

Effects of Strain, Host, Time of Harvest, and Virus Concentration on Double-Stranded RNA Analysis of Citrus Tristeza Virus

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ABSTRACT

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Major and minor double-stranded ribonucleic acids (dsRNAs) of citrus tristeza virus (CTV) could be detected and compared with confidence when all the dsRNAs purified from 1 to 2 g of infected citrus bark tissue were analyzed by polyacrylamide gel electrophoresis. Tissue from field or greenhouse-grown infected citrus must be in optimal condition for dsRNA analysis for this to be true. Four strains of CTV, selected to represent some of the biological diversity in the University of California, Riverside, collection of CTV isolates induced the accumulation of virus specific dsRNAs in *Citrus sinensis* (sweet orange), *C. aurantium* (sour orange), *C. aurantifolia* (Mexican lime), *C. limon* (lemon), *C. paradisi* (grapefruit), *C. medica* (citron), and *C. excelsa*. The most reliable dsRNA results were obtained from sweet orange, *C. excelsa*, and citron. Sour orange and grapefruit were the least reliable hosts for dsRNA analysis. The ranking of

hosts for dsRNA quantity was generally similar to that determined for antigen titer of CTV in the hosts tested. The dsRNA profiles were characteristic for each strain, particularly when extracts were from the most reliable hosts for dsRNA analysis. Sour orange, grapefruit, and lemon had a tendency to reduce the number of dsRNAs and/or their relative intensity, as well as the quantity of dsRNA. A marked effect of time of year was observed, and CTV antigen and dsRNA were detected with difficulty when daytime temperatures were highest, which in Riverside is from June to September. The results of CTV antigen and dsRNA analyses were better at other times of the year. February to April was optimal. These results will be of value if dsRNA analysis is included in surveys for strains of CTV.

Citrus tristeza virus (CTV) strains are presently differentiated by the reaction of sensitive indicator seedlings or grafted combinations of *Citrus* spp. (13,17,18,20,21,24). Virus strains that cause stunting and chlorosis of sour orange, grapefruit, and lemon are commonly referred to as seedling yellows strains (9,17,18,24). Strains are also identified by their ability to induce stem pitting in grapefruit, sweet orange, and other commercially grown *Citrus* spp. (18). These two qualities, stem pitting and seedling yellows induction, are not necessarily linked (10,18,24). Strains isolated from sweet orange in southern California are mild and are normally unable to induce the seedling yellows reaction (24), and they usually do not induce severe stem pitting of grapefruit.

The value of double-stranded ribonucleic acid (dsRNA) analysis for diagnosis of plant viruses has been reviewed (7). Previous reports have indicated that virus specific dsRNAs can be detected in extracts from bark of *Citrus* spp. infected with CTV (4,6,8,16). We have recently carried out a survey of dsRNAs isolated from sweet orange seedlings experimentally infected with 66 isolates of CTV representing seedling yellow, stem pitting, and typical southern Californian isolates (5) and have concluded that typical California isolates appear to lack a readily detectable dsRNA with a molecular weight (MW) of 0.5×10^6 that is detectable in plants infected with seedling yellows and stem-pitting strains. DsRNAs other than the expected replicative form of the full-length genome may be useful for making distinctions between strains of this and other RNA plant viruses (6,7,12,23).

While performing previous experiments, we became aware of the need to better characterize the conditions that would favor detection of dsRNAs in extracts from both field and greenhouse-grown *Citrus* spp. infected with CTV. This is a timely objective because there is a new interest in surveying the citrus industry in southern California for severe strains of CTV. Seedling yellows

strains have been detected in California in the past (24). A survey has recently been completed at the University of California, Riverside (UCR), during which some trees infected with seedling yellows strains were found and eradicated (14). Another survey is about to begin in commercial groves. Both surveys rely on indexing in indicator seedlings.

This report summarizes results of experiments designed to evaluate the effect of isolation method, host (seven *Citrus* spp.), and strain (four strains) on dsRNA quantity and complexity and CTV antigen concentration in plants grown in greenhouse conditions and the effect of time of harvest of tissue from naturally infected sweet orange in the field.

MATERIALS AND METHODS

Hosts for CTV. Seedlings of *Citrus sinensis* (L.) Osb. 'Madam Vinous' (sweet orange), *C. aurantium* L. (sour orange), *C. aurantifolia* (Christm.) Swing. (Mexican lime), *C. limon* (L.) Burm. f. (lemon), *C. paradisi* MaCf. 'Duncan' (grapefruit), *C. medica* L. 'Seedling 861-S1' (citron), and *C. excelsa* Wester were bud-graft inoculated (three seedlings per strain) with each of four strains of CTV. Bark tissue was removed from inoculated seedlings at 6 wk and 4 mo after inoculation.

Strains of CTV. Four strains, designated A, B, C, and D for this study, were selected for greenhouse experiments from a collection of over 100 strains maintained by C. N. Roistacher and D. J. Gumpf at UCR. They were selected to represent some of the diversity encountered among CTV strains in the collection. Some properties of these strains are indicated in Table 1.

Strain T505 (A) was collected from a Valencia sweet orange in 1977 in the Central Valley of California. It has been indexed for citrus psorosis virus and citrus exocortis viroid, with negative results. It has given typical CTV reactions in Mexican lime (vein clearing with some stem pitting) in numerous tests over the last 9 yr. It is notable for being biologically typical of isolates obtained from southern California, except for the degree to which it can stem pit sweet orange seedlings.

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Strain 19V (B) was isolated from a Shamouti sweet orange in the UCR citrus collection, which was weak and small at the time of virus isolation, and later showed severe die-back.

Strain SY560 (C) was isolated from *C. macroptera* in the UCR citrus collection. The tree was in decline when the virus was isolated, and it died subsequently. The strain has been indexed for citrus psorosis virus, citrus vein enation virus, and citrus exocortis viroid, with negative results. It is notable for its severe reaction in Mexican lime and *C. excelsa*, and for classical seedling yellows reactions in grapefruit, lemon, and sour orange.

Strain 565V (D) was isolated from a Hart's Tardiff sweet orange in 1968, from Los Angeles County, CA. It was originally contaminated with citrus psorosis virus but was indexed free of citrus exocortis viroid. It is notable for the severe stem pitting it induces in grapefruit, in the absence of a seedling yellows reaction.

Strains identified by the letter V (B and C) were transmitted to Mexican lime by the aphid *Aphis gossypii* Glover before placement in the UCR CTV collection.

Collection and dsRNA analysis of field samples. Eight Valencia sweet orange trees known to be infected with CTV were selected from an experimental grove located at UCR. Young green twigs were removed from different positions around each tree at monthly intervals from November 1984 to November 1985 and used as a source of bark for dsRNA analysis. A standard sample, consisting of bark of greenhouse-grown citron infected with strain C, was collected at the beginning of the experiment. An aliquot of frozen powdered bark of this standard sample was always extracted and analyzed for dsRNA along with each set of eight field samples.

Isolation and dsRNA. The final purification was a modification of a general scheme for dsRNA (15,19,23) adapted for use with *Citrus* spp. infected with CTV. Bark from three seedlings infected with a single strain of CTV was pooled. For field trees, bark from twigs of an individual tree was pooled. Two grams of bark tissue was ground to a powder in liquid nitrogen, and 4 ml of double-strength STE [single strength STE is 0.1 M sodium chloride, 0.05 M tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediamine-tetraacetic acid (EDTA), pH 6.8] was added to the powder, together with 0.6 ml of 10% sodium dodecyl sulfate, 0.2 ml of bentonite (25 mg/ml), and 6 ml of STE saturated phenol. The mixture was shaken for 30 min and then centrifuged at 8,000 g for 15 min.

The aqueous phase (5 ml) obtained by centrifugation was adjusted to 16% ethanol and percolated through a column of Whatman CF-11 cellulose powder (1 g of dry weight, approximately 5 ml of wet volume) in the presence of STE buffered 16% ethanol. Columns were washed with 30 ml of STE buffered 16% ethanol and the dsRNA contained in the washed column was eluted with 6 ml of ethanol-free STE. The first 2 ml of eluate was discarded because it contained little or no dsRNA. The dsRNA in the collected eluate was precipitated by the addition of three volumes of 95% ethanol and 0.2 ml of 3 M sodium acetate, pH 5.5, and storage at -20 C for 24 hr. The precipitated dsRNA was collected by centrifugation and resuspended in 30-60 µl of 0.004 M Tris, 0.02 M sodium acetate, 1 mM EDTA, pH 7.8 (electrophoresis buffer).

DsRNA samples were loaded onto channels of a 6% polyacrylamide gel (acrylamide:bisacrylamide = 40:1, v/v) cast for a vertical slab-gel electrophoresis apparatus (gel dimensions were 83 mm × 63 mm × 1.5 mm), and electrophoresed for 3 hr at constant voltage (100 V, approximately 60 mA). Electrophoresed gels were stained with ethidium bromide (60 ng/ml), and placed on a transilluminator (wavelength of 254 nm) and photographed (10-20 sec, f5.6) using Polaroid type 57 black and white film with appropriate filters (4). Molecular weights of CTV dsRNAs were estimated by the graphical method of Bozarth and Harley (3), in comparison with previously used standards (4,23).

Quantification of CTV antigen. Bark from three seedlings infected with a single strain of CTV was pooled. Bark tissue was ground to a powder in liquid nitrogen and 0.5 g of the powder was resuspended in 5 ml of phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 2.0% polyvinylpyrrolidone. The relative amount of CTV antigen in different dilutions (undiluted,

i.e., 1/10, and at 1/100 and 1/500) of the samples was determined by enzyme-linked immunosorbent assay (ELISA). Rather than following the original direct ELISA (1), an indirect test was used whereby plates were coated with goat polyclonal CTV antibody (Ab) IgG (1.0 µg/ml), and trapped antigen (six replicates of each dilution of each sample) was reacted with a rabbit polyclonal CTV Ab IgG (1.0 µg/ml), followed by alkaline-phosphatase conjugated goat anti-rabbit Ab IgG (0.4 µg/ml). Substrate (*p*-nitrophenol phosphate, disodium) at a concentration of 0.6 mg/ml was added and incubated for 20 min at room temperature. Absorbance at 405 nm was measured on a microtiter EIA plate reader (Model EL 3071, Bio Tek Instruments, Inc., Burlington, VT).

RESULTS

Choice of dsRNA purification method. Previously published methods (4,15,19,23) that used 7 g of tissue gave good recovery of virus specific nucleic acids that are presumed to be dsRNAs of CTV. They had the same sensitivity to ribonuclease and deoxyribonuclease as has been reported for other dsRNAs purified by this method in previous studies (4,15). These methods were destructive in that harvesting this amount of bark tissue from greenhouse-grown citrus seedlings left insufficient amounts for subsequent testing of individual plants. A modification of this method, which used smaller starting weights and smaller amounts of cellulose powder, was used for this study. The dsRNA recovered from 1 to 2 g of bark tissue from plants maintained in optimum condition for dsRNA analysis was sufficient, and dsRNAs were detected readily after gel electrophoresis (Figs. 1-3).

Effect of strain on dsRNA pattern. Results for all four strains propagated in sweet orange are shown in Fig. 1. Each gel channel in this and subsequent figures was loaded with the dsRNA that was recovered from 1 g of infected bark tissue, unless otherwise indicated. A slow, migrating major dsRNA was detected in all samples. This dsRNA has a molecular weight (MW) of 13.3×10^6 and is assumed to be the replicative form (RF) of CTV genomic ssRNA (MW = $5.4-6.5 \times 10^6$) (2,4). Another major dsRNA, which had a MW of 0.8×10^6 , was detected for all four strains. Major dsRNAs are defined as having a visually assessed intensity in stained gels equal to or greater than the 0.8×10^6 dsRNA common to all strains. Three of the four strains (strains B, C, and D) had a readily detected minor dsRNA with a MW of 0.5×10^6 . Other major or minor dsRNAs were detected (MWs between 0.8 and 13.3×10^6) and some of these were common and others unique to specific strains. The four strains have been analyzed on six different occasions in sweet orange. The differences described and illustrated were reproducible, and it was possible to diagnose these four strains in sweet orange.

Effect of host on dsRNA. Results for strain C in seven hosts are shown in Figure 2. The 13.3×10^6 MW dsRNA was detected in all samples, but recovery was least from sour orange and grapefruit. The additional dsRNAs characteristics of strain C were most readily detected in sweet orange, *C. excelsa*, citron, Mexican lime, and to a lesser extent in lemon, grapefruit, and sour orange. The 0.5×10^6 MW and the 0.8×10^6 MW dsRNAs were detected in five

TABLE 1. Comparison of host reactions to four strains of CTV used to determine the effect of host on dsRNA analysis

Strain	UCR collection identity	Reaction on indicator seedlings ^a			
		Sour orange	Grapefruit	Lemon	Sweet orange
A	T505	nr ^b	nr	nr	mild SP
B	19v	nr	nr	nr	mild SP
C	SY560	SY	SY	SY	nr
D	565v	nr	SP	nr	nr

^a A summary of host reactions observed in 1980 and 1983 when these isolates were last indexed by C. N. Roistacher, UCR.

^b Abbreviations: nr = no reaction; SP = stem pitting (plentiful, unless otherwise noted); SY = chlorosis and/or stunting typical of the seedling yellows reaction.

hosts but were not detected as readily in lemon, Mexican lime, and citron as they were in sweet orange and *C. excelsa*. The 0.5 and 0.8×10^6 MW dsRNAs were barely detected in grapefruit and were not detected in sour orange. Strain C was chosen for Figure 2 to illustrate an additional point. One of the strain specific dsRNA segments (MW = 1.7×10^6) was nearly as prominent as the major dsRNA (MW = 13.3×10^6) and more prominent than the other consistently detected CTV dsRNA (MW = 0.8×10^6) in extracts from sweet orange, *C. excelsa*, citron, and Mexican lime. It was not present in detectable amounts in lemon and grapefruit, even though the 13.3×10^6 MW and the 0.8×10^6 MW dsRNAs were detected in these hosts. This was the most marked effect of host on the number of prominent dsRNAs detected, and then only for this one dsRNA segment.

Results for strain A and B (Fig. 3) were similar to those for strain C. Strain D differed from the other three strains in that dsRNAs were detected readily in grapefruit and sour orange. Only minor variations in the dsRNA profiles are discernible when results in *C. excelsa*, citron, and Mexican lime are compared. The greatest variation in these hosts was for strain A, and the major effect was the relative amounts rather than presence or absence of specific bands. Sweet orange, *C. excelsa*, and citron were the best hosts for detection of the 0.5×10^6 MW dsRNA associated with infection by strains B, C, and D. Results for the earlier harvest were similar to those illustrated and described for the later harvest. An overall ranking of hosts is proposed in Table 2.

Effect of host on ELISA values and comparison with dsRNA detection. A summary of results of ELISA testing of the same plants used for the dsRNA analysis is shown in Figure 4. It is clear that virus titer was not the same in all host types. When values for all four strains were summed, sweet orange and *C. excelsa* had the

highest cumulative values ($A_{405nm} = 1.8$ and 1.6 , respectively), grapefruit and lemon had intermediate values ($A_{405nm} = 1.1$ and 0.9 , respectively), and sour orange and Mexican lime had the lowest values ($A_{405nm} = 0.7$ and 0.3 , respectively). In a separate experiment a complete block design was used to place samples in microtiter plates and the data were analyzed by Duncan's multiple range test. The mean values (A_{405nm}) for all values for all strains in sweet orange were 0.26 , and this was significantly different at 1% level to the values in lemon (0.16), *C. excelsa* (0.15), and grapefruit (0.15). These values in their turn were significantly different at 1% level from those in Mexican lime (0.12) and sour orange (0.10). A proposed ranking is summarized in Table 2. It is generally similar but not identical to that determined for dsRNA detection. The relatively low ranking for Mexican lime by ELISA, compared with the dsRNA ranking of this host, was somewhat weighted by the disproportionately low ELISA value for strain C in Mexican lime compared with values for this high titer strain in other hosts. Citron, which was one of the better hosts for recovery of dsRNA, was not tested by ELISA in this experiment but has given consistently high ELISA readings for strains A and C in other experiments.

Strain D was detected readily in all hosts, which parallels results for dsRNA detection for this strain. Other strains were barely detected in sour orange and were detected with difficulty in some other hosts, depending on the strain, at the sap dilution used for Figure 4. No obvious correlations with dsRNA results were apparent for these minor variations. The highest dilution of sap ($1/500$) was chosen for Figure 4 to emphasize the differences in antigen titers. At higher concentrations of antigen ($1/10$ and $1/100$ dilutions), all hosts gave strong positive results for CTV, when compared with results for noninoculated plants of the same species.

Effect of harvest date on dsRNA and ELISA results. The dsRNA recovered from 2 g of bark collected from one of eight field trees naturally infected with CTV is illustrated in Figure 5. The

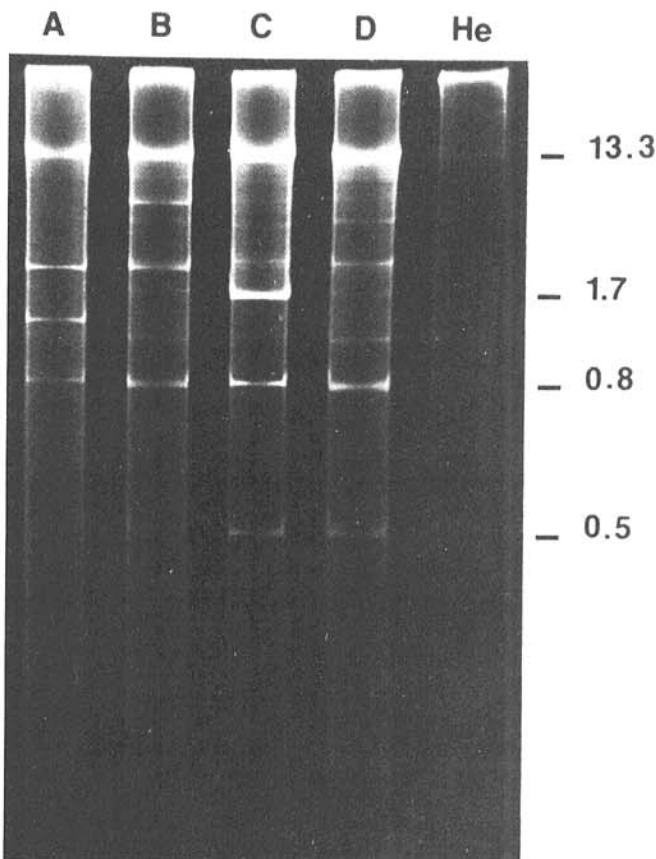


Fig. 1. Double-stranded RNAs of strain A (A), strain B (B), strain C (C), and strain D (D) of citrus tristeza virus (CTV) extracted from experimentally infected sweet orange seedlings 4 mo after inoculation. All the dsRNA from 1 g of infected (A–D) or healthy (He) bark tissue was electrophoresed through a 6.0% polyacrylamide gel, and stained with ethidium bromide. Molecular weights ($\times 10^6$) are indicated on the right.

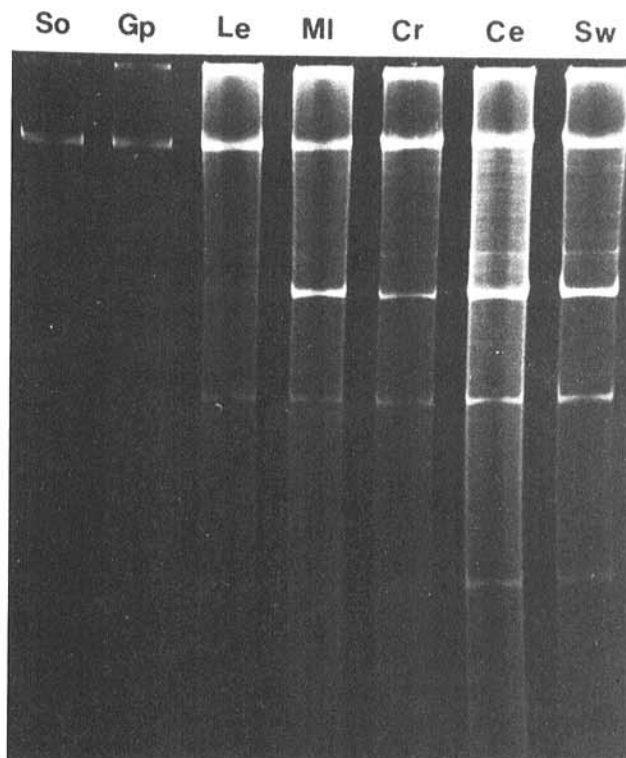


Fig. 2. Double-stranded RNAs of one strain (strain C) of citrus tristeza virus (CTV) extracted from experimentally infected seedlings of sour orange (So), grapefruit (Gp), lemon (Le), Mexican lime (MI), citron (Cr), *Citrus excelsa* (Ce), and sweet orange (Sw) seedlings 4 mo after inoculation. All the dsRNA from 1 g of infected bark tissue was electrophoresed through 6.0% polyacrylamide gel and stained with ethidium bromide.

dsRNAs associated with CTV infection of greenhouse-grown sweet orange seedlings were readily detected in all eight field samples collected in February. The quality of dsRNA illustrated is typical for field isolates from the area and includes the lack of a 0.5×10^6 MW dsRNA. A similar result was obtained for samples harvested from these same trees in each of the months from November 1984 to April 1985. By contrast, less or no dsRNA was detected in samples from these same trees collected from May to October 1985. The dsRNA results are rated in Table 3. A replicate sample of bark harvested at the beginning of the experiment from seedlings experimentally infected with strain C gave similar results in each of the monthly analyses (data not shown). A reextraction of bark harvested from a field tree in February was included in the analysis of eight field trees harvested in September. The results for the February bark were as good as when first analyzed. Samples from the north, south, east, and west sides of two trees were analyzed in February 1985. No effect of branch position on dsRNA recovery or complexity was detected.

The antigen titer of CTV in field trees is compared with the ranking of dsRNA results in Table 3. Antigen titer was high in November and December, declined in January and February, was

high again in March through June, then declined to almost undetectable amounts in July through October at the dilution used. There was a good overall agreement between antigen titer and dsRNA results, with an interesting lack of specific agreement. The recovery of CTV in the spring following low temperatures in December and January was detected first in the dsRNA results (February) and a month later (March) in the ELISA results. Similarly, the decline of CTV at the onset of warmer temperatures in April and May was noticed first in the dsRNA results (May) and was followed one month later (June) by a change in the ELISA results.

DISCUSSION

DsRNAs of different strains of CTV can be detected from 1 to 2 g of bark of infected *Citrus* spp. collected from field trees or from greenhouse-grown plants. In our experience this constitutes a nondestructive harvest and will permit subsequent analysis of the same greenhouse seedling or branch of a field tree at a later date. The four strains could be distinguished by dsRNA analysis, and the complexity of the dsRNA for each isolate was quite stable when purified from those hosts that give the best recovery of dsRNA, namely sweet orange, *C. excelsa*, and citron. The time of year may be important in determining when samples should be collected from sweet orange trees in the field. Sampling when temperatures are at their highest should be avoided. These observations confirm results of preliminary experiments (6,8).

TABLE 2. Overall ranking of hosts for dsRNA analysis and antigen titer for four CTV strains

Ranking	dsRNA ^a	ELISA ^b
Good	Sweet orange	Sweet orange
	<i>Citrus excelsa</i>	<i>C. excelsa</i>
	Citron	Citron ^c
	Mexican lime	
	Grapefruit	
Fair	Lemon	Lemon
	Sour orange	Mexican lime
Poor		Sour orange

^a Subjective ranking based on visual comparison of stained polyacrylamide gels illustrated in Figures 1-3. Factors used included the absolute level of detection of the major 13.3×10^6 dsRNA and the relative abundance of this dsRNA or dsRNAs with lower MWs. Weight was given to results of Figure 2 in determining final rank order.

^b Ranking based on comparison of six replicates of a composite sample of bark from three seedlings of each host, excluding citron.

^c Arbitrary assignment based on values obtained with strains A and B, which are standard isolates maintained in citron in our laboratory and are regularly tested by enzyme-linked immunosorbent assay.

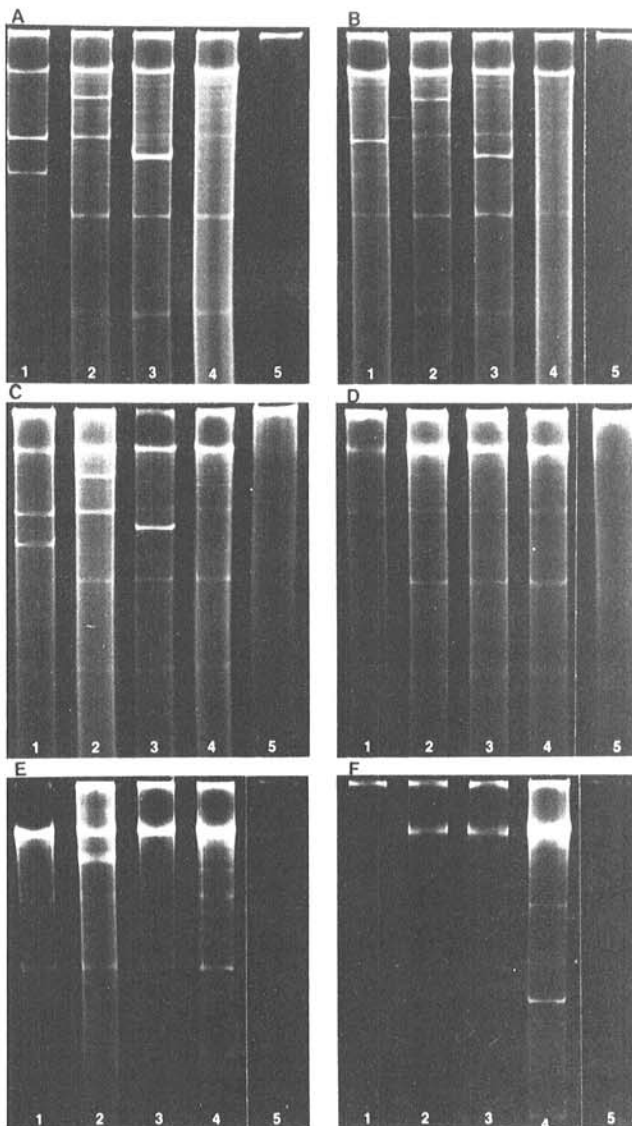


Fig. 3. Double-stranded RNAs of four strains (strains A-D, gel columns 1-4, respectively) of citrus tristeza virus (CTV) extracted from experimentally infected seedlings of *Citrus excelsa* (A), citron (B), Mexican lime (C), lemon (D), grapefruit (E), and sour orange (F) 4 mo after inoculation. Extract from noninoculated plants is in gel column 5. All the dsRNA from 1 g of infected bark tissue was electrophoresed through 6.0% polyacrylamide gels, and stained with ethidium bromide.

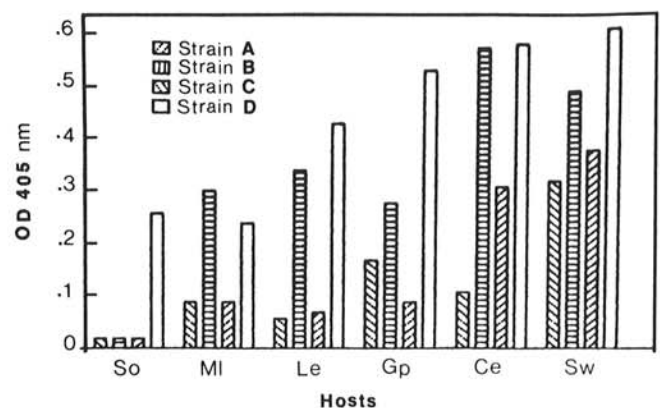


Fig. 4. Detection of CTV antigen by indirect ELISA in sap (diluted 1/500) from experimentally infected seedlings of sour orange (So), Mexican lime (MI), lemon (Le), grapefruit (Gp), *Citrus excelsa* (Ce), and sweet orange (Sw) 4 mo after inoculation. Each value is the mean of six replications for each sample. Values for samples from noninoculated seedlings of any species never exceeded 0.01 at the sap dilution used.

Additional experiments will be needed to determine if all the dsRNAs detected for a given strain are truly CTV specific, or whether some represent unsuspected viral or satellite-like agents. They are presumed to be virus specific based on the absence of detectable amounts of dsRNA in noninoculated plants. The likelihood of there being other viruses in the infected plants is not too great, however. Two of the isolates were transmitted by aphids before their entry into the UCR CTV collection, and so any second virus would also have to be aphid transmissible. The two other viruses indexed negative for tested viruses (see Materials and Methods).

It is less likely that reliable results can be obtained from two other commercial sources, lemon and grapefruit, which would normally be included in disease surveys. These hosts may affect the number and relative intensities of dsRNA of some isolates. It will be interesting to see if these changes are permanent on subculture of selected isolates after passage through grapefruit and lemon.

Antigen titer in plants sampled was a fairly good indicator that

TABLE 3. Comparison of CTV antigen titer and dsRNA recovery from field trees for 12 mo

Year	Month	Temperature ^a	Antigen titre ^b	dsRNA recovery ^c
1984	Nov.	20/7	0.88 ± 0.13	+++
1984	Dec.	16/7	0.63 ± 0.14	+++
1985	Jan.	16/6	0.44 ± 0.14	+++
1985	Feb.	20/7	0.26 ± 0.15	++++
1985	Mar.	20/7	0.70 ± 0.31	++++
1985	Apr.	26/11	0.90 ± 0.16	++++
1985	May	26/11	0.88 ± 0.15	++
1985	June	33/15	0.53 ± 0.22	++
1985	July	36/18	0.22 ± 0.11	+
1985	Aug.	35/16	0.08 ± 0.06	+
1985	Sep.	29/15	0.08 ± 0.05	+
1985	Oct.	27/12	0.08 ± 0.05	++
1985	Nov.	20/7	nd ^d	+++

^a Mean monthly maximum/minimum air temperatures (C) at Riverside, CA.

^b Means and standard deviations of enzyme-linked immunosorbent assay values (absorbance at 405 nm) for a 1/50 dilution of young green bark from eight field-grown sweet orange trees. The mean values for four CTV-infected and four noninfected greenhouse grown seedlings of sweet orange were 0.93 ± 0.09 and 0.01 ± 0.01, respectively.

^c Visual evaluation of dsRNA results for the eight trees used for estimation of antigen titer, in comparison with an internal standard (strain C in citron). Results for one tree are illustrated in Figure 5. The intensity of stained dsRNAs recovered from field samples was compared with that recovered from an equal aliquot of a standard sample in each monthly purification. Results were rated as greater than (++++), equal to (+++), or less than (++) the standard. A fourth rating (+) was reserved for those months when even the major dsRNA (MW = 13.3 × 10⁶) was barely detectable.

^d nd = Not determined.

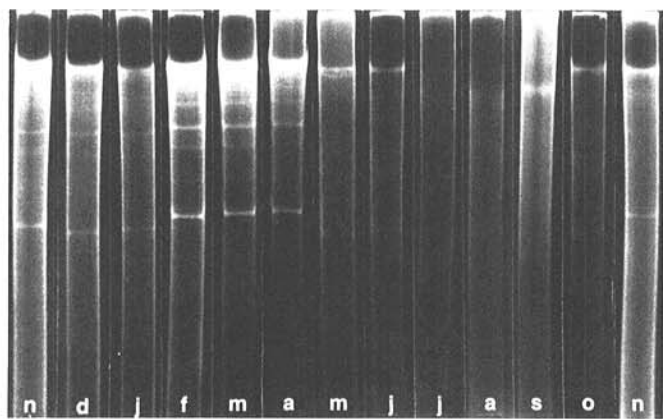


Fig. 5. Double-stranded RNAs of citrus tristeza virus (CTV) extracted from bark of a naturally infected sweet orange tree harvested from the field at monthly intervals. Months are identified by the first letter of their names, and are in chronological order from November 1984 to November 1985. All the dsRNA from 2 g of infected bark tissue was electrophoresed through 6.0% polyacrylamide gels, and stained with ethidium bromide.

dsRNA results would be optimal, in that those hosts that had the highest antigen titer, including unusual situations such as strain D in sour orange, were those that gave the best dsRNA results. The ranking of hosts by their antigen titer was similar but not identical to that in a previous report (11). The changes in antigen titer and dsRNA recovery in field trees were also generally parallel.

The observation that decline or increase in amount of CTV was noted 1 mo earlier by dsRNA analysis than by ELISA suggests that CTV dsRNA recovered by cellulose chromatography is not entirely an end product of virus replication. It appears to be actively produced before new virion accumulation and is required for sustained virion production.

The development of techniques that can be used in surveys designed to detect a particular strain of a plant virus, in a crop that is uniformly infected with other strains of the same virus, is a difficult task. It would normally be approached through the use of either strain-specific antisera, none of which are currently available for CTV, or specific indicator hosts. Our results indicate that dsRNA analysis may also be useful for this purpose. Another approach would be the use of nucleotide sequence specific DNA probes (22).

Two surveys for virulent strains of CTV have been initiated in southern California, where most sweet orange trees are infected with CTV strains that do not cause severe diseases on currently used rootstocks. The test that has been used is primary indexing on grapefruit seedlings. Subsequent indexing of those isolates that cause a seedling yellows reaction or stem pitting of grapefruit is performed on sour orange, lemon, and sweet orange seedlings. It normally takes 6–9 mo to complete this serial indexing. The first survey of 20,000 trees of several commercial and noncommercial *Citrus* spp. at UCR has been completed (14). A second survey will involve trees of several commercially grown *Citrus* spp. throughout southern California.

Results from the present study suggest that dsRNA results can be optimized if attention is paid to dsRNA purification method, host, time of year, and overall CTV titer in infected trees. dsRNA analysis may have some role to play in surveys because there is some indication from the results for four strains in this study and for 66 strains in another study (5), that it may be possible to differentiate and group strains on the basis of dsRNA results. More work needs to be done, however, before concluding that dsRNA results for a single strain will ever be sufficient information on which to predict precise biology. Tests on thousands of field sweet orange trees by this method would not be practical for most agencies involved in routine surveys. A likely use of dsRNA analysis would be to retest sweet orange trees initially diagnosed as being infected with severe strains and to rapidly test adjacent trees in the suspect grove for incidence and distribution of strains with a dsRNA pattern similar to the one present in the tree sampled in the original survey. In southern California this would best be done in the spring months.

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