Etiology

Identification of Small Single- and Double-Stranded RNAs Associated with Severe Symptoms in Beet Western Yellows Virus-Infected *Capsella bursa-pastoris*

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ABSTRACT

Falk, B. W., and Duffus, J. E. 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa-pastoris*. Phytopathology 74:1224-1229.

An isolate (ST9) of beet western yellows virus (BWYV) that caused severe stunting symptoms in infected plants of *Capsella bursa-pastoris* was compared with two isolates (ST1 and STFL) that caused typical symptoms. Yields of purified virions were 10 times greater from *C. bursa-pastoris* infected with ST9 than with ST1 or STFL. Purified ST9 virions contained two single-stranded (ss-) RNAs of 1.9 and 0.93×10^6 M_r whereas ST1 and STFL virions each had only one ss-RNA of 1.9×10^6 M_r. The ST9-infected plants also contained two prominent double-stranded RNAs (ds-RNAs) that were not found in ST1- or STFL-infected plants. These RNAs were 2.2 $\times 10^6$ M_r and smaller than 4.6×10^5 M_r. Both new ds-RNAs were present in much higher concentrations than were the other ds-RNAs from the ST9- or STFL-infected plants. No differences in proteins or densities were detected between the ST9 and the STFL or ST1 virions. The association of the extra RNAs with the ST9 isolate and the resulting severe symptom development on *C. bursa-pastoris* is discussed.

Additional key words: luteovirus, satellite.

Beet western yellows virus (BWYV) is a member of the luteovirus group (29), and is one of the most widespread and economically important plant viruses in the world. BWYV is serologically related to the RPV isolate of barley yellow dwarf virus (BYDV), to potato leafroll virus (PLRV), and to several other lesser known luteoviruses such as carrot red leaf virus (32). Recently, several luteoviruses have been characterized and found to contain a single species of single-stranded RNA (ss-RNA) of $1.8–2.0 \times 10^6$ M_r (relative mass) (2,17,25,28,31), and there is evidence that the ss-RNA of PLRV is "plus" polarity and has a genome-linked protein of M_r 7,000 (23). In 1977 we reported that two ss-RNAs of 1.9 and 0.9×10^6 M_r, were associated with purified virions of the ST9 isolate of BWYV (7). This conflicts with the data for other luteoviruses and other BWYV isolates that have been characterized (17) in that none have been found to have a lower M_r species that might be similar to the 0.9×10^6 M_r species found with the ST9 BWYV isolate. We originally used the ST9 isolate for our studies on BWYV RNAs because early work showed that it was much easier to purify substantial amounts of this isolate than of other BWYV isolates (J. E. Duffus, *unpublished*). However, it also was observed that the ST9 isolate caused very severe, somewhat atypical symptoms in infected *Capsella bursa-pastoris*, Medic., the common indicator for BWYV infection.

Therefore, we reinvestigated whether two ss-RNAs were associated with BWYV virions and report that the extra 0.9×10^6 M_r RNA is associated only with a severe isolate, ST9.

MATERIALS AND METHODS

Virus and vector maintenance. Virus isolates used in this study have been previously described. The ST1 isolate was originally

isolated from field-infected radish (*Raphanus sativus* L.) plants in California (6). The ST9 isolate was isolated from naturally infected broccoli (*Brassica oleracea* var. *botrytis*) in California (7). The STFL isolate is the spring yellows isolate obtained from naturally infected lettuce (*Lactuca sativa* L.) in Florida (9). All were maintained in *C. bursa-pastoris* by aphid transfer. Viruses that were used as controls were maintained by mechanical transfer. Tobacco mosaic virus (TMV) was kept in *Nicotiana tabacum* 'Xanthi,' and brome mosaic virus (BMV) in *Avena sativa* L. as previously described (10). Turnip yellow mosaic virus (TYMV) was obtained from P. R. Desjardins, University of California, Riverside, and maintained in *Brassica pekinensis*. Cucumber mosaic virus (CMV) was isolated from naturally infected celery (*Apium graveolens* L.) in Florida and maintained in *Capsicum annuum*.

The green peach aphid, *Myzus persicae* Sulzer, was used for all BWYV transmissions. Nonviruliferous *M. persicae* were reared on radish (*Raphanus sativus* L. 'White Icicle') or pepper (*Capsicum annuum* L.) in a growth room at 16 hr light and 26 C. Virus transmissions were done as described previously (6,8,9) by giving groups of aphids 24-hr acquisition access periods (AAP) to detached virus-infected leaves in sealed petri dishes. Groups of aphids were then transferred and caged on test seedlings of *C. bursa-pastoris* for 48- to 72-hr inoculation access periods (IAP). When the IAP was complete, cages were removed and plants were sprayed with Ambush®.

Several plant species were tested as hosts for ST9 and STFL by both aphid and mechanical inoculation experiments. Mechanical inoculations were done with suspensions in 0.03 M phosphate, pH 7.0, and with Carborundum as an abrasive. Three weeks after inoculation, recoveries were made from aphid-inoculated test species back to *C. bursa-pastoris* and the symptom type on *C. bursa-pastoris* was used to determine whether the test species were hosts for STFL or for the severe type of ST9 (characterized by severe symptom development on *C. bursa-pastoris*). Mechanically inoculated species were tested as hosts for ST9 by analysis of double-stranded (ds-) RNAs by polyacrylamide gel electrophoresis.

Virion purification. Virions of all three BWYV isolates were purified from infected *C. bursa-pastoris* by using modifications of methods used for other luteoviruses (3,30,32). Fresh or frozen tissues were homogenized in two volumes of extraction buffer (0.1 M citrate, 0.05 M MgCl₂, 0.2% thioglycollic acid, pH 6.0) in a Waring blender. Driselase (Plenum Scientific) was added to a concentration of 1.0% to the unfiltered homogenate and this was stirred at room temperature for 6–7 hr. Chloroform:butanol (1:1) was added to a concentration of 25% (v/v) and the solution was stirred vigorously for 1 min. The emulsion was centrifuged at 5,500 g (max.) in the Beckman JA-14 rotor for 10 min. The aqueous phase was removed and polyethylene glycol 6000 (PEG) and sodium chloride were added to 8% and 1.2% (w/v), respectively. After it was stirred for 10 min, the solution was kept overnight at 4 C. Virions were then pelleted by centrifugation at 5,500 g (max.) in the JA-14 rotor. Pellets were resuspended in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine (suspending buffer) and incubated at 4 C for 1 hr. Samples were centrifuged at 7,700 g (max.) in the Beckman JA-20 rotor for 10 min. The supernatant was saved and the pellet was resuspended as before, centrifuged, and this supernatant was added to the first supernatant. The supernatants were layered onto 1-ml cushions of 20% sucrose (in suspending buffer) and centrifuged in the Beckman type 40 rotor at 145,000 g (max.) for 2 hr. The pellets were resuspended in 1–2 ml of suspending buffer and layered onto sucrose density gradients (5, 10, 10, 10 ml of 10, 20, 30, and 40% [w/v] sucrose in suspending buffer, respectively) and centrifuged for 3.5 hr at 141,000 g (max.) in the Beckman SW 28 rotor. Gradients were fractionated with an ISCO® model 640 density gradient fractionator. The virion-containing zones were saved and used for further analysis. The virions of the STFL and ST9 isolates also were compared by equilibrium density gradient centrifugation in cesium chloride (CsCl) and cesium sulfate (Cs₂SO₄) gradients. Samples were centrifuged for 18 hr at 150,000 g (max.) at 20 C using the Beckman SW 50.1 rotor. Gradients were fractionated into 0.3-ml fractions and the refractive indices for each fraction were determined. CsCl densities were determined from a table of physical constants and Cs₂SO₄ densities were calculated as described by Ifft et al (18).

Protein and nucleic acid analysis. Virion proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by using the system of Laemmli (20). Sample preparation and electrophoresis conditions were as previously described (11).

Nucleic acids were extracted from purified virions by using SDS and phenol (8). Nucleic acids were precipitated with 2.5 volumes of ethanol and 0.1 M sodium acetate at −20 C and resuspended in sterile distilled water containing 0.1% *n*-lauroyl sarcosine (NLS) and stored at −20 C until use. Electrophoresis of virion RNAs was done by using the glyoxal-dimethyl sulfoxide (DMSO) denaturing system (24), except that buffers and the gel contained 20 mM phosphate, pH 7.0, and 1 mM EDTA. Electrophoresis was on 3-mm-thick, horizontal 1% agarose gels for 2 hr at 100 V (3.3 V/cm) at room temperature. After electrophoresis the gels were soaked in 0.05 M sodium hydroxide for 20 min, 0.5 M ammonium acetate for 20 min, and then 0.1 M ammonium acetate for 1 hr (22). Nucleic acids were stained for 1 hr with ethidium bromide (400 ng/ml). Nucleic acids were visualized by exposing the gel to ultraviolet radiation (302 nm) and photographed using Polaroid Type 665 positive/negative film and Wratten 23A and 9 filters.

Double-stranded RNAs were extracted from freshly harvested or frozen tissues essentially using the method of Dodds (5) except that ss- and ds-RNAs were separated using columns of Cellex N-1 cellulose (Bio-Rad Laboratories). After elution from the columns, the ds-RNA fraction was treated with DNase (Sigma) at 4 µg/ml in 0.2 M NaCl, 0.01 M MgCl₂, 0.1 M tris, pH 7.3, at room temperature for 20 min. The ds-RNAs were precipitated with 2.5 volumes of ethanol, resuspended in sterile distilled water containing 0.1% NLS, and stored frozen. Some ds-RNA samples were contaminated with ss-RNAs after Cellex chromatography and therefore were further purified by incubating overnight at 4 C in 2 M LiCl. Samples were then centrifuged at 12,000 g, the pellet was discarded, and ds-RNAs were ethanol precipitated from the 2 M LiCl supernatant fraction.

The ds-RNAs were analyzed by electrophoresis on "mini slabs" (IDEA Scientific) composed of 5% polyacrylamide using the tris-sodium acetate buffer system of Loening (21). Electrophoresis was for 3 hr at 75V (constant voltage) at room temperature. After electrophoresis, ds-RNAs were stained by soaking the gel for 30 min in 1 mM EDTA, pH 7.0, containing 40 ng/ml ethidium bromide. Gels were photographed as for the virion RNAs.

Ribonuclease A treatment of ST9-infected shepherd's purse ds-RNAs in high and low salt (33) also was used to confirm the ds-RNA nature of these RNAs. Double-stranded RNA samples, either in 3 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0) or sterile H₂O, were incubated at room temperature for 15 min with RNase A (1 µg/ml). Samples were adjusted to contain 5 µg/ml of proteinase K and 0.05% SDS and incubated for an additional 10 min to digest RNase A. The undigested ds-RNAs were precipitated by adding ethanol and analyzed by electrophoresis in 5% gels.

RESULTS

Transmission of the virus isolates. The ST9 isolate of BWYV caused severe twisting and stunting of infected *C. bursa-pastoris* (Fig. 1), and if plants were kept 4–5 wk after inoculation, infection with ST9 often caused death of the plants. This is in contrast to the symptoms normally associated with BWYV infection in *C. bursa-pastoris* (6,9). Infection with ST1 or STFL isolates resulted in the more typical leaf curling, hardening, and overall plant yellowing.

Because of the severe symptoms associated with the ST9 isolate, care was necessary to ensure that this isolate was transmitted before it killed test plants of *C. bursa-pastoris*, particularly when very small seedlings were infected. Also, BWYV isolates are very efficiently transmitted by *M. persicae* (6), but it was observed here that transmissions using the ST9 isolate did not always result in 100% of the infected test plants showing the severe symptom type. There were always some plants of *C. bursa-pastoris* from the ST9

transmission experiments that showed more typical symptoms of infection by BWYV, while typically 50–75% of the test plants gave the severe reaction. Aphid transmissions were done from the plants showing "typical" symptoms and, in three experiments, none of the "typical" plants subsequently gave rise to the severe symptom type. Therefore, the ST9 isolate was maintained only by transferring from severely stunted *C. bursa-pastoris*.

When plants of *Lactuca sativa, Solanum nigrum, Brassica oleracea*, var. *botrytis, Cichorium endivia, Cichorium intybus*, or *Nicotiana clevelandii* were inoculated with ST9 and subsequently used as source plants for transmission back to *C. bursa-pastoris*, only typical BWYV symptoms developed on the back-inoculated *C. bursa-pastoris*. When radish was used as the test host, however, no severe symptoms (when compared with radishes infected with ST1 and STFL) developed on ST9-infected radishes, but severe

Fig. 1. Plants of *Capsella bursa-pastoris* showing severe and typical symptoms associated with infection of the ST9 and STFL isolates of beet western yellows virus (BWYV). Healthy *C. bursa-pastoris* is on the left.
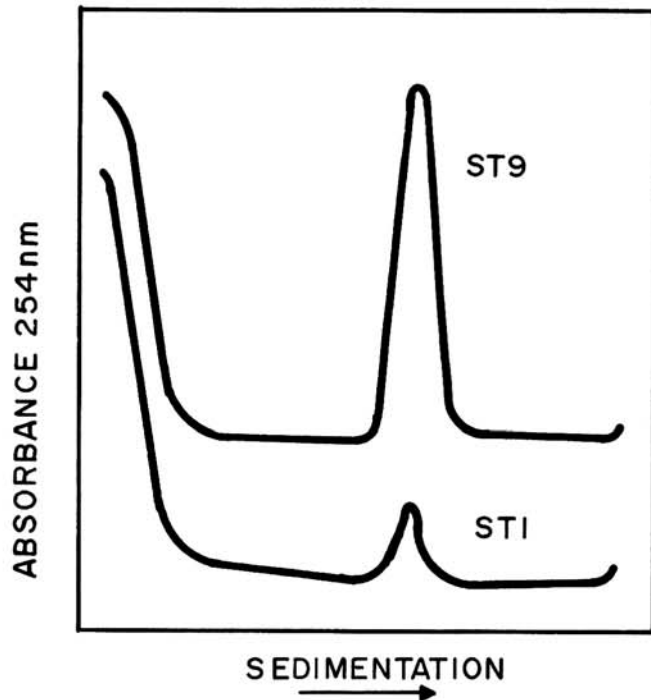

Fig. 2. Absorbance profile (254 nm) for fractionated rate-zonal sucrose density gradients of the ST9 and ST1 isolates of beet western yellows virus. Virions were purified from 50 g of *Capsella bursa-pastoris* infected with each isolate. Gradients were 10–40% sucrose and were centrifuged 3.5 hr in the Beckman SW 28 rotor.

symptoms developed on back-inoculated plants of *C. bursa-pastoris* when ST9 infected radishes were used as source plants. Thus, radish was a host for ST9 and did not develop the severe reaction.

No mechanical transmission of ST9 was achieved to any of the above mentioned plant species as was determined by ds-RNA analysis of the mechanically inoculated species.

Virion purification. Virions of all three BWYV isolates were easily purified from infected *C. bursa-pastoris* by using the Driselase® procedure. When the purified preparations were analyzed by rate-zonal sucrose density gradient centrifugation, no obvious sedimentation differences for ST9 and STFL or ST1 were apparent, but the ST9-infected plants always yielded about 10 times the amount of virions per gram of tissue as the STFL- or ST1-infected plants (Fig. 2). The yields were estimated by spectrophotometry using the extinction coefficient of 7, used for CRLV (32). ST9 yields were 5–10 mg/kg in 14 experiments, while STFL and ST1 yields were about 0.5–1 mg/kg. Also, when purified ST9 was fed to *M. persicae* through Parafilm membranes, and the aphids subsequently placed on healthy seedlings of *C. bursa-pastoris*, infected plants showed the severe symptom type typical for ST9-infection.

Purifications also were done from plants inoculated with ST9 but which failed to develop severe symptoms and showed typical BWYV symptoms. The virion yields from these plants were always very similar to ST1 and STFL. The estimated yields for ST1 and STFL correspond with other luteoviruses such as BYDV and PLRV (2,3,15,30). Similar yields were obtained by using either freshly harvested or frozen tissues of infected *C. bursa-pastoris*.
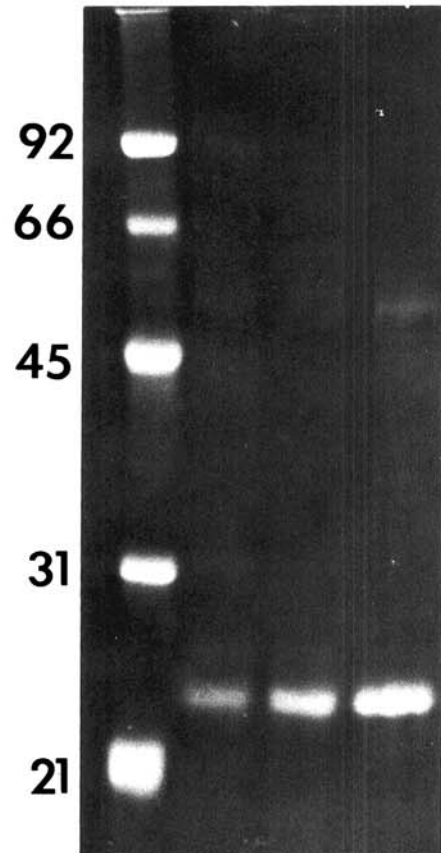

Fig. 3. SDS gel showing capsid proteins of the ST9 (9), STFL (FL), and ST1 (1) isolates of BWYV after SDS-polyacrylamide gel electrophoresis. Electrophoresis was in a 5% stacking gel, 12% resolving gel for 1 hr at 200 V. Molecular weight markers (P) from top to bottom are phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor. Relative masses $(M_r) \times 10^{-3}$ shown at left.

The purified virions of ST9 and ST1 were examined by electron microscopy and isometric particles 25 nm in diameter were present in both preparations. There were no obvious morphological differences between the virions of these two isolates.

Both ST9 and STFL had densities of 1.41 g/cc in CsCl gradients. For Cs_2SO_4 gradients, both STFL and ST9 sedimented in single bands at a density of 1.355 g/cc. Both STFL and ST9 had peak areas in Cs_2SO_4 gradients that were 9 times as large as those in CsCl gradients when equal amounts of purified virions were placed on each gradient type, indicating that some virion degradation occurred in the CsCl gradients.

Virion protein and nucleic acid analysis. The capsid proteins of purified virions of STFL, ST1, and ST9 were compared by SDS-PAGE. The proteins of all three isolates were indistinguishable by SDS-PAGE. All had a major protein band at 24,000 M_r (24K) (Fig. 3). Minor bands were also occasionally detected at 56,000 M_r (56K). No similar bands were seen in preparations from healthy plants.

Agarose gel electrophoresis of virion RNAs showed two RNAs for the ST9 isolate and one each for the STFL and ST1 isolates (Fig. 4). The two ST9 RNAs always appeared to be approximately equal in concentration, both here with denaturing and under the nondenaturing conditions used previously (7). Under the denaturing conditions used here, the relative masses of the RNAs were estimated to be 1.9×10^6 M_r and 0.93×10^6 M_r for ST9, 1.9×10^6 M_r for STFL, and 1.9×10^6 M_r for ST1 when mobilities were compared to the TMV (2.2×10^6 M_r) (27) and BMV (1.09, 0.99, 0.75, and 0.28×10^6 M_r) marker ss-RNAs.

We also compared the ds-RNAs of these isolates to determine if a corresponding difference existed in the numbers of ds-RNAs. The yields of detectable amounts of ds-RNAs for the BWYV isolates were very low compared to those of the CMV and TYMV standards. We typically used ds-RNAs from 0.1–0.2 g of TYMV- and CMV-infected tissues 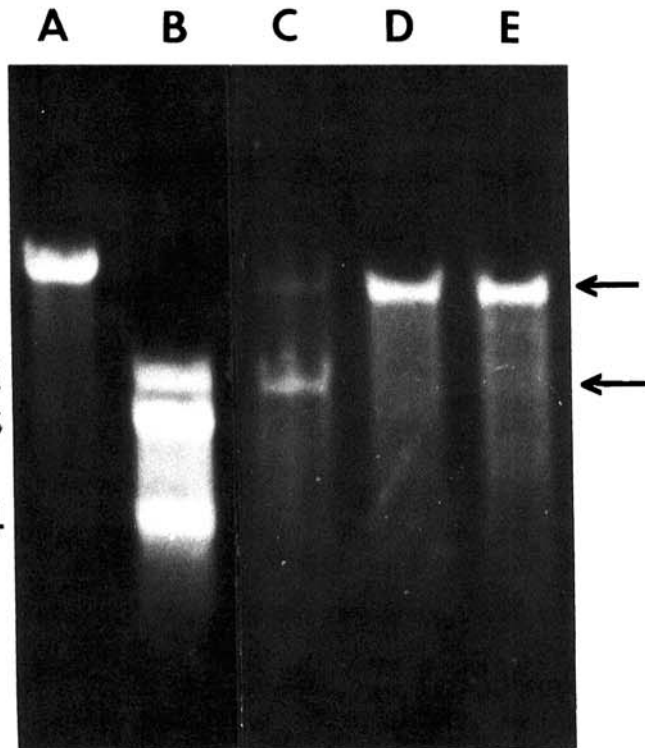for each gel run and obtained intensely staining ds-RNAs that could be used as markers. In contrast, to detect and clearly resolve the ds-RNAs for the STFL isolate, it was necessary to use the ds-RNAs extracted from at least 6–10 g of infected *C. bursa-pastoris*, and the ethidium bromide stained ds-RNAs still did not fluoresce as intensely as those from 0.2 g of TYMV or CMV-infected tissues (Fig. 5). Four major ds-RNA bands were resolved in the STFL extracts. The relative masses of these ds-RNAs were estimated under nondenaturing conditions by using the TYMV and CMV ds-RNAs as markers. The four STFL ds-RNAs were 3.6, 1.8, 1.4, and 1.0×10^6 M_r. However, two of the bands (1.8 and 1.0×10^6 M_r) corresponded to bands sometimes also found in extracts from healthy *C. bursa-pastoris*. The pattern of ds-RNAs detected in extracts from ST9-infected *C. bursa-pastoris* was significantly different from that from STFL-infected *C. bursa-pastoris* in that there were two very intensely fluorescing bands (2.2×10^6 M_r and $<4.6 \times 10^5$ M_r, the smallest marker ds-RNA available to us) in ST9 extracts that were not detected in the STFL or healthy extracts. These were in addition to the 3.6×10^6 ds-RNA that also was clearly resolved for ST9. The concentrations of the two extra ds-RNAs were much greater than all other detectable ds-RNAs. They were easily detected when the ds-RNAs from only 1 g of infected tissue were analyzed. Also, the 2.2×10^6 M_r ds-RNA was so concentrated compared to the 3.6×10^6 ds-RNA that it obscured other ds-RNAs in close proximity and there was always a series of slightly smaller ds-RNA bands immediately below it that did not allow resolution of other ds-RNAs that may be present in ST1 or healthy extracts. When ds-RNAs were isolated from plants of *C.*
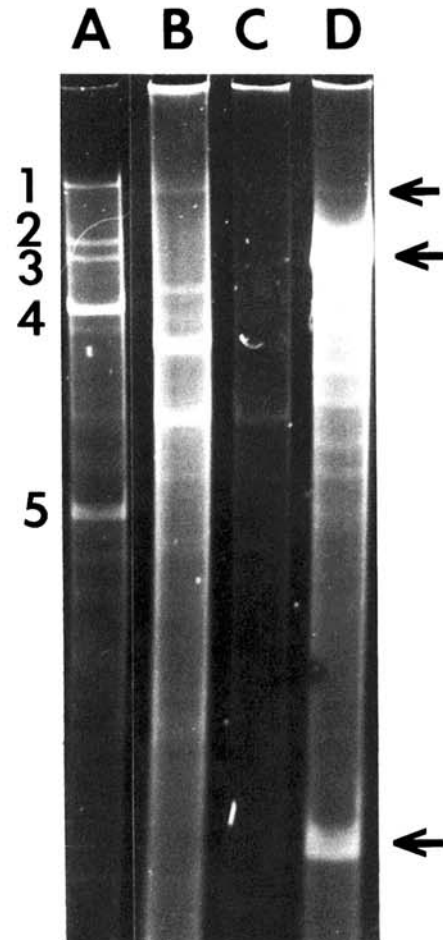

Fig. 4. A 1% horizontal agarose gel after denaturing electrophoresis of virion RNAs. RNAs were denatured with glyoxal and DMSO and electrophoresed for 2 hr at 100V. Lanes A and B show tobacco mosaic virus (number 1 at left) and brome mosaic virus (numbers 2, 3, and 4 at left) ss-RNAs, respectively. Lanes C, D, and E show the virion ss-RNAs of the ST9, STFL, and ST1 isolates of beet western yellows virus, respectively. The upper arrow shows the position of the BWYV 1.9×10^6 ss-RNA and the lower arrow refers to the ST9 0.93×10^6 ss-RNA.

Fig. 5. A 5% polyacrylamide slab gel showing ds-RNAs. Lane A shows a mixture of TYMV (band 1) and CMV (bands 2–5) ds-RNAs. Lane B shows ds-RNAs extracted from 10 g of STFL-infected *Capsella bursa-pastoris*. Lane C shows ds-RNAs extracted from 10 g of healthy *C. bursa-pastoris*. Lane D shows ds-RNAs extracted from 10 g of ST9-infected *C. bursa-pastoris*. Top arrow shows the 3.6×10^6 ds-RNA, middle arrow shows the 2.2×10^6 ds-RNA, and bottom arrow shows the $<4.6 \times 10^5$ ds-RNA.

bursa-pastoris that had been inoculated with ST9 they did not give severe symptoms but showed only typical BWYV symptoms. The 2.2×10^6 and $<4.6 \times 10^5$ M_r ds-RNAs were not detected, but the ds-RNA pattern was indistinguishable from that of the STFL-infected *C. bursa-pastoris*.

The ds-RNA nature of these RNAs also was confirmed by ribonuclease A treatment and 2 M LiCl fractionation. All of the RNAs shown in Fig. 5 were soluble in 2 M LiCl. Also, the ST9 ds-RNAs were resistant to RNase A when incubated in 0.45 M NaCl, but were digested when incubated with RNase in water (Fig. 6).

DISCUSSION

The ST9 isolate of BWYV shares many properties with the ST1 and STFL isolates shown here, yet differs significantly in other interesting properties. Most virion properties of all three isolates were indistinguishable. These included the number and relative mass of capsid proteins as determined by SDS-PAGE; serological properties; virion morphology; and density and sedimentation in equilibrium and rate-zonal density gradients, respectively. However, the ST9 isolate causes severe symptoms in *C. bursa-pastoris* and in some cases death of infected plants, while both STFL and ST1 gave typical BWYV symptoms. Yields of purified virions from ST9-infected *C. bursa-pastoris* were always 10 times

Fig. 6. A 5% polyacrylamide slab-gel showing ds-RNAs from TYMV-infected Chinese cabbage and ST9-infected *Capsella bursa-pastoris* after RNase A treatment in high and low salt. Lines A and B are TYMV and ST9 ds-RNAs, respectively, after RNase A (1 μg/ml) treatment in 3 × SSC. Lanes C and D are TYMV and ST9 ds-RNAs, respectively, after RNase A treatment in water. The upper arrow shows the position of TYMV ds-RNA, the middle and lower arrows show the positions of the ST9 2.2×10^6 ds-RNA and the $<4.6 \times 10^5$ ds-RNA, respectively.

greater than the yields from plants of *C. bursa-pastoris* infected with the STFL or ST1 isolates. Tests using double antibody sandwich enzyme-linked immunosorbent assay (ELISA) with antiserum prepared to ST1 also gave significantly stronger reactions for *C. bursa-pastoris* infected with ST9 compared to those infected with STFL or ST1 (*unpublished*).

The most interesting differences detected between ST9 and the other two isolates were the 0.93×10^6 M_r virion ss-RNA found in nucleic acid preparations from ST9 virions, and the extra ds-RNAs associated with ST9-infected *C. bursa-pastoris*. The ds-RNA pattern for STFL resembles the ds-RNA pattern for various isolates of the related luteovirus, BYDV (13). It seems likely that the 3.6×10^6 M_r ds-RNAs represent the genomic replicative RNAs and correspond to the 1.9×10^6 M_r virion ss-RNAs for both the STFL and ST9 isolates, and the 2.2×10^6 M_r ST9 ds-RNA probably corresponds to the 0.93×10^6 M_r virion ss-RNA. We detected no virion ss-RNA that might correspond to the smallest very prominent ds-RNA found in ST9-infected *C. bursa-pastoris*, yet this may not be unexpected based on results with other viruses (16,33). Also, even though the size estimates given here for the BWYV ds-RNAs are not exactly twice those for the virion ss-RNAs, nondenaturing size estimates for ds-RNAs have been demonstrated not to correspond accurately to glyoxal-DMSO denatured estimates for the counterpart ss-RNAs (12), and our size estimates were within an acceptable range. However, nucleic acid hybridization experiments are necessary to determine if the ds-RNAs are in fact the ds-RNA counterparts or replicative forms of the virion ss-RNAs.

Although the host range experiments reported here were limited, they suggest some interesting possibilities for the ST9 isolate. Of the species tested here only *C. bursa-pastoris* was a host that reacted showing severe symptoms, increased virion yield, the virion associated 0.93×10^6 M_r ss-RNA, and the prominent ds-RNAs of 2.2×10^6 and $<4.6 \times 10^5$ M_r.

Radish also was a host, as determined by aphid transmissions from ST9-infected radish back to *C. bursa-pastoris*; in four experiments, however, no symptoms were noted that were obviously different from radishes infected with ST1 or STFL isolates. Also, in two experiments no increased virion production was found in ST9-infected radishes, nor were the extra ss- and ds-RNAs detected (*unpublished*). Also of interest is that we failed to infect broccoli (*B. oleracea* var. *botrytis*) with ST9 in these experiments (as determined by transmissions from ST9-infected broccoli back to *C. bursa-pastoris*) even though ST9 was originally recovered from broccoli. This might be explained, however, by cultivar differences.

At least two possibilities exist that might partially explain the characteristics associated with the ST9 isolate of BWYV. The ST9 severe symptoms on *C. bursa-pastoris* could be the result of a mixed infection, BWYV and coinfecting virus. If this were true, however, we would expect to detect capsid proteins for the second virus and no virion capsid proteins, other than those of BWYV, were detected by SDS-PAGE. Also, in density gradients no peaks other than those for BWYV were obtained. This would not be the case even for a satellite virus situation like tobacco necrosis virus and satellite (26). Also, if a coinfecting virus lacked its own functional protein coat, but that relied on a helper virus for a protein coat such as lettuce speckles mottle virus (LSMV) (8,10), it would not be detected by SDS-PAGE. However, LSMV can infect plants without the helper virus, BWYV. We obtained no evidence that suggested this might be the case for ST9. Mechanical inoculations from ST9-infected *C. bursa-pastoris* were made to several plant species, including *C. bursa-pastoris* and radish, and these plants were analyzed for ds-RNAs characteristic of ST9. None were detected (*unpublished*). Similarly, when plants of *C. bursa-pastoris* infected with STFL were mechanically inoculated with ST9, no severe symptoms developed suggesting that "severe" associated RNAs could not be mechanically transmitted to plants already infected with a typical strain of BWYV. Thus, the possibility of the presence of a coinfecting virus in ST9-infected plants seems unlikely.

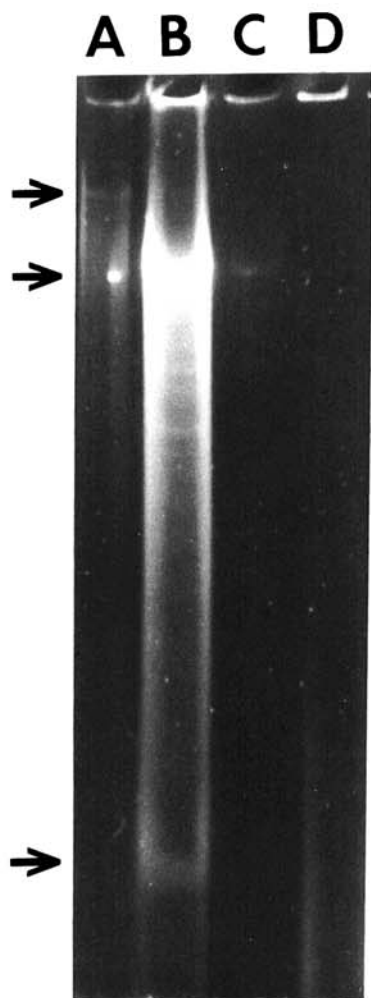Alternatively, the ss- and ds-RNAs associated with the ST9

isolate might represent a satellitelike RNA. The "extra" RNAs associated with ST9 have some similarities to satellite RNAs. Symptom type is associated with the ST9 RNAs as is the case with TCV and some CMV satellites (1,19). Loss of severe symptoms in *C. bursa-pastoris* during the transmission of ST9 was accompanied by a loss of the "extra" ss- and ds-RNAs and a decrease in virion production. Small satellite ds-RNAs like the ds-RNA form of CARNA 5 also are produced in very high amounts in infected plants (4) as are the ST9 "extra" ds-RNAs. The 0.93×10^6 ss-RNA, however, is much larger than other recognized satellite RNAs such as for TCV (1) or CMV (19), and the virion titer is greater in ST9-infected plants of *C. bursa-pastoris* which is not the case with the other satellite RNAs.

Because of the lack of mechanical transmission of luteoviruses or of their RNAs, the experiments necessary to determine if the $0.93 \times 10^6 M_r$ ss-RNA is a satellite cannot be done by using the RNAs and whole plants as have been done with other satellite RNAs (1,14,19,26). However, hybridization studies should determine if the extra ST9 RNAs share any homology with the BWYV genomic RNA, or if they are unique.

LITERATURE CITED

1. Altenbach, S. B., and Howell, S. H. 1981. Identification of a satellite RNA associated with turnip crinkle virus. Virology 112:25-33.
2. Brakke, M. K., and Rochow, W. F. 1974. Ribonucleic acid of barley yellow dwarf virus. Virology 61:240-248.
3. D'Arcy, C. J., Hewings, A. D., Burnett, P. A., and Jedlinski, H. 1983. Comparative purification of two luteoviruses. Phytopathology 75:755-759.
4. Diaz-Ruiz, J. R., and Kaper, J. M. 1978. Isolation of viral double-stranded RNAs using a LiCl fractionation procedure. Prep. Biochem. 8:1-7.
5. Dodds, J. A. 1980. Revised estimates of the molecular weights of ds-RNA segments in hypovirulent strains of *Endothia parasitica*. Phytopathology 70:1217-1220.
6. Duffus, J. E. 1960. Radish yellows, a disease of radish, sugar beet, and other crops. Phytopathology 50:389-394.
7. Falk, B. W., Duffus, J. E., and Morris, T. J. 1977. Two RNA species isolated from purified beet western yellows virus. Proc. Am. Phytopathol. Soc. 5:148.
8. Falk, B. W., Duffus, J. E., and Morris, T. J. 1979. Transmission host range and serological properties of the viruses that cause lettuce speckles disease. Phytopathology 69:612-617.
9. Falk, B. W., and Guzman, V. L. 1981. A virus as the causal agent of spring yellows of lettuce and escarole. Proc. Fla. State Hortic. Soc. 94:149-152.
10. Falk, B. W., Morris, T. J., and Duffus, J. E. 1979. Unstable infectivity and sedimentable ds-RNA associated with lettuce speckles mottle virus. Virology 96:239-248.
11. Falk, B. W., and Tsai, J. H. 1983. Assay for maize stripe virus-infected plants using antiserum produced to a purified noncapsid protein. Phytopathology 73:1259-1262.
12. Galinski, M. S., Chow, K.-C., Rohrman, G. F., Pearson, G. D., and Beaudrew, G. S. 1982. Size determination of *Orygia pseudostuga* cytoplasmic polyhedrosis virus genomic RNA. Virology 122:328-335.
13. Gildow, F. E., Baltinger, M. E., and Rodrow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.
14. Gonsalves, D., Provvidenti, R., and Edwards, M. C. 1982. Tomato white leaf: The relation of an apparent satellite RNA and cucumber mosaic virus. Phytopathology 72:1533-1538.
15. Hammond, J., Lister, R. M., and Foster, J. E. 1983. Purification and some properties of an isolate of barley yellow dwarf virus from Indiana. J. Gen. Virol. 64:667-676.
16. Henriques, M.-I., and Morris, T. J. 1979. Evidence for different replicative strategies in the plant tombusviruses. Virology 99:66-74.
17. Hewings, A. D., and D'Arcy, C. J., 1983. Characterization of two luteoviruses. Phytopathology 73:789-790.
18. Ifft, J. B., Voet, D. H., and Vinograd, J. 1961. The determination of density distributions and density gradients in binary solutions at equilibrium in the ultracentrifuge. J. Phys. Chem. 65:1138-1145.
19. Kaper, J. M., and Waterworth, H. E. 1977. Cucumber mosaic virus-associated RNA 5: causal agent for tomato necrosis. Science 196:429-431.
20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. Nature (Lond.) 227:680-685.
21. Loening, U. E. 1967. The fractionation of high-molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. 102:251-257.
22. Long, E. O., and Dawid, I. B. 1979. Expression of ribosomal DNA insertions in *Drosophila melanogaster*. Cell 18:1185-1196.
23. Mayo, M. A., Barber, H., Robinson, D. J., Tamada, T., and Harrison, B. D. 1982. Evidence that potato leafroll virus RNA is positive-stranded, is linked to a small protein and does not contain polyadenylate. J. Gen. Virol. 59:163-167.
24. McMaster, G. K., and Carmichael, G. C. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. PNAS 4835-4838.
25. Mehrad, H., LaPierre, H., and Yot, P. 1979. RNA in potato leafroll virus. FEBS (Fed. Eur. Biochem. Soc.) Lett. 101:169-174.
26. Murant, A. F., and Mayo, M. A. 1982. Satellites of plant viruses. Ann. Rev. Phytopathol. 20:49-70.
27. Murant, A. F., Taylor, M., Duncan, G. H., and Raschke, J. H. 1981. Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. J. Gen. Virol. 53:321-332.
28. Rowhani, A., and Stace-Smith, R. 1979. Purification and characterization of potato leafroll virus. Virology 98:45-54.
29. Shepherd, R. J., Francki, R. I. B., Hirth, L., Hollings, M., Inouye, T., MacLeod, R., Purcifull, D. E., Sinha, R. C., Tremaine, J. H., Valenta, V., and Wetter, C. 1976. New groups of plant viruses approved by the International Committee on Taxonomy of Viruses, September 1975. Intervirology 6:181-184.
30. Takanami, Y., and Kubo, S. 1979. Enzyme-assisted purification of two phloem-limited plant viruses: tobacco necrotic dwarf and potato leafroll. J. Gen. Virol. 44:153-159.
31. Takanami, Y., and Kubo, S. 1979. Nucleic acids of two phloem-limited viruses: tobacco necrotic dwarf and potato leafroll. J. Gen. Virol. 44:853-856.
32. Waterhouse, P. M., and Murant, A. F. 1981. Purification of carrot red leaf virus and evidence from four serological tests for its relationship to luteoviruses. J. Gen. Virol. 57:191-204.
33. Zelcer, A., Weaber, K. F., Balazs, E., and Zaitlin, M. 1981. The detection and characterization of viral-related double-stranded RNAs in tobacco mosaic virus-infected plants. Virology 113:417-427.