Natural Spread of Citrus Ringspot Virus in Texas and its Association with Psorosis-like Diseases in Florida and Texas*

L.W. Timmer and S.M. Garnsey

Citrus ringspot virus (CRSV), as originally described by Wallace and Drake (1968), was recovered from a tree with a psorosis-like bark lesion and CRSV has been associated with psorosis-like bark lesions on grapefruit trees in Texas (Timmer, 1974). Previously, it was impossible to distinguish the effects of psorosis A, psorosis B, CRSV, concave gum, and similar viruses because all were believed to be only graft-transmissible and were often present in trees as mixed infections. Recently, we have demonstrated that CRSV is mechanically transmissible from citrus to citrus and to a wide range of herbaceous hosts and that isolates from Florida, Texas, and California characteristically produce local lesions on *Chenopodium quinoa* Willd. (Garnsey, 1975; Garnsey *et al.*, 1976; Garnsey and Timmer, 1980; Timmer *et al.*, 1978).

Natural spread of CRSV in Texas was indicated in previous work (Timmer, 1974) and a ringspot-like virus which causes bark lesions spreads rapidly in Argentina (Pujol and Beñatena, 1965; Pujol, 1966; Timmer and Beñatena, 1977). Citrus ring pattern virus is naturally spread in Iran (Dehyar and Habashi, 1974). Psorosis and certain other viruses are carried in pollen (Vogel and Bové, 1976), but the means of natural spread of psorosis, CRSV, or possibly related viruses has not been demonstrated.

The purpose of this study was: 1) to survey field trees in Texas and Florida for CRSV to correlate its presence with field symptoms, particularly to psorosis-like bark lesions; and 2) to investigate natural spread of CRSV in Texas. A preliminary report of this study has been published (Timmer and Garnsey, 1978).

MATERIALS AND METHODS

To assay for CRSV by mechanical inoculation, young symptomatic citrus leaves, or other tissues as indicated, were ground with a mortar and pestle at a ratio of 1 g of tissue/10 ml of cold 0.05 M Tris-HCl [tris (Hydroxymethyl) aminomethane] buffer with 0.5% 2-mercaptoethanol, pH 8.0 (TME) and the extract applied with a cotton swab to carborundum-dusted *C. quinoa* leaves. Appearance of chlorotic to necrotic local lesions in 3-7 days with no subsequent development of systematic symptoms indicated the presence of CRSV. The only other citrus virus that produces local lesions on *C. quinoa* is tatter leaf, which can be differentiated from CRSV by distinctive symptoms on citrus and a systemic mottle in *C. quinoa* (Semancik and Weathers, 1965).

Grapefruit orchards at the Texas A&I University Citrus Center, Weslaco, were observed twice a year for the presence of psorosis-like bark lesions and foliar symptoms of CRSV. Container-grown Star Ruby grapefruit which had apparently become naturally infected also were inspected periodically and indexed for CRSV.

Seed was collected from infected field trees planted in the greenhouse to test for seed transmission of CRSV and psorosis. Seeds from symptomatic fruit were tested whenever possible. Seedlings were transplanted to pots when 15-20 cm tall and observed for symptoms for about 1 year. Plants were pruned periodically to force new growth. Plants with suspected symptoms were assayed by graft transmission to Madam Vinous sweet orange or by mechanical transmission to *C. quinoa* to confirm infection.

*Florida Agricultural Experiment Station Journal Series No. 1760.

Two CRSV-infected nucellar Hudson grapefruit trees in Texas were used to determine the distribution of CRSV in host tissues. Each tissue source was assayed on at least eight fully expanded leaves of a single *C. quinoa* plant. Flower and leaf tissues were assayed in April 1978 at full bloom. Fruit and bark tissue from a single source were assayed in June 1978 when fruit was about 5 cm in diameter. Petals and anthers from a Washington navel orange tree in Florida were also assayed on *C. quinoa.*

Soil was collected from beneath two 7-year-old Hudson grapefruit trees and one 16-year-old Redblush grapefruit tree infected with CRSV for soil transmission tests of CRSV. The three samples were divided and one-half of each was autoclaved. One seed flat and three pots were filled with each half-sample. Each flat was seeded with 144 sour orange seed and three sour orange seedlings about 20 cm tall were planted in each pot. Plants were observed for 15 months and pruned periodically to force new growth. At the end of the experiment, soil and roots were examined for plant parasitic nematodes.

Field collected insects were caged on at least three CRSV-infected citrus seedlings with symptomatic young growth to test for insect transmission. After an acquisition feeding of at least 4 days, insects were transferred to healthy Madam Vinous sweet orange and/or Mexican lime seedlings for an inoculation feeding of at least 4 days. Nymphs and early instars were used for most attempts, but a few adults were included in most experiments. Inoculated plants were sprayed with insecticide, transferred to the greenhouse, and observed for symptoms for at least 1 year.

RESULTS

Survey. To survey Texas trees (table 1), bark or leaves from affected field trees were grafted into grapefruit, sweet orange, or Mexican lime seedlings. Symptomatic young leaves from these seedlings were assayed for CRSV on *C. quinoa.* Trees which indexed negative were assayed again from subsequent flushes of growth. All nucellar trees with bark lesions, presumed to be

TABLE 1
ASSAY OF TEXAS CITRUS TREES WITH VARIOUS SYMPTOMS FOR CITRUS RINGSPOT VIRUS BY LOCAL LESIONS PRODUCED ON *CHENOPODIUM QUINOA*

Variety	Approx. age, years	Field symptoms	Greenhouse symptoms on citrus	No. positive/ no. tested on *C. quinoa*
Old-line*				
Redblush grapefruit	10-30	bark lesions†	ringspot	19/19
Duncan grapefruit	6	bark lesions	ringspot	1/1
Redblush grapefruit	25	bark lesions	fleck + spot	0/1
Redblush grapefruit	3	fleck	fleck + oak-leaf	0/1
Redblush grapefruit	1	none	fleck	0/1
Pineapple orange	15	bark lesions	ringspot	2/2
Valencia orange	12	bark lesions	fleck	0/1
Nucellar*				
Redblush grapefruit	15-20	bark lesions	ringspot	15/15
Hudson grapefruit	6-8	bark lesions	ringspot	3/3
Hudson grapefruit	6-8	ringspot	ringspot	3/3
Star Ruby grapefruit	6	bark lesions	ringspot	1/1

* Most of the old-line trees were from commercial orchards in Texas and were probably derived from infected propagative material, whereas all of the nucellar trees were from orchards of the Texas A&I University Citrus Center, Weslaco and probably were naturally infected.

† Most trees with bark lesions showed occasional foliar symptoms of ringspot in the field.

naturally infected, and most old-line trees with bark lesions tested positive for CRSV by this procedure (table 1). The CRSV was not recovered from four trees showing mild flecking symptoms, and two of those did not have bark lesions.

To survey trees in Florida, young leaves were collected from the spring growth flush on field trees and assayed directly on *C. quinoa*. Some trees were indexed on indicator seedlings by graft inoculation and symptomatic tissue from those plants was also assayed on *C. quinoa*. The CRSV was recovered from most of the tested field trees with bark lesions (table 2). Many of these trees were old and severely affected by scaly bark. The CRSV was invariably detected in trees which showed epinasty, leaf drop, and shoot necrosis in new growth and from most trees which showed strong flecking symptoms. It was recovered from spring-flush growth of affected trees in successive years. Indexing of CRSV-infected trees on Duncan grapefruit seedlings in the greenhouse produced typical CRSV symptoms. Trees which showed only mild flecking or oak-leaf patterns in young leaves, concavities on the trunk, and no bark lesions tested negative for CRSV.

Natural spread. Trees surveyed in Texas orchards were propagated from nucellar, virus-free budwood sources (table 3). The nucellar trees in orchards D-4 and C-4 were interplanted between old-line grapefruit trees, some of which were infected with CRSV. The old-line trees in orchard C-4 were removed when the nucellar trees were 10 years old. All other orchards were solid plantings of nucellar trees. The number of trees with bark lesions or ringspot symptoms increased slowly over 8 years, but did not exceed 2 per cent in any orchard (table 3). No obvious pattern of spread was detected. In some orchards, infected trees were concentrated in one area but adjacent infected trees were not found. All trees with bark lesions detected prior to 1975 indexed positive for CRSV when assayed on *C. quinoa*. Occasional trees with bark lesions indexed since 1975 were also positive for CRSV.

Symptoms of CRSV were observed in some recently budded Star Ruby grapefruit trees on trifoliate orange and Troyer citrange rootstocks grown in 6-liter containers in half-shade at the Texas A&I University Citrus Center. During the fall of 1978, all container stock in this 10 x 15-m shaded area was inspected frequently for ringspot symptoms and all suspicious plants

TABLE 2
ASSAY OF FLORIDA CITRUS TREES* WITH VARIOUS SYMPTOMS FOR CITRUS RINGSPOT VIRUS BY LOCAL LESIONS PRODUCED ON *CHENOPODIUM QUINOA*

	No. positive/no. tested on *C. quinoa*			
Field symptoms	Sweet orange	Grape-fruit	Temple + tangerine	Total
Fleck only	4/6	0/1	——	4/7
Oak-leaf only	0/3	——	0/1	0/4
Fleck + oak-leaf	0/2	——	——	0/2
Bark scaling only	1/3	0/1	0/1	1/5
Bark scaling + fleck	8/8	5/6	1/1	14/15
Bark scaling + oak-leaf	1/2	——	0/1	1/3
Bark scaling + fleck + oak-leaf	3/7	0/1	0/3	3/11
Fleck + trunk concavities	——	0/1	——	0/1
Oak-leaf + trunk concavities	0/4	——	——	0/4
Oak-leaf + fleck + trunk concavities	1/2	0/1	——	1/3

* Trees from commercial orchards, mostly 15 years or older. Assays made directly from spring flush growth of field trees.

TABLE 3
NUMBER OF TREES WITH BARK LESIONS OR RINGSPOT SYMPTOMS IN TEXAS GRAPEFRUIT ORCHARDS PROPAGATED FROM NUCELLAR, VIRUS-FREE SOURCES

Orchard	No. of trees	Present age	Cumulative number of trees with bark lesions or ringspot symptoms in:								Per cent infected
			1971	'72	'73	'74	'75	'76	'77	'78	
D-4	886	16	0	1	5	6	7	9	10	11	1.2
C-5	992	18	0	1	1	1	5	6	7	7	0.7
B-5	676	17	2	6	6	7	8	8	8	8	1.2
C-4	276	17	0	1	1	1	1	4	4	4	1.4
2	182	18	1	1	2	2	2	2	2	2	1.1
2N	256	8	0	4*	4	4	4	5†	5	5	2.0
3S	125	7	0	1*	1	1	1	1	1	1	0.8

* Foliar ringspot symptoms only.
† Three of the trees with foliar ringspot symptoms developed bark lesions.

were assayed on *C. quinoa*. All trees in the shaded area had been propagated from budwood sources which indexed virus-free, but several Troyer citrange seedlings which had been graft inoculated with CRSV were present. Six of 20 trees on trifoliate orange rootstock and 7 of 38 trees on Troyer citrange were found to be infected within a 3-month period. Most showed symptoms after they were pruned to initiate branching. All infected plants were confined to 4 of the 22 rows of pots. In one case, there were four infected trees in a row and in another, there were two adjacent infected trees. None of the following trees in the same area showed symptoms of CRSV: 14 recently budded Star Ruby grapefruit on Columbia sweet lime and Swingle citrumelo rootstocks; 20 recently budded Parsons Special mandarin on Columbia sweet lime and Troyer citrange rootstocks; 50 sour orange seedlings; and about 200 2-year-old, old-line Redblush grapefruit on sour orange rootstock.

Tests for seed transmission. Transmission of CRSV through seed was tested by growing seedlings from the following sources (the number of seedlings tested in parentheses): a 16-year-old nucellar Redblush grapefruit with bark lesions (184); two 7-year-old nucellar Hudson grapefruit with bark lesions (108 and 81); a 7-year-old nucellar Hudson grapefruit with foliar ringspot symptoms (98); a nucellar 5-year-old Star Ruby grapefruit with bark lesions (242); and a 25-year-old sour orange seedling with flecking symptoms which had probably been inadvertantly graft-inoculated with psorosis (150). All of the source trees, except for the sour orange, which was not tested, indexed positive for CRSV on *C. quinoa*. None of the seedlings developed symptoms of CRSV.

When tissues from various parts of the tree were tested for CRSV on *C. quinoa*, the average numbers of local lesions per leaf were: petals—23; stamens—40; anthers—0; pistils—4; nearly mature leaf tissue—16; young bark—5; lesion bark from the trunk—0; fruit flavedo—1; albedo—0; columella—0. Extracts of petals from the Florida source produced abundant local lesions on *C. quinoa*, but the anthers produced none.

Tests for soil transmission. None of the citrus seedlings grown beneath CRSV-infected trees developed symptoms. *Tylenchulus semipenetrans* Cobb was the only plant parasitic nematode observed on the roots of these seedlings. The average number of *T. semipenetrans* larvae from all soils which had not been autoclaved was 10,800/100 cm^3 of soil in the flats and 1,500/100 cm^3 of soil in the pots.

Tests for insect transmission. The following insects, which are commonly present on citrus in Texas, were tested as possible vectors of CRSV. The number of experiments conducted and the approximate number of insects used in each experiment are given in parentheses: planthopper — *Metcalfa pruinosa* (Say) (2;50); leafhoppers — *Homalodisca* sp. (4;20), *Oncometopia* sp. (1;15), *Gyponana* sp. (2;20), *Scaphytopius* sp. (2;15); green citrus aphid — *Aphis spiraecola* Patch (6;>100); and citrus mealy bug — *Planococcus citri* (Risso) (1;>100). None of the receptor plants exposed to inoculation by these various insects developed symptoms of CRSV.

DISCUSSION

There is a strong association between bark lesions on citrus trees and the presence of CRSV in those trees. Most old-line trees with bark lesions in Florida and Texas were found to carry the virus. The presence of CRSV in nucellar grapefruit trees with bark lesions in Texas indicates that CRSV is capable of causing bark lesions since these trees appear to be free of other viruses (Timmer, 1974). We cannot conclude, however, that CRSV is synonymous with psorosis A because we have been unable to recover CRSV from a few trees in Texas and Florida which have bark lesions. Graft inoculation of indicator seedlings from these trees generally produces mild spotting and flecking in

contrast to the severe shock symptoms, chlorotic spots, ringspots, and severe fleck which follow inoculation of indicator plants from trees infected with CRSV. Bark lesions on trees which do not yield CRSV may be caused by psorosis A or by CRSV which was not detected because of the nonuniform distribution of the virus (Timmer and Garnsey, 1979). Since trees with trunk concavities and mild fleck and oak-leaf symptoms have never yielded CRSV, there appears to be no association between CRSV and concave gum virus. Psorosis B, as originally described by Fawcett and Bitancourt (1943) may be synonymous with CRSV. Field symptoms of CRSV and psorosis B are identical. Nucellar trees with CRSV have rapidly expanding bark lesions characteristic of psorosis B and extracts from three California isolates of psorosis B obtained from C.N. Roistacher produced local lesions on *C. quinoa* (Garnsey and Timmer, 1980).

Slow natural spread of CRSV occurs in Texas and more rapid spread of a similar virus occurs in Argentina (Timmer and Beñatena, 1977). The means of spread has not been established. Soil transmission is not indicated because the naturally infected orchard trees are widely scattered and because infected, container-grown trees were found. Seed transmission was not detected and the apparent low concentration of CRSV in reproductive tissues reduces the likelihood that spread occurs by this means. It is possible that the spread of CRSV is mechanical. All of the older orchards (table 3) have been mechanically hedged and/or topped at least twice and the virus may have been carried on the machine blades. The infection of container stock could have occurred by means of clippers used in pruning and forming these nursery trees, although clippers were disinfested with 0.5% NaOCl prior to working on these trees. The virus is readily transmitted by the stem slash technique using buffered extracts of symptomatic young leaf tissue, but we have been unable to demonstrate transmission on clippers or other tools (Garnsey and Timmer, 1980). Accidental graft transmission is unlikely. The nucellar field trees infected with CRSV do not carry exocortis viroid, as would be expected if they had been propagated from or formed a root graft with an infected old-line bud source (Timmer, 1974). Differential transmission of CRSV and exocortis by mechanical means between grapefruit trees is probable since exocortis viroid is not readily mechanically transmitted to or from grapefruit (Garnsey and Weathers, 1972). The infected container trees were budded from a source which had been indexed and inspected frequently for CRSV symptoms. However, it is possible that a single branch of the mother tree had become infected and that CRSV was present in one or two of the budsticks used to propagate the container stock. In limited tests, insect transmission was not demonstrated, but the possibility that it occurs cannot be eliminated. The slow rate of natural spread in Texas indicates that the vector or means of spread is probably inefficient.

LITERATURE CITED

DEHYAR, K., and M. HABASHI
1974. Citrus ring pattern virus in the Caspian Sea area of Iran. Iranian J. Plant Path. 10: 17-20.

FAWCETT, H.S., and A.A. BITANCOURT
1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33: 837-64.

GARNSEY, S.M.
1975. Two mechanically transmissible viruses in navel orange selections introduced from Algeria. Plant. Dis. Rep. 59: 689-93.

GARNSEY, S.M., and L.W. TIMMER
1980. Mechanical transmissibility of citrus ringspot virus isolates from Florida, Texas, and California, p. 174-79 this volume.

GARNSEY, S.M., and L.G. WEATHERS
1972. Factors affecting mechanical spread of exocortis virus, p. 105-11. *In* Proc. 5th Conf. IOCV. Univ. Florida Press, Gainesville.

GARNSEY, S.M., C.O. YOUTSEY, G.D. BRIDGES, and H.C. BURNETT
1976. A necrotic ringspot-like virus found in a 'Star Ruby' grapefruit tree imported without authorization into Florida. Proc. Fla. State Hort. Soc. 89; 63-67.

PUJOL, A.R.
1966. Difusión natural de psorosis en plantas citricas. Inst. Nac. Técnol. Agropecuar., Concordia, Ser. Téchnica No. 8, 15 p.

PUJOL, A.R., and H.N. BEÑATENA
1965. Study of psorosis in Concordia, Argentina, p. 170-74. *In* Proc. 3rd Conf. IOCV. Univ. Florida Press, Gainesville.

SEMANCIK, J.S., and L.G. WEATHERS
1965. Partial purification of a mechanically transmissible virus associated with tatter leaf of citrus. Phytopathology 55: 1354-58.

TIMMER, L.W.
1974. A necrotic strain of citrus ringspot virus and its relationship to citrus psorosis virus. Phytopathology 64: 389-94.

TIMMER, L.W., and H.N. BEÑATENA
1977. Comparison of psorosis and other viruses causing leaf flecking in citrus, p. 930-35. *In* 1977 Proc. Int. Soc. Citriculture. Lake Alfred.

TIMMER, L.W., and S.M. GARNSEY
1978. The distribution of citrus ringspot virus in Texas and Florida citrus. Phytopathology News 12: 199-200.

TIMMER, L.W., and S.M. GARNSEY
1979. Variation in the distribution of citrus ringspot and psorosis viruses within citrus hosts. Phytopathology 69: 200-03.

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: 1054-58.

VOGEL, R., and J.M. BOVÉ
1976. Transmision de maladies infectieuses d'agrumes a agrumes par le pollen d'arbres applique sous l'ecorce de plantes saines. C.R. Acad. Sci. Paris, Ser. D 283: 1409-12.

WALLACE, J.M., and R.J. DRAKE
1968. Citrange stunt and ringspot, two previously undescribed virus diseases of citrus, p. 177-83. *In* Proc. 4th Conf. IOCV. Univ. Florida Press, Gainesville.