Detection of dsRNA in Grapevines Showing Symptoms of Rupestris Stem Pitting Disease and the Variabilities Encountered

OSSMAT I. AZZAM, Graduate Research Assistant, and DENNIS GONSALVES, Professor, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456; and DEBORAH A. GOLINO, Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

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Rupestris stem pitting (rSP), a graft-transmissible grapevine disease, can be identified only by its reaction (pitted wood) on inoculated *Vitis rupestris* 'St. George.' DsRNA was extracted from grapevines from California and Canada that indexed positive for rSP on St. George. Two distinct dsRNA species (B and C) (M_r = 5.3 × 10^6 and 4.4 × 10^6, respectively) were detected from the stem tissue of rSP-positive samples. Although similar dsRNA species (B and C) were detected in extracts of grapevines from New York, the association of dsRNA B and C with rSP in New York samples was not consistent. Also, eight different dsRNAs, known to be associated with the powdery mildew fungus, *Uncinula necator*, were detected in leaves of New York samples. In New York, the dsRNAs were not observed in leaves or stem samples collected from June through late August during the 1988 and 1989 growing seasons, suggesting that dsRNA detection in the grape tissue is variable throughout the season. We suggest that dsRNA species B and C are associated with rSP disease. The inconsistent results with New York samples are discussed.

Rupestris stem pitting (rSP) is a graft-transmissible grapevine disease first recognized in 1976 in *Vitis vinifera* L. selections imported from western Europe and Australia into California (8). The disease is diagnosed by stem pitting symptoms induced on the trunk of graft-inoculated *V. rupestris* Scheele 'St. George.' Based on this observation, Goheen (8) defined rSP as a disease that induces stem pitting on the wood cylinder below, but not above, the point of inoculation on St. George rootstock, and which does not cause foliar symptoms. Moreover, the agent does not produce any apparent symptoms on the grape virus indicators LN-33 or Cabernet franc. According to Goheen (8), rSP causes reduced vine growth in comparison with a healthy vine. The causal agent of the disease has not been identified.

Rupestris stem pitting was not considered a potential concern in New York State until 1983 when vines of the grape selections NY65.533.13 (JS23-416 × Gewurztraminer) and *Vitis* interspecific hybrid, Colobel, developed stem pitting along their woody cylinders (B. Reisch, R. Pool, and A. C. Goheen, *personal communication*) and tested positive for rSP by indexing onto St. George. Because these vines had never been grafted, the possibility of natural spread of rSP was suggested. Therefore, efforts were made to check the distribution of the disease in upstate New York, including commercial vineyards and nurseries in the Finger Lakes Region. Indexing results showed that 170 of 257 tested vines were positive for rSP (P. Deth and D. Gonsalves, *unpublished*). Such high incidence of rSP demonstrated the need for the study of this disease and the development of a rapid method by which infected vines could easily be recognized.

The study of rSP is difficult because the causal agent has not been mechanically transmitted, and indexing on St. George takes 2–3 yr to complete. Therefore, our goal was to find a method that would reliably and rapidly diagnose rSP. Because the agent is suspected to be a virus, we chose to use dsRNA analysis. This technique has been widely used for detection of a spectrum of plant virus diseases (5,14).

We report the consistent detection of two high molecular weight dsRNA species from phloem cane tissue of rSP-diseased grapevines from California and Canada and to a lesser extent from New York. Additionally, we detected other dsRNA species in leaves of healthy and rSP-diseased grapevines from New York. In a concurrent publication (1), we show that these dsRNA species are associated with the grape powdery mildew fungus, *Uncinula necator* (Schwein.) Burrill.

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MATERIALS AND METHODS

Selection of vines infected with rSP for dsRNA analysis. Selected grapevines from California were originally indexed by Goheen at the University of California, Davis, and found to be positive for rSP and free from other known viruses. The California samples were obtained from vines grown at Foundation Plant Materials Service (FPMS), University of California, Davis, and were designated according to the cultivar and the rSP isolate number. These included: Pinot noir-4 (rSP 102), Pinot noir-6 (rSP 103), Thompson seedless-4 (rSP 105, two separate vines), Sylvanner (rSP 104), Anglianico (rSP 107), Gewurztraminer-10 (rSP 100), and Pinot gris-1A (rSP 106). Canadian samples were supplied by William Lanterman, Saanichton Plant Quarantine Station, Sidney, British Columbia, and included Pinot noir (0390), DeChaunac, Pinot gris, Gutedel, and 125-AA C11. Healthy Pinot noir samples were also obtained from both locations. In addition, healthy Sylvanner, Anglianico, and Thompson seedless-4 were tested.

Based on the 1983 survey results, we selected the following New York rSP-positive grapevines for dsRNA screening: Gamay Beaujolais, Price, Pinot gris, Concord, DeChaunac, NY65.533.13 (JS 23-416 × Gewurztraminer), and Colobel. Bud inocula from these vines had induced distinct pitting on St. George in 1986 (P. Deth and D. Gonsalves, *unpublished*). In addition, four vines that had indexed negative were selected—Villard blanc, Muscat Ottonel, Urbana, and NY65.533.13 (heat-treated vine). Leaf and stem samples were checked for grapevine leafroll associated virus (GLRaV) type III (18) and tobacco ringspot virus by enzyme-linked immunosorbent assay (ELISA) and were found to be negative. Similarly, all leaf samples tested negative for tomato ringspot virus by ELISA. These vines were maintained in a vineyard at the New York State Agricultural Experiment Station at Geneva, and cuttings from each cultivar were propagated and maintained in a greenhouse.

Mature leaf samples were collected from the field during mid- to late summer, divided into 15-g samples, and stored at −20 C until they were processed. Stem phloem samples were scraped from 1-yr-old canes, packed in 10-g samples, and stored at −20 C.

DsRNA extraction. The method followed was that reported by Kurppa and Martin (12) and modified by Hu et al (11). Aliquots of 20–30 μl of purified dsRNA were electrophoresed through 6% polyacrylamide gels (30:0.8, acrylamide/bisacrylamide) in a vertical slab minigel apparatus (10 × 10 cm) in electrophoresis buffer (0.04 M Tris, 0.02 M sodium acetate, 0.02 M EDTA, pH 7.8). Electrophoresis was carried out at 20 mA for 11–12 hr. Gels were stained in ethidium bromide (50 ng/ml) for 10 min, destained in distilled water for 5 min, and photographed with Polaroid 667 film or 55 film (with a negative) with a red filter. Some dsRNA samples were treated with DNase (10–12 μg/ml) as described by Gildow et al (7) and RNase (10–30 μg/ml) in the presence of 0.3 M NaCl or 3× STE (1× STE = 0.1 M NaCl, 0.05 M Tris, and 0.01 M EDTA, pH 6.8) and in the presence of water or 1× STE after electrophoresis (5).

DsRNA of citrus tristeza virus (CTV) (M_r = 13.3 × 10^6) from infected citrus bark tissue provided by S. Garnesy, USDA-ARS, Orlando, FL; tobacco mosaic virus dsRNA (TMV) (M_r = 4.3 × 10^6) from infected tobacco leaf tissue provided by M. Zaitlin, Cornell University, Ithaca, NY; maize rough dwarf virus dsRNA (MRDV) (M_r = 2.88, 2.50, 2.35, 2.35, 2.12, 1.75, 1.45, 1.25, 1.18, and 1.1 × 10^6) provided by G. Boccardo, Laboratorio di Fitovirologia applicata del CNR, Turin, Italy; and cucumber mosaic virus dsRNA (CMV) (M_r = 2.54, 2.30, 1.64, and 0.75 × 10^6) were used as molecular weight markers. Molecular weights of dsRNA species were estimated with the method of Bozarth and Harley (3).

RESULTS

DsRNA detection in vines infected with rSP from California and Canada. Because the etiology of rSP is not known, the definition of rSP is based on symptoms that are induced on the indicator St. George (8). Thus, it is important to use material that represents, as much as possible, the original material from which rSP was discovered. Some of our vines infected with rSP from California came from the original vines that had indexed postive for rSP. For example, California Pinot noir-4 and -6 represent two of the vine sources in which rSP disease was first observed (A. C. Goheen, *personal communication*). By this approach, any dsRNA species detected from tissues of these vines would likely be associated with rSP disease.

DsRNA was extracted from 13 samples collected from Canada and California. Two distinct dsRNA species, B and C (Figs. 1 and 2), were detected in stem extracts from six of eight and three of five rSP-diseased vines from California and Canada (Table 1). The estimated molecular weights of B and C were 5.3 and 4.4 × 10^6 (Figs. 1 and 2), respectively. In some samples, dsRNA species C was barely detectable in agarose gels (Fig. 1, lanes 2 and 5) but could be detected in other samples that were electrophoresed in polyacrylamide gels (Fig. 2). Species B and C were not detected in leaf extracts of vines infected with rSP, nor in stem or leaf extracts of rSP-negative vines from both locations. In addition to dsRNA species B and C, we detected low molecular weight RNA species (0.6–0.7 × 10^6) in both healthy and rSP-diseased vines in 1% agarose gels (Fig. 1). RNase treatment suggested that these smaller species were single-stranded RNA of host origin (*unpublished*). The dsRNA analysis test was repeated two to five times based on the availability of grape canes.

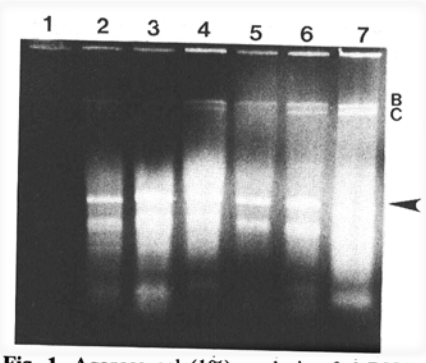

Fig. 1. Agarose gel (1%) analysis of dsRNA extracted from phloem tissue from canes of grapevines infected with rupestris stem pitting (rSP) and healthy grapevines. **(Lane 1)** healthy *Vitis vinifera* 'Pinot noir' (0396-Canada), **(lane 2)** rSP-positive Pinot noir (0390-Canada), **(lane 3)** healthy Pinot noir-4 (California), **(lane 4)** rSP-positive Pinot noir-4 (California), **(lane 5)** rSP-positive Pinot noir-6 (California), **(lane 6)** rSP-positive hybrid Colobel-256 (New York), and **(lane 7)** rSP-positive hybrid Colobel-257 (New York). The electrophoresis was performed for 1 hr at 8 V/cm and the gel was stained in ethidium bromide. DsRNAs are associated with rSP. In addition, low molecular weight species (arrow) were detected in samples of diseased and healthy vines. Digestion with RNase suggested that they were ssRNA of host origin (*unpublished data*).

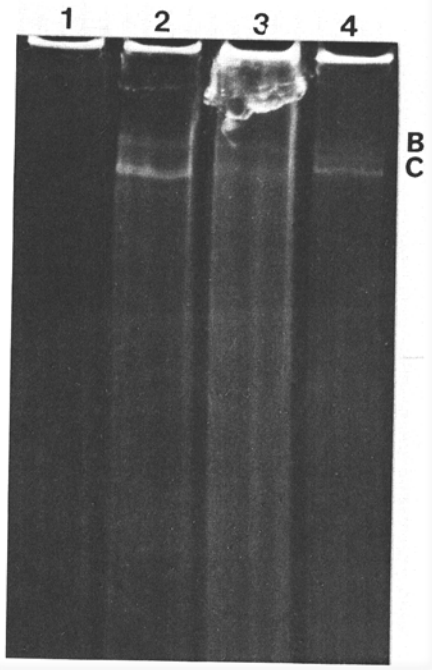

Fig. 2. Polyacrylamide gel (6%) electrophoretic analysis of dsRNA extracted from phloem tissue from canes of rupestris stem pitting (rSP) infected and healthy grapevines. **(Lane 1)** healthy *Vitis vinifera* 'Pinot noir' (0396-Canada), **(lane 2)** rSP-positive Pinot noir (0390-Canada), **(lane 3)** rSP-positive hybrid Colobel-256 (New York), and **(lane 4)** rSP positive Pinot noir-4 (California). Electrophoresis was performed for 11 hr at 20 mA and the gel was stained in ethidium bromide.

Detection of dsRNA species from New York samples. According to the 1983 indexing results, the seven rSP-positive vines from New York showed an average rating of 2/3 (0/3 = no pitting and 3/3 = severe pitting) for pitting symptoms on the woody indicator St. George. Symptoms were not observed on LN-33. The four negative samples did not show symptoms on St. George or LN-33.

Initial samples were collected in the fall of 1987, and dsRNA was extracted from mature leaves and phloem stem tissue using 15 and 10 g per sample, respectively. Surprisingly, a number of dsRNA species were detected in extracts of stem and leaves. These dsRNA species were labeled A–F based on their estimated molecular weights (Fig. 3). For convenience, species E and F refer to groups of dsRNAs with similar sizes.

Table 1. Detection of dsRNA in leaf and stem samples of field-grown grapevines from California and Canada that indexed positive or negative for rupestris stem pitting (rSP)

rSP sample	dsRNA species[a] Leaf	Stem
California		
rSP-negative		
Pinot noir-13	–	–
Sylvanner	–	–
Anglianico	–	–
Thompson seedless	–	–
rSP-positive		
Pinot noir-4	–	B,C
Pinot noir-6	–	B,C
Thompson seedless-4(1)	–	B,C
Thompson seedless-4(2)	–	B,C
Pinot gris-1A	NA	B,C
Gewurztraminer -10	NA	–
Sylvanner	–	B,C
Anglianico	–	–
Canada		
rSP-negative		
Pinot noir-0396	–	–
rSP-positive		
Pinot noir-0390	–	B,C
Pinot gris	–	B,C
Gudetel	NA	B,C
125 AA CL 1	NA	–
DeChaunac	–	–

[a] – = dsRNA was not detected; B,C = rSP-associated dsRNA species B (M_r = 5.3 × 10^6) and C (M_r = 4.4 × 10^6) were observed; NA = not available.

Molecular weights of species A and D (seen mostly from leaf samples) were estimated as 6.3 and 3.4 × 10^6, respectively. The molecular weights of the smaller dsRNA species were estimated at 2.1, 1.9, and 1.75 × 10^6 for species E and 1.1, 1.05, and 0.95 × 10^6 for species F. Species B and C were identical in migration to the dsRNA species observed for the California and Canada samples (Figs. 1 and 2).

The nature of the dsRNA species from the leaf and stem samples was confirmed by DNase and RNase treatments. Leaf dsRNAs were intact after the digestion with 10 μg/ml of DNase and they were resistant to 10 μg/ml of RNase treatment in 0.3 M NaCl. However, these dsRNAs were digested in water in the presence of 10 μg/ml of RNase. DsRNA species isolated from stem samples were resistant to DNase at 12 μg/ml and to 30 μg/ml of RNase in the presence of 3× STE buffer solution, but they were digested in 1× STE buffer containing the same concentration of RNase.

Results of the dsRNA analyses from the New York samples are summarized in Table 2. DsRNA species B and C were observed only in the stem tissue of Colobel, which indexed positive for rSP, and Villard blanc, which indexed negative. On the other hand, dsRNA species A and D–F were detected almost exclusively from leaf extracts. Thus, the dsRNA patterns observed in a cultivar, such as Colobel (rSP positive), differed depending on whether leaf or stem samples were tested (Fig. 3). DsRNA species A and D–F were also observed in leaf tissue from a heat-treated NY65.533.13. In addition, dsRNA species D was detected in both Villard blanc and Muscat Ottonel, which indexed negative for rSP, and species F was seen in Villard blanc and Concord, one vine negative and one vine positive for rSP. However, these different dsRNAs were not detected in leaves or stem tissue of all other tested samples (Table 2).

Comparative migration rates of species B and C and their isolation from only stem tissue suggested that dsRNA species B and C from Colobel and Villard blanc were similar, if not identical, to dsRNA species from rSP-diseased vines from California and Canada (Figs. 1 and 2, Table 1). However, dsRNA species A and D–F appeared to be unique to New York samples. A concurrent work (1) showed that these were from conidia and cleistothecia of the grape powdery mildew fungus, *U. necator*.

Seasonal variation in dsRNA analysis of Colobel. Because dsRNA species B and C appeared to be associated with a graft-transmissible agent (Table 1, Figs. 1 and 2) and species A and D–F associated with *U. necator* (1), it was of interest to trace the detectability of these dsRNA species during different growth stages of the grapevine. Colobel (rSP positive), its sister vines, and Villard blanc (rSP negative) were used in these experiments. During the 1988 and 1989 growing seasons, samples were collected at monthly intervals from field-grown vines and dsRNA preparations were analyzed. The results are summarized in Table 3. In 1988, dsRNA species were not detected in samples collected in June and July. DsRNA species (B and C) were first detected in stem samples collected from Colobel in late August and from Villard blanc in October. DsRNA species A and D–F were detected only in Colobel leaves collected in September and October. In 1989, dsRNA species were not detected in Colobel and Villard blanc leaves or stems from June through late August, with the exception of a single sample of Colobel-256, where dsRNA species B and C were detected in the stem tissue in June.

Distribution of dsRNA in Colobel. To assess the variability of dsRNA within different parts of a vine at a given time, leaf and stem samples were collected from terminal and basal sections of a single cane of Colobel-256 in October, when dsRNA concentration was high. Analysis of these samples showed that dsRNA (Fig. 4) concentration was variable. The highest concentrations of dsRNA species (A and D–F) were observed from terminal leaf samples. The concentration of dsRNA was lower in the basal leaf sample, although the dsRNA species were easily detected. DsRNA species B and C were detected in extracts of basal stem tissue but not in terminal stem tissue. In this experiment, species E and F were observed in both stem samples.

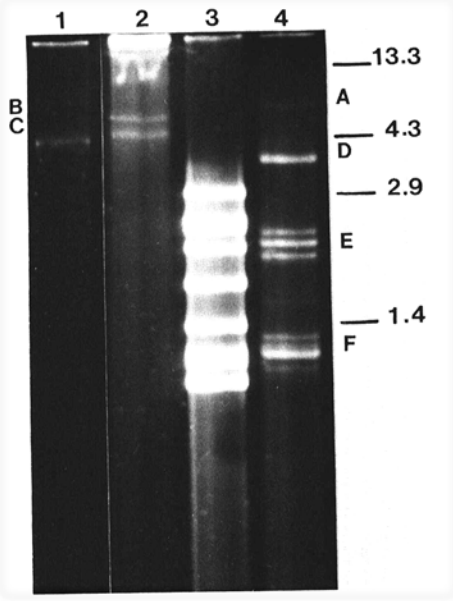

Fig. 3. Polyacrylamide gel (6%) electrophoretic analysis of dsRNA extracted from stem and leaf tissue of grapevines infected with rupestris stem pitting (rSP) from New York. **(Lane 1)** tobacco mosaic virus-infected tobacco, **(lane 2)** rSP-positive Colobel-257 stem tissue, **(lane 3)** purified dsRNA of maize rough dwarf virus as a molecular weight marker, and **(lane 4)** rSP-positive Colobel-257 leaf tissue. Electrophoresis was performed for 11 hr at 20 mA and the gel was stained in ethidium bromide. Bands on the gel are labeled A–F in order of increasing electrophoretic mobility and the molecular weights (× 10^6) of the markers are listed on the right. DsRNA species A and D–F are associated with the powdery mildew fungus, *Unicula necator*, and species B and C (lane 2) are associated with rSP.

Table 2. Detection of dsRNA species in stem and leaf tissue collected in fall 1987 from 11 selected field-grown grapevines that indexed positive or negative for rupestris stem pitting (rSP) in New York

	DsRNA species[a]											
	A		B		C		D		E		F	
Sample tested	S	L	S	L	S	L	S	L	S	L	S	L
rSP negative												
Villard blanc	–[b]	–	+	–	+	–	–	+	–	–	–	+
Muscat ottonel	–	–	ND	–	ND	–	–	+	–	–	–	–
Urbana	–	–	–	–	–	–	–	–	–	–	–	–
NY65.533.13[c]	–	+	–	–	–	–	–	+	–	+	–	+
rSP positive												
Colobel	–	+	+	–	+	–	–	+	–	+	–	+
Concord	–	–	–	–	–	–	–	–	–	–	–	–
Price	–	–	–	–	–	–	–	–	–	–	–	–
Pinot gris	–	–	–	–	–	–	–	–	–	–	–	–
DeChaunac	–	–	–	–	–	–	–	–	–	–	–	–
Muscat ottonel	–	–	–	–	–	–	–	–	–	–	–	–
NY 65.533.13	–	–	–	–	–	–	–	–	–	–	–	–
Gamay beaujolais	–	–	–	–	–	–	–	–	–	–	–	–

[a] A–F designate the different dsRNA species seen on polyacrylamide gel analysis. S = stem, L = leaf.

[b] + = Designated dsRNA species was detected, – = dsRNA species was not observed detected in at least two extractions. Positive tests were repeated two to five times. ND = test was not done. DsRNA species A and D–F are known to be associated with the grape powdery mildew fungus, *Uncinula necator* (1).

[c] Heat treated.

Table 3. Detection of dsRNA from leaves and stems of field-grown rupestris stem pitting (rSP) indexed[a] grapevines from New York during 1988 and 1989 growing seasons

rSP sample	1988								1989							
	25 July		27 Aug.		24 Sept.		29 Oct.		28 June		24 July		28 Aug.		24 Sept.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Villard blanc	–[b]	–	–	–	–	–	–	B,C	–	–	–	–	–	B,C	–	B,C
Colobel-256	–	–	–	B,C,F	A,D–F	B,C	A,D–F	B,C	–	B,C	–	–	–	B,C	A,D–F	B,C
Colobel-257	–	–	–	B,C,F	A,D–F	B,C	A,D–F	B,C	–	–	–	–	–	B,C	A,D–F	B,C
Colobel-265	–	–	–	B,C,F	A,D–F	B,C	A,D–F	B,C								

[a] Villard blanc indexed negative and Colobel indexed positive for rSP.

[b] – = dsRNA species were not detected; A, B, C, and D–F = these specific dsRNAs were detected. Note that species B and C were consistently found in stem but not leaf tissue. DsRNA species A and D–F were found to be associated with the grape powdery mildew fungus, *Uncinula necator*. DsRNA species B and C were consistently associated with rSP infected grapevines from California and Canada.

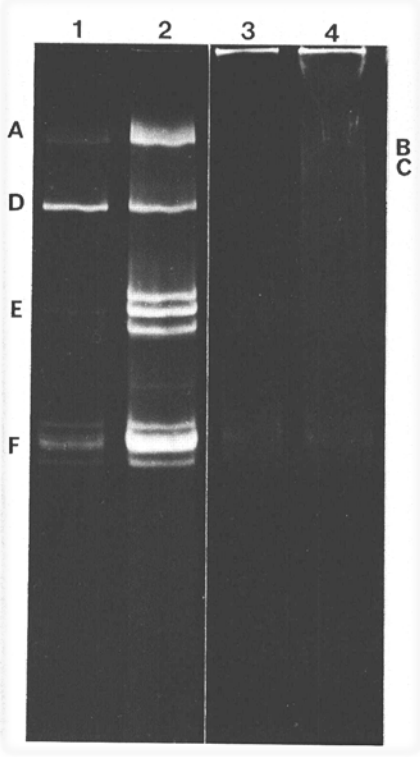

Fig. 4. Polyacrylamide gel (6%) electrophoretic analysis of dsRNA extracted from different tissue types of a grapevine (Colobel-256) infected with rupestris stem pitting (rSP). **(Lane 1)** dsRNA purified from basal leaves, **(lane 2)** dsRNA from terminal leaves, **(lane 3)** dsRNA from terminal stem tissue, and **(lane 4)** dsRNA from basal stem. Note the faint rSP-associated dsRNA species B and C in lane 4. DsRNA species A and D–F are associated with the powdery mildew fungus, *Uncinula necator*. Samples were analyzed by electrophoresis for 11 hr at 20mA and the gel was stained in ethidium bromide.

DISCUSSION

In this investigation, we showed the consistent presence of two distinct dsRNA species with molecular weights of 5.3 and 4.4 $\times$ 10^6 in stem tissue of rSP-diseased vines from California and Canada. The rSP-affected samples (Pinot noir-4 and -6) from California are among the original vines from which rSP was first described. In addition, the California and Canada rSP-affected vines that were tested had been thoroughly indexed for rSP and found to be free from other grape viruses. Thus, our results suggest that dsRNA species B and C are associated with rSP disease.

However, dsRNA species B and C were not consistently detected from grapevines from New York that indexed positive for rSP. These observations may be attributable to the low concentration of dsRNA found in some samples, to the fact that the pitting symptoms seen on the inoculated St. George indicators were caused by a multitude of agents not yet determined (10), to the uneven distribution of these dsRNA species in grapevines, or to the general difficulty encountered in extracting nucleic acid from woody tissue. Grapes are known to accumulate high levels of nucleic acid-phenolic complexes which may preclude nucleic acid extraction (15, 16). Because several grape cultivars with different levels of phenolic compounds were used in this study, variability in the test would be expected. The same reason may explain why dsRNA species B and C were not detected in leaf samples. In general, leaves have higher levels of phenolic compounds than stem tissue. Furthermore, our data in Table 3 suggest that detection of dsRNA within a cultivar depends on plant phenology.

The finding that species B and C were also detected in the rSP-negative Villard blanc could be attributed to two reasons. First, the biological indexing was done in 1983 while the dsRNA analysis was done in 1987. Therefore, it is possible that during these 4 yr, the field-grown vine Villard blanc got infected with rSP. The second reason might be that the distribution of the causal agent of rSP is unevenly distributed in grapevines. Therefore, the budwood selected for indexing may have been free of the rSP agent. The uneven distribution of viruses in grapevines has been documented with tomato ringspot in New York (9).

The failure to detect dsRNA species A and D–F in tissues of vines infected with rSP from California suggested that they were not associated with the rSP disease. Indeed, further work (1) showed that they are associated with the grape powdery mildew fungus, *U. necator*.

Our results on dsRNA species associated with rSP are relatively consistent with those of Walter and Cameron (17). Their work on the rSP-infected Pinot noir California samples (M. H. Walter, *personal communication*), showed the presence of a single dsRNA species (M_r = 5–6 $\times$ 10^6) in 1% agarose gels. In most of our experiments, 6% polyacrylamide gels were used, and we detected two distinct dsRNA species—M_r = 5.3 $\times$ 10^6 (species B) and 4.4 $\times$ 10^6 (species C). This difference in dsRNA size could be attributable to the use of different electrophoretic procedures. Dodds et al (6) reported that 6% polyacrylamide gels, as used in this study, are of higher resolution than agarose gels, which Walter and Cameron (17) used in their investigation. However, when electrophoresis was performed in 1% agarose gels, our dsRNA preparations still had two dsRNA species. The fact that we detected two species instead of one could be attributable to the use of different dsRNA purification methods or, simply, dsRNA species C could represent a degradation of species B or another agent. Further studies are needed to determine the relation, if any, between our observed dsRNAs and those observed by Walter and Cameron (17).

Recently, Monette et al (13) reported the presence of one major dsRNA species of M_r = 0.24 $\times$ 10^6 (359 base pairs) in samples infected with rSP. They detected this dsRNA from in vitro shoot tip cultures but not from bark or leaf tissue of field-grown samples. In contrast, we showed the presence of two high molecular weight dsRNA species associated with field-collected bark tissue of vines derived from a number of those in which Monette et al detected the low molecular weight dsRNA. Also, we did not detect the dsRNA observed by Monette et al in 1% agarose or in 6% polyacrylamide gels. The reason might be that we did not use in vitro shoot tip cultures or that the dsRNA concentration was too low to be detected in the field-grown samples used in our work.

Our data suggest that rSP is not related to closteroviruses associated with GLRaV and corky bark. DsRNAs associated with these diseases are much larger (M_r = 8–10 $\times$ 10^6) than dsRNA species B and C. We did not detect closteroviruslike particles with GLRaV purification procedures (*unpublished*). Moreover, the symptoms caused by these diseases on St. George, LN-33, and Caber-

net franc are very different than those caused by GLRaV and corky bark.

It is possible that rSP dsRNA species B could be produced by a virus with the same molecular weight as that of grape virus A (GVA). This virus is reported to have a single-stranded RNA genome of 2.6×10^6 (2) and a predicted dsRNA form of 5.2×10^6. In fact, GVA is a small closterovirus about 800 nm long isolated first from a grapevine showing stem pitting symptoms (4). However, we were not able to detect any closterovirus-like particles from samples infected with rSP, and our several efforts to transmit the agent to *Nicotiana clevelandii* A. Gray, which supports GVA, have failed.

The etiology of rSP is still not known. However, the detection of dsRNA species that are associated with rSP-diseased vines from California, the place where rSP was first described (8), lays the ground work to compare these dsRNAs with those observed by Walter and Cameron (17) and Monette et al (13). A logical approach is the cloning of the dsRNA species and using the cDNA clones or their transcripts as hybridization probes.

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