Etiology

A Carlavirus Associated with Blueberry Scorch Disease

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We wish to thank the Washington State Blueberry Commission and the British Columbia Blueberry Cooperative Association for financial support.

Accepted for publication 27 June 1988.

ABSTRACT

Martin, R. R., and Bristow, P. R. 1988. A carlavirus associated with blueberry scorch disease. Phytopathology 78:1636-1640.

Blighting of blossoms and a few leaves during peak bloom were the primary symptoms of a newly recognized disease of highbush blueberry (*Vaccinium corymbosum*) in Washington. Marginal chlorosis of leaves produced on older wood and overall reduction in plant vigor were additional symptoms observed on diseased bushes. The cultivars Berkeley, Atlantic, Collins, Herbert, and Pemberton were particularly susceptible. Blight symptoms were produced on previously healthy plants after they were graft-inoculated with wood from diseased plants. Bundles of viruslike particles in leaf tissue were associated consistently with infected plants and were present in previously healthy plants after they were graft-inoculated from infected plants. Viruslike particles purified from infected Pemberton blueberry plants were 690 nm long × 14 nm in diameter; the coat protein had a relative molecular mass of 35,200 daltons and a single RNA molecule of 8,400 bases. Antiserum prepared against these viruslike particles distinguished between diseased and healthy bushes in the field by enzyme-linked immunosorbent assay (ELISA). In indirect ELISA, the purified viruslike particles reacted with antisera to nine different carlaviruses but not with antiserum to turnip mosaic virus, a potyvirus. Association of these particles with diseased bushes leads us to believe that these viruslike particles are probably the causal agent of this newly recognized disease of highbush blueberry, for which we propose the name blueberry scorch disease. For the purpose of this paper, we refer to the virus as blueberry scorch virus (BBScV), recognizing that the virus may be a new member of the carlavirus group or a strain of a current member of this group.

A previously unreported disease of highbush blueberry was first observed in 1980 in Berkeley blueberries near Puyallup, WA. The disease which is known as blueberry scorch has since been observed in other blueberry cultivars. The symptoms of blueberry scorch first appear in late April to early May during bloom. Symptoms in some cultivars consist primarily of a blossom blight with a few necrotic leaves near the blighted flower clusters and marginal chlorosis of leaves produced on older wood; other cultivars only exhibit the marginal chlorosis on the leaves of the older wood; and some cultivars remain symptomless. The blighted blossoms often are retained throughout the summer but fail to develop into fruit. Blighted leaves may be retained for a short while but most leaves drop within 3–4 wk of the onset of symptoms. In cultivars that exhibit blossom blight, the twigs with the blighted flower clusters often die back 4–10 cm. The symptoms may appear on one or several branches or an entire bush. Affected bushes develop symptoms every year. Infected Berkeley plants can be killed in 3–6 yr, whereas infected Pemberton plants survive much longer, showing a progressive decline.

The symptoms of blueberry scorch can be confused with frost injury, bacterial blight, mummy berry blossom blight, and Botrytis blossom blight (2). Blueberry scorch disease has no relation to the

slope of the land and occurs whether or not there is frost. Bacterial blight, which was a serious problem in the spring of 1986 in the Pacific Northwest, can be controlled with fixed copper sprays which have no effect on blueberry scorch; moreover, scorch occurs whether or not environmental conditions are favorable for the development of bacterial blight. Mummy berry and Botrytis blighted blossoms and petioles usually are associated with masses of spores, whereas in the case of scorch, no spores are consistently associated with the blighted blossoms.

In this paper, the disease is called blueberry scorch disease and the causal virus is designated blueberry scorch virus (BBScV). Here we describe the purification of a virus from blueberry scorch-diseased blueberry plants, partially characterize this virus, and describe conditions necessary to detect this virus by enzyme-linked immunosorbent assay (ELISA) in infected blueberry leaves.

MATERIALS AND METHODS

Virus source. Naturally infected Pemberton blueberry plants from a field near Puyallup, WA, were used as a source for virus purification. Material used for virus purification tested negative for tomato ringspot virus in ELISA, did not show any symptoms of blueberry shoestring virus (13), and in thin-sectioned material did not contain caulimoviruslike inclusions characteristic of blueberry red ringspot virus-infected blueberry (8) or rods arranged in tetrads as observed in blueberry mosaic-diseased blueberry (7). After harvesting, the infected leaves were stored at 4 C for 1–5 days.

Transmission. Three plants of each of the following hosts were inoculated mechanically with infected Pemberton blueberry leaves homogenized in 0.05 M phosphate, pH 7.0, containing 2% polyvinylpyrrolidone (PVP, mol. wt. 44,000) with and without 0.5% nicotine: *Beta vulgaris* L. 'Detroit Dark Red,' *Brassica juncea* (L.) Czern. 'Florida Broadleaf' and 'Tender Green,' *B. pekinensis* (Lour.) Rupr. 'Pe-tsai,' *Callistephus chinensis* (L.) Nees 'Powder Puff,' *Catharanthus roseus* (L.) G. Don, *Chenopodium amaranticolor* Coste & Reyn., *C. capitatum* (L.) Asch., *C. murale* L., *C. quinoa* Willd., *Cucumis sativus* L. 'Straight 8,' *Datura stramonium* L., *Gomphrena globosa* L., *Lactuca sativa* L. 'White Boston,' *Montia sibirica* (L.) T. Howell 'Sibirica,' *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. glutinosa* L., *N. rustica* L., *N. sylvestris* Speg. & Comes, *N. tabacum* L. 'Harrownova,' 'Havana 425,' 'Samsun,' 'White Burley,' and 'Xanthi,' *Petunia hybrida* Hort. Vilm. Andr. 'Coral Satin,' *Phaseolus vulgaris* L. 'Black Turtle,' 'Bountiful,' 'Pinto,' 'Red Kidney,' and 'Top Crop,' *Physalis pubescens* L. (*P. floridana*), *Pisum sativum* L. 'Improved Laxton,' *Plantago lanceolata* L., *Trifolium incarnatum* L., *T. pratense* L. 'Double Cut,' *T. repens* L., *Verbesina encelioides* (Cav.) Benth. & Hook., *Vigna unguiculata* (L.) Walp. subsp. *unguiculatat*, *Vicia faba* L. 'Windsor Long Pod,' and *Zinnia elegans* Jacq. Freshly purified virus was used also as inoculum for mechanical transmission tests to the same indicator hosts. All herbaceous plants were assayed for virus infection by electron microscopy using the leaf-dip method or by ELISA.

Symptomless scion wood from diseased plants, taken from July through September, was used to graft 2-yr-old Collins, Bluecrop, Jersey, and Blueray blueberry plants. Grafted plants were maintained in a greenhouse or screenhouse for 2 mo and then overwintered in screenhouses. Plants were observed for symptoms weekly during the growing season and were tested for virus infection by ELISA.

Electron microscopy. Tissue samples were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, post-stained in osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812. Sections were stained in lead citrate and uranyl acetate. Purified virus preparations and antibody-trapped particles were stained with 2% phosphotungstic acid, pH 7.0 (11).

Virus purification. Leaves of infected Pemberton blueberry plants were homogenized (5 ml per gram of tissue) in 0.1 M sodium borate, pH 8.2, containing 2% (w/v) PVP-44, 0.1 M ethylenediaminetetraacetic acid (EDTA), 0.5% (v/v) nicotine alkaloid, and 0.1% (v/v) 2-mercaptoethanol. After being squeezed through cheesecloth, the expressed sap was centrifuged at 10,000 *g* for 10 min. The supernatant was centrifuged at 110,000 *g* for 90 min, and the pellets were resuspended in 0.01 M potassium phosphate, pH 7.5, containing 0.1% (v/v) PVP-44 and 1mM EDTA. After low-speed centrifugation at 10,000 *g* for 10 min, the sample was centrifuged through a 30% sucrose cushion at 110,000 *g* for 2 hr. The pellets were resuspended in phosphate buffer as above, and CsCl was added to a density of 1.28 g/ml (3.86 g/10 ml of final volume). The sample then was centrifuged for 16 hr at 247,000 *g*, and the opalescent band was drawn off with a needle and syringe. This band was centrifuged at 170,000 *g* for 1 hr and resuspended in 0.01 M phosphate buffer, pH 7.5.

Electrophoresis. The relative molecular mass of the coat protein was determined by polyacrylamide gel electrophoresis on a vertical 12% gel with a 4% stacking gel. The samples were boiled for 5 min in 0.06 M Tris-Cl, pH 6.8, with 10% glycerol, 2% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol, and 0.00125% bromophenol blue before they were loaded onto the gel. The gel was 7 cm wide, 8 cm long, and 0.75 mm thick. Electrophoresis was at 10 mA/gel until the dye moved through the stacking gel, then at 20 mA/gel for 45 min. After electrophoresis, the gel was fixed and stained with Coomassie Brilliant Blue R-250 in 25% methanol and 7% acetic acid and then destained in 25% methanol and 7% acetic acid.

For RNA extraction, the purified virus was resuspended in 0.1 M Tris-Cl, pH 8.9, with 1% SDS, extracted twice with phenol-chloroform (1:1, v/v), and precipitated with 1/10 volume of 2 M sodium acetate, pH 5.4, and 2 volumes of absolute ethanol overnight at −70 C. The RNA was washed twice with 70% ethanol and quantitated spectrophotometrically using an extinction coefficient of 25 $(mg/ml)^{-1}$ cm^{-1} at 260 nm. Approximately 75 ng of the RNA per lane was electrophoresed at 75 V for 1.5 hr on a 10×7 cm 1% agarose gel containing 5 mM methylmercuric hydroxide and post-stained with ethidium bromide after the addition of 2-mercaptoethanol (10). RNA standards consisted of an RNA ladder from 9.5 to 0.3 kb (Bethesda Research Laboratories, Bethesda, MD). These RNA standards were loaded at approximately 100 ng/band/lane. The U1 strain of tobacco mosaic virus (TMV) RNA (100 ng) was run as an additional standard. This strain of TMV has been sequenced and it is known that the RNA is 6,395 bases (5).

Serology. A New Zealand White rabbit was immunized by four weekly intramuscular injections of purified virus emulsified with an equal volume of Freund's incomplete adjuvant. After a 3-wk rest period, the rabbit was given a fifth intramuscular injection. Using an extinction coefficient of 3.0 $(mg/ml)^{-1}$ cm^{-1} at 260 nm, 400 μg of virus in 0.5 ml of buffer was used for each injection. Antiserum was obtained from blood collected 10 days after the final injection. The titer of the antiserum was determined in agar gel double diffusion tests without SDS or other degrading compounds and in indirect ELISA tests. The results of the gel double diffusion tests were obtained after the plates were incubated at room temperature for 48 hr.

Serological relationships with other viruses were determined in indirect ELISA tests. The indirect ELISA test involved coating polystyrene microtiter (Flow Laboratories, Mississauga, Ont.) plates with purified virus in phosphate buffered saline (PBS) at 1 μg/ml for 1 hr at 37 C. After washing, the plates were blocked with PBS-Tween containing 0.1% nonfat dried milk powder (blocking buffer) for 1 hr at room temperature. Then 125 μl of whole antisera at a 1/100 dilution 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. Twenty-five microliters of the 1/100 dilution in row A was transferred to row B and mixed. Then 25 μl from row B was transferred to row C, etc., until seven fivefold dilutions were carried out directly in the microtiter plate. After incubating for 1 hr at 37 C, the plates were washed and goat anti-rabbit alkaline phosphatase conjugated antiserum (BioCan, Mississauga, Ont.) was added at a 1:5,000 dilution (the manufacturer's recommended dilution) in blocking buffer and incubated for 1 hr at 37 C. The plates were then washed and substrate was added. The plates were read on an automatic ELISA plate reader (Titertek, Flow Laboratories, Mississauga, Ont.) 1 hr after the substrate was added

when titering the BBScV antiserum and 2 hr after the substrate was added when the heterologous carlavirus antisera were used to examine relationships.

Detection of virus in plants. ELISA was used to detect BBScV in blueberry plants. Direct double antibody sandwich ELISA protocol (1) was used except that the tissue was homogenized in 0.1 M borate, pH 8.0, containing 2% PVP, 0.5% nicotine, and 0.1% nonfat dried milk powder. Young and old leaves from 48 Pemberton blueberry bushes (13 healthy and 35 diseased bushes) were collected and tested by ELISA to determine which leaves were the most reliable tissue for virus detection.

RESULTS

Transmission. All herbaceous hosts listed in the "Materials and Methods" section were inoculated mechanically with homogenates of infected Pemberton blueberry leaves. Freshly purified virus also was used as inoculum. All inoculated plants remained symptomless for 6 wk and indexed negative by ELISA or by leaf-dip electron microscopy.

Graft transmissions from infected Berkeley into Jersey, from infected Pemberton into Blueray, Bluecrop, Jersey, and Collins and from infected Collins into Collins were successful (Table 1). The grafted Collins showed symptoms 6 wk after grafting as did one of the Bluecrop plants. The Jersey, Blueray, and other Bluecrop plants did not exhibit any symptoms. All grafts with disease-free scions were negative (Table 1). The Collins plants that were successfully graft inoculated died during the following summer.

TABLE 1. Graft transmission of blueberry scorch disease

Source cultivar	Indicator cultivar	No. plants showing symptoms	No. plants ELISA positive[a]
Berkeley	Jersey	0/2[b]	1/2
Pemberton	Blueray	0/2	1/2
Pemberton	Bluecrop	1/2	1/2
Pemberton	Jersey	0/2	1/2
Pemberton	Collins	1/2	1/2
Collins	Collins	2/3	2/3
Healthy Collins	Collins	0/3	0/3

[a] Leaves of enzyme-linked immunosorbent assay (ELISA) positive samples had A_{405} values (mean of three wells) greater than the A_{405} values (mean plus three standard deviations) of leaves tested from four healthy samples.
[b] Numerator is the number of plants that developed symptoms or were ELISA positive; denominator is the number of plants grafted and tested by ELISA.

Purification. Early attempts at virus purification using several published procedures for other carlaviruses failed to yield any viruslike particles. With the procedure described in the "Materials and Methods" section, a single opalescent band was observed in CsCl gradients. When nicotine, PVP, or EDTA was removed from the extraction buffer, no opalescent bands were observed in the CsCl gradient nor were any viruslike particles observed in negatively stained preparations from these purification attempts. If the PVP was reduced to 1% or the EDTA reduced to 50 mM in the extraction buffer, the quantity of viruslike particles was greatly reduced. After dialysis to remove the CsCl, the purified BBScV particles had an ultraviolet absorption spectrum typical of rod-shaped viruses, with a maximum absorption at 260 nm and a minimum at 244 nm. The A_{260}/A_{280} ratio was 1.2 (corrected for light scattering).

Electron microscopy. Bundles of viruslike particles were observed in thin sections of infected material and were not seen in comparable healthy material. Bundles (Fig. 1A) and single particles (not shown) were observed in cytoplasm. The purified preparations contained large numbers of rod-shaped particles (Fig. 1B). The size of the particles (determined from measurements of 100 particles trapped onto antiserum-coated grids from leaf sap of blueberry scorch-diseased bushes) was 690 nm in length × 14 nm in diameter. Trapped particles ranged from 675 to 720 nm.

Electrophoresis. The coat protein of BBScV migrated as a single component in 12% polyacrylamide gels with a relative molecular mass of 35,200 ± 300 daltons (mean of four determinations) (Fig. 2A).

The nucleic acid of BBScV migrated as a single band of 8,400 ± 200 bases (mean of four determinations) (Fig. 2B). RNA of the U1 strain of TMV electrophoresed in the same system was estimated to be 6,300 bases. The U1 strain of TMV is 6,395 bases (5).

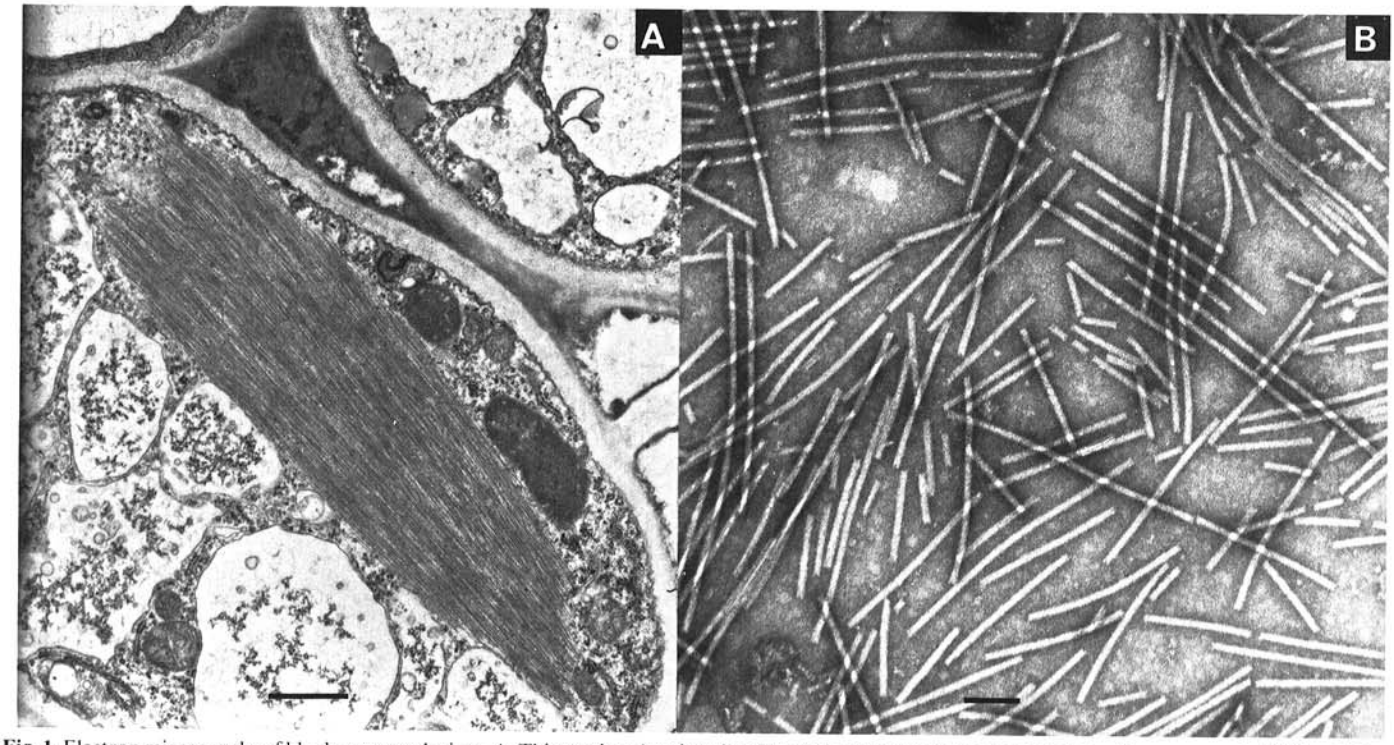

Fig. 1. Electron micrographs of blueberry scorch virus. **A,** Thin section showing virus bundle in diseased Pemberton blueberry (bar represents 1,000 nm). **B,** Negatively stained virus particles from purified preparations (bar represents 100 nm).

Serology. Antiserum produced against BBScV had a dilution endpoint titer of 1/1,024 in agar gel double diffusion tests when no SDS or other degrading compounds were added. In indirect ELISA, the BBScV antiserum had a titer of 1/7,812,500. Antisera to nine other carlaviruses were tested, in indirect ELISA, for reactivity against BBScV. The reciprocal dilution endpoints of these antisera when reacted with purified BBScV and turnip mosaic virus coated directly on microtiter plates at 1 μg/ml are shown in Table 2.

Detection of virus in plants. When blueberry tissue was homogenized in standard ELISA grinding buffer (1), the virus could not be detected in the leaf sap. The pH of the sap after grinding in this buffer was 3.7. Several buffers and additives were tried to help maintain the pH of the sap close to neutrality after grinding the blueberry tissue (Table 3). As a result of these tests, 0.1 M borate, pH 8.0, containing 0.05% Tween-20 (v/v), 2% PVP-44 (w/v), 0.1% nonfat dried milk powder (w/v), and 0.5% nicotine (v/v) was used as our routine grinding buffer for detecting BBScV in blueberry tissue.

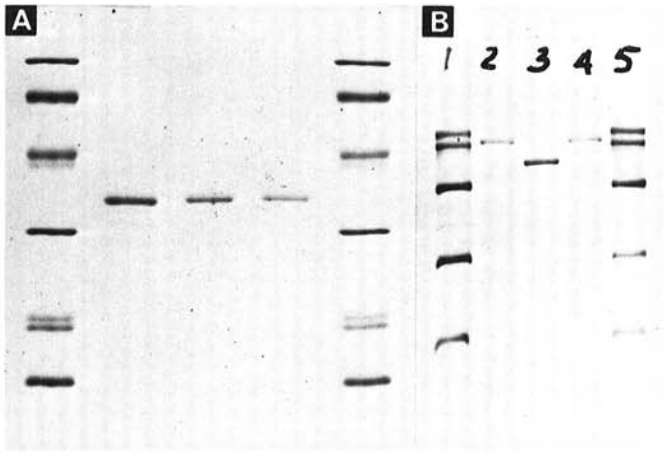

Fig. 2. Electrophoresis of blueberry scorch virus components. **A,** Polyacrylamide gel electrophoresis of blueberry scorch virus coat protein. Gel was run at 20 mA for 45 min. The outside lanes are markers with relative molecular masses: phosphorylase B, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,400 daltons (low-molecular-weight protein standards, Bio-Rad, Richmond, CA). The three center lanes are blueberry scorch virus purified from three different cultivars of blueberry (from left to right, Berkeley, Pemberton, and Stanley). **B,** Gel electrophoresis of blueberry scorch virus RNA under denaturing conditions in a 1% agarose gel containing 5 mM methylmercuric hydroxide. Electrophoresis was at 75 V for 1.5 hr on a 10×7 cm gel. After electrophoresis, the methylmercuric hydroxide was reduced with 2-mercaptoethanol and the nucleic acid was stained with ethidium bromide. Outside lanes are RNA ladder (Bethesda Research Laboratories, Bethesda, MD) with relative molecular masses of (from top to bottom) 9.5, 7.5, 4.4, 2.4, and 1.4 kilobases; center lane is 100 ng of U1 strain of tobacco mosaic virus RNA; and remaining lanes are blueberry scorch virus RNA.

TABLE 2. Reciprocal endpoint titers (mean of four determinations) of several carlavirus antisera reacted against purified blueberry scorch virus and turnip mosaic virus in indirect enzyme-linked immunosorbent assay[a]

Antisera to:	Endpoint with blueberry scorch virus	Endpoint with turnip mosaic virus
Blueberry scorch	1.5×10^6	1×10^2
Turnip mosaic	1.0×10^2	3×10^5
Carnation latent	3.0×10^5	1×10^2
Helenium virus S	1.5×10^6	1×10^2
Potato virus S	6.2×10^4	1×10^2
Dandelion carlavirus	1.5×10^6	1×10^2
Potato virus M	3.0×10^5	1×10^2
Chrysanthemum B	3.0×10^5	1×10^2
Elderberry carlavirus	6.2×10^4	1×10^2
Red clover vein mosaic	1.2×10^4	1×10^2
Pea streak	2.0×10^3	1×10^2

[a] Purified virus in phosphate buffered saline used at 1 μg/ml to coat microtiter plates.

In an attempt to optimize the detection of BBScV by ELISA, young and old leaves of field-infected plants were tested in late June from each of 48 different Pemberton blueberry bushes and the A_{405} values were compared. Young leaves were between one-half and three-fourths expanded and the old leaves were mature leaves produced near the center of the bushes. Of these 48 bushes, 35 exhibited symptoms of blueberry scorch disease and 13 were symptomless. With the young leaves, some of the diseased bushes indexed negative for BBScV by ELISA and there was a continuum of A_{405} nm values (Fig. 3A). With the old leaves, taken from the same bushes, there was a perfect correlation between the presence of scorch disease symptoms in bushes and positive ELISA results from these bushes. The A_{405} nm values of healthy and diseased bushes (based on symptoms) divided into two distinct populations (Fig. 3B). To determine a cutoff value for healthy reactions, we used the mean A_{405} value of the older leaves from healthy bushes plus three standard deviations. Similar results were obtained when older and younger leaves from BBScV-infected and healthy plants (as determined by ELISA on older leaves) of the symptomless variety Stanley were used.

DISCUSSION

We have shown that BBScV, which was purified from infected blueberry bushes, belonged to the carlavirus group. The virus bundles observed in thin-sectioned leaf material, the particle size, and the relative molecular masses of the coat protein and RNA were all typical for members of the carlavirus group (9). Also, the indirect ELISA results showed that BBScV was related serologically to nine other carlaviruses tested. Because these were one-way tests, it was not possible to determine if BBScV was a new member of the carlavirus group or a strain (blueberry strain) of an existing member of this group. This is the first report of a carlavirus from blueberry.

Detection of BBScV in blueberry tissue by ELISA required a stronger buffer system than the standard ELISA grinding buffer. When blueberry tissue was homogenized in standard ELISA buffer (that is, 0.01 M PBS-Tween, PVP, ovalbumin), the pH after grinding was 3.7. In this system, our antiserum gave less than threshold A_{405} values for some known infected plants and at best borderline positive values (mean of healthy values plus three standard deviations) for some diseased plants. By using a stronger grinding buffer similar to that used with grapevine tissue (3), ELISA values for diseased tissue were 5–20 times those of healthy plants. A similar buffer system to maintain the pH of the leaf sap at or near neutrality may be useful in the detection of blueberry red

TABLE 3. Effect of grinding buffer on detection by enzyme-linked immunosorbent assay of blueberry scorch virus in infected leaves of Pemberton blueberry with chlorotic margins

Grinding buffer	pH after grinding 1 g/10 ml of buffer	Mean (A_{405}) of eight samples[a]		
		Infected	Healthy	Threshold[b]
0.01 M PBS-TPB[c]	3.7	0.109	0.073	0.085
0.01 M PBS-TPB + nicotine[d]	6.3	0.797	0.068	0.080
0.15 M PBS-TPB	5.7	0.674	0.071	0.080
0.15 M PBS-TPB + nicotine	6.8	0.653	0.047	0.064
0.1 M borate (pH 8.0)-TPB	4.1	0.049	0.038	0.044
0.1 M borate (pH 8.0) -TPB + nicotine	6.4	0.887	0.051	0.063

[a] Samples were powdered in liquid N_2; then 1 g of tissue was homogenized in 10 ml of each buffer.
[b] Threshold = mean of healthy A_{405} values plus three standard deviations.
[c] PBS-TPB = phosphate buffered saline containing 0.5% Tween 20, 2% polyvinylpyrrolidone (mol. wt. 44,000) and 0.1% nonfat dried milk powder.
[d] 0.5 ml of nicotine per 100 ml of PBS-TPB.

ringspot virus, which can only be detected in leaf tissue during the early spring or late summer (4,6). Also, as with blueberry red ringspot virus, older leaves were a more reliable source of tissue for detection of BBScV by ELISA than were young leaves.

Detection of BBScV was optimized by harvesting mature leaves from blueberry plants. In the Pacific Northwest, mature leaves are available starting in June. Leaf tissue collected during May leads to variable A_{405} values and fails to give a clear distinction between known healthy and infected bushes. We were able to detect BBScV in infected bushes without having any background problems through September.

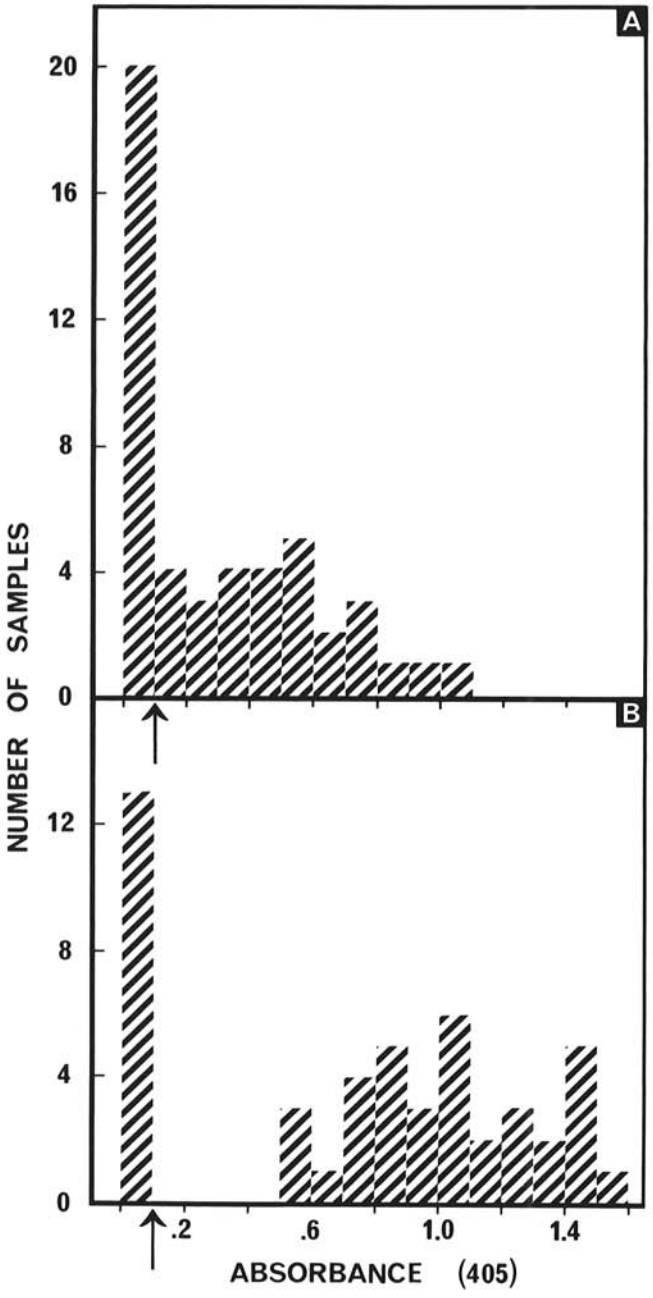

Fig. 3. Enzyme-linked immunosorbent assay results of blueberry scorch virus tests on young and old leaves from each of 48 Pemberton blueberry plants (35 plants had blueberry scorch disease symptoms and 13 were symptomless). Bars represent number of samples that had A_{405} values shown on the x-axis. **A,** Young leaves. **B,** Old leaves. Arrow indicates threshold value for positive reaction (mean plus three standard deviations of the A_{405} values of older leaves from healthy bushes).

The ELISA procedure described here provides a method to differentiate between BBScV-induced blossom blight and blossom blight resulting from other causes, that is, frost injury, bacterial blight, botrytis blossom blight, and mummy berry blossom blight. Proper diagnosis of the cause of blossom blight at an early stage is essential for implementation of a cost-effective disease management program.

The symptoms of blueberry scorch are similar to those of Sheep Pen Hill disease reported in New Jersey (14), except that the autumn foliar line pattern associated with Sheep Pen Hill disease has not been observed with blueberry scorch disease. Electron microscopy of blueberry scorch-diseased tissue showed rod-shaped particles in thin-sectioned material and in purified preparations. Electron microscopy of Sheep Pen Hill-diseased tissue showed similar particles (12) in leaf-dip preparations. However, in thin sections of Sheep Pen Hill-diseased tissue, particles appeared as tetrads when observed in cross section similar to those reported in blueberry mosaic-infected tissue (7). It is possible that the Sheep Pen Hill disease is caused by a combination of two viruses: the carlavirus that we have found associated with blueberry scorch and the virus that has been observed in blueberry mosaic-infected tissue.

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