Transmission, Field Spread, Cultivar Response, and Impact on Yield in Highbush Blueberry Infected with *Blueberry scorch virus*

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ABSTRACT

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Scorch disease caused by *Blueberry scorch virus* (BlSV) spreads rapidly and radially from foci of infection. Healthy potted blueberry plants became infected when placed next to diseased field bushes from early May through mid-August. The aphid *Fimbriaphis fimbriata*, collected from infected field bushes, transmitted BlSV to healthy blueberry plants in controlled tests and was regarded as the most important means by which bushes in commercial fields became infected. The rate of spread in the symptomless cv. Stanley appears to be the same as the rate of spread in the cv. Pemberton, which exhibits blight and dieback. Most field bushes showed symptoms during the year following inoculation, but a few did not show symptoms until the second or third year. Many (30 out of 59) cultivars and selections infected with BlSV exhibited severe blighting of flowers and young leaves and dieback of twigs. Three cultivars showed only chlorosis of leaf margins. The virus was also detected in numerous cultivars (26 out of 59) that exhibited no symptoms, and they were considered tolerant of BlSV. The virus had no effect on germination of pollen from several cultivars. BlSV reduced yield in 'Pemberton', with the loss being related to the number of years bushes displayed symptoms. Yield was reduced by more than 85% in the third year of symptom expression. The virus did not significantly reduce the yield of six tolerant cultivars that were infected with the virus but displayed no symptoms.

Additional keywords: carlavirus, cultivar reaction, pollen germination, tolerance, vector.

Scorch disease of highbush blueberry (*Vaccinium corymbosum* L.), caused by *Blueberry scorch virus* (BlSV; ITCV decimal code 14.0.1.0.003) (4), was observed originally on 'Berkeley' bushes in a commercial field near Puyallup, WA, in 1980 (1,13). Since then, BlSV has been detected in several other commercial fields in southwestern Washington and the Willamette Valley in western Oregon (12,15), but not in plantings in either northwestern Washington or the Fraser River Valley of neighboring southwestern British Columbia (12,15). Strains of BlSV cause the sheep pen hill disease (SPHD) in New Jersey (5,16).

Symptoms caused by BlSV range from complete necrosis (blighting) of flowers and young leaves and twig dieback in some cultivars (sensitive) to no visible damage in other cultivars (tolerant). A few cultivars show leaf-margin chlorosis in combination with the necrosis of flowers and leaves, but others show only leaf-margin chlorosis. Leaves on the interior of infected bushes are more likely to show this leaf-edge yellowing. Newly emerging leaves that blight turn black along the midrib and wilt, while older leaves turn tannish orange. The blighting of flowers occurs just as the corolla tubes are about to open and results in a characteristic scorched appearance (1,14). Blighted flowers are initially brown and then tan and bleach to gray over time. They are retained through the summer and may be present the following spring if not removed by pruning during the dormant season. Severely blighted bushes bare little fruit. In cultivars with severe blossom blight, the twigs often die back 4 to 10 cm from the shoot apex. Twig dieback causes lateral buds below the point of necrosis to grow and produce branches later in the season. Over a period of several years, infected bushes become very twiggy and no longer resemble healthy plants of the same cultivar. The difference is especially striking just before harvest. Branches of healthy 'Pemberton' bushes droop under the weight of ripe fruit (13), whereas infected bushes have an upright habit because the branches are shorter and the fruit load is markedly reduced. The productivity of cultivars that exhibit symptoms declines each year, and plants of some cultivars, such as Berkeley, eventually die (1).

The objectives of this study were to determine the rate of disease spread in commercial fields, identify vectors of the virus, and assess the impact of BlSV on both sensitive and tolerant cultivars. Tests were included to determine if BlSV reduced pollen germination, because a high level of fruit set is critical for good production. A preliminary report of a portion of this work has been published (3).

MATERIALS AND METHODS

Enzyme-linked immunosorbent assay (ELISA) testing. Double-antibody sandwich (DAS)-ELISA was used to detect BlSV in blueberry plants (6,13). All reagents were used at 100 µl per well in Corning flat-bottom microtiter plates (Corning, Inc., Corning, NY) except the blocking step, which was at 200 µl per well. Coating antibody (polyclonal, prepared against purified BlSV) was diluted in 0.01 M carbonate buffer, pH 9.6. Plates were coated for 4 to 6 h at room temperature. They were then blocked with phosphate-buffered saline, pH 7.4, containing 0.05% Tween-20 and 0.1% nonfat dried milk (PBS-Tween-Smp), and incubated at room temperature for 1 h. Leaf samples (three to four leaves per bush, with each leaf from a different main branch) were collected directly into Plexiglas trays containing 96 compartments arranged in the same 8 × 12 format as the microtiter plates. Filled trays were placed into a cooler with gel ice and transported to the lab, where they were held at approximately 5°C until the leaves were processed. Leaf samples

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were homogenized (approximately 1:15, wt/vol) in 0.05 M borate buffer, pH 8.0, containing 0.5% nicotine, 0.05% Tween-20, 2% polyvinylpyrrolidone (molecular weight = 44,000), and 0.1% nonfat dried milk. The extracted leaf sap was added to the plates and incubated overnight at 4°C. Plates were then washed three times with 0.5× PBS, followed by the addition of BlSV antibody conjugated to alkaline phosphatase and diluted 1:1,000 in PBS-Tween-Smp. Plates were incubated for 2 h at 28°C and washed as above. Substrate (*p*-nitrophenylphosphate at 0.5 mg/ml in 10% diethanolamine, pH 9.8) was added and plates were incubated at room temperature. Absorbance of each well at 405 nm (A_{405}) was read with a microtiter plate reader (MR5000; Dynatech Laboratories, Chantilly, VA; or Molecular Devices, Sunnyvale, CA). All samples were tested in duplicate wells. Leaves from known healthy and infected plants were included in every plate as negative and positive controls, respectively. Absorbance values were considered positive if the A_{405} values were greater than five times the values obtained for healthy checks.

Field spread. The locations of infected field bushes were mapped over an 8-year period in adjacent blocks of 'Berkeley' and 'Pemberton' and over 2 years in a third block of 'Stanley'. Bushes were on a 2.4 × 2.4-m spacing (8 × 8 ft). Infected bushes were initially identified by symptom expression and, beginning in 1987, by ELISA and symptoms.

Time of transmission. All field trials (except where noted) were conducted on the commercial farm in the Puyallup Valley where blueberry scorch disease was originally reported (1,13) and the WA1 strain of the virus was isolated (5). Healthy potted 'Berkeley' blueberry plants (2 to 3 years old, 45- to 75-cm-tall plants in 7.6-liter pots) were used as trap plants to detect the spread of BlSV over time on the commercial farm. Each plant was placed next to an infected (positive for BlSV by ELISA) field bush. Pots were fastened to steel fence posts so the canopy of the trap plant was (i) within 15 cm of and (ii) at the same height (approximately 1.4 m above the soil) as the midpoint of the fruiting area of mature field bushes. Sets of plants were placed in the field for 2-week periods beginning at early bloom and continuing until early leaf fall. Sets of 13 trap plants each were used in a 1.58-acre block of 'Pemberton' and 'Stanley' bushes in 1988, but sets of nine plants were used when the experiment was repeated in 1989. Soil in the pots was kept moist at all times. After the 2-week field exposure, trap plants were inspected for aphids, and any aphids presented were preserved in 70% ethanol for later identification (1989 only). Trap plants were then sprayed with malathion insecticide (7.8 ml of Malathion 8EC per liter sprayed to runoff) and maintained in a screenhouse through the winter. The following spring, new growth was monitored for blight symptoms, and leaves were assayed for BlSV by ELISA. Each year, one set of trap plants was never exposed to field plants and served as the check.

Transmission by aphids. Leaves with feeding aphids were collected from naturally infected 'Berkeley' and 'Pemberton' field bushes. In the greenhouse, a small dry paint brush was used to transfer aphids from the collected leaves to leaves of small healthy blueberry plants that had been propagated by tissue culture. The transfer was completed by lightly touching a feeding aphid with the brush and, when the aphid withdrew its stylet from the leaf, the brush was used to transfer it directly to a spot near the midrib on the lower surface of a leaf on a small potted plant. Each transfer took only a few seconds. A separate plant was used for each aphid. Aphids were confined to a 2.6-cm^2 area with a small cage constructed from balsa wood, clear plastic pipe (7 mm in length), and fine saran insect screen (style no. 5100900; Chicopee Mfg. Co., Cornelia, GA) (Fig. 1). 'Berkeley', 'Bluecrop', 'Blueray', and 'Jersey' test plants (eight plants per cultivar) were infested on 12 August 1988. Additional plants of each cultivar, without aphids, served as checks. One week after the initial transfer, and then twice weekly, the aphid and any progeny were transferred to new leaves on the same plant. After 27 days, all aphids were removed and preserved in 70% ethanol for identification. The plants were sprayed with acephate insecticide (2.64 ml of Orthene 75S + 0.26 ml of Latron B-1956 per liter of water sprayed to runoff) and maintained in a screenhouse for the following 2 years. Each spring, new leaves from the test plants were assayed for BlSV by ELISA.

The experiment was repeated in 1989, except that (i) groups of potted plants were infested with aphids collected on three dates earlier in the summer (21 and 28 June and 18 July); (ii) leaves with aphids were collected from naturally infected 'Pemberton' and 'Dixi' ('Dixi' bushes were on the adjoining farm to the west) field bushes; and (iii) 'Bluecrop', 'Bluejay', 'Blueray', and 'Duke' test plants were used in the greenhouse. Ten test plants of each cultivar were infested on each of the three dates. As above, aphids and progeny were transferred to a new leaf every 3 to 4 days for a total of 21 to 27 days. For one group of 40 plants infested on 18 July, all aphids were removed on 7 August and fresh aphids collected from infected field bushes were placed on each trap plant on 8 August to increase the likelihood of transmission. Thus, aphids remained on this group of plants for a total of 43 days.

In 1991, blueberry leaves with aphids were collected on four dates (24 June and 2, 10, and 23 July) from naturally infected 'Stanley' field bushes. The leaves were placed in cages (8 to 12 leaves per cage; approximately 100 aphids per cage) with potted 'Berkeley' test plants. Spent leaves were removed just before new leaves were added to the cages on each date. Another six test plants were kept in cages without aphids to serve as checks. Check plants were also sprayed periodically with malathion (same rate used in greenhouse studies). In late September, all plants were sprayed with the insecticide and moved into the screenhouse for 2 years.

Cultivar response to BlSV. Potted plants (2 to 3 years old) of 59 blueberry cultivars or selections were inoculated with BlSV by grafting. Plants were obtained from commercial nurseries and tested for BlSV before use. Scion wood was cut from the current year's growth of naturally infected field bushes. Two bottle grafts were made per plant, each on a separate main branch. Check plants were self-grafted. There were four plants per treatment for each cultivar. Plants were grafted during the summer and kept in the greenhouse until fall, when they were moved to the screenhouse after being sprayed with malathion insecticide. Plants were observed for symptoms and leaves were tested for BlSV by ELISA the following spring and every year for 3 years.

The reaction of several cultivars and selections to natural infection was evaluated in experimental plantings at Washington State University's Puyallup Research and Extension Center (WSU-Puyal-

Fig. 1. Cage used to confine an aphid and its progeny to the abaxial surface of a leaf on a potted test plant.

lup) and on the commercial farm where the disease was first recorded and the two farms adjoining it to the east and west. Some of the cultivars were the same as those inoculated by grafting, and the selections were from several breeding programs.

Viability of pollen. Fresh pollen was seeded onto the surface of 9% sucrose agar to test its viability using procedures previously described (2). Pollen was collected from newly opened flowers of both healthy and infected plants. Sources of pollen included field bushes and graft-inoculated potted plants with self-grafted plants serving as checks. Pollen was also obtained from one potted plant and 'Berkeley' field bushes (commercial field near La Center, WA) infected with both BlSV and *Blueberry shock virus* (BlShV).

Impact of BlSV on yield. The yield of healthy 'Pemberton' bushes was compared with that of naturally infected bushes showing blight symptoms for 1, 2, or 3 years. Each infected bush (symptoms plus ELISA positive for BlSV) was paired with an adjacent healthy (symptomless plus ELISA negative for BlSV) bush. All ripe berries were harvested by hand on three dates (16 July and 8 and 25 August 1987).

To assess the impact of BlSV on yield of selected tolerant cultivars ('Concord', 'Jersey', 'Olympia', 'Washington', and two selections from a private breeding program of 'N51G' and 'U-254'), pairs of adjacent bushes (located on the adjoining farm to the east) were identified for harvest. One bush in each pair was healthy (ELISA negative for BlSV) and the other infected (ELISA positive for BlSV). Plants were harvested by hand on four dates (26 July, 9 and 23 August, and 9 September 1988). All ripe berries were removed at these times. Ten to twelve pairs of bushes were harvested per cultivar.

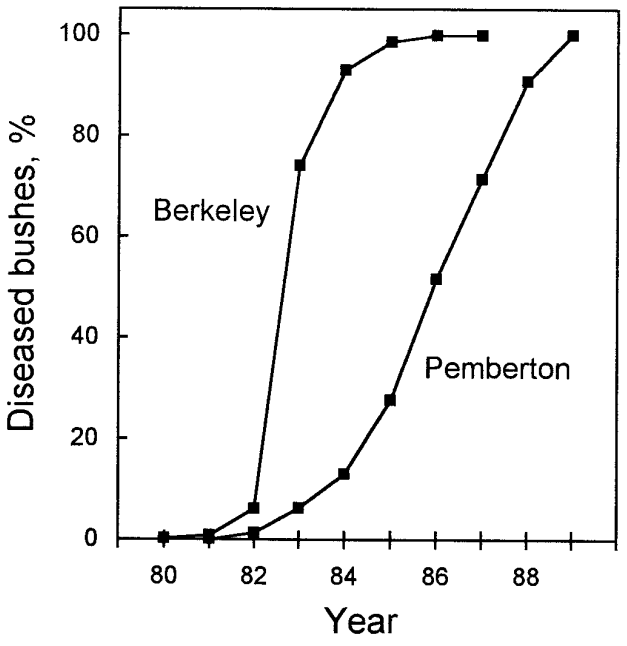

Fig. 2. Natural spread of *Blueberry scorch virus* in adjacent blocks of established 'Berkeley' and 'Pemberton' bushes (413 and 238 bushes, respectively) in a commercial planting near Puyallup, WA. Diseased bushes were identified by symptoms.

TABLE 1. Spread of *Blueberry scorch virus* (BlSV) in bushes of 'Pemberton' exhibiting scorch symptoms compared with symptomless bushes of 'Stanley'

	Bushes ELISA[x] positive for BlSV (%)	
Year	'Pemberton'[y]	'Stanley'[z]
1987	78.2	78.8
1988	95.0	100.0
% Increase	16.8	21.2

[x] Enzyme-linked immunosorbent assay.
[y] Block contained 238 bushes.
[z] Block contained 104 bushes.

RESULTS

Natural spread. Symptoms of the disease were first recorded on one 'Berkeley' bush in the southeastern corner of a block of 413 mature 'Berkeley' bushes on a commercial field near Puyallup, WA, in 1980. The disease spread radially, and plotting disease incidence over time produced a sigmoidal curve (Fig. 2). Within 3 years, the disease had spread into an adjacent block of 238 'Pemberton' bushes. There were several infection foci in the 'Pemberton' block, so the radial pattern of spread within that cultivar was not as apparent. During the initial 4 to 5 years, the number of infected plants nearly doubled each year. The rate of spread in 'Pemberton' was close to that in 'Berkeley'. This same rapid rate of spread was recorded in a commercial planting of 575 'Berkeley' bushes near Albany, OR, where leaf tissue was tested by DAS-ELISA to identify diseased bushes. Disease incidence rose from 0% in 1987 to nearly 80% in 1991 when that planting was taken out.

Each year, 4 to 8% of the 'Pemberton' bushes testing positive for BlSV for the first time remained symptomless. These symptomless bushes had a latent period of 1 or 2 years. Often, flowers and leaves on one or a few branches bore symptoms, but the rest of the plant remained free of symptoms. When a number of threeleaf samples were taken from different main limbs of individual plants, not all of the samples tested positive. It took some time for the virus to move systemically throughout the plant following infection. Occasionally, bushes with symptoms tested negative in ELISA; however, upon resampling, they tested positive.

Disease spread illustrated in Figure 2 was based on only symptom expression. Once the ELISA test was developed, we discovered that 'Stanley' bushes on this and two adjoining farms were also infected but symptomless. Between 1987 and 1988, the number of ELISA-positive 'Stanley' bushes increased by 21.2% compared with 16.8% for 'Pemberton' over the same time period (Table 1). Thus, the rate of spread in the symptomless cv. Stanley was similar to that of 'Pemberton', which exhibits symptoms.

TABLE 2. Transmission of *Blueberry scorch virus* (BlSV) to and aphid colonization of healthy trap plants ('Berkeley') placed next to diseased field bushes

Year	Exposure period	Positive/exposed[x]	Colonized/exposed[y]
1988	4 May–20 May	1/13	13/13
	20 May–3 June	3/13	13/13
	3 June–17 June	1/13	13/13
	17 June–1 July	1/13	11/13
	1 July–15 July	1/13	12/13
	15 July–29 July	2/13	10/13
	29 July–12 August	0/13	9/13
	12 August–26 August	0/13	9/13
	26 August–7 September	0/13	8/13
	7 September–23 September	0/13	6/13
	23 September–7 October	0/13	10/13
1989	14 April–28 April	0/9	3/9
	28 April–12 May	0/9	8/9
	12 May–26 May	3/9	9/9
	26 May–9 June[z]	4/9	2/9
	9 June–23 June	1/9	3/9
	23 June–7 July	3/9	7/9
	7 July–21 July	3/9	8/9
	21 July–4 August	3/9	8/9
	4 August–18 August	4/9	6/9
	18 August–1 September	0/9	4/9
	1 September–15 September	0/9	3/9
	15 September–29 September	0/9	5/9
	29 September–13 October	0/9	0/9

[x] Number of trap plants positive for BlSV/number exposed.
[y] Number of trap plants colonized by aphids/number exposed.
[z] Malathion insecticide applied to both field bushes and trap plants on 28 May.

TABLE 3. Transmission of *Blueberry scorch virus* (BlSV) by natural and graft inoculation, and cultivar response

Cultivar	Natural inoculation	Graft inoculation	Symptom expression[v]
Northern highbush			
Atlantic	+[w]	n.t.	B, C
Berkeley*[x]	+	+	B, C
Bluechip	+	+	N
Bluecrop*	+	+	N
Bluegold	+	+	N
Bluehaven	+	+	B
Bluejay*	+	+	B
Blueray*	+	+	B
Bluetta *	+	+	N
Burlington	n.t.	+	N
Collins*	+	+	B, C
Concord	+	n.t.	N
Coville	n.t.	+	N
Darrow*	+	+	B
Dixi	+	+	B, C
Duke*	n.t.	+	N
Earliblue*	+	+	B, C
Elizabeth	+	n.t.	B
Elliott*	n.t.	+	B
Friendship	n.t.	–	N
Gem	+	n.t.	B
Herbert	+	+	B, C
Ivanhoe	+	n.t.	N
Jersey*	+	+	B
Lateblue	+	n.t.	N
Meader	+	+	B
Nelson	n.t.	+	N
Nui	n.t.	+	N
Olympia	+	+	C
Patriot*	+	+	C, T
Pemberton*	+	+	B, C
Puru	n.t.	+	N
Rancocas	+	+	N
Reka	n.t.	+	N
Rubel	+	+	B, O
Sierra	n.t.	+	N
Spartan*	+	+	B
Stanley	+	n.t.	C
Sunrise	n.t.	+	N
Toro*	n.t.	+	N
Washington	+	+	N
Weymouth	+	+	B, C
1613-A (Hardiblue)[y]	+	+	N
N51G (Eberhardt)[y]	+	+	C
Southern highbush			
O'Neal	n.t.	+	N
Half-high			
Northblue	n.t.	+	N
Northcountry	n.t.	+	N
Northland	+	+	B
Northsky	n.t.	+	N
Selections[z]			
G-11	+	n.t.	B
G-130	+	n.t.	B
G-352	+	n.t.	B
G-353	+	n.t.	B
MU-698	+	n.t.	B
MU-6833	+	n.t.	B
MU-7016	+	n.t.	B
NC-1522	+	n.t.	B
R-18	+	n.t.	B
U-254	+	n.t.	N

[v] N = no symptoms; B = blighting of flowers and leaves; C = leaf-edge chlorosis; O = oak-leaf line pattern on leaves in autumn; and T = minor twig dieback.
[w] + = Enzyme-linked immunosorbent assay (ELISA) positive for BlSV; – = ELISA negative for BlSV; and n.t. = not tested.
[x] * = Pollen from healthy and infected plants tested for viability.
[y] Name in parentheses is that used in the Pacific Northwest.
[z] G = USDA-Beltsville; MU = Maine-US; NC = North Carolina State University; R = unknown; and U = private breeding program (J. Eberhardt, Olympia, WA).

Time of transmission. Transmission to trap plants took place between 6 May and 29 July 1988 and between 12 May and 18 August 1989 (Table 2). During 1988, only 11.5% of the trap plants became infected compared with nearly 40% in 1989. This occurred even though a higher proportion of the trap plants were colonized by aphids during the period of transmission in 1988 (92.3% in 1988 versus 58.6% in 1989). Transmission corresponded with the pres-

Fig. 3. Oak-leaf line pattern on a naturally infected 'Rubel' bush in early October.

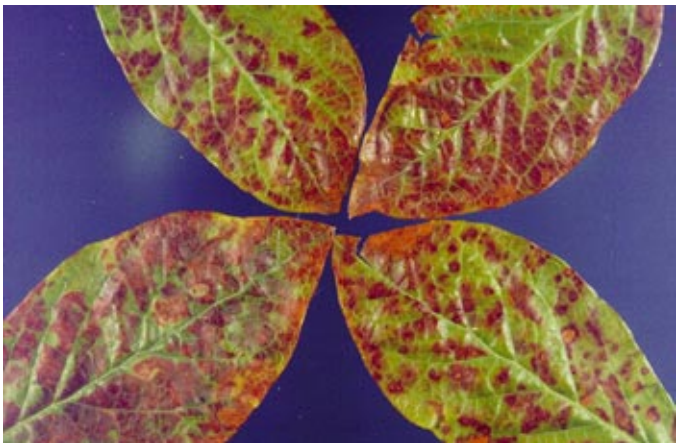

Fig. 4. Foliar symptom in mid-May on a 'Berkeley' plant infected with both *Blueberry scorch virus* and *Blueberry shock virus* (plate 130 in literature citation 14).

ence of aphids on the trap plants. All infected field plants immediately adjacent to trap plants were infested with aphids during both years. In late May 1989, the cooperating grower applied malathion insecticide to all bushes on the five-acre farm, including the set of trap plants in the field at that time. The aphid population crashed and then recovered (Table 2). Transmission to trap plants followed a similar pattern but lagged behind the recolonization of the trap plants by aphids.

Transmission by aphids. Only one 'Bluecrop' plant tested positive for BlSV when a single aphid was placed on each test plant. Transmission over all four cultivars (the other three were 'Berkeley', 'Blueray', and 'Jersey') was 3% (1 out of 32). All of the aphids used were from the genus *Fimbriaphis*. When they could be identified to species, they were *F. fimbriata* Richards. None of the plants became infected when the test was repeated in 1989, even though aphids were collected and trap plants were infested earlier in the summer to better correspond with when transmission occurred in the field. In the 1991 experiment, the six test plants in cages where numerous aphids were introduced supported large populations of aphids by 23 July. All six of these plants tested positive for BlSV the following spring, while the six check plants remained free of BlSV.

Cultivar response to BlSV. Of the 42 cultivars inoculated by grafting, only 'Friendship' remained free of the virus after 3 years in the screenhouse plus 1 year in the field (Table 3). Slightly more than half (21 out of 41) of the cultivars that became infected remained symptomless and were considered tolerant. Symptoms on the remaining cultivars ranged from leaf-edge chlorosis to severe blighting of both flowers and young leaves.

In field plantings, 43 cultivars and selections were found to be naturally infected (Table 3). Most of them (26 out of 43) were among those cultivars inoculated by grafting. Of the remaining seven cultivars and 10 selections, only three were symptomless. 'Rubel' was the only cultivar or selection to produce the oak-leaf line pattern described for plants infected with the SPHD strain of BlSV (Fig. 3). 'Berkeley' plants that were infected with both BlSV and BlShV developed blotchy orangish red spots on some of the leaves that were not blighted (Fig. 4).

Pollen viability. The viability of pollen from all 15 cultivars tested in vitro was not adversely affected by BlSV. Pollen from 'Berkeley' bushes infected with both BlSV and BlShV was just as viable as pollen collected from either healthy or BlShV-infected bushes.

Impact of BlSV on yield for a cultivar with scorch symptoms. Yield was reduced by one-third the first year 'Pemberton' bushes exhibited symptoms (Table 4). Yields were even lower for bushes that had displayed symptoms for 2 and 3 years. Yield losses were the result of a combination of fewer flowers and blighting of flowers. The midpoint of harvest for BlSV-infected bushes was 5 days earlier than that for healthy bushes, as more than 90% of the fruit harvested from infected bushes came from the first two pickings. By comparison, only 80% of the fruit was harvested from the healthy bushes in the first two pickings. Branches of healthy bushes were nearly bent to the ground under the weight of ripe fruit. In contrast, bushes infected for several years with BlSV were upright, because less fruit was produced on short twiggy growth.

TABLE 4. Yield of healthy 'Pemberton' field bushes and adjacent bushes exhibiting symptoms of *Blueberry scorch virus* (BlSV) for 1, 2, or 3 years

Number of years with blight symptoms	Yield[x], kg per bush
0 (healthy)[y]	19.00 a[z]
1	13.13 b
2	5.24 c
3	3.26 d

[x] All ripe berries harvested on three dates (16 July and 8 and 25 August).
[y] Plants without symptoms and enzyme-linked immunosorbent assay negative for BlSV.
[z] Mean separation by Student-Newman-Keuls test ($P < 5\%$). Means followed by a different letter are significantly different.

Impact on yield of cultivars that remain symptomless. BlSV had no significant impact on the yield of all six tolerant cultivars tested (Table 5). Two cultivars, N51G (known locally as Eberhardt) and Olympia, showed leaf-margin chlorosis, but no blighting of flowers and leaves. This mild leaf symptom associated with BlSV did not have a marked impact on yield. There was no shift in the midpoint of harvest for any of the six tolerant cultivars.

DISCUSSION

This work provides the first direct evidence of aphids transmitting BlSV from blueberry to blueberry. Hillman et al. (10), using unidentified aphids collected from blueberry bushes in Burlington County, New Jersey, were able to transmit the SPHD strain of BlSV from infected *Chenopodium quinoa* plants to healthy *C. quinoa* plants when 20 to 50 aphids were placed in a cage holding one infected and two healthy plants. Our early attempts to transmit BlSV using single aphids of *F. fimbriata* on small blueberry plants were largely unsuccessful. Apparently, BlSV is transmitted inefficiently by this aphid, and like most other carlaviruses that are transmitted by aphids, BlSV is nonpersistent in its vector. *Elderberry virus* (ECV) (18) and *Hop mosaic virus* (HoMV) (8) are transmitted by the aphids *Myzus persicae* and *Phorodon humuli*, respectively, and both are nonpersistent. Although BlSV may be inefficiently transmitted by individual aphids, the rate of field spread is high when aphid populations remain unchecked. At the field site where disease spread was followed (Fig. 2), the abaxial surface of leaves was sticky with honeydew by mid-June and sooty mold grew by late July in most years. Only 2 of the 29 carlaviruses vectored by aphids are reported to be transmitted in a semipersistent manner. Whiteflies (family Aleyrodidae) are known to transmit two carlaviruses (9,11), but these insects are not known to be pests of blueberry or other ericaceous plants.

SPHD of blueberry is caused by a strain of BlSV (16). Cavileer et al. (5) demonstrated that more than one strain of BlSV can exist in a field and that the two east coast strains studied were more closely related to one another than they were to a west coast stain. Strain differences most likely account for differences in symptom expression between the east and west coasts. East coast strains have been transmitted to both *C. quinoa* and *C. amaranticolor* by mechanical means (5), but those from the west coast could not be transmitted to any herbaceous host (13). The fall line pattern in leaves (often an oak-leaf line pattern) is frequently associated with SPHD but has only been observed on 'Rubel' on the west coast. In New Jersey, 'Bluecrop' exhibits symptoms but is symptomless on the west coast, suggesting that the reaction of some cultivars may be strain related.

For blueberry production to be economically viable, a very high percentage of flowers must set fruit, and berries must reach a reasonable size (7). The number of seeds per berry is positively correlated with fruit size (17). We hypothesized that, if BlSV inter-

TABLE 5. Impact of *Blueberry scorch virus* (BlSV) on the yield of infected but symptomless blueberry cultivars

Cultivar	Yield, kg per bush		
	Healthy[w]	Infected[x]	% Change
Concord	4.967	5.151	+3.7 ns[y]
N51G (Eberhardt)[z]	3.705	3.618	–2.4 ns
Jersey	7.311	7.110	–2.8 ns
Olympia[z]	6.381	5.499	–13.8 ns
U-254	0.489	0.454	–7.2 ns
Washington	0.720	0.569	–20.9 ns
Mean	3.929	3.734	–5.0 ns

[w] Enzyme-linked immunosorbent assay (ELISA) negative for BlSV.
[x] ELISA positive for BlSV.
[y] Analysis of variance, $P < 5\%$. ns = Not significant.
[z] Cultivars with leaf-edge chlorosis only.

fered with pollen germination (especially in tolerant cultivars), fruit set or fruit size might be reduced. BlSV, either alone or in combination with BlShV (2), had no significant impact on pollen viability in in vitro tests. Hence, the presence of large numbers of infected plants of tolerant cultivars should not impact yield because of pollen quality.

The existence of tolerant cultivars creates a dilemma in efforts to control the scorch disease. While tolerant cultivars remain productive once infected, they are a source of inoculum for further disease spread. Eppler (8) suggested that tolerant cultivars of hops, when infected with HoMV, were a more important source of inoculum than were sensitive plants that show symptoms. BlSV may spread to other plants in the planting, to plants used to replace infected sensitive plants, to plants used to expand acreage, or to plants in nearby fields. When new plants are introduced, cultivar selection is limited to tolerant ones. In addition to being tolerant, the selected cultivar often needs to have a similar ripening period as the cultivar being replaced. Most commercial blueberry plantings contain several varieties with staggered but overlapping ripening periods to give a longer harvest season and to make efficient use of labor and equipment. To date, many of the newest cultivars (those currently desired for planting) are sensitive to BlSV or have not been tested.

In commercial plantings, scorch disease is only known to occur in northern highbush types. The reactions of many cultivars of this type to BlSV are presented in this report. BlSV was also transmitted to a few southern and half-high types by grafting. Since 1989, when the National Clonal Germplasm Repository in Corvallis, OR, began testing blueberry accessions for BlSV, 11 northern highbush, 9 rabbiteye, and 3 southern highbush accessions in their field collection have tested ELISA positive (J. D. Postman, *personal communication*). Six of the eleven infected northern highbush accessions were obtained from a cultivar/selection trial at WSU-Puyallup in 1981. Some of those plants were probably infected at that time and were the source of inoculum for natural spread of BlSV in the field collection at the Repository. Many of the remaining plants in the trial at WSU-Puyallup were positive for BlSV when tested for the first time in 1988. All of the northern highbush plants that did not come from Puyallup and the rabbiteye plants were virus free when they went into the field collection, based on testing of duplicate plants maintained in the screenhouse at the Repository. In the field collection, the rabbiteye ('Baldwin', 'Bluebelle', 'Brightwell', 'Briteblue', 'Centurion', 'Climax', 'Coastal', 'Ethyl', and 'Tifblue') and southern highbush ('Croatan', 'Murphy', and 'Woodward') plants testing positive for BlSV were in the same rows as infected highbush plants. It is not known if any of the rabbiteye or southern types exhibited symptoms. The susceptibility of rabbiteye and southern highbush types points out the importance of preventing the introduction of BlSV into regions where these types are grown.

Roguing based on the appearance of symptoms is not a practical method of disease control, because a low percentage of infected plants do not exhibit symptoms until the second year after natural inoculation. This lag between inoculation and symptom expression was the reason why roguing failed to halt the spread of HoMV in European hop yards (8). For roguing to be successful, all infected plants (both sensitive and tolerant cultivars) must be identified and destroyed before BlSV is spread by the aphid vector. For Washington conditions, identification and destruction must occur before early May, when transmission first occurs. From a practical perspective, it is unlikely that growers would remove infected bushes of tolerant cultivars as long as they remained productive.

The aphid *F. fimbriata* appears to be the main vector of BlSV between plants in Pacific Northwest blueberry fields, but other species have not been tested. All reported attempts to transmit the disease mechanically from blueberry to blueberry have been unsuccessful (13). Healthy blueberry bushes in fields adjacent to fields with infected bushes are at risk of becoming inoculated with the virus unless aphid populations are controlled. Field studies to assess the effectiveness of aphid control on disease spread have not been conducted.

Sources of infected blueberry bushes other than those in adjacent commercial plantings exist and must be considered if effective measures to control aphids are to be implemented. When blueberry farms are taken out of production, the plants are frequently sold rather than destroyed. The sale of symptomless but infected bushes provides rapid dispersal of BlSV (and its aphid vector) to new sites. Although commercial growers are aware of this potential source of inoculum, a small number of bushes sold to a homeowner may constitute a focus of inoculum that is unrecognized but has the potential to initiate large-scale infections in nearby commercial blueberry plantings.

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LITERATURE CITED

1. Bristow, P. R., and Martin, R. R. 1987. A virus associated with a new blight disease of highbush blueberry. (Abstr.) Phytopathology 77:1721-1722.
2. Bristow, P. R., and Martin, R. R. 1999. Transmission and the role of honeybees in field spread of blueberry shock ilarvirus, a pollen-borne virus of highbush blueberry. Phytopathology 89:124-130.
3. Bristow, P. R., Windom, G. E., and. Martin, R. R. 1992. Period of transmission of blueberry scorch carlavirus and blueberry shock ilarvirus in the field. (Abstr.) Phytopathology 82:1085.
4. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J., eds. 1996. Plant Viruses Online: Descriptions and Lists from the VIDE Database. Published by the Australian National University, Canberra, Australia.
5. Cavileer, T. D., Halpern, B. T., Lawrence, D. M., Podleckis, E. V., Martin, R. R., and Hillman, B. I. 1994. Nucleotide sequence of the carlavirus associated with blueberry scorch and similar diseases. J. Gen. Virol. 75: 711-720.
6. 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.
7. Eck, P. 1988. Blueberry Science. Rutgers University Press, New Brunswick, NJ.
8. Eppler, A. 1997. The mosaic disease of hops (*Humulus lupulus*): Disease patterns. Meded. Fac. Landbouwwet. Rijksuniv. Gent 62/3b:969-976.
9. Francki, R. I. B., Milne, R. G., and Hatta, T. 1985. Carlavirus. Pages 173-182 in: Atlas of Plant Viruses Vol. I. CRC Press, Boca Raton, FL.
10. Hillman, B. I., Lawrence, D. M., and Halpern, B. T. 1995. Characterization and detection of blueberry scorch carlavirus and red ringspot caulimovirus. J. Small Fruit Vitic. 3:83-93.
11. Koenig, R. 1982. Carlavirus group. CMI/AAB Descriptions of Plant Viruses. Vol. 16, No. 259. Commonw. Mycol. Inst./Assoc. Appl. Biol., Richmond, Surrey, England.
12. MacDonald, S. G., and Martin, R. R. 1990. Survey of highbush blueberries for scorch viruses. Can. Plant Dis. Surv. 70:95-96.
13. Martin, R. R., and Bristow, P. R. 1988. A carlavirus associated with blueberry scorch disease. Phytopathology 78:1636-1640.
14. Martin, R. R., and Bristow, P. R. 1995. Scorch. Pages 51-52 in: Compendium of Blueberry and Cranberry Diseases. F. L. Caruso and D. C. Ramsdell, eds. The American Phytopathological Society, St. Paul, MN.
15. Martin, R. R., and MacDonald, S. G. 1988. Survey of blueberry scorch virus and tomato ringspot virus in highbush blueberry in B.C., Washington and Oregon. Can. Plant Dis. Surv. 68:91-92.
16. Martin, R. R., MacDonald, S. G., and Podleckis, E. V. 1992. Relationship between blueberry scorch and sheep pen hill viruses of highbush blueberry. Acta Hortic. 308:131-138.
17. Moore, J. N., Reynolds, B. D., and Brown, G. R. 1972. Effect of seed number, size and development on fruit size in cultivated blueberries. HortScience 7:268-269.
18. Van Lent, J. W. M., Wit, A. J., and Dijkstra, J. 1980. Characterization of a carlavirus in elderberry (*Sambucus* spp.). Neth. J. Plant Pathol. 86:117-134.