Mechanical Transmissibility of Citrus Ringspot Virus Isolates From Florida, Texas, and California*

S.M. Garnsey and L.W. Timmer

Citrus ringspot virus (CRSV) was first described in California (Wallace and Drake, 1968). Subsequently, ringspot-like diseases have been described from many citrus-growing areas (Timmer and Beñatena, 1977; Timmer and Garnsey, 1980). Timmer and Garnsey (1979*b*) suggested that CRSV may be synonymous with psorosis B as originally described by Fawcett and Bitancourt (1943). The natural spread of CRSV, or a similar virus, in Argentina (Timmer and Beñatena, 1977) and Texas (Timmer, 1974; Timmer and Garnsey, 1979*b*, 1980), and the discovery of CRSV in an unauthorized importation of Star Ruby grapefruit in Florida (Garnsey *et al.*, 1976) have renewed interest in CRSV.

The California isolate of CRSV was not mechanically transmitted (Wallace and Drake, 1968; Desjardins *et al.*, 1969), and thus apparently differed from CRSV isolates in Florida and Texas which were mechanically transmitted (Garnsey, 1975; Garnsey *et al.*, 1976; Timmer *et al.*, 1978). Erratic distribution of CRSV within citrus hosts (Timmer and Garnsey, 1979*a*) and instability of the virus in extracts (Garnsey *et al.*, 1976) have affected transmission success (Timmer *et al.*, 1978). The chlorotic to necrotic local lesions produced on mechanically inoculated *Chenopodium quinoa* Willd. have been used for diagnostic purposes (Timmer *et al.*, 1978; Timmer and Garnsey, 1979*a*, 1980), but a direct correlation between these lesions and CRSV in citrus has not been established. Problems have been encountered in transmitting CRSV from herbaceous hosts to citrus (Timmer *et al.*, 1978; Garnsey and Timmer, unpublished) and in establishing a correlation between symptoms in citrus and noncitrus hosts.

In this paper, we: 1) report mechanical transmission of the California isolate of CRSV and several isolates of psorosis B to herbaceous hosts and mechanical transmission of additional Florida and Texas isolates of CRSV from citrus to citrus; 2) correlate symptoms in *C. quinoa* with those in citrus; 3) report differences among citrus hosts in receptivity to mechanical inoculation with CRSV; and 4) report factors affecting the stability of CRSV preparations.

MATERIALS AND METHODS

Tests were conducted in air-cooled or air-conditioned, partly shaded glasshouses in Orlando and Gainesville, Florida, and Weslaco, Texas. Unless noted otherwise, temperatures were usually between 21 and 27°C. Supplemental light (Grolux, Wide Spectrum) was supplied to herbaceous plants in winter. Plants were grown in sterilized potting mix, fertilized and sprayed as needed to maintain healthy, vigorous growth. All indicator plants were grown from seed, except Etrog citron and Eureka lemon plants which were grown from rooted cuttings of clonal, virus-free sources.

For routine purposes, inocula were prepared by grinding leaf tissue in cold 0.05M TME buffer (Tris [Tris-(hydroxymethyl)aminomethane] pH 8.0 plus 0.5 per cent 2-mercaptoethanol) with prechilled mortars and pestles. Inocula were applied immediately with cotton swabs to leaves predusted with 500-

*This paper reports the results of research only. Mention of a pesticide or proprietary product in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended.

mesh Carborundum. In some cases, inocula were also applied by a stem-cut technique (Garnsey and Whidden, 1973).

Citrus ringspot virus isolates used included: 1) CRSV-4, an isolate from Star Ruby grapefruit in Florida (Garnsey *et al.*, 1976); CRSV-5, a previously undescribed ringspot isolate from an old-line Florida navel orange tree, which had been mechanically transmitted from citrus to citrus and from citrus to herbaceous hosts and is similar, but not identical to CRSV-2 (Garnsey, 1975); 3) TXR-1, the necrotic citrus ringspot isolate originally described from Texas (Timmer, 1974); 4) three other similar isolates from Texas, coded TXR-2, TXR-11, and TXR-12 (Timmer and Garnsey, 1979*a*); 5) the California isolate of CRSV (CaCRV) (Wallace and Drake, 1968); and 6) three isolates of psorosis B from California (P-208, P-251-B, and P-250-2-A). All research on Texas and California isolates in Florida was done in the quarantine facility of the Florida Department of Agriculture and Consumer Services. Isolates from California and Texas were introduced in infected budwood or leaves and graft-transmitted to healthy orange or grapefruit seedlings which served as the source of inocula.

RESULTS

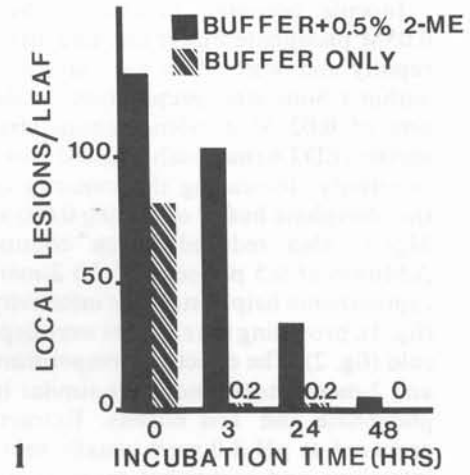
Preparation of inoculum. Inconsistent, sporadic mechanical transmission from citrus to herbaceous hosts was obtained initially with many isolates. In some tests, the virus was transmitted readily; in other similar tests it was transmitted poorly or not at all (Timmer *et al.*, 1978). It gradually became apparent that several factors were affecting transmission success. We found that the virus often is not uniformly distributed in systemically infected plants and that only young citrus tissues with severe symptoms yield highly infectious inocula (Timmer and Garnsey, 1979*a*). Generally, better results were obtained with tissues collected from recently infected plants with shock-phase symptoms than with tissues from chronically infected plants.

Inocula prepared in cold, neutral 0.05M phosphate buffer lost infectivity rapidly and were often not infectious within 1 hour after preparation. Addition of 0.02 M ethylenediaminetetraacetate (EDTA) markedly reduced initial infectivity. Increasing the molarity of the phosphate buffer or adding 0.005M $MgCl_2$ also reduced lesion counts. Addition of 0.5 per cent (V/V) 2-mercaptoethanol helped stabilize infectivity (fig. 1), providing the extracts were kept cold (fig. 2). The effects of temperature and 2-mercaptoethanol were similar in phosphate and Tris buffers. Extracts prepared at pH 8.0 were usually more infectious and somewhat more stable than extracts prepared at pH 7.0.

Mechanical transmission from citrus to citrus. In addition to the two Florida isolates (CRSV-2 and CRSV-4) previously transmitted mechanically from citrus to citrus (Garnsey, 1975; Garnsey *et al.*, 1976), we transmitted CRSV-5, TXR-1, and the California psorosis B isolate P-208 during the course of host range tests.

In separate transmission tests, the CRSV-4 isolate was readily transmitted by stem-slash inoculation with inocula prepared in TME buffer, but not by direct knife transfer (knife contaminated by cutting infected plants and then used to make inoculation cuts). The TXR-1 isolate was not transmitted with clippers contaminated by cutting infected *Citrus excelsa* plants and then clipping sweet orange, Duncan and Hudson grapefruit, *C. excelsa*, Alemow, Etrog citron, and trifoliate orange plants.

Mechanical transmission from citrus to herbaceous hosts. Three isolates from Florida (CRSV-2, CRSV-4, and CRSV-5) and four isolates from Texas (TXR-1, TXR-2, TXR-11, and TXR-12) were mechanically transmitted to numerous herbaceous hosts from citrus. All of these isolates produced chlorotic to necrotic local lesions in inoculated *Chenopodium quinoa* (fig. 3). Other hosts and symptoms have been described in detail (Timmer *et al.*, 1978). In



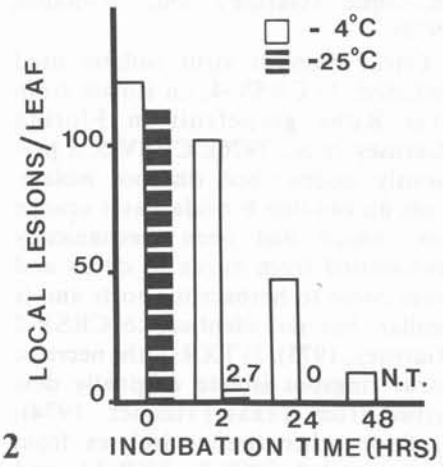


Fig. 1. Effect of 2-mercaptoethanol on infectivity of extracts of CRSV-infected tissue. Extracts prepared in 0.05M potassium phosphate buffer with and without 0.5 per cent 2-mercaptoethanol (2-ME). Inoculum prepared from uniform aliquots of tissue at 1/25 (W/V) dilution. Lesion counts are means from 6 leaves on different *Chenopodium quinoa* plants. Separate aliquots were assayed at each time interval indicated to avoid effects of Carborundum or receptor plant extracts on the virus. Extracts incubated at 4°C.

Fig. 2. Effect of incubation temperature on infectivity of extracts of CRSV-infected tissue. Extracts prepared in TME buffer (0.05 Tris + 0.5 per cent 2-mercaptoethanol, pH 8.0) and incubated as indicated. Other conditions as indicated in fig. 1.

addition, numerous uncharacterized field isolates from Texas and Florida were transmitted to *C. quinoa* (Timmer and Garnsey, 1978, 1980).

Local lesions were formed on *C. quinoa* plants mechanically inoculated with CaCRV and the three isolates of psorosis B from California.

For all isolates, lesion numbers and appearance were somewhat variable. In addition to condition and preparation of inocula, the condition of the receptor plants was also important. We used supplemental light (16-hr. photoperiod) and moderate light intensity to produce *C. quinoa* plants with large, succulent leaves. Conspicuous lesions formed most readily on leaves which were nearly fully expanded. Fewer lesions formed on younger, partly expanded leaves, and these lesions remained chlorotic and small. On older, mature leaves, lesions often coalesced and became mostly necrotic. No systemic symptoms were seen on *C. quinoa*, and CRSV was recovered only from local lesion areas.

Mechanical transmission form herbaceous hosts to citrus. We could not transmit any isolate of CRSV from *C. quinoa* to citrus, even with inocula prepared from excised local lesions which were highly infectious to *C. quinoa*. However, we transmitted CRSV from *C. quinoa* to *Gomphrena globosa* L. and then back to citrus. We made several successive single lesion transfers of CRSV-4, CRSV-5, TXR-1, and TXR-2 in *C. quinoa*, and then transmitted these isolates sequentially to *G. globosa* and then to citrus. The foliar symptoms in citrus produced by these single lesion isolates from *C. quinoa* were the same as those produced by the original cultures (fig. 4).

Susceptibility of citrus hosts to mechanical inoculation with CRSV. Although we had earlier transmitted CRSV-2 from several herbaceous hosts back to citrus (Garnsey, 1975) and had

Fig. 3. Local lesions on leaf on *Chenopodium quinoa* mechanically inoculated with isolate CRSV-4 Photographed 6 days after inoculation.

transmitted the CRSV-4 isolate mechanically directly from citrus to citrus with little difficulty (Garnsey *et al.*, 1976), we had little success transmitting CRSV-4, CRSV-5, and several Texas isolates back to citrus in sporadic initial tests. The difficulty was apparently due to our initial choice of receptor plants (usually Duncan grapefruit or sour orange), although warm summer conditions and less-than-optimum titer in the *G. globosa* plants used as inocula sources may have contributed to the problem.

Transmission of CRSV-4, CRSV-5, and TXR-1 from *G. globosa* to some citrus hosts was readily achieved in more intensive tests done under ideal fall weather conditions. The inocula were prepared from inoculated leaves of *G. globosa* plants with strong symptoms and caused numerous local lesions on *C. quinoa* assay plants. Etrog citron, Mexican lime, and *C. excelsa* plants were much more receptive than sweet orange, sour orange, and Duncan grapefruit to CRSV infection via

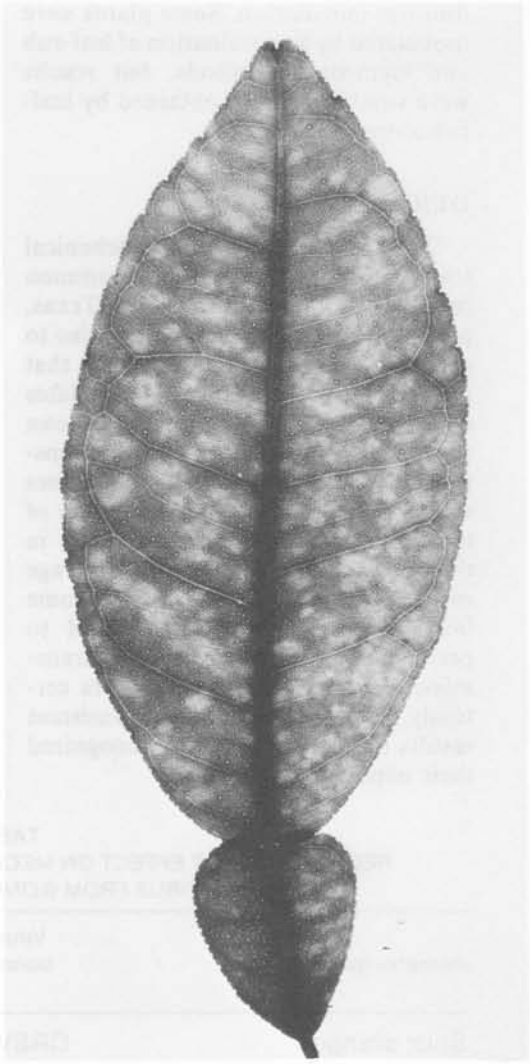


Fig. 4. Systemic symptoms on Duncan grapefruit seedling infected with isolate CRSV-4 which had been transmitted from citrus to *Chenopodium quinoa*, transferred twice in *C. quinoa* as a single lesion culture, then transmitted sequentially to *Gomphrena globosa*. Etrog citron, and Duncan grapefruit. All transfers by mechanical inoculation, except the final transmission between Etrog citron and Duncan grapefruit was by graft.

mechanical inoculation (table 1). All plants were in comparable stages of growth when inoculated and received uniform inoculation. Some plants were inoculated by a combination of leaf-rub and stem-slash methods, but results were similar to those obtained by leaf-rub alone.

DISCUSSION

Our results show that mechanical transmissibility is a property common to CRSV isolates from Florida, Texas, and California and, apparently, also to psorosis-B isolates. We believe that many, if not all, of the CRSV isolates described from other areas can probably also be mechanically transmitted with appropriate techniques under proper conditions. Instability of the virus, erratic virus distribution in the host, fluctuation in titer with stage of infection and low receptivity of some hosts have probably contributed to previous failures in mechanical transmission of CRSV. These factors certainly caused failures and inconsistent results in our tests until we recognized their importance.

We believe that the local lesion symptom in *C. quinoa* is caused by CRSV, since single-lesion cultures from *C. quinoa* produced typical CRSV leaf and shoot symptoms in young citrus indicators.

Differences among citrus hosts in susceptibility to virus infection via mechanical inoculation have been reported with other viruses (Garnsey and Weathers, 1972; Garnsey, 1974). Even though Duncan grapefruit and sour orange show excellent symptoms, this is not a reliable measure of susceptibility to mechanical inoculation. Etrog citron is apparently a good receptor plant for most citrus viruses, although it may not show severe symptoms.

At this point, we are not sure if mature tree symptoms, such as bark lesions, are associated with CRSV or another psorosis component present in the tree. The mechanical transmission of psorosis B to *C. quinoa* further suggests that at least some of the ringspot-like symptoms described for that disease are due to the presence of CRSV and that CRSV may be more widely distributed in older citrus

TABLE 1
RECEPTOR PLANT EFFECT ON MECHANICAL TRANSMISSION OF CITRUS RINGSPOT VIRUS FROM *GOMPHRENA GLOBOSA* TO CITRUS

Receptor plant	Virus isolate	Plants inoculated* (no.)	Plants infected (per cent)
Sour orange	CRSV-4	13	0
Duncan grapefruit	CRSV-4	10	10
Sweet orange	CRSV-4	13	23
Mexican lime	CRSV-4	15	67
Etrog citron	CRSV-4	19	79
Citrus excelsa	CRSV-4	13	85
Sour orange	CRSV-5	2	0
Etrog citron	CRSV-5	2	100
Sweet orange	TXR-1	11	0
Mexican lime	TXR-1	15	33

* Results from several experiments. Results for CRSV-4 and CRSV-5 obtained at Orlando, Florida. Results for TXR-1 at Weslaco, Texas. Plants inoculated by leaf-rub or a combination of leaf-rub and stem-slash methods. All receptor plants had essentially comparable flushes of succulent new growth at time of inoculation.

plantings than originally suspected (Timmer and Garnsey, 1978, 1980). We believe that CaCRV and the psorosis-B isolates can also be mechanically transmitted to additional herbaceous hosts and back to citrus, but only limited work was possible in this study.

The ability to transmit mechanically the different CRSV isolates and to detect them by local lesion assay on *C. quinoa* will enable further study on this group to provide a better understanding of its relationship to the psorosis complex. It is also possible that mechanical transmission (as a contaminant) could be involved in some of the observed natural spread of CRSV, although we have not yet obtained experimental evidence for that.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of R. Whidden, G. Whistler, and S. Villarreal; the use of the quarantine facility of the Florida Department of Agriculture and Consumer Services; the assistance of C.N. Roistacher in providing isolates of CaCRV and psorosis B, and photography by R. Smith.

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