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Effect of Host Passage on dsRNAs of Two Strains of Citrus Tristeza Virus

T. Jarupat, J. A. Dodds, and C. N. Roistacher

ABSTRACT. Two strains of CTV (T505 and SY560) were maintained in sweet orange and reliable, reproducible dsRNA profiles were obtained in several analyses in stained 6.0% polyacrylamide gels. The positions of some dsRNA segments in electrophoresed gels were the same for both strains and others were unique for each strain. When these strains were graft inoculated from sweet orange to sweet orange, no effect on dsRNA profiles was observed. When the strains were grafted from sweet orange to grapefruit, the quantity of dsRNA was less than in sweet orange, particularly for dsRNAs other than the replicative form (RF) (MW = 13.3 x 10^6), and the number and intensity of stained bands were altered in a reproducible way.

A dsRNA (MW = 1.4 x 10^6) specific for T-505 in sweet orange was not detected in grapefruit and it did not return to detectable levels in subsequent graft inoculation back to sweet orange, *Citrus excelsa*, lemon or grapefruit. A dsRNA (MW = 1.7 x 10^6) specific for SY-560 in sweet orange was barely detected in grapefruit. On subsequent graft inoculation from grapefruit, this dsRNA returned to readily detectable amounts in sweet orange and *C. excelsa*, but remained barely detectable or undetectable in subinoculated grapefruit and lemon.

These results suggest that these two strains of CTV are probably mixtures, and that the ratios of virus components in such mixtures is affected by the host used to propagate the strain.

The detection of disease specific dsRNAs in extracts from plants infected with RNA viruses has been used as a means to identify some virus groups, individual viruses, and even virus strains (8, 12, 17). Some virus groups, such as the potyviruses, are not well suited to this kind of analysis, whereas the closteroviruses are one group that normally gives good results with this method. Descriptive analysis of dsRNAs of citrus tristeza virus (CTV), beet yellows virus, carnation necrotic fleck virus (4, 5, 6, 7, 11), grape leafroll virus (13) have been obtained from relatively small tissue wieghts (0.1 g to 7.0 g).

When chromatography on cellulose powder is used for rapid purification, optimum results for CTV are obtained when attention is paid to host species, past environment, virus strain, and gel medium used for analysis. Sweet orange, citron, and *Citrus excelsa* were hosts in which reproducible dsRNA results could be obtained for CTV (5). Other hosts, particularly grapefruit and sour orange, gave much poorer yields. Two dsRNAs (MW = 13.3 x 10^6 and 0.8 x 10^6) have been consistently detected for all CTV strains (5, 6, 7). One of these (MW = 13.3 x 10^6) is presumed to be the replicative form (RF) of CTV, since it is twice the size of the genome (1). The pattern of other dsRNAs resolved by polyacrylamide gel electrophoresis differed for each of four strains.

The ability to detect CTV dsRNA from field trees has been reported (6). In a subsequent study, recovery of dsRNA from samples collected from sweet orange in the field was variable, and was not reliable when sampling was done in the hottest part of the growing season (5). Detection of expected dsRNAs from field samples was most reliable in the spring in southern California.

In the course of these studies, we became aware of apparent reproducible changes in dsRNA patterns for a given strain, which depended on the host used to propagate the virus. The objective of this study was to determine the effect of host passage on CTV dsRNA patterns. This involved passage of two strains of CTV from a reliable host (sweet orange) to a host that induced change (grapefruit) and then reinoculation of sweet orange and other hosts to determine if change was transient or not.

MATERIALS AND METHODS

Hosts. Madam Vinous sweet orange, *C. excelsa*, Duncan grapefruit, and lemon were used as experimental hosts for CTV. They were trained to grow as a single shoot, with one or two plants in each 3-liter pot.

Strains. Two strains of CTV, T505 and SY560, were used. Strain T505 was collected from a Valencia sweet orange in 1977 in the Central Valley of California. It indexed negative for citrus psorosis virus and citrus exocortis viroid. 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 biologically typical of isolates obtained from southern California, except that it can cause mild stem pitting of sweet orange seedlings. Strain SY560 was isolated from *C. macroptera* in the University of California, Riverside (UCR) citrus collection. The source tree was in decline when the virus was isolated and it died subsequently. The strain indexed negative for citrus psorosis virus, citrus vein enation virus, and citrus exocortis viroid. It produced a severe reaction in Mexican lime and *C. excelsa*, and a classical seedling yellows reaction in grapefruit and sour orange.

Inoculation. Experimental plants were inoculated with each CTV strain by bud-grafting from infected plants to three healthy seedlings of each citrus species. The source of inoculum was either the original sweet orange seedlings used to maintain the strains, the experimentally infected plants, or non-inoculated plants.

Inoculation paths for each strain. Strains T505 and SY560 were inoculated to either sweet orange or grapefruit on the first host passage, and were inoculated from one of these to sweet orange, grapefruit, *C. excelsa* or lemon on the second passage. The plants used for the first passage had been infected for 6 months at the time they were used as inoculum for the second passage. The inoculation paths are summarized in table 1.

Times of harvest. Shoots were removed from new flush growth at 6, 10 and 14 months after inoculation. Green bark was used for dsRNA isolation.

dsRNA analysis. Each dsRNA sample was isolated from a pool of 2.0 g of bark tissue from three plants. The dsRNA was purified by two cycles of CF-11 cellulose chromatography and analyzed by electrophoresis on 6.0% polyacrylamide gels as previously described (5). The dsRNA recovered from 1.0 g of tissue was analyzed on a single gel channel. Electrophoresed gels were stained with ethidium bromide (60 ng/ml) and photographed.

RESULTS

Comparison of dsRNA profiles of strains T505 and SY560 in sweet orange. The dsRNAs of these strains

TABLE 1
A SUMMARY OF THE PATH OF INOCULATIONS USED TO TEST THE EFFECT OF HOST PASSAGE ON dsRNAs OF TWO STRAINS OF CTV, AND A KEY TO THE POLYACRYLAMIDE GELS WHICH WERE USED TO DETECT AND DESCRIBE THE dsRNAs ISOLATED FROM THE PLANTS ANALYZED

	Fig. legend[y]	
Inoculation path[z]	T505[x]	SY560[x]
Sweet orange	1a	2a
Sweet orange	1b	2b
Sweet orange	1c	2c
Grapefruit	1d	2d
C. excelsa	3a	3c
Lemon	3f	3h
Grapefruit	1e	2e
Sweet orange	1f	2f
Grapefruit	1g	2g
C. excelsa	3b	3d
Lemon	3g	3i

[z]Strains T505 and SY560 were originally in sweet orange, and were inoculated to either sweet orange or grapefruit on the first host passage, and were inoculated from one of these to sweet orange, grapefruit, *Citrus excelsa* or lemon on the second passage.
[y]The numbers and letters refer to gel channels illustrated in fig. 1, 2, and 3, e.g. 1b refers to the gel channel labeled b in fig. 1.
[x]T505 and SY560 are codes which identify the two strains of CTV used.

have been described previously (5, 7). Results obtained in this study (fig. 1, 2, and 3) were similar to the previous results. A major dsRNA (MW = 13.3 x 10^6) and a minor dsRNA (MW = 0.8 x 10^6) were common to both isolates. A dsRNA (MW = 1.4 x 10^6) was characteristic of strain T505, and two dsRNAs (MW = 1.7 and 0.5 x 10^6) were characteristic of strain SY560.

Effect of host passage on strain T505. The dsRNAs associated with infection of sweet orange by this strain are illustrated in fig. 1a. When this strain was passed sequentially through sweet orange, no change was detected in either the first (fig. 1b) or second (fig. 1c) passage. This was also true for this inoculation path when the host for the second passage was *C. excelsa* (fig. 3a) or lemon (fig. 3f). Sweet orange, *C. excelsa* and lemon induced no obvious temporary or permanent change in dsRNA patterns of this strain.

By contrast, when grapefruit was used for the first passage (fig. 1e), one of the dsRNAs (MW = 1.4 x 10^6, and designated as band X in fig. 1) was not detected as readily, if at all, in comparison with the staining intensity of the other dsRNAs which characterize strain T505. This result was repeated when strain T505 from the first passage in sweet orange (fig. 1b) was inoculated to grapefruit at the second passage (fig. 1d). The dsRNA of interest (band X in fig. 1) was still not detected in grapefruit (fig. 1g), and neither was it detected in sweet orange (fig. 1f), *C. excelsa* (fig. 3b), or lemon (fig. 3g) when these hosts were used for the second passage and when the source for the first passage was grapefruit.

Passage of strain T505 through grapefruit appears to result in the loss of a specific dsRNA band, not only in the host in which this effect is seen, but also in other hosts which are

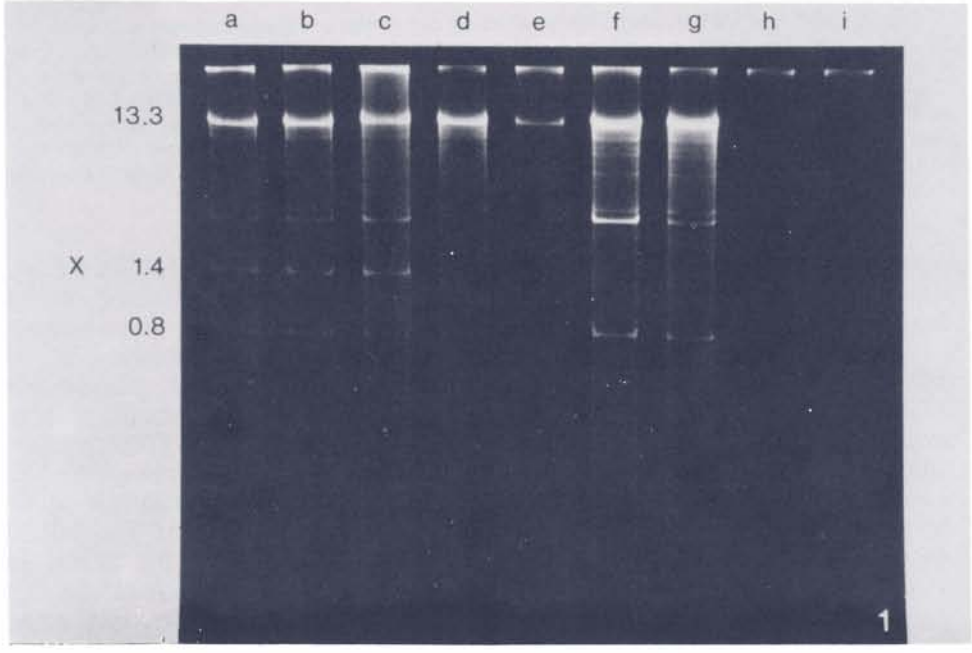

Fig. 1. DsRNA of CTV strain T505 purified by two cycles of cellulose chromatography from 1.0 g of tissue. The hosts were either sweet orange (a, b, c, f, and h) or grapefruit (d, e, g and i) and the analysis was on the original source plant (a), the first (b, and e), or the second (c, d, f, and g) passage. The band marked x was affected by host passage. Uninfected plants were included as controls (h, i). Purified dsRNAs were electrophoresed on 6.0% polyacrylamide gels and stained with ethidium bromide. Direction of electrophoresis is from top to bottom. Molecular weights were estimated graphically by the method of Bozarth and Harley (2).

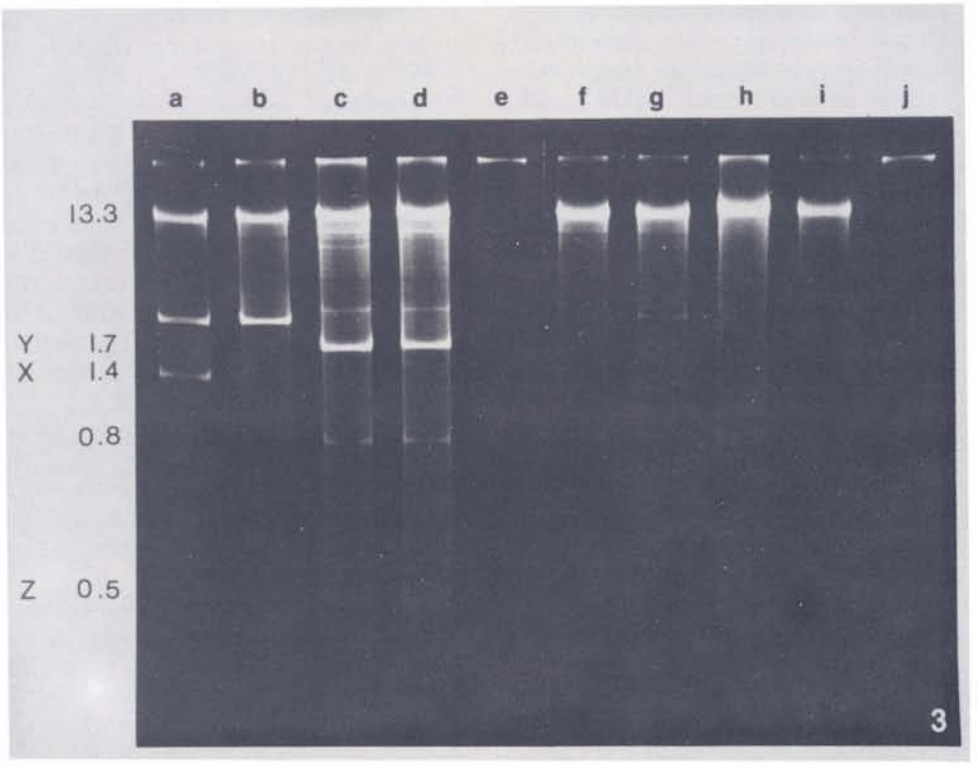

Fig. 2. DsRNA of CTV strain SY560 purified by two cycles of cellulose chromatography from 1.0 g of tissue. The hosts were either sweet orange (a, b, c, f, and h) or grapefruit (d, e, g and i) and the analysis was on the original source plant (a), the first (b and e) or the second (c, d, f, and g) passage. The band marked y was affected by host passage. The band marked z distinguishes strain SY560 from strain T505. Uninfected plants were included as controls (h, i). Purified dsRNAs were electrophoresed on 6.0% polyacrylamide gels and stained with ethidium bromide. Direction of electrophoresis is from top to bottom. Molecular weights were estimated graphically by the method of Bozarth and Harley (2).

capable of expressing this dsRNA, when they are inoculated with the modified strain.

Effect of host passage on strain SY560. The dsRNAs associated with infection of sweet orange by this strain are illustrated in fig. 2a. When this strain was passed sequentially through sweet orange, no change was detected in either the first (fig. 2b) or second (fig. 2c) passage. This was also true for this inoculation path when the host for the second passage was *C. excelsa* (fig. 3c). Sweet orange, and *C. excelsa* appear to induce no obvious temporary or permanent change in dsRNA patterns of this strain.

By contrast, when grapefruit was used for the first passage (fig. 2e), one of the dsRNAs (MW = 1.7 x 10^6, and designated as band Y in fig. 2) was not detected as readily in comparison with the staining intensity of the other dsRNAs which characterize strain SY560. This result was repeated when strain SY560 from the first passage in sweet orange (fig. 2b) was inoculated to grapefruit at the second passage (fig. 2d). The dsRNA of interest (band y in fig. 2) was detected weakly in grapefruit (fig. 2g), but was readily detected in sweet orange (fig. 2f), and *C. excelsa* (fig. 3d) when these host were used for the second passage and when the source for the first passage was grapefruit.

A dsRNA (MW = 0.5 x 10^6) that distinguished strain SY560 from strain T505 is designated as band Z in

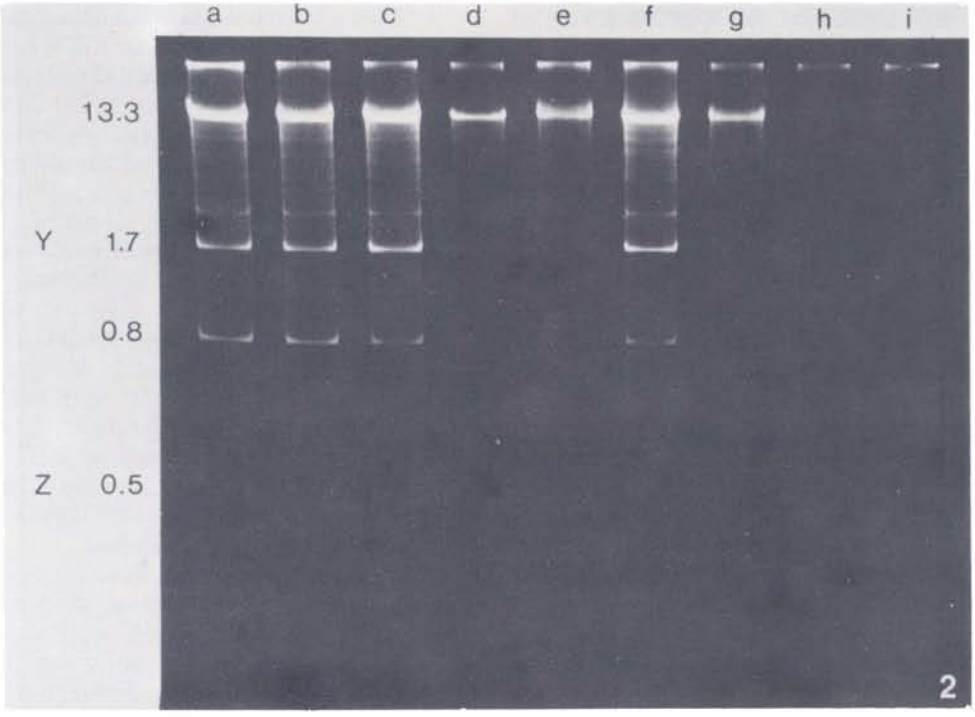

Fig. 3. DsRNA of CTV strains T505 (a, b, f and g) and SY560 (c, d, h, and i) purified by two cycles of cellulose chromatography from 1.0 g of tissue. The hosts were either *Citrus excelsa* (a-e) or lemon (f-j) and the analysis was on the second passage from plants inoculated from sweet orange (a, c, f, and h) or grapefruit (b, d, g, and i). The bands marked x and y were affected by host passage. The band marked z distinguishes strain SY560 from strain T505. Uninfected plants were included as controls (e, j). Purified dsRNAs were electrophoresed on 6.0% polyacrylamide gels and stained with ethidium bromide. Direction of electrophoresis is from top to bottom. Molecular weights were estimated graphically by the method of Bozarth and Harley (2).

fig. 2 and 3. It was detected in all hosts tested at each passage, but detection was weak in grapefruit (fig. 2d, 2e, and 2g) and lemon (fig. 3i, and 3g) when the overall recovery of dsRNA was not as high as that from sweet orange (fig. 2a, 2b, 2c, 2f) or *C. excelsa* (fig. 3c and 3d).

The strain SY560 specific dsRNA (MW = 1.7 x 10^6) that was prominent in sweet orange and *C. excelsa*, but which was weak in grapefruit, was also difficult to detect on second passage in lemon (fig. 3h and 3i), regardless of whether sweet orange or grapefruit was used for the first passage.

Passage of strain SY560 through grapefruit and lemon appears to result in the repression of a specific dsRNA band in the host in which this effect is seen, but the loss is not permanent because it reappears in other hosts which are capable of expressing this dsRNA, when they are inoculated with the modified strain.

General effect on dsRNA yield in grapefruit. All gels illustrate the amount of dsRNA recovered from 1.0 g of bark tissue. There did appear to be a negative effect of grapefruit on dsRNA yield (fig. 1d, 1e, 2d, 2e, 2g), especially in a reduction in the amount of dsRNAs other than the 13.3 x 10^6 dsRNA (the replicative form, RF) in comparison with the RF dsRNA. This confirms previous results (5). A similar effect previously reported for lemon (5) was not so apparent in the current study.

DISCUSSION

The results presented indicate that while dsRNA results are gener-

ally consistent for a given strain of CTV in a given citrus host, there are effects of host on dsRNA patterns observed in stained polyacrylamide gels. In one case, involving strain T505, evidence was obtained for a significant repression of a specific dsRNA in grapefruit. The dsRNA did not return upon subsequent infection of hosts that can support its detection. Whether this repression is permanent cannot be determined until subsequent transfers are tested for eventual reappearance of this dsRNA.

Results for strain SY560 are not so clear-cut. The MW = 1.7 x 10^6 dsRNA appeared to be repressed in grapefruit. This dsRNA was reported to migrate only slightly faster than a minor dsRNA in a previous study (7), and the observed repression may not be what it seems. Another interpretation of the results is that the 1.7 x 10^6 dsRNA is repressed to near non-detectable levels, and the band that remains is actually a different, MW = 1.8 x 10^6 dsRNA common to several isolates of CTV. The interpretation of the level of repression of the 1.7 x 10^6 dsRNA in grapefruit is a separate consideration from the observation that this dsRNA was able to return to normal levels of detection in sweet orange regardless of the host used for the first passage.

There have been several biological results which establish that CTV can exist as mixtures of components in individual plants (3, 9, 10, 14, 16). The effect of host passage on virulence of CTV isolates, and the selection of protecting isolates is a topic of a companion paper (15). Results of this study provide physical evidence to indicate that even well-characterized strains of CTV may not be single entities, but may consist of multiple components capable of being expressed to relatively different levels depending on the host into which the mixture is inoculated. The possibility that some hosts could even exclude a detectable component of the mixture is further established.

These observations have consequences for cross-protection studies, in that they reinforce the likelihood that strains useful for protection in one host may not be suitable in another host, especially if that host can induce demonstrable change in the quality of the isolate.

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