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Author
Roistacher, C. N.

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DISEASES INDUCED BY VIROIDS AND VIROIDLIKE PATHOGENS

The Cachexia and Xyloporosis Diseases of Citrus—A Review

C. N. Roistacher

ABSTRACT. The cachexia and xyloporosis diseases of citrus were reviewed by Childs in the first and third conferences of the IOCV (11, 14). These are both thorough and excellent reviews and the detailed chronology of research to 1966 is not repeated here. This review will focus on the disease of xyloporosis as illustrated and defined by Reichert and Perlberger (26) and the cachexia disease as defined by Childs (6, 7, 8). The similarities and differences in symptoms between the two diseases will be reviewed. Also, recent studies on indexing, mechanical transmission and viroid nature of the cachexia agent will be discussed. Finally, comments on nomenclature and terminology, plus arguments for a precise nomenclature for the two diseases will be presented.

XYLOPOROSIS AND CACHEXIA

Xyloporosis. Reichert and Perlberger (26) reported a new disease problem of citrus in Israel based on field observations between 1928 and 1933. This disease appeared in many trees grafted on Palestine sweet lime, the predominant rootstock used in Israel at that time. The authors defined three characteristic stages of the disease. In the first stage, small conoid pits with corresponding small pegs in the bark made their appearance as early as one year after budding. Symptoms appeared mostly beneath the budunion. In the second stage, the wood and bark were discolored, the bark depressed and the depressions coalesced. Swelling sometimes occurred at the union. Pits in the wood were reported to be very numerous "and lie so near each other that the wood seems to be perforated like a sieve. The third stage of the disease begins with a brown discoloration of the bark which extends over about half the trunk, with splits in the bark and the discolored parts become blackish and peel away in pieces." These symptoms are well illustrated in 10 figures. After the last stage the leaves became small and yellow, with the tree showing high fruit set "and the tree would die or was so debilitated that it was usually pulled."

The authors reported that the disease was widespread and no differences in disease incidence could be observed from the coastal to the hot interior valleys. "We may therefore conclude that no primary importance is to be attached to climatic conditions as a factor."

Some pertinent points and conclusions may be reached from this study:

1) Sweet lime was the primary variety affected—usually as a rootstock, but also as unbudded seedlings.

2) Sweet orange, grapefruit and lemons as scions were not affected, but the disease was passed to mandarin.

3) Symptoms appeared in sweet lime as early as 1 year after budding. In one 3-yr-old grove, 43% of the trees were diseased and 24% of these were severely affected.

4) The authors believed the disease to be nonparasitic. No transmission studies or suggestions were made that the disease was transmissible or came from infected budwood.

Cachexia. Between 1950 and 1956, Childs and Childs and co-workers published a series of papers on a disease of tangelos and mandarins (6, 7, 8, 9, 10). They called the disease cachexia (from the Greek: kakos = bad and hexis = condition). In these studies the following points were established:

1) Symptoms of vein chlorosis, tree stunting, cankers, phloem discoloration and stem pitting were consistently present. Phloem discoloration in the tangelo scion was distinct, and diagnostic and stem pitting consisted of "bumps and projections on the bark that fit into depressions in the wood." (6). The symptoms were well described and illustrated (6, 8, 21).

2) The disease was transmissible.

3) Many scions such as sweet orange, grapefruit and lemon acted as symptomless carriers.

4) Orlando tangelo seedlings were excellent indicator plants for the disease and could show symptoms within 3 yr after inoculation.

5. Different tangelo varieties as scions or seedlings reacted differently to inoculation. There was great variation in scion susceptibility, with Orlando tangelo appearing most susceptible.

6) There appeared to be strains of the causal organism, which was presumed to be a virus.

7) Indexing was proposed for detection of the disease, and for the selection of disease-free budwood.

8) The rootstocks primarily affected by the disease were mandarins and kumquats and hybrids of these species.

9) Tristeza and psorosis viruses did not cause the disease.

Xyloporosis and cachexia—similarities and differences. Childs (7, 8), viewing a preserved specimen of Bahia navel on sweet lime stock from Brazil stated that the symptoms were similar enough to be the same disease. Childs (10) began cross-inoculation tests to sweet lime and tangelo and found that cachexia symptoms were consistently produced on Orlando tangelo seedlings through inoculation with buds from infected trees. Buds from trees on xyloporosis-affected sweet lime rootstocks in Florida induced typical cachexia symptoms when propagated on Orlando tangelo seedlings and "in reverse procedure typical xyloporosis symptoms were induced in sweet lime rootstocks when budded with buds from cachexia-affected Orlando tangelo trees." He felt that differences in symptom expression may be a matter of host response. Childs wrote that **"the relationship between cachexia of Orlando tangelo and xyloporosis of sweet lime in Palestine would not be definitely settled until cross inoculation studies of the Orlando tangelo and Palestine forms of xyloporosis are made."** In his observations he suggested that symptoms of the two diseases are not identical. e.g. "that phloem discoloration which is characteristically pronounced in cachexia-affected Orlando tangelo trees is not present in xyloporosis-affected trees." Also the wood pitting symptoms reported for xyloporosis on Palestine sweet lime are not present or typical in tangelo.

Similar observations have been made by Grant, *et al.* (17), Calavan, *et al.* (1) and Roistacher (personal observations). Calavan, *et al.* (1) stated "current experiments have not yet shown whether cachexia as observed in California is identical with or distinct from xyloporosis."

There is some confusion as to just what xyloporosis is. Fawcett (15, 16) in reports of a visit to Palestine in 1930 mentions a disease condition new to him on sweet lime rootstock. He believed the condition due to the effect of the sun on the bark. Katzprowsky (18), described a new disease of sweet lime in Palestine in 1931. "Just below the union of the bud—one or more small depressions are seen in the bark about the middle or the end of the second year after budding. The depression may proceed and form a flat and later sunken area all along the stock instead of the normal round shape. When the bark is raised, brownish dots are visible. During the following winter the leaves are turned yellow by the cold winds and they drop." Katzprowsky believed that the pinholes and cracks as well as the brownish gum mixture on the cambium were the effects of dehydration. "*After a long survey the writer came to the conclusion that we are dealing here with a phenomenon of dehydration of sweet lime stock by drying winds.*" (italics by Katzprowsky).

In 1953, Reichert, *et al.* (28) inoculated sweet lime seedlings with tissue from xyloporosis-infected plants and found no evidence of symptoms 2 yr later. No mention of the results of this study was made in subsequent publications (29, 30), although the authors stated that the experiment was being continued "for further observations on the possible appearance of wood pitting symptoms." Reichert regarded the little leaf disorder and stubborn disease as identical to xyloporosis (27, 29). Also, in his observations of stubborn disease in Arizona and California he reports "A great number of trees were examined for their bud union condition and the nature of the fruit, and it was found that all trees of grapefruit and other citrus varieties affected with stubborn showed typical xyloporosis pitting and lopsided fruit, both symptoms typical of xyloporosis." Reichert concluded that xyloporosis was widespread in California and Arizona.

Grant, *et al.* (17) found it difficult to transmit cachexia to sweet lime. Buds taken from seven widely separated field sources of cachexia were used to inoculate 26 Orlando tangelo seedlings and 25 Colombian sweet lime seedlings. At 2.5 yr, 24 of the 26 Orlando tangelo seedlings showed definite symptoms, but none of the 25 sweet lime seedlings showed symptoms. However, Childs (14) reported that after a few more years symptoms did appear in the sweet limes inoculated by Grant, *et al.* Calavan, *et al.* (1) had similar results on Palestine sweet lime. The lack of symptoms in the sweet lime after 2.5 yr contrasts to the intense and severe symptoms reported for xyloporosis by Reichert and Perlberger in the original observations where trees 3 to 4 years from budding were severely affected.

Reichert and Bental (30), observed 200 15-yr-old Clementine mandarin trees on Palestine sweet lime stock and reported three types of diseases: cachexia type, xyloporotic type and an inverse pitting type. They concluded there were three separate diseases.

Olson et al. (23) concluded that "xyloporosis of Palestine sweet lime and cachexia of Orlando tangelo are caused by the same virus. The sweet lime, however, appears not to be a specific indicator for xyloporosis since it shows bark splitting symptoms in the presence of exocortis virus". Olson et al. felt that the Orlando tangelo was the preferable indicator plant in Texas.

Childs, *et al.* (13) also showed that two tangelo varieties and three sweet lime varieties were inferior to Orlando tangelo as an indicator for cachexia. After 5.5 yr, the two tangelo varieties showed 17/35 positive reactions with an intensity rated as 1.7 on a rating scale of 1 to 10. The three sweet lime varieties showed 6/45 positive reactions with an average intensity rating of 1.5. However, the Orlando tangelo showed 108/108 positive reactions with an average intensity rating of 7.1. These observations were made 3 to 4 yr after inoculation and again demonstrated the poor response of sweet limes to inoculation with cachexia-infected tissue.

In the early years of the Citrus Variety Improvement Program (CVIP) in California, candidate trees were budded to four Orlando tangelo and four Palestine sweet lime seedlings. Positive severe, moderate and mild cachexia controls were included each year. The tests were terminated when the mild controls became positive in Orlando tangelo, usually about 4 to 6 yr or more after inoculation. The bark was completely peeled away and the entire trunk examined for gumming and pitting. Observations showed very poor response in Palestine sweet lime stocks to all three categories of cachexia, whereas the Orlando tangelo stocks usually responded positively and in proportion to the severity of the inoculum source. It was noted, however, that many non-inoculated trunks of Palestine sweet lime showed varying degrees of pitting and some were rather severe. Tristeza was not present in the area of observation. Based on their studies, Calavan, *et al.* (2) concluded "it is apparent that wood pitting per se does not constitute proof of the presence of xyloporosis. It follows that it is impossible to diagnose xyloporosis on the basis of slight wood pitting alone under local conditions." Palestine sweet lime was abandoned as an indicator in all future tests in the CVIP.

In the presence of tristeza, infected sweet lime will pit severely (3, 19). Wood pitting has been observed by others in non-inoculated Palestine sweet lime seedlings or trees (2, 10). Purple scale can induce wood pitting (17). Carpenter and Furr (5) found pitting in 60 out of 136 non-infected seedlings of various hybrids and conoid pits were found in six 3-year-old nucellar Palestine sweet lime trees. Patt and Hiller (25), found pits on many varieties of citrus in Israel, i.e., on non-inoculated seedlings of Chinnoto sour orange, Egyptian lime, rough lemon, Palestine sweet lime and Troyer citrange. The Shamouti orange, Palestine sweet lime and rough lemon showed very slight to severe pitting on practically all rootstocks. It is apparent from these reports and observations that sweet lime should not be used as an indicator for cachexia and that it is difficult and unreliable to diagnose xyloporosis on the basis of wood pitting alone.

Seed transmission. Studies by Childs (10) suggested possible seed transmission of cachexia since symptoms were found in non-inoculated seedlings. However later studies by Olson (24), and by Childs (12), indicated no seed transmission. Since cachexia is a viroid, similar to citrus exocortis viroid (CEV) and other citrus viroids recently reported (34, 36), seed transmission is expected to be rare.

Indexing. Childs (8) first proposed that the Orlando tangelo could be used as an indicator for cachexia, thus pointing out a means to avoid the disease in propagative budwood. Indexing on Orlando tangelo seedlings was subsequently adopted in the Florida Citrus Budwood Certification Program, the California CVIP, and by others. Calavan and Christiansen (3), tested many tangelo and mandarin varieties and hybrids for susceptibility to cachexia and found that the Parson's Special mandarin, Owari satsuma and Sunshine and Wekiwa tangelos gave the most severe reaction under cachexia-infected scions. The reaction of Palestine sweet lime was mild except under grapefruit tops. Orlando tangelo reacted strongly to inoculum from cachexia-infected Marsh grapefruit, moderately to inoculation from Wekiwa tangelo and rarely and very mildly to inoculation from Willowleaf mandarins.

These authors concluded that: 1) in the absence of symptoms 4 yr after inoculation, the index remained inconclusive; 2) eight or more indicator plants should be used. 3) symptoms might be variable, and 4) indexing should be done in a tristeza-free environment.

Salibe (35) suggested a more rapid means of indexing for cachexia by forcing a bud of Orlando tangelo on a large 1- to 2-yr-old seedling of Rangpur lime, and obtained cachexia symptoms in 1 yr. Following up on this work, Calavan and Christiansen (3) and Roistacher, *et al.* (31) found that buds from selected seedling lines of Parson's Special mandarin, when forced on a vigorous rootstock such as rough lemon, would show excellent symptoms in the Parson's Special mandarin at the bud union and at the cut-back joints in 1 yr or less. Parson's Special mandarin was superior to Orlando tangelo as an indicator. This more rapid index was confirmed by Vogel and Bove (37), who induced excellent syptoms in 6 months with Parson's Special mandarin on Volkamer lemon rootstock.

Nauer and Roistascher (20) tested 63 selections of mandarins and mandarin hybrids as seedling indicators at two locations in an effort to find a more rapid indicator for cachexia. None of the seedlings reacted to an inoculation with a severe isolate of cachexia viroid under good conditions for symptom development.

Parson's Special mandarin buds propagated on a rough lemon, and rapidly forced or pushed after inoculation are used as the standard indicator for indexing for cachexia in the Citrus Clonal Protection Program in California. Extremely mild-reacting positive controls are included in each index test. Indexing is done in a warm glasshouse where test plants are held for 1 yr. When a substantial number of candidate trees are tested, as many as 12 mild-positive control plants are used. Small bark patches are carefully peeled back from a small 3-sided U-cut and the wood and bark are examined for symptomatic gumming. If 8 or 9 of the 12 mild positives show symptoms, all test plants are peeled and the experiment is finalized. If few or no symptoms are observed in the controls, the bark is replaced and secured with budding tape, and the plants removed to the field, planted at close spacing and observed for another year or until at least 8 or 9 of the 12 mild positive controls show symptoms. The mild positive control plants usually will become symptomatic within 1 yr in the field. For best results, it is important that field temperatures be warm or hot.

Mechanical transmission. The appearance of symptoms typical of cachexia in unbudded seedlings has been reported by many workers (10, 21, 26, 33). Transmission via seed is rare if it occurs at all (8, 9, 10, 11, 24, 25). Aphid transmission was shown to be negative (22). Roistacher, *et al.* (33) observed the appearance of two unexplained cachexia infections in non-inoculated Parson's Special mandarin indicator plants in the glasshouse. Experiments to determine mechanical transmissibility of cachexia were then done. All of the 55 citron receptor plants became infected when they were inoculated by knife cuts from a positive cachexia-infected citron. A quick dip of the knife blade in a 1% sodium hypochlorite solution completely prevented transmission. Transmission was determined by grafting buds from the test citron plants to Parson's Special mandarin indicator plants. It is evident from these studies that cachexia is highly transmissible by cutting with infected knives from citron to citron.

Viroid nature of the cachexia disease agent. The similarities between the cachexia agent and CEV have been reviewed (34). Both pathogens are highly transmissible by knife cuts from citron to citron—both pathogens are inactivated on knife blades by sodium hypochlorite disinfestant—both pathogens appear to be uniformly distributed within seedlings or trees in a wide citrus host range, and many of these hosts act as symptomless carriers—both pathogens can reduce the size and vigor of scions regardless of rootstocks and do not appear to be directly destructive to the bud unions—both pathogens are difficult to eliminate from scion budwood by thermotherapy (4), however both pathogens are readily eliminated from micro shoot tips by shoot tip grafting *in vitro* (32).

Recent studies by Semancik, *et al.* (36) have confirmed the viroid nature of the cachexia agent. Nucleic acid extracts of cachexia-infected citron tissue were analyzed by sequential polyacrylimide gel electrophoresis (PAGE). Results showed an RNA band of about 300 nucleotides displaying the characteristic circular and linear forms of the viroid. This band was eluted, mechanically transmitted by razor cuts to citron and the mature new growth citron was bud-inoculated to Parsons Special mandarin indicator plants. Results positively confirm that the cachexia disease could be reproduced by inoculation with this viroid. When budwood of seven cachexia isolates was inoculated into citron and the new growth citron tissue analyzed by electrophoresis, all seven isolates showed this distinct viroid band. This specific viroid had not been found associated with CEV or any known citrus viroid isolates. No homology to CEV or citrus variable viroid was detected in molecular hybridization tests against cDNAs made to this viroid.

DISCUSSION AND CONCLUSION

Xyloporosis should be defined as the condition outlined and described by Reichert and Perlberger (26) and confined to the observations and illustrations given by these authors. Specifically:

1) It is a problem associated with sweet lime.
2) Early symptoms are conoid pits in the wood with a brown color at the base of the pits and with corresponding pegs in the bark. Later symptoms show discoloration in the wood and bark with depressed areas in the outer bark plus numerous pits. Final stages show discoloration of the bark extending to half the trunk with discolored parts turning black. These symptoms are well illustrated (26).
3) Symptoms appear within 1 yr after scions are grafted, and up to 75% of the trees may show symptoms within 4 yr.
4) Climatic conditions are not a factor in disease incidence or development.
5) Sweet orange, grapefruit and lemon scions are not affected but symptoms "can pass to mandarin."

Transmission of xyloporosis, as defined above, has never been done to reproduce these types of symptoms. However, the comment on passage of the symptoms from infected sweet lime to mandarin could suggest transmission. The association of this disease with little leaf and stubborn diseases adds somewhat to the confusion of exactly what xyloporosis is. Attempts to transmit cachexia disease to sweet lime have in general failed to reproduce the symptoms described above (2, 9, 10, 13, 17, 23, 38). Transmission tests in Israel also failed to reproduce the above symptoms (28). Therefore, the name xyloporosis should be used for the specific conditions and symptoms on **sweet lime** as illustrated and described in detail by Reichert and Perlberger in 1934 (26).

The cachexia disease of citrus appears to be specific. It is a disease which is transmitted by grafting or by mechanical transmission. Mandarins, kumquats, and hybrids of these, plus *Citrus macrophylla*, are symptomatic and highly susceptible. Symptoms are specific, showing strong gum formation with or without pitting. Pits are generally large, more like lumps and projections that coincide and fit into the depressions in the wood. Gumming of varying intensity is usually associated with the pits. The presence of needle-like xyloporosis-type pitting is rare. The disease can be routinely detected on Orlando tangelo or Parson's Special mandarin thus permitting diagnosis to aid in selecting disease-free budlines or for elimination of the pathogen by shoot tip grafting. All evidence indicates that the cachexia disease is neither seed transmitted nor vector transmitted.

The causal agent of cachexia has now been shown to be a viroid of about 300 nucleotides. A specific RNA has been eluted and transmitted mechanically to citron and then bud transmitted to Parson's Special mandarin indicator plants which developed cachexia symptoms. This discovery suggests the possibility of developing a rapid nucleic acid hybridization index test. Cachexia is highly mechanically transmissible, similar to exocortis, and is readily disseminated via budwood in symptomless carriers. The development of a rapid test for cachexia would ultimately aid in the elimination of this viroid from propagative budwood. Direct PAGE analysis of citron or cucumber tissue (36) 6 to 8 weeks after inoculation could also considerably reduce the lengthy incubation period required for the Parson's Special mandarin indexing test.

Citrus cachexia viroid (CCaV) should be the preferred name associated with the specific citrus viroid IIb (36) which is in turn associated with the disease that induces specific symptoms on Parson's Special mandarin (and presumably Orlando tangelo) indicator plants. Xyloporosis should be used to designate the *specific* condition or complex associated with sweet lime as originally described by Reichert and Perlberger (26). Factors other than the cachexia viroid were probably of primary importance in causing xyloporotic pitting.

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