Sources of Genetic Resistance to Red Ringspot Virus in a Breeding Blueberry Population

M.K. Ehlenfeldt¹ and A.W. Stretch²

U.S. Department of Agriculture, Agricultural Research Service, Blueberry and Cranberry Research Center, Chatsworth, NJ 08019

A.D. Drape³

U.S. Department of Agriculture, Agricultural Research Service, Small Fruit Laboratory, Beltsville, MD 20705

Additional index words. *Vaccinium*, genetic resistance

Abstract. A group of 1031 genotypes representing 245 different crosses from a joint U.S. Dept. of Agriculture-New Jersey Agricultural Experiment Station blueberry breeding program was evaluated for blueberry red ringspot virus (BBRRSV) symptoms after 8 years of field exposure. Among 41 parents represented by 10 or more progeny, significant differences were observed in offspring BBRRSV expression. The species *Vaccinium lamarckii* Camp. (4x) and *V. amoenum* Ait. (6x) and the cultivars Woodard (6x) and Earliblue (4x) seem to have high frequencies of alleles for BBRRSV resistance. Significant differences were also found among 21 different crosses. The most resistant cross was 'Elizabeth' x 'Earliblue', which had a 23% BBRRSV incidence. Progeny evaluation revealed that none of the parents involved produced families in which all plants were resistant; hence, resistance to this virus may be under polygenic control.

Blueberry red ringspot is a virus-associated blueberry disease that produces characteristic red rings, blotching, or both on young stems, leaves, and fruit of infected plants (Hutchinson, 1950; Hutchinson and Varney, 1954). The disease diminishes fruit quality by reducing berry size and increasing deformities (A.W.S., unpublished data); yield losses as high as 25% have been documented (D.C. Ramsdell, unpublished). Blueberry red ringspot virus (BBRRSV) is most common in New Jersey and Arkansas (Kim et al., 1981), but it also has been reported in Michigan, Connecticut, Massachusetts, New York, North Carolina, and Oregon (Converse and Ramsdell, 1982; Ramsdell et al., 1987). In New Jersey, BBRRSV spreads readily, whereas in other states, such as Michigan, BBRRSV is not transmitted in the field; rather, the virus occurs only when infected stock is introduced (Kim et al., 1981). BBRRSV field transmission in New Jersey is typically from bush to bush within a row, but it does not seem to spread by mechanical contact. The suspected BBRRSV vector in New Jersey is the mealybug (probably *Dysmicoccus* spp.) due to the regular occurrence of soil-dwelling mealybugs in areas of active BBRRSV spread (A.W.S., un-

published data). Some empirical evidence exists regarding cultivar susceptibility. 'Blueray', 'Bluetta', 'Burlington', 'Cabot', 'Coville', 'Darrow', 'Earliblue', and 'Rubel' are considered susceptible. 'Bluecrop' has good field resistance, and 'Jersey' may be immune (Ramsdell et al., 1987).

A long-term field plot in New Jersey was used to evaluate BBRRSV resistance, and the genetic nature of that resistance, in a wide range of genotypes that had been exposed to BBRRSV inoculum.

The group of 1031 genotypes evaluated represents 245 different crosses selected from a joint U.S. Dept. of Agriculture-New Jersey Agricultural Experiment Station blueberry breeding program from 1976 to 1983. The plants were the products of a wide range of crosses: conventional highbush (*Vaccinium corymbosum* L.), highbush x species, rabbiteye (*V. ashei* Reade) x highbush, and rabbiteye x species. These genotypes were the most promising horticultural types from the original crosses but were not otherwise selected. They were growing in a 0.32-ha plot at the Rutgers Blueberry and Cranberry Research Center near Chatsworth, N.J. The plot consisted of fourteen 100-m-long rows; there was ≈1.5 m between plants and 3.0 m between rows. All plants were exposed to BBRRSV for a minimum of 8 years. They were evaluated in Fall 1991 for foliar and stem BBRRSV symptoms. Three evaluators examined plants for symptoms and rated them as positive or negative, without regard to severity of symptom expression. After collecting data, we plotted the infected plants on a field map to ensure that infection was not limited to specific areas and that all plants had received similar exposure. Exposure potential was estimated by determining the maximum and average interval between infected plants. Exposure homogeneity was evaluated by a chi-square contingency table comparing quarter sections of the plot with a null hypothesis of no difference.

Families were evaluated by cross and common parent to see if any combination of parents or single parent was a useful source of BBRRSV resistance for breeding. Parental data were pooled for plants that had more than 10 progeny in the experimental block. Cross data were also compared for cross combinations that had more than 10 progeny. Overall comparisons were made using chi-square statistics. Intergroup multiple comparisons were

Table 1. Blueberry red ringspot disease frequency among progeny, grouped by cross. Minimum family size is 10.

Cross	Blueberry red ringspot		
	Positive	Total	Percent ²
Elizabeth x Earliblue	7	30	0.23 a
Elizabeth x Bluecrop	4	12	0.33 ab
MU 652 x 290-2	5	11	0.45 ab
Elizabeth x G-128	20	42	0.48 ab
Elizabeth x Blueray	8	15	0.53 a-c
Elizabeth x June	6	11	0.55 a-c
G-124 x G-107	12	21	0.57 a-c
G-179 x 290-2	20	33	0.61 a-c
Bluecrop x G-107	8	13	0.62 a-c
Berkeley x G-124	7	11	0.64 a-c
G-111 x G-107	16	25	0.64 a-c
272-3 x G-363	8	12	0.67 a-c
Bluecrop x G-111	11	15	0.73 a-c
G-156 x 290-2	20	27	0.74 a-c
G-179 x US 117	9	12	0.75 a-c
Elizabeth x Berkeley	11	14	0.79 a-c
US 71 x Elizabeth	16	19	0.84 a-c
G-361 x G-358	17	20	0.85 a-c
G-120 x US 69	20	23	0.87 bc
G-136 x NJUS-14	30	30	1.00 bc
G-136 x NJUS-6	21	21	1.00 c

Received for publication 28 Apr. 1992. Accepted for publication 29 Oct. 1992. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Research Geneticist.

²Research Plant Pathologist.

³Research Geneticist (retired). Current address: 604 E. Park Dr., Payson, AZ 85541.

completed by chi-square using a distribution table based on Sidák's multiplicative inequality, which takes into account the number of comparisons and degrees of freedom and results in a conservative evaluation of differences (Sokal and Rohlf, 1981).

The infection rate in the experimental plots was 65.7%. The maximum distance between any two infected plants was eight and the average distance was 2.0 plants. Of the symptomless plants, 93% had only a single plant between them and the nearest infected plant. It is noteworthy that the group of eight contiguous noninfected plants was comprised of three crosses, all of which had US 64 ('Bluecrop' × Maine lowbush selection ME3205) as a parent. Because the total progeny number was <10, this parent was not evaluated in any of the additional statistics, but may be a good source of BBRRSV resistance. Infection was homogeneous across the field ($\chi^2=0.118$, $P=0.73$).

Significant differences were found among the 21 crosses represented ($\chi^2=81.077$, $P\leq 0.001$) (Table 1). Among these crosses, three subgroups were separated. The best single cross was 'Elizabeth' × 'Earliblue', with a BBRRSV incidence of 23%. In general, the rankings of the crosses reflected the relative rank of the parents. 'Elizabeth', which was used extensively as a parent, seemed to be a neutral partner, producing families with high and low frequencies of BBRRSV.

None of the genotypes produced all resistant progeny (Table 2). Significant differences were found for the 41 parents evaluated ($\chi^2=219.5$, $P\leq 0.001$). Multiple comparison analysis resolved 11 subgroups in the total group. Genotypes included in the lowest incidence subgroup were tetraploid and hexaploid species, tetraploid cultivars and selections, and hexaploid cultivars. Among the most promising parents were 4x *V. lamarkii* (BBRRSV frequency = 0.10) and *V. amoenum* (0.33). In the "a" group were the tetraploid cultivars Earliblue (0.43), Blue-ray (0.53), Bluecrop (0.54), June (0.55), Elizabeth (0.56), Berkeley (0.69), Weymouth (0.71), and Darrow (0.76), and the hexaploid cultivars Woodard (0.33) and Tifblue (0.56).

Among the parental clones evaluated, none produced all resistant progeny. However, two species, 4x *V. lamarkii* and 6x *V. amoenum*, may provide high frequencies of BBRRSV-resistance alleles. Since highbush blueberry is a polysomic tetraploid (Krebs and Hancock, 1989), a triplex or quadriplex condition would be needed to produce all resistant progeny, if resistance were inherited as a single dominant gene. Partial dominance with a threshold level required for resistance or polygenic inheritance is, however, more likely in these genotypes. This idea is supported by the fact that 'Earliblue' and 'Blue-ray' produced many BBRRSV-resistant progeny, yet both 'Earliblue' and 'Blue-ray' are susceptible.

We were not able to determine whether the resistance we observed is true resistance or lack of field expression. This determination is

Table 2. Blueberry red ringspot disease frequency among progeny, pooled by parent.

Common parent	Species makeup ^z	Blueberry red ringspot		
		Positive	Total	Percent ^y
<i>Vaccinium lamarkii</i>				
Camp. (4x) ^x	---	1	10	0.10 a
Woodard (6x)	AS	14	43	0.33 ab
<i>V. amoenum</i> Ait. (6x) ^w	---	16	46	0.35 a-c
<i>V. constablaei</i> Gray ^w	---	12	28	0.43 a-d
Earliblue	C	21	49	0.43 a-d
US 40	C, AS	10	22	0.45 a-e
G-128	C	21	45	0.47 a-f
G-124	C	21	44	0.48 a-f
<i>V. brittonii</i>				
Porter ex Bicknell (4x) ^x	---	9	18	0.50 a-g
Blue-ray	C	8	15	0.53 a-g
Bluecrop	C	28	52	0.54 a-g
US 67	C, D	7	13	0.54 a-g
June	C	6	11	0.55 a-g
Tifblue (6x)	AS	36	64	0.56 a-g
Elizabeth	C	105	186	0.56 a-h
US 74	C, D	8	14	0.57 a-i
G-107	C	37	62	0.60 a-i
290-2	AS, C, D	61	101	0.60 a-j
MU 652	C	16	26	0.62 a-k
G-111	C	30	45	0.67 a-k
Berkeley	C	24	35	0.69 a-k
Weymouth	C	15	21	0.71 a-k
G-179	C	46	64	0.72 a-k
G-156	C	23	32	0.72 a-k
272-3	C	14	19	0.74 a-k
G-363	C, AN	13	17	0.76 a-k
Darrow	C	13	17	0.76 a-k
<i>V. atrococcum</i>				
(Gray) Heller (4x) ^{w,v}	---	37	48	0.77 a-k
US 117	C, D	32	40	0.80 b-k
Patriot	C	31	37	0.84 c-k
US 71	C, AT	26	31	0.84 c-k
G-361	C	17	20	0.85 d-k
G-358	C	20	23	0.87 e-k
G-120	C	27	30	0.90 f-k
US 69	C, AT	37	41	0.90 g-k
NJUS-6	C, AT	32	32	1.00 h-k
US 68	C, AT	11	11	1.00 i-k
G-136	C	68	68	1.00 jk
CH89-49	C	10	10	1.00 k
NJUS-14	C, AN	25	25	1.00 k

^zAN = *V. angustifolium*, AS = *V. ashei*, AT = *V. atrococcum*, C = *V. corymbosum*, D = *V. darrowii*.

^yValues with different letters are significantly different at $P=0.05$ using a chi-square test evaluated by Sidák's multiplicative inequality corrections.

^xClassified as *V. angustifolium* by Vander Kloet (1988).

^wClassified as *V. corymbosum* by Vander Kloet (1988).

^vColchicine-derived 4x.

hindered by the number of clones involved in this evaluation and the poor reliability of currently available serological tests. The nature of true resistance also may be complex if BBRRSV is vector-borne, because resistance may be either pathogen or vector resistance. This evaluation of inheritance patterns may form the base for further genetic studies needed to clarify the true nature of BBRRSV resistance in blueberries.

Literature Cited

- Converse, R.H. and D.C. Ramsdell. 1982. Occurrence of tomato and tobacco ringspot viruses and of dagger and other nematodes associated with cultivated highbush blueberries in Oregon. Plant Dis. 66:710-712.
- Hutchinson, M.T. 1950. Can you recognize the symptoms of stunt disease? Proc. 19th Annu. Blueberry Open House 19:9-11.
- Hutchinson, M.T. 1954. Ringspot: A virus disease of cultivated blueberry. Plant Dis. Rptr. 38:260-263.
- Kim, K.S., D.C. Ramsdell, J.M. Gillett, and J.P. Fulton. 1981. Virions and ultrastructural changes associated with blueberry red ringspot disease. Phytopathology 71:673-678.
- Krebs, S.L. and J.F. Hancock. 1989. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63: 11-18.
- Ramsdell, D.C., K.S. Kim, and J.P. Fulton. 1987. Red ringspot of blueberry, p. 121-126. In: R.H. Converse (ed.). Virus diseases of small fruits. U.S. Dept. Agr., Agr. Res. Serv., Wash., D.C. Hdbk. 631.
- Sokal, R.R. and F.J. Rohlf. 1981. Biometry. 2nd ed. W.H. Freeman, New York.
- Vander Kloet, S.P. 1988. The genus *Vaccinium* in North America. Agr. Canada, Ottawa. Publ. 1828.