Resistance to Blueberry Shoestring Virus in Southern Highbush and Rabbiteye Cultivars

Theresa Acquaah and D.C. Ramsdell
Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

J.F. Hancock
Department of Horticulture, Michigan State University, East Lansing, MI 48824

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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), northern 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 ‘Elliott’ (50%) and ‘Bluecrop’ (6%) had the highest infection rates, followed by rabbiteye ‘Brightwell’ (12%), ‘Misty’ (2%), and northern highbush ‘Bluecrop’ (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 nonparametric statistics (Steel and Torrie, 1960).

BBSSV was purified from infected blueberry blossoms using the method of Ramsdell and Stage-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. The inoculum was used for infection onto 20 young plants, each cultivar using a piece of sponge and 320-mesh V. ashei. Three changes of 1 ml purified anti-BBSSV-IgG in a coating buffer were added to the virus solution made a pool of alkaline phosphatase (Sigma Chemical Co., St. Louis). Two microliters of a 25% solution of glutaraldehyde was added to the mixture and allowed to incubate 4 h at room temperature. The excess glutaraldehyde was removed by dialyzing the solution against 100 mL per well with a 1:1000 dilution of purified anti-BBSSV-IgG in a coating buffer and 9.6% NaCl, including an overnight incubation. After dialysis, bovine serum albumin was added at a final concentration of 1 mg/ml and the conjugate was stored at 4°C.

Flat-bottomed polystyrene microtiter plates (Dynatech Co., Alexandria, VA) were coated with 200 μl per well with a 1:1000 dilution 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 37°C. Table 1. Percentage of plants testing ELISA positive for 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.

Table 1. Percentage of Plants Testing ELISA Positive for 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.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cultivar</th>
<th>M (%)</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern highbush</td>
<td>Bluecrop</td>
<td>2.5 a</td>
<td>0.0 a</td>
</tr>
<tr>
<td></td>
<td>Blueray</td>
<td>50.0 b</td>
<td>3.8 a</td>
</tr>
<tr>
<td></td>
<td>Elliott</td>
<td>64.6 b</td>
<td>3.8 a</td>
</tr>
<tr>
<td>Southern highbush</td>
<td>Georgiagem</td>
<td>2.5 a</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Misty</td>
<td>2.5 a</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>O'Neal</td>
<td>12.5 a</td>
<td>8.8 a</td>
</tr>
<tr>
<td>Rabbiteye</td>
<td>Brightwell</td>
<td>0.0 a</td>
<td>0.0 a</td>
</tr>
<tr>
<td></td>
<td>Climax</td>
<td>36.3 b</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Premier</td>
<td>10.0 a</td>
<td>3.8 a</td>
</tr>
</tbody>
</table>

*93% Vaccinium corymbosum, 6% V. angustifolium.
*75% Vaccinium corymbosum, 25% V. darrowi.
*86% Vaccinium corymbosum, 6% V. darrowi, 6% V. ashei, 1% V. angustifolium, 1% V. tenellum.
*85% Vaccinium corymbosum, 3% V. darrowi, 4% V. ashei, 11% V. angustifolium.
*100% Vaccinium ashei.
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 ‘Elliot’ and ‘Bluecap’ 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 northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop’. After aphid inoculation, rates of infection were very low in northern highbush ‘Bluecrop'. 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.

**Literature Cited**


