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Search for Resistance to Blueberry Shoestring Virus in Highbush Blueberry Cultivars[1]

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Abstract

Plants of 28 highbush blueberry cultivars (*Vaccinium corymbosum* L.) were rub-inoculated with 1.0 mg/ml purified BBSSV and were tested by the enzyme-linked immunosorbent assay (ELISA) 7 times over two years. All cultivars showed high rates of infection ranging from .38 to 100%. 'Bluejay' and 'Burlington' had the lowest values, while 'Bluecrop' had one of the highest, even though 'Bluecrop' is thought to be field resistant. When aphids were allowed to move from diseased to healthy 'Jersey' and 'Bluecrop' plants, both cultivars incurred high rates of infection.

Blueberry shoestring virus (BBSSV) has been reported in most regions where the highbush blueberry, *Vaccinium corymbosum*, is grown commercially (4). It is the most important blueberry virus disease in Michigan and in several fields planted to cv. 'Jersey' more than 50% of the bushes are infected (2,5).

The disease has been well characterized, but its spread is difficult to control (4). Visible symptoms can take up to four years to be expressed and the characteristic red coloration of leaves and stems can be mimicked by sun scalding, winter damage and nutrient deficiency. Unrecognized, but diseased plants can serve as a constant inoculum source which can be spread by aphids.

The aphid vector, *Illinoia pepperi* (Mac G.), can be partially controlled to limit spread; however, not all aphids are killed by insecticide application and sub-lethal doses stimulate increased movement. Also, the insecticides kill a number of important natural predators (1).

The most effective protection against BBSSV would be to plant cultivars resistant to the aphid and virus. A field survey was previously conducted for aphid resistance on 16 cultivars in southwest Michigan and significant differences were found among cultivars, but the most resistant individuals still supported substantial aphid populations (3). This study was undertaken to screen a number of blueberry cultivars for resistance to the virus.

Materials and Methods

Dormant 1-year-old cuttings of 27 cultivars were planted March, 1981, in a completely randomized design in a greenhouse at Michigan State University, East Lansing, Michigan. The plants were grown in 19 cm diameter pots in a soil mix of 1:1 (v/v) Canadian sphagnum peat moss and agricultural grade coarse perlite. Plants were fertilized and irrigated according to previously described procedures (5). Greenhouse temperatures were allowed to fluctuate naturally except during December, January and February when they were maintained between 0-10 C to induce dormancy.

Representatives of each cultivar were rub-inoculated with 1 mg/ml of BBSSV purified from blueberry blossoms in April, 1982 and 5 mg samples of leaves per plant were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of virus in October, 1982; April, July, August, 1983 and April, 1984. Twenty-five

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blossoms per plant were also evaluated in April, 1984. All plants which did not test ELISA positive after the first inoculation were re-inoculated in April, 1984 and ELISA tested the following September and October. Inoculation and ELISA procedures are described elsewhere (5). Individuals were considered ELISA positive if their absorbance equaled or was greater than the mean A_{405} value of three healthy blueberry control values plus two standard deviation units.

In the spring of 1984, five aphids were transferred from laboratory stocks to six 1-year-old infected 'Jersey' plants and were allowed to feed and reproduce for 2 months. Three similarly aged 'Jersey' and 'Bluecrop' plants were then placed alternately around each infected 'Jersey' plant. The aphids were allowed to move from source to trap plants for one week before being destroyed and their numbers were counted daily, Two months later, the trap plants were tested for the presence of BBSSV by ELISA. Each set of one diseased 'Jersey' plant and six healthy trap plants was maintained in a separate 1 m^3 screenhouse in a greenhouse at Michigan State University, East Lansing, Michigan.

Results and Discussion

After rub-inoculating the plants twice and ELISA-testing them seven times, most cultivars showed infection rates between 60 and 100% (Table 1). 'Bluejay' and 'Burlington' appeared to be the most resistant with percentages of 44 and 38%, respectively. The largest range of values was seen after the first inoculation, but previous studies have shown that multiple samples must be made to get accurate representations of susceptibility (5).

At least 80% of the 'Bluecrop' rub-inoculted with 1mg/ml virus were diseased, even though 'Bluecrop' has shown symptoms in the field only once (Nelson, pers. comm.). This suggest that 'Bluecrop' has simply escaped infection, is tolerant, or is resistant to lower levels of inoculum than used in the screen.

'Bluecrop' may be more tolerant than 'Jersey' and rarely shows external symptoms, although one of us (DCR) has ELISA tested symptomless 'Bluecrop' plants adjacent to highly diseased 'Jersey' without detecting virus. Still, the virus has an extremely patchy distribution within plants (5) and could have been missed due to sampling errors.

Table 1. Cumulative percentage of plants testing 'ELISA' positive at least once after one or two leaf-rub inoculations of 1.0 mg/ml purified BBSSV.

Cultivar	Number of plants	First Inoculation[z]	Second Inoculation[y]
Atlantic	3	67	100
Berkeley	12	17	80
Bluecrop	12	42	80
Bluehaven	7	14	60
Bluejay	9	22	44
Bluetta	11	64	64
Burlington	11	27	38
Collins	5	40	83
Concord	6	50	78
Coville	10	0	67
Darrow	11	9	89
Dixie	12	33	90
Earliblue	11	45	100
Elizabeth	11	45	100
Elliot	12	0	80
Jersey	10	60	80
GN-87	12	17	100
Herbert	11	27	100
June	12	0	80
Lateblue	12	42	87
Northland	8	25	100
Patriot	11	36	100
Pemberton	11	0	100
Rancocas	12	8	100
Rubel	10	40	100
Spartan	12	42	100
Stanley	10	30	100
1316A	9	33	67

[y]April 1984
[z]April 1982

Table 2. Mean number of *Illinoia pepperi* aphids found on diseased source and test plants after 6 days.

Day	'Jersey' source (infector) plant	'Jersey' test plant	'Bluecrop' test plant
0	203.1	—	—
1	162.2 a[z]	40.4 ab	22.4 b
2	88.3 a	76.5 a	41.0 b
3	75.5 a	86.9 b	53.4 b
4	43.8 a	84.1 c	55.8 ab
5	25.2 a	89.2 c	48.5 b
6	27.0 a	79.3 c	54.5 b
$\bar{x}$	89.3 a	71.0 a	45.9 b

[z]Letters denote significant separation across columns by Duncan's multiple range test, 5% level.

If 'Bluecrop' is resistant to lower levels of inoculum, these levels must be very low, since high rates of infection occurred when aphids were allowed to move from diseased source to trap plants. Fifty-eight percent of the 'Bluecrop' plants tested ELISA positive, while 33% of the 'Jersey' were positive. These patterns arose even though 'Jersey' plants supported more aphids than those of 'Bluecrop' (Table 2). Individuals of the aphid vector *I. pepperi* have been shown to accumulate from 1.5 to 6.0 mg of virus in controlled tests (2).

It is possible that 'Bluecrop' has had sufficiently limited contact with the disease in the field to escape infection. 'Bluecrop' plants have been widely planted in Michigan only the last 10-15 years and most are not in close proximity to diseased fields. Where diseased and healthy fields are adjacent, spread is still probably limited because aphids normally move only a few meters down the row (1). Mechanical harvesters have been shown to spread aphids widely, but 'Jersey' and 'Bluecrop' have different harvest seasons.

In conclusion, no cultivar appeared to be immune to BBSSV, not even 'Bluecrop' which has rarely shown symptoms in the field. 'Bluejay' and Burlington' showed the most resistance, but at least 1/3 of clones were infected under high inoculation rates and 'Burlington' has shown shoestring symptoms in the field (4). We are now screening native *N. corymbosum* for resistance and are developing methods to measure levels of tolerance.

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About The Cover Artist

Lynda Eades Chandler is a professional botanical illustrator with a B.S. degree in Ornamental Horticulture from the University of Florida. She has illustrated for **HortScience**, Dover Publishing Co., Prentice-Hall Publishing Co., the U.S.D.A. Plant Taxonomy Lab, and U.S.D.A.-A.P.H.I.S. Lynda is a member of the Guild of Natural Science Illustrators, and is married to Craig K. Chandler, a research horticulturist at the Ohio Agricultural Research and Development Center, Wooster, Ohio.