Local Lesion Isolate of Citrus Ringspot Virus Induces Psorosis Bark Scaling[1]

S. M. Garnsey, and L. W. Timmer

ABSTRACT. A citrus ringspot virus isolate (CRSV-6) was isolated from a sweet orange tree with severe psorosis bark symptoms in Lake County, Florida. Sweet orange seedlings graft-inoculated with CRSV-6 developed necrotic shock symptoms in young shoots, flecking and chlorotic blotches in young leaves, mature leaf symptoms, and bark lesions. The CRSV-6 isolate was mechanically transmitted to *Chenopodium quinoa* and single-lesioned twice in this host. Several single-lesion isolates were transmitted to *Gomphrena globosa* and produced typical chlorotic blotches and spots. Isolates were transmitted mechanically from *G. globosa* to Etrog citron, and subsequently by graft transmission from citron to navel sweet orange trees on sour orange rootstock. The graft-inoculated sweet orange trees quickly developed leaf and twig symptoms similar to those induced by the original field source. Trunk lesions developed 9 to 12 months after inoculation. This is the first demonstration that an infectious agent mechanically transmissible through *C. quinoa* and *G. globosa*, and back to citrus, can induce psorosislike bark lesion symptoms.

Index words. mechanical transmission, psorosis B, herbaceous hosts.

The psorosis complex of citrus consists of several diseases which produce chlorotic patterns in young citrus leaves and varying bark and/or wood symptoms (7, 11). The causal agents have never been well characterized, but are assumed to be virus or viruslike. Crinkly leaf and infectious variegation were once included in the psorosis complex based on leaf symptoms, but have now been associated with a distinct, well-characterized virus (7).

Bark-scaling symptoms have commonly been associated with psorosis A and psorosis B (3, 12). Bark symptoms of psorosis B are similar to those of psorosis A, but are more rampant and are frequently associated with fruit and mature leaf symptoms (3). Bark-scaling symptoms are frequently not expressed on young propagations of psorosis-infected budwood or in psorosis-inoculated indicators, even though these show distinct leaf symptoms. Inoculum taken from lesion areas on branches may produce more severe reactions in indicator plants than inoculum from nonlesion areas. Wallace suggested that psorosis B was not a distinct disease, but a reaction of healthy plants to lesion inoculum of psorosis A (12).

A mechanically transmitted viruslike agent identified as citrus ringspot virus has been recovered from trees with severe bark scaling in several citrus-growing areas (4, 5, 8). An association of citrus ringspot to psorosis B was suggested based on fruit and mature leaf symptoms (9), but this has been questioned (11). Despite a close association between the presence of mechanically transmissible citrus ringspot virus isolates in field trees and bark scaling, no causal relationship has been demonstrated. The Texas citrus necrotic ringspot isolate is the best characterized isolate of ringspot, and when transmitted to herbaceous hosts and back to citrus reproduced all the original foliar and fruit symptoms (10). Bark lesions, however, were not readily observed in young citrus trees infected with either the original or mechanically transmitted subisolates of the Texas source (coded CRSV-4 in Florida).

In this study, we report the first direct association of a mechanically transmissible agent to lesion bark symptoms of psorosis.

[1]Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

METHODS AND MATERIALS

Original isolate. The origin of the citrus ringspot isolate used in this study was an old navel orange tree propagated on sour orange rootstock near Leesburg, Florida. This tree (coded LC-15) showed extensive bark scaling on the trunk and scaffold limbs. The canopy was thin and distressed on limbs with extensive bark lesions, and leaf flecking and oak leaf patterns were observed in young leaves. Glasshouse-grown sweet orange plants propagated on sour orange rootstocks were inoculated with bark lesion inoculum from LC-15. The inoculated plants showed severe necrotic shock symptoms, various leaf patterns including ringspots, and bark lesions so extensive that twig or shoot dieback frequently occurred. No bark lesions were observed in the sour orange portion of the trunk. Leaf piece and lesion bark subinoculations reproduced the same syndrome. This isolate was subsequently designated as Florida citrus ringspot virus isolate no. 6 (CRSV-6). Subisolates from this source which have been mechanically transmitted to citrus and herbaceous hosts have been identified with a subletter as indicated.

Glasshouse conditions. Plants were maintained in a partially shaded glasshouse equipped with evaporative coolers as previously described (5). Mechanical transmission experiments were normally conducted during periods when temperatures could be maintained from 21 to 26 C. Some inoculated plants were incubated in an air-conditioned, clear plastic cell within the glasshouse which was maintained near 24 C. *Chenopodium quinoa* Willd. plants were grown under supplemental lighting (16-hr photoperiod) to inhibit flowering and promote succulent, vigorous growth. Light quality and intensity factors were not recorded, but bush bean (*Phaseolus vulgaris* L.) and cowpea plants, (*Vinca unguiculata* (L.) Walp.), were slightly etiolated when grown under these same conditions.

Transmission. Unless noted otherwise, standard procedures as previously described (5) were used to mechanically transmit the virus isolates used. Mechanical transmissions to citrus receptor plants were made only to soft, partially expanded young flush leaves. Inoculations to *C. quinoa* and *Gomphrena globosa* L. plants were made to leaves of varying ages on each plant.

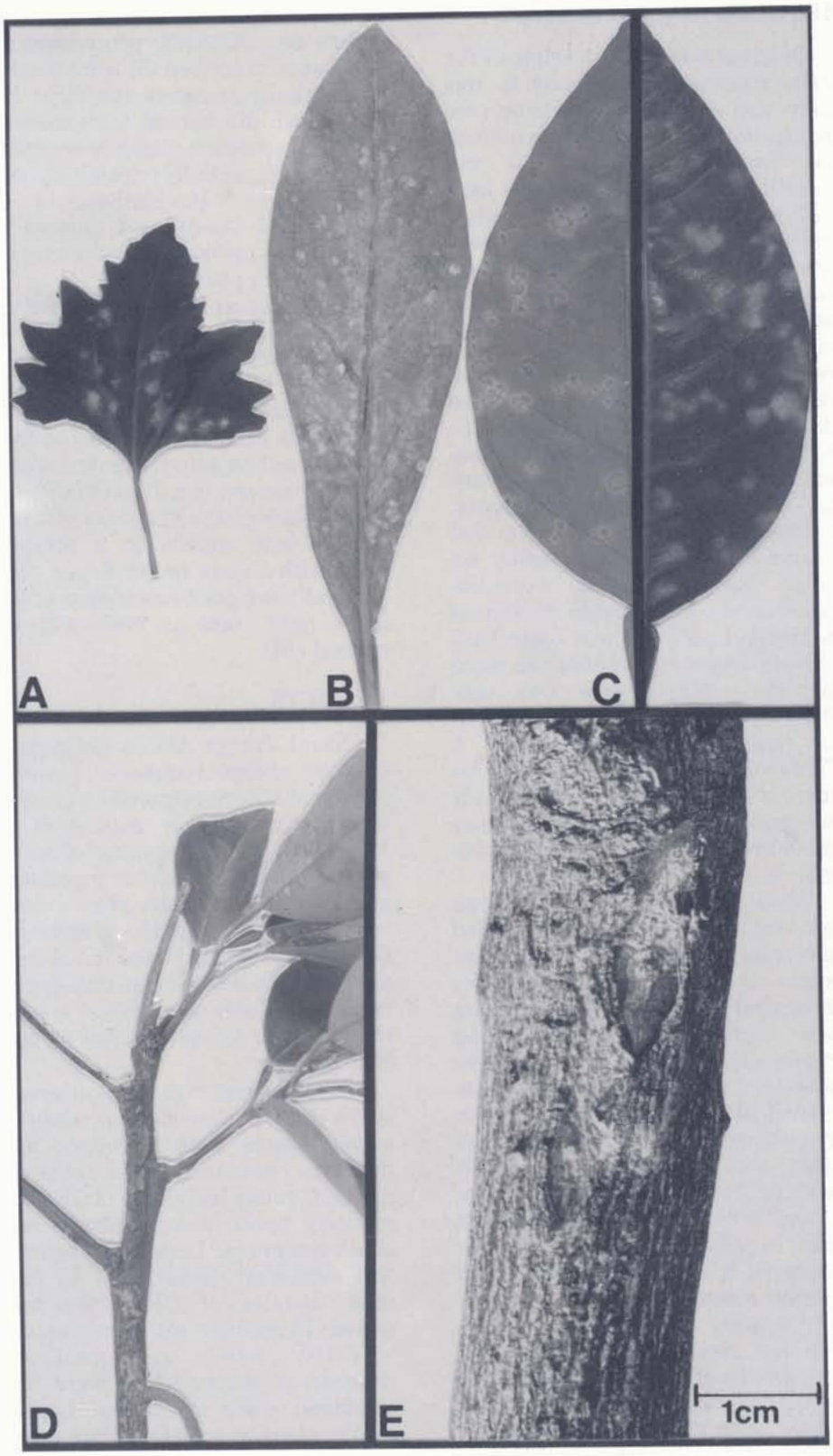
Single-lesion cultures of CRSV-6 were selected from leaves of *C. quinoa* with well-defined and well-separated local lesions 10 to 14 days postinoculation. Individual lesions (2 to 3 mm in diam) were dissected from the leaf and rapidly triturated with a sterile glass rod in a drop of buffer on a cold glass plate. The drop of lesion extract was applied to a receptor plant with a glass rod or finger tip.

Leaf piece graft-inoculation procedures used were as previously described (10).

RESULTS

Navel orange plants propagated on sour orange rootstock showed a rapid and severe response to graft inoculation with lesion tissue from the LC-15 tree. Leaf symptoms often appeared 12 to 16 days after inoculation and twig lesions were often evident within 3 months in the glasshouse. Often, most or all of the navel scion died and only sour orange rootsprouts remained. These sour orange sprouts showed foliar symptoms, but no bark lesions.

Local lesions (fig. 1a) appeared 7 to 10 days postinoculation when *C. quinoa* plants were inoculated with inoculum prepared from chlorotic mottled, young leaf tissue of CRSV-6-infected navel orange plants with shock symptoms. Lesion development was somewhat slower than for most other isolates of CRSV we have tested. In common with other isolates of CRSV, lesions were most pronounced on leaves which were fully expanded when inoculated. Lesions on older leaves were also more necrotic than those on younger leaves.


A
B
C
D
E
1cm

Single lesions from different leaves were reinoculated to healthy *C. quinoa* plants. The area of the *C. quinoa* leaf inoculated with one drop of local lesion inoculum generally developed numerous lesions which coalesced. The tissue in the coalesced lesion area was harvested and used to inoculate *G. globosa* plants or additional *C. quinoa* plants.

The local lesion isolation procedure was repeated from *C. quinoa* lesion inoculum. First and second generation local lesion isolates increased in *G. lobosa* (fig. 1b) were then mechanically inoculated to young Etrog citron and Mexican lime receptors. One first generation (coded CRSV-6a) and three second generation local lesion cultures (coded CRSV-6b, -6c and -6d) from different lesions were successfully transmitted. The infected Etrog citron and Mexican limes showed ringspot, necrotic etch, leaf mottle and shock reactions typical of ringspot infection.

Tissues from Etrog citron leaves systemically infected with the different local lesion isolates were used to graft-inoculate two healthy navel orange plants from each source. There were two citron inoculum source plants for CRSV-6b, c and d. The sequence of transfers is summarized in table 1.

All 14 graft-inoculated navel orange plants showed shock and foliar symptoms similar to those produced by the original source in the glasshouse and many developed twig lesions within a few months. After approximately 6 months in the glasshouse, the plants were transplanted to large containers and placed outside. Bark-scaling lesions began appearing on the trunks of some plants 9 to 10 months after inoculation. After 21 months, at least one plant inoculated with each of the three second generation lesion sources of LC-15 (CRSV 6b-d) showed some or all of the following: bark scaling on the trunk or a major limb (fig. 1e), lesions on smaller mature green twigs (fig. 1d); twig necrosis; and mature leaf symptoms (fig. 1c). Symptoms varied in intensity from plant to plant, but the variation between replications of the same treatment was apparently as great as that between treatments. Twelve of 14 plants inoculated showed bark lesions on the main trunk. Fruit symptoms were observed on two plants.

No similar symptoms were observed on healthy control plants used for each stage of the transfer sequence described (table 1).

DISCUSSION

The results reported indicate that an infectious agent causing bark lesions in sweet orange is mechanically transmissible and is associated with a syndrome of other symptoms in herbaceous and citrus hosts associated with citrus ringspot virus infection.

The transfer procedures and sequence used suggest that the bark lesions observed were caused by the citrus ringspot virus present in the original tree. However, if the bark-scaling component infected *C. quinoa* and *G. globosa* latently, then cotransfer of two separate components could have occurred. Absolute coidentity of the bark-scaling agent to ringspot will require purification and further characterization studies of CRSV-6.

Recent studies with the Texas isolate of CRSV (CRSV-4) indicate that CRSV-4 is multicomponent and may have rather unique properties (2).

Fig. 1. A) Local lesions on leaf of *Chenopodium quinoa* 10 days after inoculation with citrus ringspot isolate number 6 (CRSV-6); B) Symptoms of CRSV-6 infection in inoculated leaf of *Gomphrena globosa* 14 days after inoculation (symptomatic *Gomphrena* tissue was used to prepare inoculum to reinfect citrus); C) Mature leaf symptoms of citrus ringspot in navel orange leaf infected with CRSV-6, lower surface on left, upper surface on right; D) Bark lesions on young twig of navel orange tree infected with single lesion isolate of CRSV-6 (note dead shoots indicating necrotic shock response), and E) Bark scaling on main trunk of navel orange tree infected with a single lesion isolate of CRSV-6.

TABLE 1
TRANSMISSION SEQUENCE OF THE BARK-SCALING COMPONENT OF CITRUS RINGSPOT VIRUS INTO A LOCAL LESION HOST AND BACK TO CITRUS

Step	Donor source	Receptor plant	Transmission procedure
1	Sweet orange	*C. quinoa*	Mechanical—bulk[z]
2	*C. quinoa*	*C. quinoa*	Mechanical—single lesion
3	*C. quinoa*	*C. quinoa*	Mechanical—single lesion
4	*C. quinoa*	*G. globosa*	Mechanical—bulk
5	*G. globosa*	Etrog citron	Mechanical—bulk
6	Etrog citron	Sweet orange	Graft—leaf piece

[z]Leaf inoculation with inoculum prepared from whole leaf source.

The CRSV-6 isolate has not been extensively studied, but there is evidence that it has physical properties similar to CRSV-4 (1). The symptoms produced by the two isolates were similar except for the bark lesions produced by CRSV-6.

The successful demonstration of mechanical transmission of a bark-scaling agent was probably facilitated by selection of a parent isolate which produced extensive bark lesions rapidly on small plants.

Although bark scaling is commonly considered a specific symptom of psorosis infection, it is more likely that bark scaling is not psorosis-specific, but can be induced by various agents, including psorosis and some isolates of citrus ringspot. Bark scaling typical of psorosis has been observed in several areas, including Brazil (6) and Florida (C. O. Youtsey, personal communication), where association with an infectious, graft-transmissible agent could not be demonstrated.

The CRSV-6 isolate should prove useful for further studies on how bark-scaling reactions can be induced in citrus by specific agents.

LITERATURE CITED

1. DaGraca, J. V., and R. F. Lee
1987. Formation of hybrid citrus ringspot virus isolates by component mixing. Phytopathology 77: 640 (abstr.).
2. Derrick, K. S., R. H. Brlansky, R. F. Lee, L. W. Timmer, and T. K. Nguyen
1986. Two components associated with citrus ringspot virus. Phytopathology 76: 1072 (abstr.).
3. Fawcett, H. S. and A. A. Bitancourt
1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33: 837-864.
4. Fischer, H. C., C. Casafus, L. Marmelicz, and L. Arroyo
1982. Presencia en la Argentina de una enfermedad de citrus relacionada con el "citrus ringspot virus." II Congreso Latinamericano de Fitopatologia. Buenos Aires, Argentina.
5. Garnsey, S. M., and L. W. Timmer
1980. Mechanical transmissibility of citrus ringspot virus isolates from Florida, Texas, and California, p. 174-179. *In* Proc. 8th Conf. IOCV. IOCV, Riverside.
6. Passos, O. S., Y. S. Coelho and A. P. C. Sobrinho.
1974. More information on psorosis disease in Bahia, Brazil, p. 135-136. *In* Proc. 6th Conf. IOCV. Univ. Calif., Div. Agr. Sci., Richmond.
7. Timmer, L. W., and H. N. Beñatena
1977. Comparison of psorosis and other viruses causing leaf flecking in citrus. Proc. Int. Soc. Citriculture 3: 930-935.
8. Timmer, L. W., and S. M. Garnsey
1980. Natural spread of citrus ringspot virus in Texas and its association with psorosis-like diseases in Florida and Texas, p. 167-193. *In* Proc. 8th Conf. IOCV. IOCV, Riverside.
9. Timmer, L. W. and S. M. Garnsey
1980. Ringspot. *In:* J. M. Bove and R. Vogel (eds.) Descriptions and Illustrations of Virus and Viruslike Diseases of Citrus, 2nd ed., SETCO-IRFA, Paris.

10. Timmer, L. W., S. M. Garnsey, and J. J. McRitchie
1978. Comparative symptomatology of Florida and Texas isolates of citrus ringspot virus on citrus and herbaceous hosts. Plant Dis. Rep. 62: 1054-1058.
11. Vogel, R. and J. M. Bove
1980. Citrus ringspot in Corsica, p. 180-182. *In* Proc. 8th Conf. IOCV. IOCV, Riverside.
12. Wallace, J. M.
1978. Virus and viruslike diseases, p. 67-184. *In* W. Reuther, *et al.* (eds.). The Citrus Industry, Vol. IV. Div. Agr. Sci., Univ. Calif., Berkeley.