tarocco orange or-

chards in Corsica and most orchards are affected. In certain orchards 75 per cent of the trees show the disease. Other species or varieties that show the symptoms in the field are mandarin (*C. reticulata* Blanco) trees on sour orange and ovale calabrese sweet orange on sour orange. These trees are more than 20 years old and exhibit symptoms on both the scion and the rootstock. In one grove of 1,300 seven-year-old Clementine trees on sour orange, 170 have symptoms on the sour orange part of the trunk, but no symptoms above the bud-union line. In only one grove of 10-year-old trees are both the scion and the rootstock affected. This disorder was also observed by the authors on several species or varieties of citrus in Sicily (September, 1964) and in Sardinia (April, 1966).

Methods and Results

GRAFT-TRANSMISSION OF THE DISEASE.—In 1961, before cristacortis was discovered, 4 Tarocco orange trees on sour orange were indexed on the following seedlings: Hamlin orange, Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.], Orlando tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco), and [*Poncirus trifoliata* (L.) Raf.]. Three seedlings of each test plant were inoculated and 1 uninoculated seedling was kept as a control. By the end of 1963 and after the cristacortis symptoms on the Tarocco orange trees had been recognized in the field, the results of the 1961 inoculations were as follows: All 4 Tarocco orange trees were found to carry psorosis and exocortis viruses. Three had induced on Orlando tangelo the same symptoms (Fig. 2) observed in the field on the Tarocco orange trees. None of the uninoculated test plants have shown symptoms of any kind up to the present time (September, 1966). In the meantime, the 3 inoculum source trees (Tarocco orange trees 1, 2, and 3) that have induced cristacortis on Orlando tangelo seedlings have shown cristacortis symptoms themselves on the top and on the sour orange rootstock. The fourth Tarocco tree that did not induce cristacortis on Orlando tangelo has shown no symptoms so far. The Hamlin orange seedlings and the sweet lime seedlings inoculated with bark from Tarocco trees 1, 2, and 3 have also shown cristacortis symptoms. None of the inoculated *P. trifoliata* seedlings have shown symptoms to date.

In 1964, 25 Tarocco orange trees from various locations in Corsica, some with and some without cristacortis symptoms, were indexed on seedlings of the following species or varieties: Hamlin orange, Orlando tangelo, Etrog citron [*C. medica* (L.) var. *ethrog* Engl.], and Mexican lime [*C. aurantifolia* (Christm.) Swing.]. The plants were grown in pots

in the screenhouse. All 25 were found to carry psorosis and exocortis viruses, even those that had shown no symptoms of cristacortis. No symptoms of tristeza (stem pitting or vein clearing) have been observed on the Mexican lime seedlings since they were inoculated 3 years previously. As in the 1961 inoculations, those Tarocco trees having cristacortis symptoms also induced cristacortis on Orlando tangelo and on Hamlin orange. No cristacortis symptoms have been observed to date on Mexican lime and Etrog citron.

SUSCEPTIBLE SPECIES OR VARIETIES.—In 1964, seedlings of several species and varieties of citrus were inoculated with bark patches from an affected Tarocco orange tree. Within 30 months after inoculation, typical symptoms were observed on seedlings of the following species and varieties: Golden Buckeye, Pera, and Valencia late sweet orange, Owari Satsuma, Willow-leaf, Cleopatra, and Trabut mandarin, Marsh grapefruit (*C. paradisi* Macf.), sour orange, and Rough lemon [*C. limon* (L.) Burm. f.]. On the other hand, Etrog Citron 60-13, Eureka lemon, Mexican lime, Millsweet lime, and petite Jaffa orange seedlings have not reacted in the same period. At one year after inoculation, the most severe symptoms were observed on the Orlando tangelo seedlings. All inoculated seedlings, except those of Eureka lemon and Rough lemon, showed leaf symptoms of psorosis, and the Etrog Citron 60-13 plants developed clear-cut symptoms of exocortis.

To study further the susceptibility of *Citrus* species and varieties, 3-year-old trees of 17 species or varieties grafted on sour orange rootstock, planted in the *Citrus* collection, were inoculated with 4 bark patches from a single affected Tarocco orange tree in 1964. There were from 25 to 96 trees of each variety in the collection. Two trees of each variety were inoculated and the remainder were left uninoculated as controls. The inoculations were made into the main branches of each tree; none of the trees showed cristacortis symptoms at the time of inoculation.

The results of these tests are as follows: In the 2-year period after inoculation, none of the uninoculated trees showed symptoms. On the other hand, cristacortis symptoms appeared on the inoculated trees of 15 of the 17 species or varieties. Only the inoculated Clementine and the Marsh grapefruit trees have not yet shown symptoms. Symptoms were observed on both the scion and the sour orange rootstock of the following species or varieties: Wilking mandarin, Thompson grapefruit, and Moro, Tardive Italienne, Grosse sanguine, Vernia, and Valencia late sweet oranges. Symptoms were observed only on the top (not on the stock) of the following species or varieties: Ruby grapefruit, and San-

guinello and Double fine sweet orange. Only the sour orange rootstock, of trees of the following species or varieties showed symptoms: Carvailhal mandarin, Eureka lemon, and Cadenera, Washington navel, and Hamlin sweet orange. The most severe symptoms were observed on both the scion and the rootstock of Thompson grapefruit. It may be relevant to note that the Thompson grapefruit is a new line, whereas all other species or varieties in the experiment were old lines.

CRISTACORTIS AND CACHEXIA.—To compare cristacortis and cachexia diseases, the following experiments were commenced in 1964, on Tarocco orange trees on sour orange planted at the Citrus Experiment Station in 1961. Three groups of 2 trees each, one with and one without cristacortis symptoms, were chosen. The 2 trees of the first group were cut off 5 cm above the bud-union line to force the growth of sour orange sprouts. Less than 1 year later, the sour orange suckers of affected trees showed cristacortis symptoms, whereas the sour orange suckers on symptomless trees still showed no symptoms in 1966.

In the second group, Orlando tangelo buds were grafted on the main branches of the 2 Tarocco orange trees and forced. On the healthy tree the buds grew well and developed no symptoms. However, on the affected trees the Orlando tangelo shoots grew very poorly, presented severe cristacortis symptoms 17 months after budding, and died soon thereafter.

In the third group, 2 trees were also budded with Orlando tangelo buds, but in addition were inoculated with cachexia-xyloporosis (California strain, code 114-Calavan GH 1384) at the same time. Twenty-eight months later, typical symptoms of cristacortis appeared on the Orlando tangelo shoots grown on the affected tree, but no symptoms whatsoever have appeared on Orlando tangelo shoots on the cristacortis-free, but cachexia-infected, trees. Thus, cachexia-xyloporosis has failed to produce any symptoms in the period in which cristacortis induced severe symptoms on Orlando tangelo.

Discussion and Conclusions

Numerous inoculation experiments have shown that the agent causing cristacortis symptoms on Tarocco orange trees on sour orange is easily graft transmitted, which suggests that the disorder is caused by a virus. However, the nature of the viral factor is not yet known. That the causal agent is not tristeza virus seems rather certain since Mexican lime seedlings inoculated three years ago have never shown symptoms of tristeza.

Although Orlando tangelo seedlings are very susceptible to both

cachexia-xyloporosis and cristacortis viruses, our results do not support the idea that cristacortis virus may be related to the usual strains of cachexia-xyloporosis virus. The arguments can be summarized as follows.

1) Symptoms of cristacortis on Orlando tangelo seedlings are different from those of cachexia-xyloporosis. Moreover, symptoms of cristacortis can be found on all parts of the Orlando tangelo seedlings from the small roots up to the small shoots.

2) Symptoms of cachexia-xyloporosis on infected mandarin trees on sour orange are commonly very severe above the bud-union line, but are never observed below the bud-union line on sour orange. There is abundant evidence that cachexia-xyloporosis does not produce symptoms on sour orange or sweet orange. Cristacortis, on the contrary, is characterized by pronounced symptoms on sour orange.

3) In cross sections of an Orlando tangelo seedling affected with cristacortis, gum-like material may be seen extending from the pit to the center of the section. No such symptoms were found on Orlando tangelo affected with cachexia-xyloporosis.

4) Under Corsican conditions, Orlando tangelo seedlings inoculated with a severe strain of cachexia-xyloporosis begin to show symptoms only four years after inoculation, whereas symptoms of cristacortis often appear within one year following inoculation.

At first sight, the symptoms of cristacortis resemble those of blind pocket and concave-gum viruses, and young-leaf symptoms of psorosis, especially the oak-leaf pattern, do appear on orange seedlings inoculated with bark from cristacortis-affected trees. Nevertheless, we believe that the stem-pitting symptoms are not due to a known strain of blind pocket or concave-gum virus for the following reasons: 1) Symptoms of blind pocket and concave gum on mandarin or sweet orange trees on sour orange rootstock are never found below the bud-union, whereas symptoms of cristacortis are almost always found on the sour orange rootstock as well as above the bud-union. 2) Cross sections through blind pocket- and concave-gum-affected branches exhibit more or less concentric rings of gum-like material staining the wood, whereas cross sections through cristacortis-affected branches exhibit gum-like material deposited along the rays. 3) Furthermore, with blind-pocket and concave-gum diseases, the cambial side of the bark covering the pockets or concavities remains smooth and no pegs extend into the wood, whereas cristacortis is characterized by pronounced pegs and ridges extending deep into the stem.

In Sicily, Russo and Klotz (2) described an unusual form of concave-gum disease on Tarocco orange trees on sour orange; they called it

Tarocco pit. Cristacortis and Tarocco pit are considered different disorders for the following reasons: Tarocco-pit symptoms have not been mentioned on the sour orange rootstocks of Tarocco orange trees, whereas cristacortis symptoms are virtually always present on the sour orange rootstock of Tarocco infected with cristacortis virus. Furthermore, Tarocco pit and cristacortis have different symptoms. Tarocco pit is characterized by circular pits or holes in the wood and "from these holes is extruded a material like ground cork" (2). Pits of cristacortis are several times longer than wide (Fig. 1), and no ground cork-like material is extruded through the external bark surface.

In our experience, trees affected by cristacortis usually also carry psorosis and exocortis viruses. However, there are trees on sour orange rootstock which carry psorosis and exocortis viruses, or psorosis, exocortis, and cachexia-xyloporosis viruses; yet they show no symptoms of cristacortis. Also, attempts to reproduce cristacortis by inoculating Orlando tangelo seedings on sour orange, with cachexia-xyloporosis, psorosis-A, exocortis viruses, or cachexia-xyloporosis, concave-gum, and exocortis viruses have given no cristacortis symptoms after two years. In the same experiment, however, eight out of nine Orlando tangelo seedlings inoculated with cristacortis have already shown cristacortis symptoms. Thus, further experimental work is needed to determine definitely whether cristacortis is caused by a new strain of cachexia-xyloporosis virus, a new strain of blind pocket and concave-gum virus, a complex of several virsuses, or even by a new virus, as we are presently inclined to believe.

Although first noted by the authors in 1963, it is probable that cristacortis has been present in the Mediterranean area for many years. In 1959, Reichert (1), in his survey of citrus virus diseases in the Mediterranean area, described the occurrence of what he calls xyloporosis, on sour orange rootstock, and on sweet orange. According to him, sour orange would be quite susceptible to xyloporosis. It is likely that the symptoms that Reichert noticed, and attributed to xyloporosis, are the same as those we have now called cristacortis.

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