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Resistance to Blueberry Shoestring Virus in Southern Highbush and Rabbiteye Cultivars

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***Abstract*. To determine if blueberry shoestring virus (BBSSV) is absent in the southern United States due to resistance of cultivars, we mechanically and rub-inoculated 1-year-old rooted microshoots of nine cultivars representing southern rabbiteye (*Vaccinium ashei* Reade), southern highbush (hybrids of *V. corymbosum* and *V. darrowi* Camp), and northern highbush (*V. corymbosum* L.). Leaves were sampled from plants, and enzyme-linked immunosorbent assay screened for the presence of virus over 15 months. Only a few individuals were infected after aphid inoculation, but many northern and southern cultivars became infected after mechanical inoculation. Northern highbush 'Elliot' (50%) and 'Blueray' (46.3%) had the highest infection rates, followed by rabbiteye 'Climax' (36.3%) and the southern highbush 'O'Neal' (12.5%). The lowest rates of infection were found in southern highbush 'Georgiagem' (2.5%), 'Misty' (2.5%), rabbiteye 'Brightwell' (0.0%), and northern highbush 'Bluecrop' (2.5%). Since many southern cultivars were infected by the disease, resistance likely has not excluded BBSSV from the southern United States.**

Blueberry shoestring disease was first reported as a possible virus-caused disease of cultivated blueberries in New Jersey by Varney (1957). Since then, the disease has been identified in lowbush (*Vaccinium angustifolium* Ait.) and northern highbush plants in various areas (Hancock et al., 1993; Lockhart and Hall, 1962) other than the deep South of the United States, where rabbiteye and southern highbush cultivars are grown. The northern highbush cultivars Elliott and Blueray were highly susceptible to blueberry shoestring virus (BBSSV), while 'Bluecrop' had strong field resistance (Hancock et al., 1986). We undertook these studies to determine if the disease is absent in the deep South because southern blueberries are resistant to the disease.

Materials and Methods

When ≈15 cm high, 1-year-old rooted microshoots of three cultivars each of northern highbush, southern highbush, and rabbiteye were mechanically and aphid-inoculated in May 1993 (Table 1). Micropropagation was performed according to Callow et al. (1989). Shoots ≈2 cm long were inserted 1 to 2 mm into peat and maintained under plastic tunnels to root. Treatment values were compared using the Wilcoxon signed-rank test for nonparamatic statistics (Steel and Torrie, 1960).

BBSSV was purified from infected blueberry blossoms using the method of Ramsdell and Stace-Smith (1979). For mechanical inoculation, the purified virus was diluted with 0.05 M sodium phosphate buffer, pH 7.0, to yield a viral inoculum level of 0.01 mg•ml^{-1}. The inoculum was rub-inoculated onto the abaxial side of leaves of 20 plants of each cultivar using a piece of sponge and 320-mesh (0.04 mm) carborundum as an abrasive. Ten plants of each cultivar were mock-inoculated using 0.05 M sodium phosphate buffer, pH 7.0. After inoculation, plants were placed on a greenhouse bench in a completely randomized design. The average day/night greenhouse cycles were 18 to 43C/18 to 27C (summer); 18 to 30C/18 to 21C (fall); 18 to 24C/18C (winter); and 18 to 32C/18 to 21C (spring). At 9, 12, and 15 months after inoculation, 1 g of young fully expanded leaves was screened for the virus using the double-antibody sandwich method of enzyme-linked immunosorbent assay (ELISA) (Schulte et al., 1985).

For aphid inoculation, gravid females were collected from the field and kept in a petri dish on moist filter paper. Viviparae were gathered and allowed to multiply for 2 months on healthy blueberry plants kept in a growth chamber set at 20C with a 14-h daylength. Nonviruliferous aphids were then transferred to eight symptomatic blueberry plants for a 72-h acquisition access period. Viruliferous aphids were transferred onto 20 young plants, ≈15 cm in height, of each of six cultivars ('Elliott', 'Blueray', 'Bluecrop', 'Premier', 'O'Neal', and 'Brightwell') grown in 500-ml pots, at 10 aphids per plant and caged. Aphids were allowed a 72-h inoculation access period (IAP). At the end of the IAP, aphids were killed with Orthene insecticide (Valent USA Corp., Walnut Creek, Calif.). Control plants were treated in a similar manner, except that nonviruliferous aphids were transferred directly to the healthy test plants. All plants were placed in a completely randomized design on the bench and maintained under greenhouse conditions. At 3, 6, 9, and 12 months postinoculation, 1 g of young fully expanded leaves was sampled at random and screened for the detection of the BBSSV using ELISA.

The ELISA was performed using previously established procedures (Schulte et al., 1985). The IgG fraction of BBSSV antiserum and its enzyme conjugate were prepared according to modifications of Clark and Adams (1977). One milliliter of a 1-mg purified anti-BBSSV IgG/1 ml of 0.05 M sodium-carbonate-bicarbonate buffer (pH 9.6) was mixed with 2 mg of alkaline phosphatase (Sigma Chemical Co., St. Louis). Two microliters of a 25% solution of glutaraldehyde in water was added to the mixture and allowed to incubate 4 h at room temperature. The excess glutaraldehyde was removed by dialyzing the solution against three changes of 1× PBS [phosphate buffered saline = 15 mM phosphate and 8% (v/v) NaCl], including an overnight incubation. After dialysis, bovine serum albumin was added at a final concentration of 5 mg•ml^{-1} and the conjugate was stored at 4C.

Flat-bottomed polystyrene microtiter plates (Dynatech Co., Alexandria, Va.) were coated at 200 µl per well with a 1:1000 dilution (w/v) of purified anti-BBSSV-IgG in a coating buffer (0.05 M sodium carbonate-bicarbonate buffer, pH 9.6). Plates were placed in plastic bags, sealed, and incubated for 3 h at 37C. Leaf

Table 1. Percentage of plants testing ELISA positive to blueberry shoestring virus (BBSSV) after mechanical (M) and aphid (A) inoculation. Values are the means of tests taken 3, 6, 9, and 12 months after inoculation. Means are separated with a Wilcoxon signed-rank test at $P < 0.05$.

		BBSSV positive (%)	
Type	Cultivar	M	A
Northern highbush	Bluecrop[z]	2.5 a	0.0 a
	Blueray[z]	50.0 b	3.8 a
	Elliott[y]	46.2 b	3.8 a
Southern highbush	Georgiagem[x]	2.5 a	---
	Misty[w]	2.5 a	---
	O'Neal[v]	12.5 a	8.8 a
Rabbiteye	Brightwell[u]	0.0 a	0.0 a
	Climax[u]	36.3 b	---
	Premier[u]	10.0 a	3.8 a

[z]93% *Vaccinium corymbosum*, 6% *V. angustifolium.*
[y]100% *Vaccinium corymbosum.*
[x]75% *Vaccinium corymbosum*, 25% *V. darrowi.*
[w]86% *Vaccinium corymbosum*, 6% *V. darrowi*, 6% *V. ashei*, 1% *V. angustifolium*, 1% *V. tenellum.*
[v]85% *Vaccinium corymbosum*, 3% *V. darrowi*, 4% *V. ashei*, 11% *V. angustifolium.*
[u]100% *Vaccinium ashei.*

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samples (1 g) were homogenized in PBS-PVP-OVA Tween-20 buffer (1:10 w/v) using a tissuemizer (Tekmar Co., Cincinnati).

Results and Discussion

Among inoculated plants, there was an increase in average level of infection between months 3 (8.3%) and 6 (21.0%), but after this point, infection rates stabilized (20.5% in month 9 and 23.7% in month 12). There was no significant difference between the 6th, 9th, and 12th months after inoculation according to the Wilcoxon signed paired rank test at $P < 0.05$. The relative ranking of cultivars did not shift between sampling dates. Therefore, the four dates were combined for further statistical comparisons.

Most northern and southern cultivars became infected with BBSSV after rub inoculation, although levels of susceptibility varied greatly (Table 1). Northern highbush 'Elliott' and 'Blueray' had the highest infection rates, followed by the rabbiteye 'Climax'. The most resistant types were southern highbush 'O'Neal', 'Georgiagem', and 'Misty'; rabbiteye 'Premier' and 'Brightwell'; and northern highbush 'Bluecrop'. After aphid inoculation, rates of infection were very low in all cultivars regardless of origin, but a few individuals of all the northern and southern types were infected, except for 'Brightwell' and 'Bluecrop'.

In an earlier survey of native *V. ashei*, *V. angustifolium*, *V. pallidum* Ait., *V. darrowi*, *V. myrsinites* Lamarck, *V. tenellum* Ait., and *V. corymbosum*, Hancock et al. (1993) found only northern *V. corymbosum* and *V. angustifolium* became infected with BBSSV. However, few individuals of the southern species were examined, so it was unclear whether they were immune to the disease or just escapes. In this study, the rabbiteye cultivar Climax had one of the highest infection rates, demonstrating that some clones of *V. ashei* are susceptible to the disease. The cultivars derived from *V. darrowi*, 'O'Neal', 'Georgiagem', and 'Misty' (Ehlenfeldt and Vorsa, 1993; Hancock and Goulart, 1993, 1994) proved to be resistant, but their resistance may not be derived from *V. darrowi* since they all have highly resistant 'Bluecrop' as a parent. Because 'Climax' was easily infected with BBSSV, and all the other southern types except 'Brightwell' were susceptible, overall resistance to the disease has not prevented spread into the southern blueberry production regions. Hancock et al. (1993) suggested that the higher temperatures in the South might impede development of the disease, but preliminary experiments have not shown a significant decrease in infection rates between 20 and 30C (Acquaah, 1995). The aphid vector, *Illinoia pepperi* (MacG.), is poorly adapted to high temperatures (Elsner and Whalon, 1985), is not found in the South (Kreigle, 1985), and, possibly, no other vector transmits the disease. Alternatively, the virus may have originated in the North and simply may not have had enough time to spread south.

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