Characterization of an Ilarvirus Associated with a Necrotic Shock Reaction in Blueberry

S. G. MacDonald, R. R. Martin, and P. R. Bristow

First author: former graduate student, Department of Plant Science, University of British Columbia, Vancouver; second author: research scientist, Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, British Columbia, Canada V6T 1X2; third author: associate plant pathologist, Washington State University, Puyallup Research and Extension Center, Puyallup 98371.

We thank the British Columbia Blueberry Cooperative Association, the Washington State Blueberry Commission, the Oregon State Blueberry Commission, and the Canada-British Columbia Agricultural Regional Development Subsidiary Agreement for financial support.

Accepted for publication 25 October 1990 (submitted for electronic processing).

ABSTRACT

MacDonald, S. G., Martin, R. R., and Bristow, P. R. 1991. Characterization of an ilarvirus associated with a necrotic shock reaction in blueberry. Phytopathology 81:210-214.

Blueberry bushes with scorch symptoms were found during a survey of blueberry fields in coastal British Columbia, Washington, and Oregon. Some were infected with blueberry scorch carlavirus; others in Washington and Oregon were found to contain a second virus, for which the name blueberry shock ilarvirus (BSIV) is proposed. BSIV was mechanically transmissible to several *Nicotiana* species but not to other herbaceous plants tested. BSIV was purified from *N. clevelandii* and had quasi-isometric particles approximately 27 nm in diameter, a single polypeptide with relative mass (M_r) of 27,300, and four RNA molecules (M_r 1.03, 0.84, 0.57, and 0.3 $\times$ 10^6). BSIV was serologically related to Prunus necrotic ringspot virus in gel double-diffusion tests and to Prunus necrotic ringspot virus and apple mosaic virus in indirect enzyme-linked immunosorbent assay. Because of the host range, particle size and morphology, size and number of RNA and protein components, serology, and lack of aphid transmissibility of this virus, we suggest that BSIV should be considered a new member of the ilarvirus group. In a subsequent survey using enzyme-linked immunosorbent assay, BSIV was found in blueberry plants from both western Washington and Oregon, but it has not yet been found in British Columbia.

Blueberry scorch disease first appeared in Pierce County, Washington, in 1980 and has been shown to be caused by blueberry scorch virus (BBScV), a carlavirus (9). Blueberry fields in coastal British Columbia, Washington, and Oregon were surveyed for this virus in 1987 (10). Several fields had bushes with blossom- and leaf-blighting symptoms similar to those of blueberry scorch disease, described earlier (9), but were negative for BBScV by enzyme-linked immunosorbent assay (ELISA). In addition, no BBScV could be purified from these bushes with the standard purification protocol for that virus (9). No other cause of these symptoms, such as frost injury, mummy berry blossom and shoot blight, bacterial blight, or Botrytis blossom blight, could be found. The symptoms differed slightly from those caused by BBScV infection because the blighted flower clusters and leaves did not flag, and because blighted leaves were replaced during the growing season. Moreover, plants that had blight symptoms for 1–3 yr eventually became symptomless and appeared to have recovered. In contrast, bushes infected with BBScV exhibited symptoms each year. Cultivars that showed symptoms but were negative for the carlavirus included Berkeley, Bluecrop, Bluetta, Blueray, Dixi, Earliblue, Pemberton, Jersey, and Weymouth.

This paper describes a second virus associated with blueberry scorch disease. The virus is designated blueberry shock ilarvirus (BSIV), which is a new member of the ilarvirus group. Distribution of BSIV in British Columbia, Washington, and Oregon is also reported. A preliminary report has been presented (7).

MATERIALS AND METHODS

Virus sources. The virus was originally isolated from a highbush blueberry plant (cultivar Berkeley) in Clark County, Washington, which exhibited scorchlike symptoms but was negative for BBScV; it was maintained in *Nicotiana clevelandii*. A second isolate was obtained from blueberry plants in a field in Whatcom County, Washington.

Transmission. Branches were collected from diseased bushes in January 1988, fumigated in a methyl bromide chamber, and then placed in jars of water in a greenhouse (with a 16-hr day and 8-hr night regime and daytime and nighttime temperatures of 22 and 16 C, respectively) to force them to blossom.

Blossoms were collected and homogenized in 0.05 M sodium phosphate, pH 7.0, containing 2% polyvinylpyrrolidone (mol wt 44,000) (PVP-44). They were then rubbed onto Carborundum-dusted leaves of three plants each of *Brassica juncea* L. Czerniak 'Florida Broadleaf,' *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Gomphrena globosa* L., *N. clevelandii* A. Gray, *Petunia hybrida* Hort. Vilm. Andr. 'Coral Satin,' *Phaseolus vulgaris* L. 'Top Crop,' and *Lycopersicon esculentum* Mill. 'Rutgers.' Plants mock-inoculated with this buffer served as healthy controls. The plants were observed daily for symptoms for a period of 4 wk. Plants that developed symptoms were used as inoculum for further studies.

N. clevelandii infected with BSIV was also homogenized, and the homogenate was used to mechanically inoculate three plants each of *B. juncea* 'Florida Broadleaf,' *C. amaranticolor, C. quinoa, Cucumis sativus* L. 'Straight Eight,' *G. globosa, N. clevelandii, P. vulgaris* 'Top Crop,' *L. esculentum* 'Rutgers,' *N. tabacum* L. 'Samsun,' *N. tabacum* 'Havana 425,' *N. tabacum* 'Harrownova,' *N. benthamiana* L., *N. glauca* L., *N. glutinosa* L., and *N. sylvestris* Speg. & Comes. Again, plants mock-inoculated with buffer served as healthy controls. All plants were observed daily for symptom development for a period of 4 wk and tested by ELISA for BSIV.

Symptomless scion wood from diseased plants, taken from July through September, was grafted (using bottle grafts) to 2-yr-old Collins, Bluecrop, Jersey, and Blueray blueberry plants. Grafted plants were maintained in a greenhouse or screenhouse for 2 mo, without supplemental light or heat, and then overwintered in screenhouses. Plants were observed for symptoms weekly during the growing season and tested by ELISA for virus infection.

Electron microscopy. Thin-section electron microscopy was

carried out as described previously (9). Tissue samples were fixed in 4% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, post-stained in osmium tetroxide, and embedded in Epon (Shell Chemical Co., Houston, TX). Sections were stained in lead citrate and uranyl acetate (UA). Leaf extracts, purified virus preparations, and antibody-trapped particles were stained with 2% UA or 2% phosphotungstic acid.

Virus purification. A virus purification protocol developed for tomato ringspot virus by R. Stace-Smith (*personal communication*) was used to purify BSIV. Leaves of infected *N. clevelandii* were homogenized (2 ml/g of tissue) in 0.03 M dibasic sodium phosphate and 0.02 M ascorbic acid, pH 8.0, containing 0.02 M 2-mercaptoethanol. The homogenate was expressed through nylon cloth mesh, and the sap was centrifuged at 16,000 *g* for 20 min (low-speed centrifugation). The supernatant was collected and stirred while 6 M hydrochloric acid was added dropwise to adjust the pH to 5.0; it was then left overnight at 4 C. This preparation was subjected to low-speed centrifugation, and the supernatant was collected. The virus was precipitated by adding 8% (w/v) polyethylene glycol (mol wt 8,000) plus 1% (w/v) sodium chloride and stirring for 1 hr at 4 C. The virus was pelleted by low-speed centrifugation, and the pellet was allowed to dry for 10 min. The pellet was then resuspended in at least 1/10 the original volume of 0.05 M sodium citrate, pH 7.5. After low-speed centrifugation the supernatant was centrifuged at 207,000 *g* for 2 hr, and the pellets were resuspended in 0.05 M sodium citrate (0.5 ml per 12-ml tube). After another low-speed centrifugation, up to 0.5 ml of the supernatant was layered on 10–40% sucrose gradients made in 0.05 M sodium citrate buffer in a 10-ml centrifuge tube and centrifuged in a swinging bucket rotor at 4 C for 90 min at 175,000 *g*. After centrifugation, the gradients were scanned with an Isco UA-5 absorbance-fluorescence detector (Isco, Inc., Lincoln, NE) with a 254-nm filter, and fractions with peak absorbance were collected. The collected fractions were diluted with 2 volumes of 0.05 M sodium citrate, pH 7.5, and centrifuged at 207,000 *g* for 2 hr, and the pellet was resuspended in 0.05 M sodium citrate buffer, pH 7.5.

For mechanical transmissions to healthy *N. clevelandii* plants the final pellets from the purification were resuspended in 0.05 M sodium phosphate, pH 7.0, containing 2% PVP-44. These plants were kept in the greenhouse and monitored for symptoms.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of coat protein subunit. The relative molecular mass of the coat protein was determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis on a vertical 12% gel with a 4% stacking gel (6) in a Bio-Rad MiniProtean II system (Bio-Rad, Richmond, CA). Purified virus diluted in 0.05 M Tris-Cl, pH 6.8, containing 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.00125% bromophenol blue was boiled for 5 min before it was loaded on the gel. Electrophoresis was at 10 mA per gel until the dye front moved through the stacking gel into the separating gel, and then at 20 mA per gel for 40 min. After electrophoresis, the gel was fixed and stained with 0.2% Coomassie Brilliant Blue R 250 in 25% methanol and 7% acetic acid and then destained with 25% methanol and 7% acetic acid.

Western blots were performed by running a protein gel as described above and electroblotting the separated proteins onto Immobilon-P membrane (Millipore, Bedford, MA) in 25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3, at 100 V for 1 hr at 4 C. The membrane was then blocked for 2–3 hr at room temperature in PBS-Tween (0.015 M phosphate-buffered saline plus 0.05% [v/v] Tween 20) containing 1% (v/v) nonfat dried milk powder (blocking buffer). The membrane was washed extensively in PBS-Tween and incubated in a mixture of PBS-Tween, BSIV antibody (1 μg/ml), and healthy sap (1 g in 10 ml of buffer). Excess antibody was removed with four 15-min washes of PBS-Tween. Bound antibody was detected by incubating the membrane for 1.5 hr in PBS-Tween containing ^{125}I-labeled protein A at 1–5 × 10^6 counts · min^{-1} · ml^{-1}, followed by three washes in PBS-Tween and overnight autoradiography (11).

RNA extraction. Purified virus was resuspended in 0.5 ml of 0.2 M Tris-Cl, pH 7.5, containing 0.025 M EDTA, 0.3 M NaCl, 2% SDS, and 250 μg/ml of proteinase K. Incubation at 37 C for 30 min was followed by two extractions with 0.5 ml of phenol/chloroform (1:1, v/v) and one extraction with 0.5 ml of chloroform/isoamyl alcohol (24:1, v/v). The RNA was precipitated with 1/10 volume of 3 M sodium acetate, pH 5.0, and 2.5 volumes of absolute ethanol at −20 C overnight. Approxi-

TABLE 1. Reciprocal dilution endpoint titers of ilarvirus antisera reacted against purified blueberry scorch ilarvirus and Prunus necrotic ringspot virus in indirect enzyme-linked immunosorbent assay

Antiserum	Endpoint with blueberry scorch ilarvirus	Endpoint with Prunus necrotic ringspot virus
Blueberry scorch ilarvirus	2.02×10^6	2.4×10^4
Prunus necrotic ringspot	2.7×10^4	1.5×10^5
Apple mosaic	8.1×10^3	2.4×10^4
Tulare apple mosaic	*[a]	*
Asparagus virus II	*	*
Spinach latent	*	*
Tobacco streak	*	*
Elm mosaic	*	4.5×10^3
Elm mottle	*	*
Prune dwarf virus II	*	9.0×10^2
Citrus leaf rugose	*	*
Citrus variegation	*	*
Humulus japonicus virus	3.0×10^2	...[b]

[a] Asterisk indicates no absorbance (A_{405}) at dilution of 1:100.
[b] Test not done.

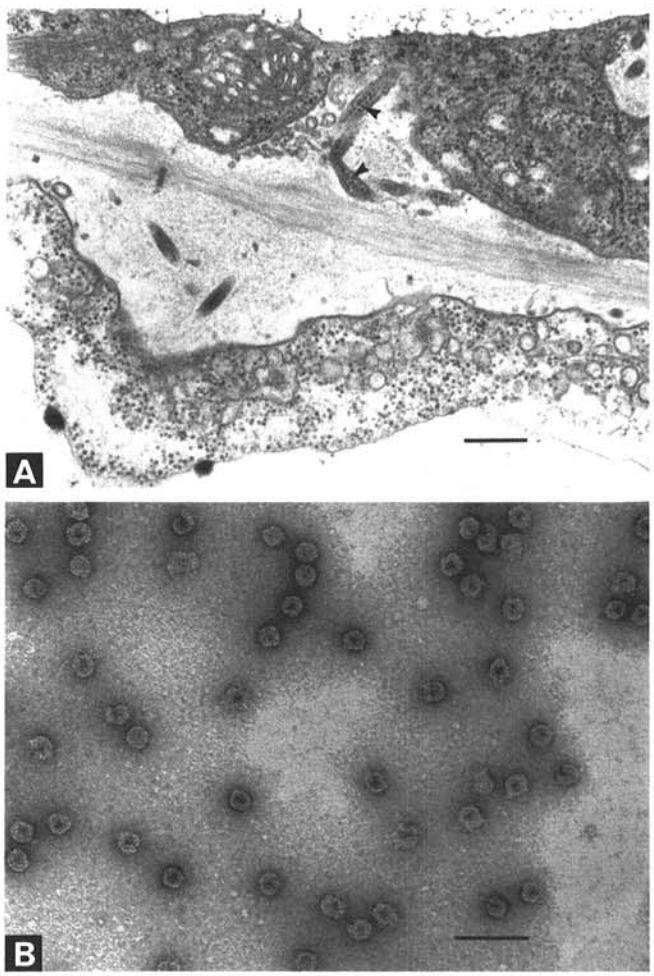

Fig. 1. A, Electron micrograph of a thin section of a leaf of *Nicotiana clevelandii* infected with blueberry shock ilarvirus showing isometric particles in the cytoplasm and in tubules (arrows) (bar represents 300 nm). **B,** Electron micrograph of negatively stained isometric virus particles from a purified preparation of blueberry shock ilarvirus (bar represents 100 nm).

mately 50 ng per band of RNA was separated by electrophoresis at 8 V/cm for 1 hr in a 1% denaturing agarose gel containing 5 mM methylmercuric hydroxide. An RNA ladder of 0.3- to 9.3-kb fragments (Bethesda Research Laboratories, Gaithersburg, MD) was used as RNA standards at approximately 100 ng per band per lane. The gel was stained with ethidium bromide after the addition of 2-mercaptoethanol. (8). The nature of the nucleic acid was determined by digestion with RNase T1 (5 μg/ml) or DNase I (RNase-free, 5 μg/ml) in enzyme buffer (50 mM Tris-Cl, pH 7.5, 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol) for 10 min at 37 C prior to electrophoresis. Tobacco mosaic virus RNA and pUC9 plasmid DNA were used as controls.

Serology. A New Zealand white rabbit was immunized with three intramuscular injections, 1 wk apart, of 0.3 mg of purified BSIV in 0.5 ml of buffer emulsified with 0.5 ml of Freund's incomplete adjuvant. After 3 mo, three additional intramuscular injections of 0.4 mg of virus, again emulsified in incomplete adjuvant, were administered. Antiserum was obtained from blood collected 10 days after the final injection. The titer of the antiserum was determined in agar double-diffusion tests (1).

Serological relationships with 12 other ilarviruses were determined in indirect ELISA tests. (The antisera, listed in Table 1, were a gift from G. I. Mink, Prosser, WA, except for the Humulus japonicus virus antiserum, which was supplied by A. N. Adams, East Malling, U.K.) The tests involved coating polystyrene microtiter plates (Flow Laboratories, Mississauga, ON) with purified BSIV or Prunus necrotic ringspot virus (PNRSV) in PBS at 1 μg/ml for 1 hr at 37 C. As a negative control, an unidentified nepovirus (currently being studied in our laboratory), purified from *N. clevelandii* by the same protocol as that used for BSIV, was coated on ELISA plates at 1 μg/ml. After being washed, the plates were blocked with PBS-Tween containing 0.1% (v/v) nonfat dried milk powder (blocking buffer) for 30 min at room temperature. Then 150 μl of a 1:100 dilution of whole antisera in blocking buffer was added to row A of each microtiter plate, and 100 μl of blocking buffer was added to rows B–H of each plate. A portion (50 μl) of the 1:100 dilution in row A was transferred to row B and mixed. Then 50 μl from row B was transferred to row C, and so on, until seven threefold dilutions had been carried out directly in the microtiter plate. After incubation overnight at 4 C, the plates were washed, and goat anti-rabbit alkaline phosphatase-conjugated antiserum (BioCan, Mississauga, ON) was added at a 1:5,000 dilution in blocking buffer. The plates were incubated for 2 hr at 37 C. and then washed, and substrate was added. The plates were read on an automatic ELISA plate reader (Titertek, Flow Laboratories, Mississauga, ON) 1 hr after the substrate was added. The absorbance (A_{405}) for each antiserum at each dilution for the negative control was subtracted from the absorbance (A_{405}) for each antiserum at each dilution obtained with BSIV or PNRSV coated directly onto the plates.

Purified BSIV was also tested against polyclonal antisera to the viruses listed in Table 1 in the Ouchterlony double-diffusion test (1). In these tests the purified virus (0.2–1 mg/ml) was tested against undiluted and diluted (1:10) samples of each antiserum.

Aphid transmission experiments. Apterous *Myzus persicae* (Sulz.) aphids from colonies maintained on *N. clevelandii* were starved for 1 hr in a petri dish lined with a wet paper towel. After being given acquisition access times of 30 sec, 1 min, 2.5 min, 5 min, 1 hr, 5 hr, and 18 hr on *N. clevelandii* infected with BSIV, six aphids per access time were transferred to healthy *N. clevelandii*.

Adult *Fimbriaphis fimbriata* (Richards) aphids originally from blueberry were fed on moist filter paper in a petri dish overnight. The young born in the petri dish were transferred to rose and strawberry to ensure a BSIV-free colony. Apterous aphids from these colonies were starved for 1 hr as described above. They were then placed on detached blueberry leaves from bushes infected with BSIV. After acquisition access times of 30 sec, 1 min, 2.5 min, 5 min, 1 hr, 5 hr, and 18 hr, six aphids per access time were transferred to healthy *N. clevelandii*.

Both species of aphids were allowed to feed on healthy *N. clevelandii* for 48 hr before the plants were fumigated with methyl bromide and placed in a greenhouse. These plants were monitored for symptoms and tested by ELISA for BSIV infection.

Detection of virus in plants. ELISA was used to detect BSIV in blueberry plants. Direct double-antibody sandwich ELISA (2) was used, except that the tissue was homogenized in 0.05 M borate

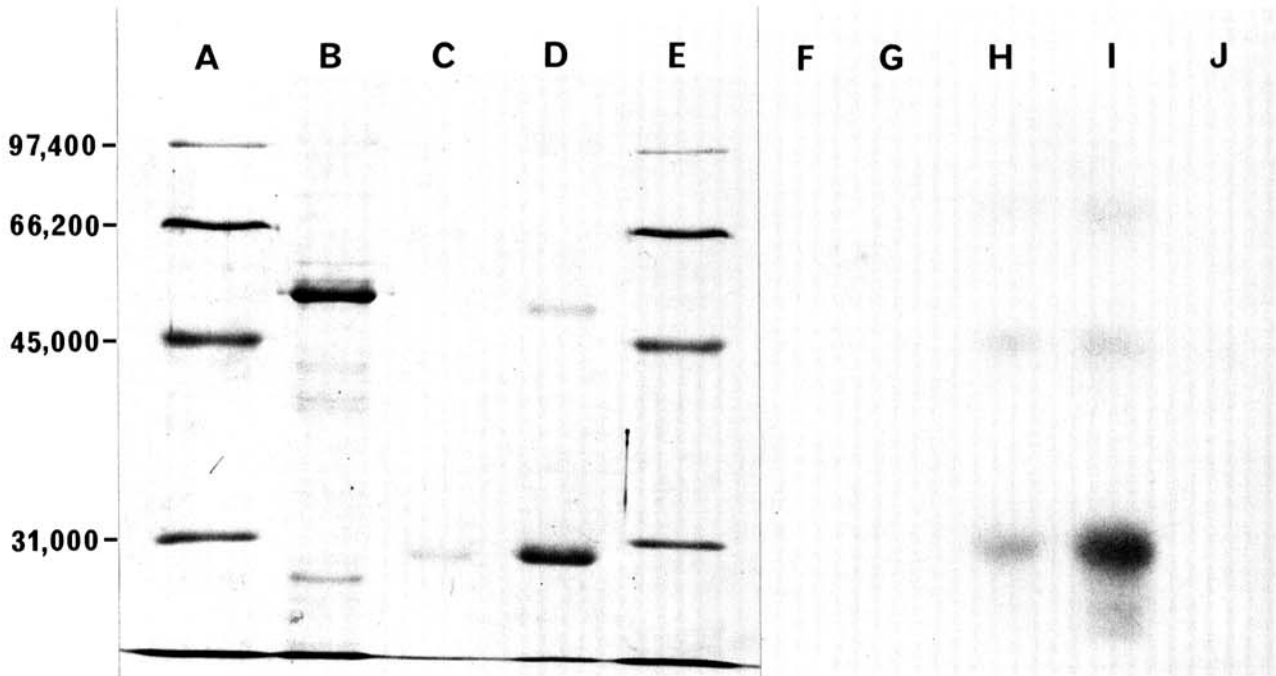

Fig. 2. Polyacrylamide gel (lanes A–E) and Western blot (lanes F–J) of the blueberry shock ilarvirus coat protein. Lanes A–E are markers with relative molecular weights given at the left; lane B is healthy *Nicotiana clevelandii* (1 g of leaf tissue homogenized in 10 ml of buffer and 10 μl loaded per lane); lanes C and D are purified blueberry shock ilarvirus at 0.5 and 2.0 μg per lane, respectively. The low-range molecular weight markers are from Bio-Rad and include phosphorylase b (mol wt 92,500), bovine serum albumin (mol wt 66,200), ovalbumin (mol wt 45,000), and carbonic anhydrase (mol wt 31,000). Lanes F–J correspond to lanes A–E from the polyacrylamide gel in a Western blot (photographed slightly smaller than the polyacrylamide gel).

buffer, pH 8.0, containing 2% (v/v) polyvinylpyrrolidone, 0.05% (v/v) Tween 20, 0.5% (v/v) nicotine alkaloid, and 0.1% (v/v) nonfat dried milk powder. Four mature leaves collected from various parts of a plant consituted a sample.

RESULTS

Mechanical transmission. Inoculated leaves of *N. clevelandii* plants developed ring spots 7 days after mechanical inoculation with homogenized blossoms of blueberries; 12 days after inoculation the infection had become systemic, with necrotic areas appearing on the young expanding leaves. These symptoms were not present on the plants mock-inoculated with buffer. Of the species tested, only *N. clevelandii, N. tabacum* 'Havana 425,' *N. benthamiana, N. tabacum* 'Samsun,' and *N. sylvestris* showed symptoms and were found to be infected by BSIV when tested by ELISA. *N. clevelandii* mechanically inoculated with purified BSIV developed symptoms similar to those of plants inoculated with homogenized blossoms of blueberry. Grafted blueberry plants developed symptoms similar to those observed in the field-infected plants as early as 6 wk postinoculation and tested positive by ELISA.

None of the healthy *N. clevelandii* plants to which aphids had been transferred from infected *N. clevelandii* or blueberry developed symptoms characteristic of BSIV infection or were positive for BSIV by ELISA.

Electron microscopy. Leaf dips of infected *N. clevelandii* revealed isometric particles, which were not seen in comparable healthy material (results not shown). Isometric particles were also seen in thin sections of infected *N. clevelandii* leaves. These particles were in the cytoplasm and were sometimes seen in tubules that appeared to be attached to the plasmodesmata (Fig. 1A). No viruslike particles were seen in leaf dips from symptomatic blueberry plants. However, isometric particles were trapped from blueberry leaves from infected plants with antiserum-coated grids using the antiserum produced to this virus (not shown).

Virus purification. After 2 hr of sucrose gradient centrifugation there were two ultraviolet-absorbing bands in the gradients, and no further bands could be resolved with further centrifugation. Fractions collected at the peak absorbance, when stained with UA and examined in the electron microscope (Fig. 1B), were found to contain quasi-isometric particles. The average diameter of these particles was 27 nm, based on the measurement of 100 virus particles. The particles ranged in size from 26 to 29 nm. (Tobacco mosaic virus was used as an internal standard.) Phosphotungstic acid stain apparently destroyed the particles, since no particles could be seen in the electron microscope when it was used. Lower yields of the virus were obtained when the purification was performed later than 7 days after inoculation of *N. clevelandii.* The virus particles had a maximum absorption at 260 nm and a minimum at 244 nm; the A_{260}/A_{280} ratio ranged from 1.46 to 1.60 (uncorrected for light scattering).

SDS-polyacrylamide gel electrophoresis of coat protein subunit. The coat protein subunits of BSIV migrated as two components in a 12% polyacrylamide gel (Fig. 2, lanes C and D). The major component had an M_r of approximately 27,300 ± 600 (3 standard deviations), and the minor component had an M_r of 51,650 ± 3,000 (3 SD). A healthy component from *N. clevelandii* had an M_r of approximately 55,000 (Fig. 2, lane B), but the large molecular weight component seen with the purified virus was shown to be viral in origin in the Western blot (Fig. 2, lane G). A band at approximately 79,000 was also detected in the Western blot (Fig. 2, lane I). These larger components may be dimers and trimers of the protein subunit.

RNA extraction. Early attempts at extracting nucleic acid from purified virus failed to yield RNA. The proteinase K treatment before extraction, however, resulted in the recovery of nucleic acid. The nucleic acid of the isometric particle migrated as four bands of M_r 1.03, 0.84, 0.57, and 0.30 × 10^6 (mean of six determinations) (Fig. 3). The nucleic acid was RNA, since it was degraded by RNase but was stable to DNase.

Serology. Antiserum produced against the blueberry isometric virus had a dilution end point of 1/64 in agar gel double-diffusion tests. When this antiserum was used in direct double-antibody sandwich to detect BSIV in *N. clevelandii*, the nonspecific reactions were higher than desired. However, diluting the conjugate in healthy sap (1 g of healthy *N. clevelandii* tissue homogenized in 10 ml of PBS-Tween containing 1% [v/v] nonfat dried milk) eliminated these nonspecific reactions. When blueberry tissue was

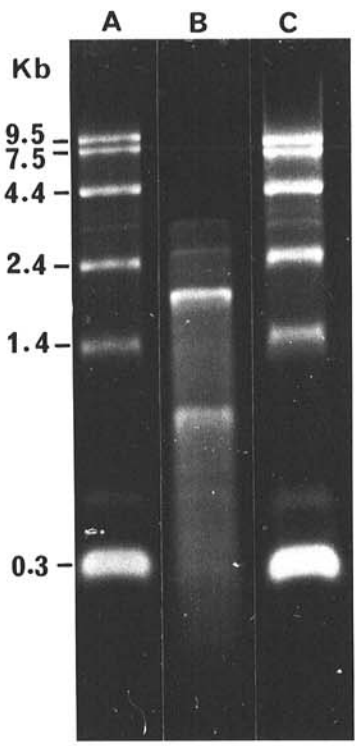

Fig. 3. A 1% agarose gel containing methylmercuric hydroxide of blueberry shock ilarvirus RNA. Lane B is RNA extracted from virus particles as described in the text. Lanes A and C are RNA markers (Bethesda Research Laboratories, Gaithersburg, MD).

TABLE 2. Results of tests for blueberry scorch virus, blueberry ilarvirus, and tomato ringspot virus in British Columbia, Washington, and Oregon for 1987 and 1989

	Farms positive for virus vs. farms tested	
	1987[a]	1989[b]
British Columbia		
Blueberry scorch virus	0/27	0/25
Blueberry ilarvirus	...[c]	0/25
Tomato ringspot virus	0/27	0/25
Washington		
Whatcom County		
Blueberry scorch virus	0/12	0/4
Blueberry ilarvirus	...	3/4
Tomato ringspot virus	1/12	0/4
Pierce County		
Blueberry scorch virus	6/7	6/8
Blueberry ilarvirus	...	3/8
Tomato ringspot virus	0/7	0/8
Clark County		
Blueberry scorch virus	1/3	1/3
Blueberry ilarvirus	...	2/3
Tomato ringspot virus	0/3	0/3
Oregon		
Blueberry scorch virus	0/6	2/15
Blueberry ilarvirus	...	3/15
Tomato ringspot virus	0/6	1/15

[a] At least 4,500 bushes were tested.
[b] At least 3,500 bushes were tested.
[c] Blueberry ilarvirus antiserum was not available in 1987.

tested, nonspecific reactions were not a problem, since infected blueberry tissue had A_{405} values 10 times those of healthy controls.

Antisera to 12 other ilarviruses were tested in indirect ELISA for reactivity against BSIV. The reciprocal dilution endpoints of these antisera, when reacted with purified BSIV and PNRSV coated directly on microtiter plates at 1 μg/ml, are shown in Table 1. PNRSV, apple mosaic virus (ApMV), and BSIV antisera reacted with BSIV in these tests, and BSIV antiserum also reacted with purified PNRSV. BSIV reacted, with spur formation, in agar gel double-diffusion tests with antiserum to PNRSV, but not with ApMV antiserum or any of the other nine ilarvirus antisera tested.

Detection of virus in plants. BSIV has been found in commercial blueberry plantings from Whatcom County in northern Washington to Linn County in the central Willamette Valley of Oregon, whereas BBScV has been found only from Pierce County in west central Washington to Linn County in Oregon (Table 2). BSIV was detected in 24 of 26 blueberry cultivars tested. Infected plants of Berkeley, Bluecrop, Bluechip, Bluetta, Bluehaven, Bluejay, Blueray, Collins, Darrow, Dixi, Earliblue, Elizabeth, Elliott, Herbert, Jersey, Lateblue, Meader, Northland, Patriot, Pemberton, Spartan, Stanley, Weymouth, and 1613-A were found, but all plants of Gem and Rancocas tested were negative for BSIV. Neither of these viruses has been found in blueberry in British Columbia.

Native vegetation surrounding blueberry fields containing plants infected with BSIV was also tested for this virus. Several plant species in the families Ericaceae and Rosaceae were included in these tests as well as other plants, both woody and herbaceous. All native plants tested by ELISA were negative for BSIV.

DISCUSSION

These results show that at least two viruses, BBScV (a carlavirus) and BSIV, cause scorchlike symptoms in blueberry. BBScV has been described previously (9), and BSIV is reported here.

BSIV has many properties that place it in the ilarvirus group of plant viruses (3,4), including quasi-isometric particles, approximately 27 nm in diameter; a single polypeptide of mol wt 27,500; four species of single-stranded RNA; mechanical transmissibility; a woody plant as its natural host; at least two particle types visible in density gradients (and perhaps more that we could not resolve); ability to produce a shock reaction in infected plants; and apparent aphid-nontransmissibility. In addition, in gel double-diffusion BSIV was distantly related to PNRSV, and in indirect ELISA BSIV reacted with antisera to PNRSV and ApMV.

BSIV should be considered a new member of the ilarvirus group (5). It has only a distant serological relatedness to PNRSV and ApMV, a narrow herbaceous host range (which excludes cucumber), and a natural host limited to blueberry.

The rate of spread of BSIV through blueberry fields is faster than expected for most ilarviruses. A field in which 40 plants had symptoms one year had an additional 60 plants infected the next year. This rapid spread may be due to the closed shape of the flowers and the amount of damage bees cause them while attempting to reach the nectar. Alternatively, pollen tube germination and growth to the style in blueberry may cause enough mechanical damage for virus transmission to occur.

BSIV has been found throughout coastal Washington and the Willamette Valley of Oregon, but not in British Columbia. It is surprising that no infected fields have been found in British Columbia, since the virus is so widespread in Washington and Oregon and planting stocks are frequently moved across the borders. Also, several infected fields in Whatcom County lie in close proximity to the Washington-British Columbia border.

The origin of this virus is unknown, but because it appeared in several areas within a few years and was not found in any of the native vegetation, it is possible that BSIV was introduced on planting stock. Many of the blueberry plants planted in the Pacific Northwest are propagated in New Jersey and Michigan. The disease has not been reported in these areas, but it would be interesting to examine commercial blueberry plantings and wild *Vaccinium* species there to determine if this virus is present.

LITERATURE CITED

1. Ball, E. M. 1961. Serological Tests for the Identification of Plant Viruses. American Phytopathological Society, St. Paul, MN. 16 pp.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Fulton, R. W. 1981. Ilarviruses. Pages 377-413 in: Handbook of Plant Virus Infections. E. Kurstak, ed. Elsevier/North Holland, Amsterdam.
4. Fulton, R. W. 1983. Ilarvirus group. Descriptions of Plant Viruses, no. 275. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England. 6 pp.
5. Hamilton, R. I., Edwardson, J. R., Francki, R. I. B., Hsu, H. T., Hull, R., Koenig, R., and Milne, R. G. 1981 Guidelines for the identification and characterization of plant viruses. J. Gen. Virol. 54:223-241.
6. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
7. MacDonald, S. G., Martin, R. R., and Bristow, P. R. 1989. Viruses capable of causing a scorch disease of highbush blueberry. Acta Hortic. 241:295-300.
8. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 541 pp.
9. Martin, R. R., and Bristow, P. R. 1988. A carlavirus associated with blueberry scorch disease. Phytopathology 78:1636-1640.
10. Martin, R. R., and MacDonald, S. G. 1988. Survey for blueberry scorch virus and tomato ringspot virus in highbush blueberry in B.C., Washington, and Oregon. Can. Plant Dis. Surv. 68:98.
11. Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. Proc. Natl. Acad. Sci. USA 76:4350-4354.