CITRUS SUDDEN DEATH

In Retrospect: Citrus Sudden Death, a Graft-Transmissible, Tristeza-like Bud Union Disease*

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When, in 1999, a new disease, now called Citrus Sudden Death (CSD), started to kill sweet orange trees grafted on Rangpur lime in the Brazilian region of Triangulo Mineiro, the problem was not at first taken too seriously, since this southwestern region of Minas Gerais State was considered marginal for growing citrus. Soon however, the disease was seen spreading into the northern part of São Paulo State. And, more importantly, the similarities between CSD and Tristeza Quick Decline (TQD) rapidly became apparent and reminded the Paulista citrus farmers of the devastating effects of TQD.

Indeed, TQD, caused by *Citrus tristeza virus* (*CTV*), is well known in Brazil, where 9 of 11 million trees grafted on sour orange succumbed to the disease in the 1940s and 1950s. When TQD first appeared in Argentina in 1930, nothing was known about the disease. Many years of research, performed within the framework of an international cooperative effort, were necessary to understand the disease before it could be controlled. These efforts led to the demonstration that TQD was graft-transmissible, caused by a virus, and transmitted in nature by a very efficient insect-vector, the aphid *Toxoptera citricida*. The disease mechanism became understood, and it was eventually established that TQD was a bud union disease in which the sour orange rootstock was affected. From there on, control of the disease became straightforward and consisted of replacing sour orange with rootstocks giving tolerant combinations with sweet orange and other citrus scion cultivars. In this way, the Paulista citrus industry could be rescued, and subsequently has become one of the largest in the world. However, this status took 50 years to achieve. Among the new rootstocks which replaced sour orange, Rangpur lime became the most popular due to its drought-resistance, and by the year 2000, 85% of the 200 million sweet orange trees in the Paulista citrus belt were grafted on this rootstock.

Realizing that the new disease, CSD, was a menace for 85% of the Paulista sweet orange trees, and that the disease was spreading across the Minas Gerais border into São Paulo State, destroying thousands of trees, it became clear that the problem had to be taken seriously, especially since it seemed to be tristeza-like. Could a tristeza-like disaster be avoided? In particular, was it possible, within the CSD-affected region, to save trees on Rangpur lime not yet affected by CSD, or possibly even recover trees already affected by it? The answers to these questions depended on understanding the nature of the disease. Was it really a tristeza-like bud union disease or was it due to abiotic factors? Several lines of

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research have provided the answers to these questions: (i) comparison of symptoms of CSD with those of TQD, (ii) epidemiology of how CSD spreads in time and in space, (iii) graft-transmission assays, (iv) pathological anatomy of the Rangpur lime bark at the bud-union of CSD-affected trees, and (v) inarching experiments. These are summarized as follows:

i) Symptoms of CSD in the affected region of Brazil were found to be very similar to those of TQD as seen in Florida in 2002 (2). In particular, for both diseases, fruits and leaves remain attached to the trees when death suddenly occurs. However, the yellow discoloration so characteristic of Rangpur lime bark from CSD-affected trees and which had proved to be of great diagnostic value does not occur in the sour orange bark from TQD-affected trees.

ii) Extensive surveys carried out by Fundecitrus showed that CSD spreads in time and space in a manner strikingly similar to the spread of *CTV*, under conditions where the aphid *Toxoptera citricida* is the vector (1). These results strongly suggested that CSD had an aerial vector such as *T. citricida* and that the causal agent was biotic.

iii) Transmission of the causal agent of CSD by graft-inoculation was successfully achieved by Fundecitrus (3). Graft transmitted agents are viroids, viruses, and endogenous, phloem- and xylem-restricted bacteria. Searches for endogenous, as well as exogenous, bacteria have produced negative results (1), and no viroids were detected in CSD-affected trees (2). Thus, the causal agent of CSD had to be a virus.

Positive graft-transmissions of the CSD agent were obtained not only with budwood- taken from 14-year-old CSD-affected trees (grafted on Rangpur lime), but also with inoculum taken from 22-year-old asymptomatic trees (grafted on Cleopatra mandarin). Both inoculum-donor trees were from the same farm, indicating that the causal agent of CSD was present not only in CSD-affected trees, but also in asymptomatic trees. The fact that symptomless trees on Cleopatra mandarin carried the CSD agent showed that trees on Cleopatra mandarin were tolerant to CSD. Indeed, in the CSD-affected region, trees on Cleopatra mandarin have never shown symptoms of CSD. The same is true for trees on Sunki mandarin, Swingle citrumelo and *Poncirus trifoliata*. However, 5- to 7 yr-old trees on Volkamer lemon with typical symptoms of CSD, including the characteristic yellow discoloration in the rootstock bark, were detected in 2003. With Volkamer lemon, progress and severity of the disease seem to be less intense than with Rangpur lime, but further studies are required to confirm these observations.

A third rootstock might be involved in CSD! Indeed, rough lemon seedlings inarched in November 2003 to CSD-affected Valencia sweet orange trees on Rangpur lime have shown the characteristic yellow discoloration ten months after inarching. Swingle citrumelo seedlings inarched to the same trees showed no symptoms 24 mo after inarching. The anatomical pathology of the inarched rough lemon seedlings is under investigation.

iv) It was shown long ago that the sour orange bark below the bud union of TQD-affected trees shows characteristic histological symptoms. The functioning phloem (FP) is greatly reduced and is characteristically affected by necrosis, collapse and obliteration of sieve tubes and companion cells, as well as by the presence of chromatic parenchyma cells. There is an excessive amount of non-functioning phloem with conspicuous necrotic areas. Medullar rays are hypertrophic and hyperplastic. Very similar anatomical alterations were seen in Rangpur lime and Volkamer lemon bark below the bud union of CSD-affected trees (2). However, in CSD the

amount of FP was not as drastically reduced as in TQD. This is probably the reason why trees on Rangpur lime begin to show symptoms of CSD only after about two years in the field, while trees on sour orange decline after about six months.

v) Inarching consists of planting one or two citrus rootstock seedlings next to a grafted tree and approach-grafting them onto the scion, above the bud union line. Many CSD-affected trees have been inarched in this way with various citrus rootstock seedlings. If CSD is a bud union disease, affected trees should show recovery when inarched with seedlings such as Cleopatra and Sunki mandarins or Swingle citrumelo, but they should not recover when inarched with Rangpur lime seedlings. This is precisely what has been observed.

Inarching with the above rootstock seedlings has given exceptionally good results, with close to 2.5 million trees inarched since 2002. Inarching serves two purposes, recovery of CSD-affected trees, and prevention of CSD symptoms. CSD-affected trees up to 10 years old could be recovered when inarched with two seedlings. Pruning of the trees before inarching adjusts the canopy size to match the deteriorated and reduced root system, extending the life of the trees by one or two years and giving the farmers more time to carry out the inarching procedure. However, pruning can also be done immediately after inarching.

Young trees begin to show mild CSD symptoms only after having been in the field for at least 2 years. Therefore, enough time is available to inarch young trees and one inarched seedling is enough to prevent CSD from developing. In the case of tristeza, young trees show severe symptoms much earlier, and this is probably the reason why inarching was not developed as a method to control TQD. Interestingly, it has been observed that with inarched trees, the initial Rangpur lime rootstock does not die and continues to provide at least mechanical support to the trees. The rootstock seedlings used to inarch the trees on Rangpur lime are not as drought resistant as the initial Rangpur lime rootstock. However, as inarching keeps the Rangpur lime rootstock alive, inarched trees might show some drought-resistance. This possibility should be confirmed.

The epidemiology studies have shown that CSD spreads very much like *CTV* and that aphids such as *T. citricida* could be responsible for this spread. Hence, it was to be expected that CSD would continue to increase within the affected regions and invade further regions of the Paulista citrus belt not yet affected. This is precisely what the surveys, carried out by Fundecitrus in 2002 (June to September) and 2003 (September to December) have shown. In São Paulo State, the disease was first restricted to the northern region, but has now progressed 60 km to the West as well as to the South within one year, and important citrus counties in the northwest and the center of the state are now infected. The number of trees that are affected or that have died in Minas Gerais and São Paulo States has been calculated to be over two million. In the years to come, the rate with which the disease will progress depends on factors such as soil and weather conditions, number of inarched and tolerant trees, density of citrus orchards and movement of infected plant material out of the affected regions. Therefore, it is essential that further surveys for CSD be carried out each year to outline the borders of the affected regions. This knowledge makes it possible to define areas of short-term risk, where inarching and/or planting tolerant trees should be carried out immediately, as well as areas of medium and long-term risk.

At this time, the causal agent of CSD is not known. All CSD-affected trees are infected with *CTV* as well as

with a CSD-associated virus (CSDaV) detected by Alellyx Applied Genomics in 2003. CSDaV is a member of the *Tymoviridae* family of viruses. As mentioned above, the epidemiology studies (2) have indicated that CSD is very probably spread by an aphid vector such as *Toxoptera citricida*. Therefore, as expected, Alellyx was able to detect the CSDaV in three aphid species known to be *CTV* vectors: *T. citricida*, *Aphis gossypii* and *A. spiraecola*. CSDaV is very probably transmitted by these aphids, in spite of the fact that no member of the *Tymoviridae* is known to be aphid-transmitted. This suggests that CSDaV might be co-transmitted with *CTV*, using *CTV* as a helper virus. In collaboration with Fundecitrus, Alellyx has also shown that CSDaV is present in citrus plants graft-inoculated with budwood from CSD-affected trees on Rangpur lime as well as from infected but CSD-tolerant trees on Cleopatra mandarin. This demonstrates that CSDaV is graft-transmissible and is present not only in symptomatic trees on Rangpur lime but also in symptomless trees on tolerant rootstocks such as Cleopatra mandarin. However, none of these interesting results proves that CSDaV is the causal agent of CSD. Further research efforts are necessary to identify CSDaV, *CTV* or the two viruses together, as the cause of CSD.

Today, after less than 4 years of intensive work, guided by tristeza-inspired hypotheses, ways to efficiently control CSD have been developed for the short term (inarching) and the long term (tolerant trees). It is now unlikely that CSD will wipe out the Paulista citrus industry as did TQD some 50 years ago. However, by losing Rangpur lime and eventually Volkamer lemon as drought resistant rootstocks, the industry might be forced to turn to irrigation, and this might be the major, if not the most beneficial consequence of CSD on the long term. Continued research efforts remain indispensable. CSD should not be neglected now that research efforts have turned to one even more serious disease which has now reached the forefront: huanglongbing. With strong research and the spirit of the musketeers: "all for one, one for all", the Paulista citrus industry will continue to be one of the best in the world.

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