Identification of Spanish Strains of Citrus Tristeza Virus by Analysis of Double-Stranded RNA

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ABSTRACT

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Double-stranded RNA (dsRNA) patterns of 24 Spanish isolates of citrus tristeza virus (CTV) representing a wide range of biological diversity were examined. Seven distinct patterns, differing by the number and/or position of the dsRNA bands, were obtained for the 24 isolates assayed. Both seasonal and host variation was observed on the recovery of the dsRNA. Greatest yields were obtained in spring and autumn from sweet orange and Dweet tangor. DsRNA recovery was not always correlated with virus titer estimated by enzyme-linked immunosorbent assay. Several strains showed host-induced variation of the dsRNA profile and, in some cases, seasonal variation. Virus titer and the amount of dsRNA recovered were not necessarily correlated with symptom severity on Mexican lime, nor could the dsRNA pattern observed be correlated with any specific biological characteristics (that is, symptom severity and aphid transmissibility). Some biologically similar CTV strains showed different dsRNA patterns, whereas other biologically different strains had the same pattern. A severe strain, T-388, recently introduced into Spain, showed a unique dsRNA profile that enabled it to be distinguished from local isolates. DsRNA analysis should be a useful tool for an eradication program in progress in Spain. Evidence for segregation of a CTV isolate by dsRNA analysis also was obtained.

Additional keywords: electrophoresis, strain segregation.

Citrus tristeza virus (CTV) presumably was introduced into Spain as early as 1930, and by 1957 it was a threatening epidemic. The uncontrolled movement of citrus budwood and the presence of three aphid species (*Aphis gossypii* Glover, *A. citricola* van der Goot, and *Toxoptera aurantii* Boyer de Fonscolombe) as vectors of the virus (13) contributed to the widespread distribution of CTV to most citrus-growing areas in the country (5).

Damage caused by tristeza consists of decline and death of sweet orange (*Citrus sinensis* (L.) Osb.), mandarin (*C. reticulata* Blanco), and grapefruit (*C. paradisi* Macf.) trees grafted on sour orange (*C. aurantium* L.), a susceptible rootstock that is predominant in the Spanish citrus industry. Many isolates of CTV found in Spain have been characterized by symptom severity induced on indicator plants and by aphid transmissibility (2; P. Moreno, *unpublished data*). None of these isolates has been shown to induce a seedling yellows reaction (12,18,27) on sour orange, lemon (*C. limon* (L.) Burm. f.), or grapefruit seedlings or to cause conspicuous stem pitting on grapefruit or sweet orange. Both reactions, seedling yellows and stem pitting, though not necessarily linked (19,24), are typical of severe CTV strains (21,22).

Recently, a severe CTV strain was discovered in Spain in an introduced satsuma cultivar (3). The presence of this severe strain, which can be vectored by *A. gossypii* (14), is a great threat to the Spanish citrus industry, and efforts are being made to eradicate it.

Biological identification of CTV strains is a long-term, troublesome operation because several different hosts need to be used and symptoms can take months to appear. Serological tests and reagents have not been able to distinguish between strains, including monoclonal antibodies obtained to Spanish CTV isolates (26) which react with most isolates assayed worldwide. Analysis of dsRNA from infected plants has proven to be a useful tool for diagnosis of plant viruses (7,11), including CTV isolates (8–10,17).

The aim of this research was to develop a quick and reliable method to distinguish the new severe CTV strain from local isolates of the virus under greenhouse and field conditions. This paper reports results obtained in dsRNA analysis of a collection of CTV isolates representative of the different citrus areas in Spain and the effect of host and sampling season on dsRNA recovery and/or dsRNA profile. Variation in dsRNA pattern among subcultures of a single CTV isolate also is shown.

MATERIALS AND METHODS

Hosts and CTV isolates. A collection of CTV isolates, representative of most citrus-growing areas in Spain, was established and maintained at the Instituto Valenciano de Investigaciones Agrarias (IVIA) free of other known citrus virus and viruslike diseases following a procedure previously described (2). These isolates were maintained in navel orange on Troyer citrange rootstock and kept in an insect-proof screenhouse. Twenty-two isolates from this virus bank, a mild isolate found later in Southern Alicante (T-385), and a severe isolate recently introduced in the country (T-388) (3) were used in this study. Biological characterization of these isolates was carried out by graft inoculation onto a set of indicator plants and by comparing aphid transmissibility under standard conditions (2,3,14).

To study the host effect on dsRNA analysis, a group of seven isolates selected by their differing dsRNA patterns were graft inoculated into five seedlings each of Mexican lime (*C. aurantifolia* (Christm.) Swing.), Eureka lemon, Dweet tangor (*C. reticulata* × *C. sinensis*), sour orange, and Pineapple sweet orange. Inoculated plants were incubated in a temperature-controlled greenhouse kept at 18–26 C.

Several subcultures obtained from isolate T-385 by graft transmission were kept on Etrog citron (*C. medica* L.) in the greenhouse.

Sampling of infected plants. Samples consisted of shoots from the latest flush. When possible, young shoots bearing fully expanded leaves were selected. Screenhouse-grown plants were

sampled individually at different seasons to determine temperature-mediated variations of virus titer and dsRNA patterns. Greenhouse-grown plants were sampled individually or pooled. Shoots from each plant or group of plants infected with the same CTV isolate were peeled, and the bark was pooled, trimmed, immediately processed, or quick frozen with liquid nitrogen and stored at −80 C.

Virus antigen titration. Relative virus antigen titer was estimated by a comparative enzyme-linked immunosorbent assay (ELISA) with the homologous antigen as the standard reference. Plant extracts were prepared by homogenizing 1 g of pooled young bark from individual plants in 10 ml of extraction buffer (0.01 M sodium phosphate buffer, pH 7.2, 0.14 M NaCl, 0.003 M NaN_3, 1% polyvinylpyrrolidone 10,000) in a polytron homogenizer. ELISA was performed by a double antibody sandwich procedure with monoclonal antibodies as previously described (26). Extracts from similar uninoculated plants were used as negative controls. The standard consisted of an extract prepared by resuspending 1 wt of freeze-dried powdered bark from a batch of plants inoculated with T-308 (the homologous antigen of the monoclonal antibodies) in 60, 120, and 240 v of extraction buffer. The plates containing the final substrate reaction were incubated until the 1:60 dilution of the standard reached absorbance values between 0.7 and 0.9 at 405 nm (usually around 1 hr). The absorbance values obtained at different seasons with each CTV isolate were compared with the corresponding values of the standard.

Purification and analysis of dsRNA. Bark samples (2–12 g) were frozen with liquid nitrogen and pulverized with a coffee grinder, and dsRNA was extracted and deproteinized by the phenol-detergent method described by Dodds et al (8,9) with minor modifications. The aqueous phase containing nucleic acids was adjusted to 16–18% ethanol and then filtered through a Millipore AP 2002500 prefilter with a Swinnex type support (Millipore Corporation, Bedford, MA). DsRNA was purified by CF-11 cellulose chromatography and collected by ethanol precipitation (9).

Electrophoretic gel analysis of dsRNA was accomplished in 5% polyacrylamide gels with a vertical mini slab-gel apparatus (80 × 70 × 0.75 mm gel size) at a constant voltage of 100 V for 3 hr. After electrophoresis, gels were stained for 15 min in 30 ng/ml of ethidium bromide, visualized on an ultraviolet (254-nm wavelength) transilluminator, and photographed with Polaroid type 667 black-and-white film. In addition, silver staining (15) was sometimes done on the same gel.

Molecular weights (MW) of dsRNAs were estimated by the graphical method of Bozarth and Harley (4) by using the following six markers: cucumber mosaic virus (MW = 2, 1.9, 1.3, and 0.55 × 10^6) (25), tobacco mosaic virus (MW = 4.3, 2.1, 0.95, and 0.42 × 10^6) (25), mycovirus of *Penicillium chrysogenum* Thom (MW = 2.18, 1.99, and 1.89 × 10^6) (6), and California isolate CTV SY560 (8) (MW = 13.3, 1.7, 0.8, and 0.5 × 10^6).

RESULTS

Improvement of the dsRNA detection method. A minimum of 5 g of fresh tissue per electrophoresis lane was necessary to readily detect dsRNA from field-infected trees and from some of the CTV isolates maintained in the screenhouse or greenhouse. Increasing the amount of fresh tissue per lane also increased the background fluorescence in the gels, thus masking the minor dsRNA bands. This problem, probably due to the presence of contaminating polysaccharides, was avoided partially by filtration of the 16–18% ethanol-adjusted extract through a Millipore glass microfiber prefilter (Millipore/Continental DI Services), using a syringe and a Swinnex type filter support (Fig. 1). This filtration also eliminated column plugging and increased the elution flow rate.

When dsRNA concentration was low, the weakly staining bands could be seen only after silver staining (Fig. 2, lanes A and B). Without prefiltering, gel lanes showed dark background after silver staining, and some bands were masked (Fig. 2, lanes C and D).

DsRNA patterns of the Spanish CTV isolates. Seven different dsRNA patterns were obtained from the 24 Spanish CTV isolates that were analyzed (Fig. 3). Five of the seven patterns (lanes A to E) correspond to the local isolates in the IVIA collection (which are representative of the different Spanish citrus areas). The other two patterns (lanes F and G) are from T-385 and T-

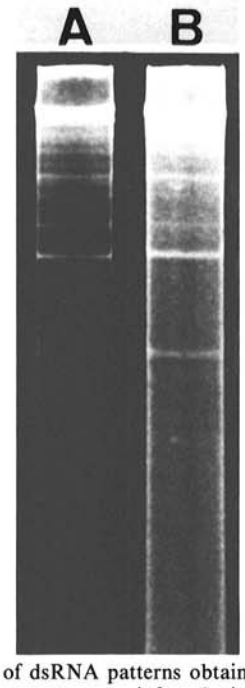

Fig. 1. Comparison of dsRNA patterns obtained from young shoots of a screenhouse-grown sweet orange infected with the T-308 citrus tristeza virus, with and without filtration of the ethanol-adjusted extract. Lane A: The ethanol-adjusted extract was filtered through a glass microfiber prefilter before CF-11 cellulose chromatography. Lane B: The extract was chromatographed without filtration.

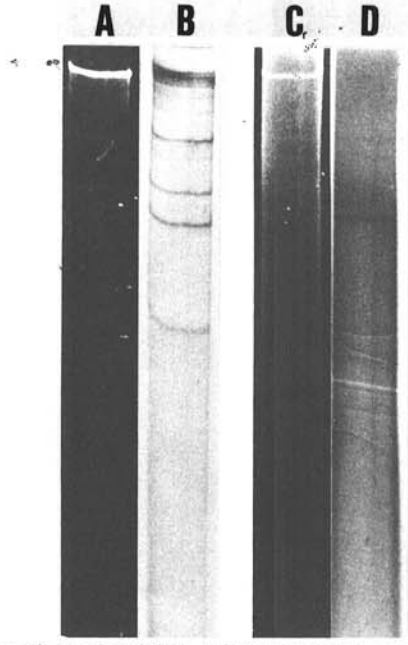

Fig. 2. Comparison of ethidium bromide and silver staining of gels for detection of minor dsRNA bands. Lanes A and B: DsRNA was purified from a citrus tristeza virus-infected field tree using the filtration step before CF-11 cellulose chromatography. The gel first was stained with ethidium bromide (lane A) and then with silver nitrate (lane B). Lanes C and D: A similar extract was chromatographed without previous filtration, and the gel was stained with ethidium bromide (lane C) and silver nitrate (lane D).

388, the two CTV isolates incorporated into the collection later and kept in the greenhouse. Isolates T-300, T-308, T-373, T-379, T-385, and T-388 all had unique patterns, whereas the remaining 17 isolates assayed showed dsRNA profiles that were essentially identical with that of T-362 (Fig. 3, lane C).

All isolates showed a strong band of 13.3 $\times$ 10^6 Da MW, corresponding to the full-length replicative form, and a readily observable 0.8 $\times$ 10^6 Da MW band. Bands of 2 and 1.2 $\times$ 10^6 Da MW were present in all isolates assayed, but their intensity was variable. For example, the 2 $\times$ 10^6 Da MW band was clearly visible in samples of T-308, T-373, T-385, and T-388 (Fig. 3, lanes B, D, F, and G) and not so intense in all the others, whereas the 1.2 $\times$ 10^6 Da MW band was strong only in T-308, T-373, and T-385 (Fig. 3, lanes B, D, and F). In addition, there were dsRNA bands specific for the following isolates: T-300 (1.9 $\times$ 10^6 Da MW), T-379 (1.8 $\times$ 10^6 Da MW), T-385 (1.85, 1.6, and 1.3 $\times$ 10^6 Da MW), and T-388 (1.5 $\times$ 10^6 Da MW). A 0.5 $\times$ 10^6 Da MW band was readily observed in T-308, T-373, and T-388, and it also was present with very low intensity in other isolates like T-385. Sometimes, the very weak bands could be seen only after silver staining (Fig. 2, lanes A and B).

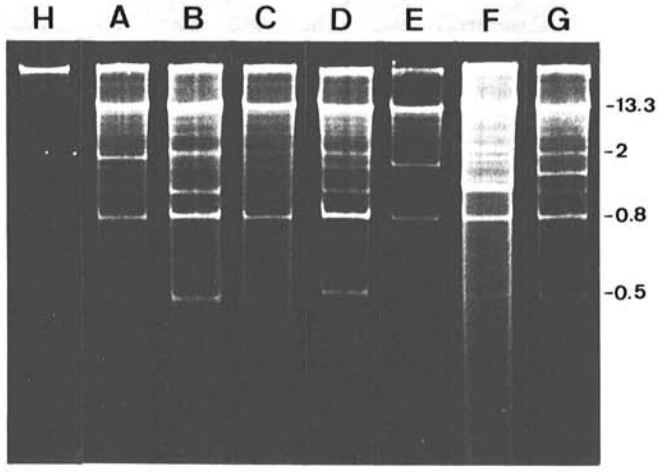

Fig. 3. DsRNA patterns obtained from a healthy sweet orange plant (lane H) and from plants infected with the following citrus tristeza virus isolates: T-300 (lane A), T-308 (lane B), T-362 or any of 17 other isolates (lane C), T-373 (lane D), T-379 (lane E), T-385 (lane F), and T-388 (lane G). Numbers on the right indicate molecular weights ($\times$ 10^6 Da), as determined from the standards (not shown).

A minimum of five extractions was performed with each isolate with sampling from two to four individual plants. The dsRNA profiles were repeatable when extractions were done from the same host and during the same season. Most of the isolates have been propagated three to 20 times along 5 yr, and their dsRNA pattern has remained unchanged with a single exception observed in T-385 (see below).

Seasonal variation of virus antigen titer and dsRNA patterns. ELISA and dsRNA analysis were performed on material harvested in January, May, September, and October on the 22 isolates maintained in the screenhouse and the two isolates kept in the greenhouse. The actual sampling dates in spring, summer, and autumn were selected to have a young flush of growth on the infected plants kept in the screenhouse. Generally, all CTV isolate samples assayed gave the highest ELISA values in the spring (beginning of May) and the lowest values at the end of summer (beginning of September). In autumn (mid-October), the ELISA values increased again, and intensities were generally the same in winter (mid-January). DsRNA patterns were clear and strong in spring and autumn, weak in summer, and very weak in winter.

The degree of seasonal variation of virus antigen titer and dsRNA recovery of seven CTV strains (showing different dsRNA patterns) are summarized in Table 1. Strains T-385 and T-388, kept under greenhouse conditions, showed only minor changes in ELISA values, and dsRNA recovery was good in each of the four sampling dates. The remaining isolates, all kept under screenhouse conditions, showed seasonal variations in virus antigen titer and intensity of the dsRNA patterns. No correlation was observed between symptom severity and antigen accumulation or dsRNA pattern intensity. For example, T-385 and T-388 showed great differences in symptom intensity induced on Mexican lime; however, antigen titer and dsRNA recovery from both strains were very similar.

The dsRNA profiles obtained for each CTV isolate at the different harvest times remained unchanged except for strains T-300 and T-385. T-300 (Fig. 4) had a very strong 1.9 $\times$ 10^6 Da MW band that was readily observed in spring and autumn, whereas in winter and summer it showed a weak triplet in the same position, which was indistinguishable from that of most Spanish isolates assayed (Fig. 3, lane C).

Influence of host on dsRNA pattern. Variation in total recovery of dsRNA also was observed in the different hosts assayed. Recovery was best from Pineapple sweet orange (Fig. 5E), Dweet tangor (Fig. 5D), and Etrog citron (data not shown). Lemon was usually a good host and yielded more dsRNA than Mexican lime (Fig. 5A versus Fig. 5B), whereas sour orange gave only the full-length dsRNA (Fig. 5C).

TABLE 1. Seasonal variation of virus antigen titer and dsRNA pattern for several citrus tristeza virus (CTV) strains

CTV strains		Spring[a]		Summer[a]		Autumn[a]		Winter[a]	
Number[b]	Symptom severity[c]	Antigen titer[d]	DsRNA pattern[e]	Antigen titer	DsRNA pattern	Antigen titer	DsRNA pattern	Antigen titer	DsRNA pattern
T-300	+++	0.789 ± 0.160	strong	0.263 ± 0.004	weak	0.497 ± 0.081	strong	0.127 ± 0.028	very weak
T-308	++++	0.907 ± 0.148	strong	0.503 ± 0.030	strong	0.961 ± 0.073	strong	1.294 ± 0.078	weak
T-311	++	0.824 ± 0.052	strong	0.122 ± 0.016	weak	0.488 ± 0.123	strong	0.460 ± 0.021	very weak
T-373	++	0.813 ± 0.095	strong	0.148 ± 0.001	weak	0.330 ± 0.053	strong	0.362 ± 0.037	very weak
T-379	++	0.599 ± 0.048	strong	0.146 ± 0.057	weak	0.284 ± 0.045	strong	0.384 ± 0.033	very weak
T-385	+	0.936 ± 0.074	strong	1.045 ± 0.005	strong	1.068 ± 0.202	strong	1.340 ± 0.038	strong
T-388	+++++	1.029 ± 0.142	strong	1.320 ± 0.066	strong	0.918 ± 0.143	strong	0.952 ± 0.105	strong

[a] Seasonal variations of antigen titer and dsRNA pattern were studied by sampling in spring (beginning of May), summer (beginning of September), autumn (mid-October) and winter (mid-January). The actual sampling dates in spring, summer, and autumn were selected to have a young flush of growth in the plants grown under screenhouse conditions. Average maximum and minimum temperatures (C) for each month were as follows: March: 21.2/6.0, April: 19.7/8.4, May: 22.2/12.1, (Spring); June: 24.9/14.7, July: 29.2/18.3, August: 29.6/18.8 (Summer); September: 29.4/16.4, October: 22.2/11.4, November: 17.7/6.3 (Autumn); December: 18.1/6.2, January: 17.5/6.7, February: 18.3/4.6 (Winter).

[b] Plants infected with T-300, T-308, T-311, T-373, and T-379 were grown in a screenhouse. Strains T-385 and T-388 were kept in a temperature-controlled greenhouse.

[c] Symptom intensity (vein clearing and stem pitting in Mexican lime): (+) very mild, (++) mild, (+++) moderate, (++++) severe, and (+++++) very severe.

[d] Ratio between the mean absorbance (A_{405}) value of each isolate and the value of the standard in the same plate. Means were obtained from four repetitions (two analyses from a pool of young bark from individual plants repeated in two consecutive years), and all values were corrected for the mean value of the negative control.

[e] DsRNA pattern was considered strong when all the bands were readily observed after staining with ethidium bromide, weak when minor bands were difficult to see with ethidium bromide staining, and very weak when minor bands could be observed only after silver staining.

Some variation in the dsRNA patterns of certain CTV isolates also was found in specific hosts. T-300 dsRNA purified from greenhouse-grown Pineapple sweet orange or Dweet tangor seedlings showed the same electrophoretic profile as that obtained from screenhouse-grown navel orange/Troyer citrange plants in spring and autumn (as shown in Fig. 4A and C), whereas purification from lemon or Mexican lime seedlings (grown in the greenhouse) yielded the same profile as that obtained from navel plants in summer and winter (as shown in Fig. 4B and D).

Minor variations also were observed in other strains. For example, when T-373 was multiplied in the five hosts, a minor band was consistently observed in Pineapple sweet orange that was not detectable in the other hosts (see arrow in Fig. 5). A similar difference was observed with T-315.

These host-induced variations of dsRNA profiles have been observed consistently in a minimum of three extractions over a 2-yr period.

Detection of isolate segregation by dsRNA analysis. A Mexican lime plant aphid-inoculated with T-385 was used as budwood source to graft inoculate several citron plants. When these plants were analyzed individually for dsRNA, a variety of patterns was observed (Fig. 6). Those patterns have remained unchanged in three successive extractions over an 8-mo period. Each of the major bands observed in the different subcultures had migration that was similar to the migration of the different bands present in the original source plants (Fig. 6).

DISCUSSION

DsRNA, including the full-length replicative form and several additional species, were isolated from plants infected with all of the CTV isolates assayed. A minimum of 5 g of tissue per lane in the electrophoresis gel was necessary to readily detect the complete dsRNA pattern of some Spanish isolates, particularly when sampling was done under unfavorable conditions (for example, field samples, during the winter, old tissue). Detection of minor bands (for example, the 0.5×10^6 Da MW band) in certain isolates required, in addition, silver staining. Dodds et al (8) reported successful detection of the dsRNA bands purifying from 1 to 2 g of tissue. Variations in dsRNA yield under favorable conditions may be due to strain differences in the rate of virus multiplication. With certain CTV isolates, we also have obtained sufficient dsRNA recovery from 1 g of tissue.

Concentration of the extracts also resulted in the concentration of contaminant polysaccharides which increased background fluorescence, when staining with ethidium bromide, or darkened gels after silver staining, and masked the presence of minor bands. This problem was avoided partially by a filtration step before fractionation on CF-11 cellulose to remove those contaminants. This step also diminished column plugging caused by flocculated materials and increased the elution rate. Though some dsRNA probably was lost when prefiltering, detection of minor bands was improved by the cleaner background, particularly when silver staining.

DsRNA analysis enabled us to identify seven different patterns among the 24 CTV isolates assayed. Six of these patterns corresponded to individual isolates, and the seventh pattern was common to the other 18 isolates tested. Differentiation of patterns was done primarily on a qualitative basis, that is, differences in the number or position of the non-full-length replicative form dsRNA bands. Differences in the intensity of certain bands also were observed among CTV isolates included in a given group by their dsRNA profile (Fig. 3, lane C). These quantitative differences, however, were observed only under optimal conditions (vigorously growing plants in greenhouse conditions), and, thus, they were considered unreliable to distinguish those isolates.

Virus antigen titer and total dsRNA recovery were not always correlated with the intensity of symptoms induced on Mexican lime. For example, T-385, the mildest isolate biologically, had an antigen titer similar to T-388, the most severe isolate by host assay, and both readily yielded detectable amounts of dsRNA from 1.5 g of tissue. Symptom intensity likewise could not be correlated with any particular dsRNA pattern. For example, isolates T-311 and T-379 which have similar biological activity gave different dsRNA patterns (Fig. 3, lanes C and E). To the contrary, isolates T-311 and T-344 were biologically different (2) but had the same dsRNA pattern (Fig. 3, lane C).

The only CTV isolate assayed that induced the seedling yellows reaction and conspicuous stem pitting on grapefruit and sweet orange was T-388 (3). This isolate had a unique dsRNA pattern which included a 0.5×10^6 Da MW band. This low-molecular-weight band has been associated previously with severe CTV strains able to induce the seedling yellows reaction or stem pitting on grapefruit (9,10). We, however, observed the production of

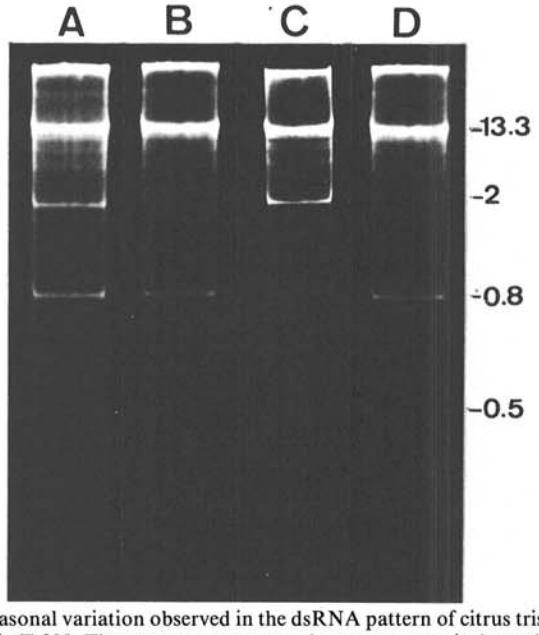

Fig. 4. Seasonal variation observed in the dsRNA pattern of citrus tristeza virus strain T-300. The same sweet orange plant was successively analyzed in spring (lane A), summer (lane B), autumn (lane C), and winter (lane D). Numbers on the right indicate molecular weights ($\times 10^6$ Da).

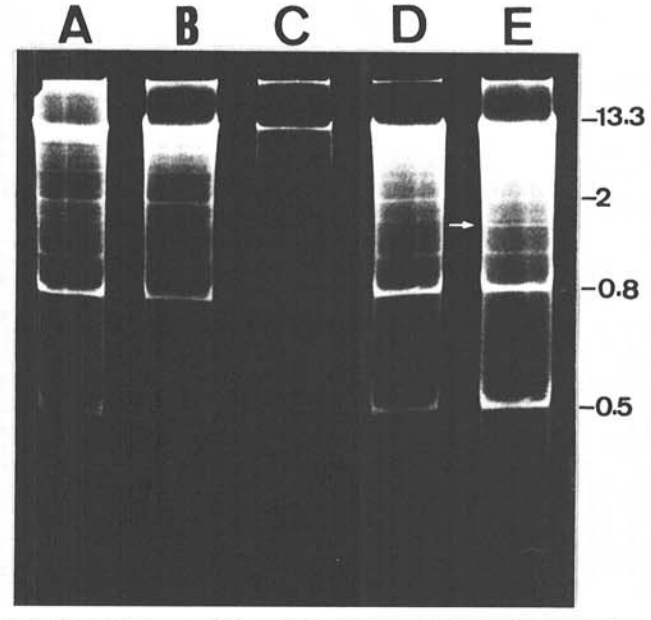

Fig. 5. Host-induced variations in dsRNA recovery and dsRNA pattern of citrus tristeza virus strain T-373. Electrophoretic profiles A–E were obtained from lemon (A), Mexican lime (B), sour orange (C), Dweet tangor (D), and Pineapple sweet orange (E). Numbers on the right indicate molecular weight ($\times 10^6$ Da). Arrow in lane E (Pineapple sweet orange) indicates a dsRNA band that is not present in extracts from other hosts.

this band by several moderate or mild Spanish isolates that were unable to induce stem pitting on grapefruit (for example, T-373, Fig. 3D) and by an isolate causing only mild pitting on Mexican lime (for example, T-385, Fig. 3G). Identification of severe strains based on the presence of the 0.5×10^6 Da MW band is not reliable among the CTV isolates currently infecting citrus in Spain. Albertini et al (1) also found this low-molecular-weight band in their CTV strain K which failed to induce symptoms even on Mexican lime.

DsRNA analysis enabled us to identify CTV strains under field or screenhouse conditions throughout the year. However, only from tissue collected in spring and autumn could the complete dsRNA profile be readily observed with ethidium bromide staining. In other seasons, silver staining was necessary to detect some minor bands. The complete dsRNA profile could be identified by ethidium bromide staining at every sampling period only for T-308, a highly multiplying CTV strain. The weakest dsRNA patterns were obtained from plants in winter, even though virus antigen titer was higher in winter than in summer (Table 1). These results differ from those reported from California (8), where better dsRNA recovery was obtained in winter than in summer. This difference probably is due to the lower average temperatures in Moncada (Valencia) during summer months compared with those same months in Riverside (California) (see Table 1 and reference 8). Under our conditions, availability of young flush tissue seems to be more important for dsRNA recovery than temperature. Usually, citrus in Valencia have a late summer flush but none in winter.

Selection of an adequate host is important to readily identify dsRNA patterns. Sweet orange, citron, and Dweet tangor were the best hosts, in agreement with results obtained by Dodds et al (8). However, under our conditions, lemon yielded more dsRNA than Mexican lime, in contrast to the results reported by these authors for the California isolates (8). Sour orange was consistently the poorest host for dsRNA analysis of all the CTV strains assayed. In all cases, we were able to detect only the full-length band. However, when samples were taken from several individual CTV-infected field trees of clementine/sweet orange/sour orange and compared for dsRNA content, the three citrus species showed patterns of similar intensity and composition (data not shown). The pattern of those trees corresponded to that of CTV isolate T-315 (data not shown), and they were located in an area where this strain was collected. This could be an indication that sour orange is able to reproduce the full dsRNA profile when receiving a continuous supply of inoculum. Alternatively, it might be a different strain having the same dsRNA profile as T-315. Dodds et al (8) found that most CTV isolates inoculated on sour orange yielded only the full-length band but that one of them did multiply well in this host and yielded the complete dsRNA profile.

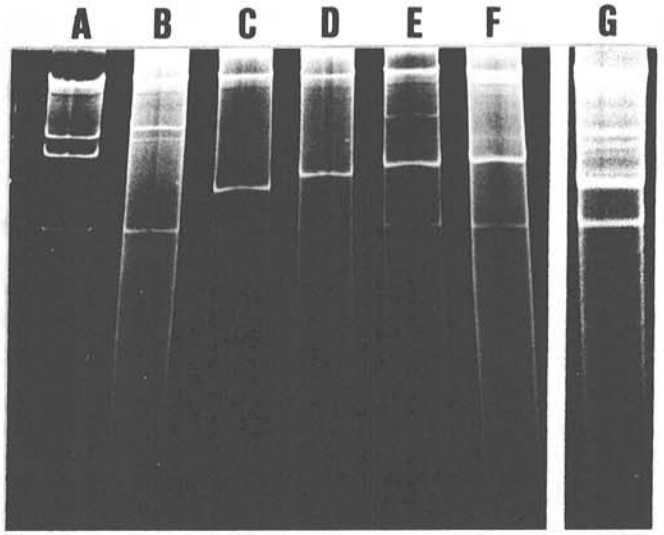

Fig. 6. Comparison of dsRNA patterns obtained from plants infected with citrus tristeza virus isolate T-385 (lane G) or several subcultures (lanes A to F) obtained from this isolate by graft inoculation.

Most of the CTV isolates assayed showed seasonal and host variation in total dsRNA recovery but no changes in the dsRNA profile. However, a few of them did show variation in their pattern depending on the season (T-300 and T-385) or the host (T-300, T-373, T-315, and T-385). These variations might be explained by the presence in each infected plant of several strains inducing specific dsRNA patterns. The ratio among the different components in the mixture might depend on the host or the environmental conditions. Segregation obtained by graft inoculation from T-385, a CTV isolate that originally had been transmitted by aphids, gives support to this hypothesis. So far, this is the only case of strain segregation that we have detected, although many propagations of the other strains, and particularly of T-300, have been made in the last few years.

Evidence for CTV strain segregation has been observed by other authors by biological methods (20,23,24). Dodds et al (10) hypothesized the presence of a mixture of strains to explain the presence, in some CTV isolates, of two dsRNA bands with slightly different mobilities in the position expected for the full-length replicative form. Jarupat et al (16) gave the same explanation for the low intensity or disappearance of specific dsRNA bands of some CTV isolates when multiplied in grapefruit. However, this is the first time that a strain separation has been documented by the dsRNA profiles obtained from the original isolate and its subcultures. These findings raise some questions about the genetic identity of what is currently known as CTV strains.

Strain T-388 is, at present, a major threat to the Spanish citrus industry, and a program to eradicate this strain is under way. This program is based mainly on the identification of plantings that were topworked with the satsuma cultivar carrying this CTV strain. However, this strain may have spread into adjacent plantings of other cultivars. The availability of a quick and reliable method of strain identification is critical to monitor the presence of T-388 in areas where local CTV strains are endemic and to avoid further spread of this severe strain because biological identification is long term (6–9 mo) and expensive. Because T-388 has a unique dsRNA pattern that enables it to be distinguished from other local strains, dsRNA analysis should be a very helpful tool for this control program.

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