A Necrotic Strain of Citrus Ringspot Virus and Its Relationship to Citrus Psorosis Virus

L. W. Timmer

Associate Professor, Texas A&I University Citrus Center, Weslaco, Texas 78596.
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ABSTRACT

A necrotic strain of citrus ringspot virus (CRSV-NS) was found in nucellar grapefruit trees (*Citrus paradisi*) in South Texas. Field symptoms observed were: flecking of young leaves, shoot necrosis, chlorotic flecks, spots, blotches, and distortion on older leaves. Mature leaf symptoms and twig lesions similar to those produced by psorosis were observed on some trees infected with CRSV-NS. Graft inoculation of seedlings of many citrus species and cultivars produced symptoms similar to those observed in the field. Graft inoculation of the peduncles of young grapefruit with rampant psorosis induced gum-impregnated spots surrounded by halos, but inoculation with CRSV-NS caused sunken chlorotic spots. Previous inoculation of 'Key' lime seedlings (*C. aurantifolia*) with psorosis virus protected them against the severe symptoms normally induced by CRSV-NS. Graft inoculation of seedlings with bark from some nucellar grapefruit trees with rampant psorosis bark lesions induced symptoms similar to those of CRSV-NS. CRSV-NS is probably a strain of the citrus psorosis virus complex.

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Citrus psorosis virus causes bark scaling and decline of citrus trees, and flecking symptoms on the young leaves. Several diseases have been described which are related to, or have symptoms in common with, psorosis. Crinkly leaf, infectious variegation, concave gum, and blind pocket were originally included in the psorosis group (5, 15, 16), but present evidence indicates that these diseases may be unrelated to psorosis (6, 13). Impietratura, an important disease in the Mediterranean area, produces young leaf symptoms similar to those of psorosis (1). In Argentina, Pujol described a naturally spread strain of psorosis (10) and eruptive gummosis (12), but the two are probably synonymous (Pujol and Timmer, *unpublished*). Citrus ringspot virus (CRSV) produces some symptoms similar to those of psorosis (17). Plants previously inoculated with nonlesion inoculum of psorosis A were protected against CRSV, but CRSV did not protect against lesion-bark inoculation of psorosis A (17). Broadbent (3) described a disease, Monak psorosis B, which is similar to CRSV. Previous infection of seedlings with crinkly leaf, psorosis A, or concave gum provided some protection against this disease (3). Weathers (18) described a mechanically transmissible strain of psorosis which protects against psorosis A lesion bark inoculation.

All of these diseases are graft-transmissible and crinkly leaf, infectious variegation, and the strain described by Weathers are mechanically transmissible (7, 18, 19). Psorosis is transmitted through the seed in Troyer and Carrizo citranges [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] (2, 11). With the exception of eruptive gummosis in Argentina (10, 12), none of these diseases is spread extensively by natural means.

Crinkly leaf and infectious variegation are known to be caused by viruses (19), but the viral etiologies of psorosis A, CRSV, etc. are presumed and the term "virus" is retained here only for custom and convenience.

This study describes a virus-like disease found affecting grapefruit (*C. paradisi* Macf.) trees in South Texas. The precise identity of the causal agent is not known; but, based on information presented here, it is called "citrus ringspot virus-necrotic strain" (CRSV-NS). This study describes the symptoms observed in the field and on inoculated indicator plants, as well as the relationship of CRSV-NS to citrus psorosis virus.

MATERIALS AND METHODS.—Seedlings for inoculation tests were grown in the greenhouse in sand culture and watered as necessary with complete nutrient solution (14). Greenhouse temp ranged from lows of 20-24 C and highs of 36-40 C in the summer to 10-14 C and 23-27 C in the winter.

Indexing for CRSV-NS was done on sweet orange (*C. sinensis* 'Madam Vinous') or lime [*C. aurantifolia* (Christm.) Swing. 'Key'] seedlings by grafting two bark patches to each of three to six seedlings for each inoculum source. Grafts were wrapped with plastic tape for 2 wk following inoculation. Seedlings were cut back to 20-30 cm after the grafts had taken and periodically thereafter to force new growth. All new growth flushes were examined for symptoms for 6-12 months following inoculations.

Indexing for psorosis was done on Madam Vinous or 'Bessie' sweet orange for exocortis on 'Etrog' citron (*C. medica* L. var. *ethrog* Engl.) 'Arizona 861' using methods described by Childs (4).

Fruit cluster inoculations were made by grafting two bark patches 10-15 cm above the fruit when fruit were 2-5 cm in diam. A single 8-yr-old nucellar 'Webb Redblush' grapefruit tree was used for each source of virus and 10 clusters on each tree were inoculated. Sources used were: (i) nonlesion bark from a tree with CRSV-NS; (ii) nonlesion bark from a nucellar Webb Redblush tree with rampant psorosis bark lesions, mature leaf symptoms, and twig lesions; (iii) nonlesion bark from an old-line red grapefruit with psorosis A bark lesions; and (iv) a self-grafted control tree.

To determine the relationship of CRSV-NS to psorosis, cross-protection tests were conducted. Twelve Key lime seedlings were inoculated with each of the following three sources: (i) CRSV-NS; (ii) nonlesion bark from a nucellar Webb Redblush with rampant psorosis lesions; and (iii) nonlesion bark from an old-line red grapefruit with psorosis A. In addition, 24 seedlings were grafted with bark from a healthy seedling. Four months later, after most of the inoculated plants had shown symptoms, challenge inoculations were made. Six of the 12 seedlings inoculated with psorosis A were challenged with CRSV-NS. Seedlings previously inoculated with CRSV-NS and rampant psorosis B were challenged with

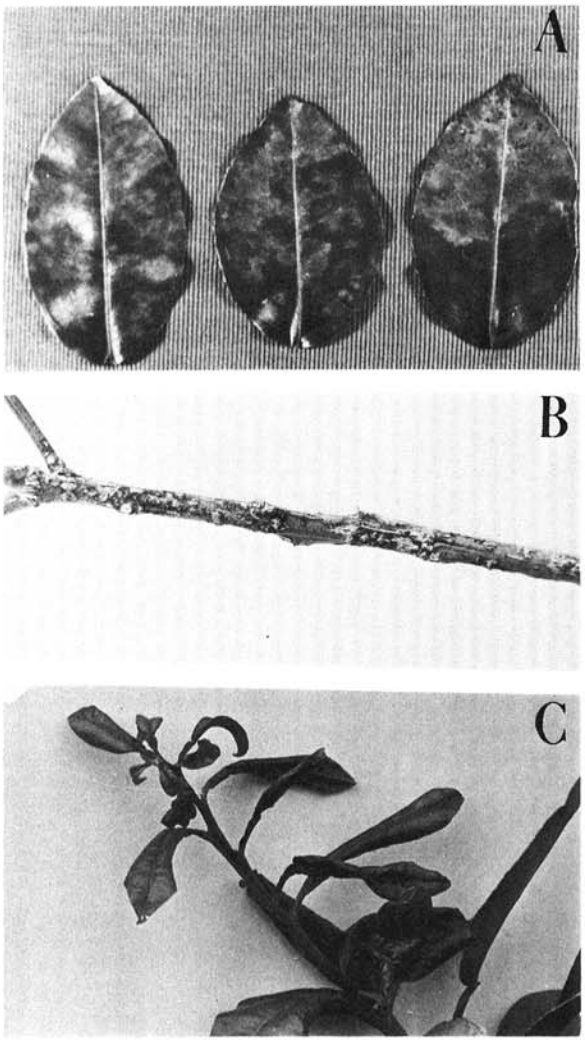

Fig. 1-A, B. Field symptoms of the necrotic strain of citrus ringspot virus (CRSV-NS): **A)** mature leaf symptoms; **B)** twig lesions; and **C)** leaf distortion.

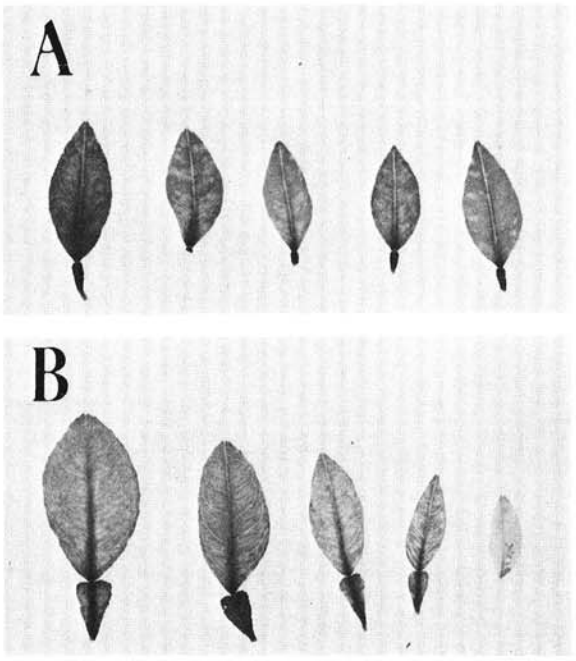

Fig. 2-A, B. Flecking symptoms on the young leaves of seedlings inoculated with the necrotic strain of citrus ringspot virus (CRSV-NS). **A)** 'Key' lime, and **B)** 'Madam Vinous' sweet orange. Note the necrotic flecks in the leaf at the right.

the other two inoculum sources. In addition, six healthy seedlings were inoculated with each virus source.

RESULTS.—To date, the presence of CRSV-NS has been established with certainty in seven grapefruit trees, which are 2.5 - 5.0 yr old. The budwood parents of these seven trees are five horticulturally seedless selections made from the seedy, red-fleshed cultivar 'Hudson' after irradiation of seed with thermal neutrons (8).

Field symptoms.—Chlorotic flecking in the young leaves was the first observable symptom on new growth. These flecks often become necrotic as the leaf ages, and the leaf may fall before it matures. Necrosis of the entire shoot is common. Tissue death proceeds unilaterally causing the shoot to curl in a characteristic manner. On some flushes of growth on certain trees, more than half of the shoots have died. Extensive shoot necrosis results in sparse foliage; infected trees are not as bushy as healthy ones of the same variety.

Chlorotic spots, blotches, and ringspots develop on many leaves, and persist after the leaves mature. On a given flush of growth, symptoms may occur on all, any part, or (occasionally) on none of the leaves. Detectable symptoms are usually present on some mature leaves of infected trees at almost any time.

Infected trees may have symptoms on mature leaves (Fig. 1-A) which resemble those of rampant psorosis. One of the infected trees, which was 4 yr old when the infection was first noted has twig lesions (Fig. 1-B) of the type usually associated with rampant psorosis in much older trees. Gum pockets similar to those produced by impietratura were found in the albedo of two fruit from this tree. Leaf distortion as illustrated in Fig. 1-C has been observed on several infected trees.

Symptoms on inoculated seedlings.—Extensive shoot necrosis usually follows inoculation of seedlings in the greenhouse. Symptoms, which have been observed as early as 10 days after inoculation, are usually produced in 4-6 wk; in one case, however, they did not appear until 10 mo after inoculation. Shoot necrosis is most common on recently inoculated plants, but continues to occur on each flush of growth long after inoculation. Flecking symptoms, as illustrated in Fig. 2, are indistinguishable from the young leaf symptom of psorosis. Oak-leaf patterns were observed only on rare occasions. Chlorotic spots, blotches, and ringspot symptoms often develop as the leaves mature. Some of the symptoms observed in the mature leaves of several cultivars are shown in Fig. 3. Leaf distortion similar to, but usually milder than that shown in Fig. 1-C, occurred on many inoculated plants.

The following species and cultivars of citrus have been inoculated with CRSV-NS: Eureka lemon [*C. limon* (L.) Burm. f.], Key lime, Duncan grapefruit, Owari satsuma (*C. reticulata* Blanco), Olinda Valencia, Madam Vinous, and Bessie sweet oranges, sour orange (*C. aurantium* L.), Orlando tangelo (*C. reticulata* × *C. paradisi*), *C. excelsa* West., Etrog citron, Troyer citrange, and the trifoliate orange (*Poncirus trifoliata*). Nearly all of these species and cultivars showed the characteristic shoot necrosis, flecking, chlorotic blotches and spots, in addition to the mild leaf distortion described above. To date, trifoliate orange is the only species on which symptoms have not been observed. Shoot necrosis has not been observed on Orlando tangelo or Etrog citron, and the symptoms were generally milder on these varieties than on the others. Symptoms were severe on all other varieties and species tested.

Symptoms on fruit of inoculated trees.—Since gum pockets were found in the fruit on a CRSV-NS-infected tree, fruit cluster inoculations were made to observe any symptoms produced, and to compare them with symptoms produced by the psorosis virus. Symptoms first appeared on fruit inoculated with rampant psorosis and CRSV-NS 5 wk after inoculation. CRSV-NS most commonly produced the sunken, chlorotic spots shown in Fig. 4-A. The rampant psorosis inoculum usually induced gum pockets surrounded by a green halo, which in turn was surrounded by a yellow halo (Fig. 4-B). With both types of inoculum, severely affected fruit often dropped prematurely. Fruit from seven of the 10 clusters inoculated with CRSV-NS, and six of the 10 clusters inoculated with rampant psorosis, showed symptoms. None of the fruit inoculated with psorosis A, or any of the self-grafted controls, showed symptoms.

Relationship of CRSV-NS to psorosis.—Cross-protection tests indicated a possible relationship between CRSV-NS and psorosis. CRSV-NS on previously uninoculated Key lime seedlings caused severe shoot necrosis along with the other symptoms described (Fig. 5-A). Psorosis A, or rampant psorosis, on previously uninoculated plants caused occasional shoot necrosis, mild flecking, and chlorotic spots on the leaves of Key lime. These plants recovered from any shock reaction, and thereafter showed only occasional flecking symptoms. Key lime seedlings previously inoculated with psorosis A, or rampant psorosis, were protected against the severe shock reaction and the other symptoms usually produced by CRSV-NS, but were stunted compared to healthy controls (Fig. 5-B, C). Since CRSV-NS affected Key lime seedlings so severely, it was impossible to determine whether CRSV-NS prevented infection by psorosis.

In addition to the seven previously described trees which are infected with CRSV-NS, 13 nucellar Webb Redblush grapefruit and one nucellar mandarin (*C. reticulata* 'Dancy') with psorosis bark lesions have been encountered. These trees range from 10-15 yr old, and most have rampant-type lesions.

There may be some relationship between CRSV-NS and the rampant psorosis bark lesions on nucellar trees. Eight of these nucellar trees with bark lesions have been indexed on sweet orange seedlings in the greenhouse with varying reactions. With three sources, inoculated seedlings showed shoot necrosis, chlorotic spots and blotches, and leaf distortion. These symptoms were identical to those produced by CRSV-NS. With two sources, only the mild flecking usually associated with psorosis A was observed. With inoculum from two of the grapefruit trees and the Dancy mandarin, no symptoms have been observed to date. When these inoculated seedlings did not show symptoms after 8 mo, they were reinoculated from the same source and, in addition, three Key lime seedlings were inoculated from the same sources. To date, none of the seedlings inoculated from

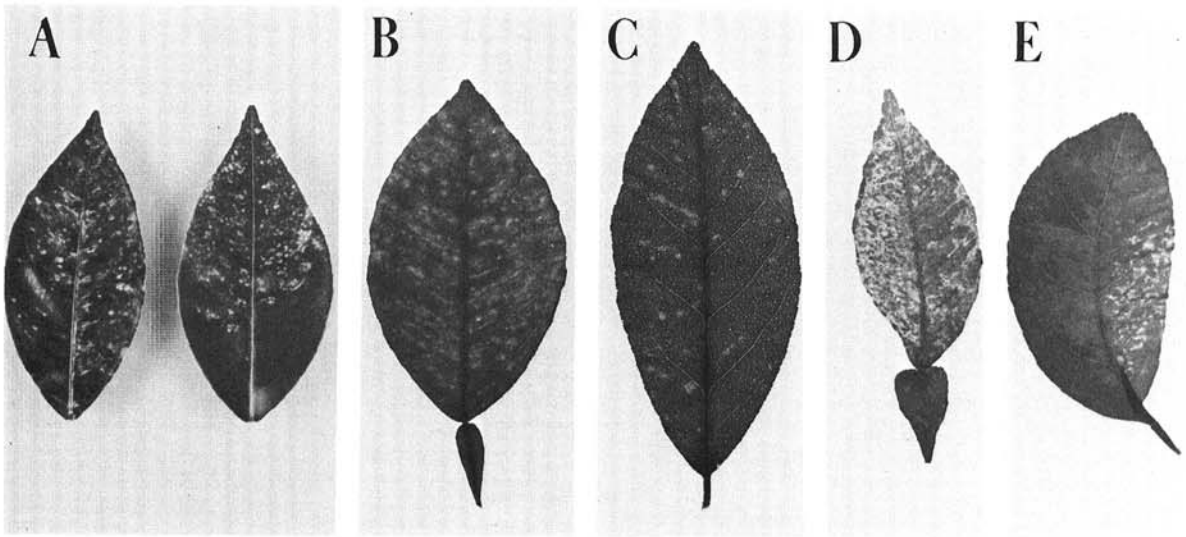

Fig. 3-(A to E). Symptoms on mature leaves of seedlings inoculated with the necrotic strain of citrus ringspot virus (CRSV-NS): **A)** and **B)** chlorotic flecks, spots, and ringspots on 'Madam Vinous' sweet orange; **C)** chlorotic spots on 'Eureka' lemon; **D)** etching and line patterns on sour orange; and **E)** chlorotic blotch and necrotic fleck on *Citrus excelsa*.

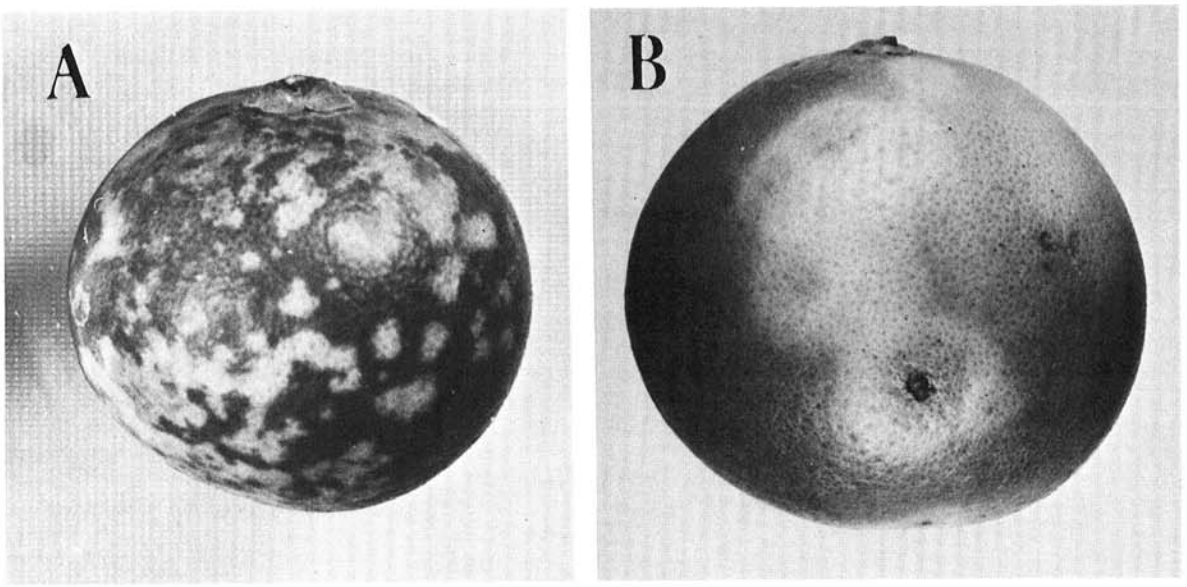

Fig. 4-A, B. Symptoms of infection by the necrotic strain of citrus ringspot virus (CRSV-NS) on inoculated fruit. **A)** Sunken chlorotic spots and blotches in grapefruit inoculated with CRSV-NS. **B)** Gum deposits surrounded by green and then by yellow halos on grapefruit inoculated with rampant citrus psorosis virus.

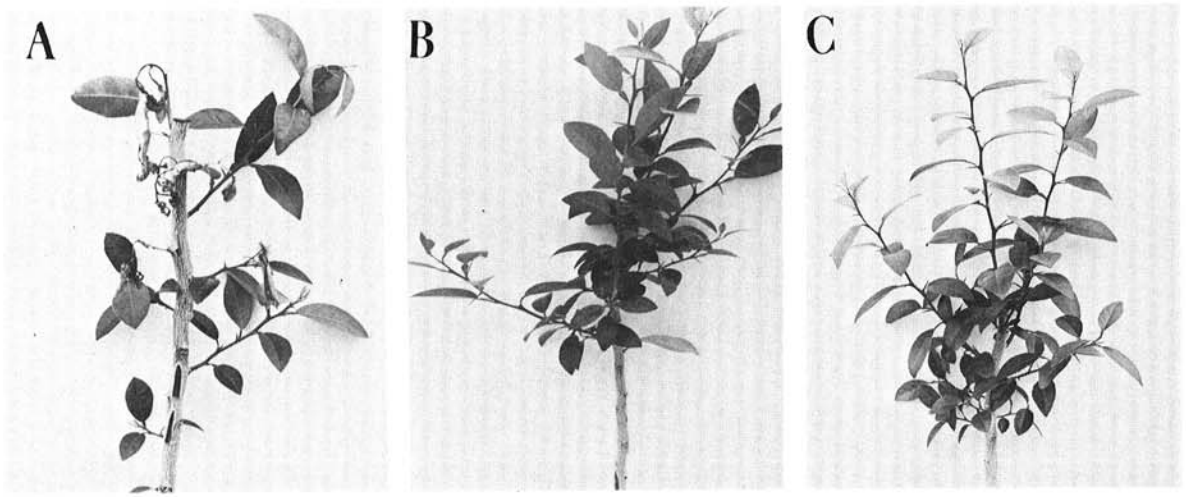

Fig. 5-(A to C). Protection of 'Key' lime seedlings against the necrotic strain of citrus ringspot virus (CRSV-NS) by prior inoculation with citrus psorosis virus. **A)** Inoculated with CRSV-NS only. **B)** Inoculated with CRSV-NS after prior inoculation with psorosis virus. **C)** Healthy control.

these three sources has shown symptoms in the greenhouse.

The nucellar grapefruit trees with psorosis bark lesions have only occasionally shown flecking symptoms in the field. After hedging, some of the trees showed flecking on the new growth. The chlorotic flecks in the young leaves later became necrotic, and the entire shoots eventually curled and died. Occasionally mature leaf and twig symptoms have occurred on these trees, but no symptoms have been observed on the fruit.

All of the field trees infected with CRSV-NS, or with rampant psorosis, are of nucellar origin and (presumably) were virus-free. The seed parent (the seedy, old-line Hudson), and the budwood parents (seedless selections of Hudson) of the seven trees infected with CRSV-NS have been indexed on Madam Vinous sweet orange (4) and apparently are free of psorosis and CRSV-NS. The budwood parents of the nucellar Webb Redblush and Dancy infected with psorosis B could not be traced in all cases and the disease status of the parents remains uncertain. It is quite likely that the seed parents of these trees carried psorosis virus.

Since these infected trees are of nucellar origin and since the parent trees, where known, are free of CRSV-NS and psorosis, infection must have occurred by means other than use of infected budwood. It is difficult to eliminate the possibility of inadvertent graft-inoculation of these trees with an old-line budwood source, or the possibility of a root graft with an infected tree in the nursery or in the field. If such had occurred, the tree would probably be infected not only with psorosis or CRSV-NS, but also with exocortis virus which is carried by most local old-line budwood sources (9). To determine whether or not these trees were also infected with exocortis, three of the trees carrying CRSV-NS and seven of the nucellar trees with psorosis bark lesions were indexed for exocortis (4); all were negative.

DISCUSSION.—The disease described here is referred to as CRSV because of its similarity to the CRSV described by Wallace and Drake (17). The designation "necrotic strain" (NS) was added because certain differences were detected between this disease and the one previously described. CRSV-NS is similar to CRSV in that both produce flecking symptoms on young leaves and chlorotic spots, blotches, and ringspot on older leaves. Both viruses severely affect a wide range of citrus varieties and hybrids.

Several differences between CRSV and CRSV-NS were apparent. Twig lesions and mature leaf symptoms which occur primarily on trees with advanced bark lesions have been associated with CRSV-NS. Leaf distortion resembling that caused by crinkly leaf also occurs on trees infected with CRSV-NS. Based upon differences in other symptoms, and in host range, CRSV-NS is not believed to be related to crinkly leaf or infectious variegation. The reaction of inoculated seedlings to the CRSV described by Wallace and Drake (17) is mild at first, and becomes more severe after serial transfer. CRSV-NS produces a severe shock reaction and shoot necrosis which continues long after inoculation. Fruit symptoms of CRSV have not been reported, but CRSV-NS causes fruit symptoms in both naturally infected trees, and in inoculated fruit clusters.

CRSV-NS resembles Monak psorosis B (3). CRSV-NS and Monak psorosis B induce similar symptoms in the leaves and in the fruit. However, Monak psorosis B caused no symptoms on Troyer citrange, Key lime, or sour orange seedlings (3) while CRSV-NS causes a severe reaction on those cultivars. CRSV-NS resembles the naturally diffused psorosis and eruptive gummosis described by Pujol (10, 12). The chief difference between these diseases is that eruptive gummosis induces profuse gumming and gum pocket formation, whereas CRSV-NS seldom causes gummosis.

The relationship of CRSV-NS to the similar diseases mentioned here remains uncertain. CRSV-NS symptoms are produced in indicator plants inoculated with bark from some nucellar trees with psorosis bark lesions. This may indicate that CRSV-NS is a component or strain of the psorosis virus complex. Broadbent (3) suggested that the psorosis complex may consist of three components, each of which causes a distinct symptom: (i) young leaf symptoms; (ii) bark lesions; and (iii) mature leaf and fruit symptoms. Psorosis B bark lesions are presumably caused by a high concn of component 2 (3). If this were the case, then trees infected by CRSV-NS would have factors 1 and 3 while the nucellar trees with bark lesions would carry component 2 with or without the presence of 1 and 3.

The manner in which these trees become infected with CRSV-NS remains uncertain. CRSV-NS has been found only in trees of nucellar origin. As far as is known, the seed and bud parents of all infected trees were free of CRSV-NS and of psorosis. If, as Broadbent (3) suggests, the psorosis complex consists of several strains, then these strains may be separating naturally in South Texas.

The economic importance of the disease is unknown. At present, only a few trees are known to be infected. If CRSV-NS became epidemic and caused bark lesions and decline of trees, it would be a potentially serious disease. The presence of a vector, or the use of infected trees as budwood sources, would increase the damage potential of the disease.

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