

Occurrence of Tomato and Tobacco Ringspot Viruses and of Dagger and Other Nematodes Associated with Cultivated Highbush Blueberries in Oregon

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ABSTRACT

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A survey was conducted in western Oregon of virus and mycoplasma-like diseases of cultivated highbush blueberries detectable by enzyme-linked immunosorbent assay. Plants in 4/16 fields were found with tomato ringspot virus and 1/16 with tobacco ringspot virus. *Xiphinema americanum*, a vector of both viruses, was found in 4/10 fields sampled, including 2 of the 4 fields in which tomato ringspot was found. Tomato ringspot virus was found in cucumber bait plants planted in soil containing *X. americanum* from the root zone of a blueberry plant infected with tomato ringspot virus. Known reference isolates of blueberry shoestring and blueberry leaf mottle viruses and of *Spiroplasma citri* were readily detectable by enzyme-linked immunosorbent assay but were not found in the blueberries surveyed. Mosaic disease, identified by its characteristic symptomatology, was also found occasionally infecting cultivated highbush blueberries in Oregon.

Additional key words: blueberry stunt disease, *Vaccinium corymbosum*

Cultivated highbush blueberries (*Vaccinium corymbosum* L.) occupy 222 ha in the Willamette Valley of western Oregon. Planting stock is obtained from local and out-of-state nurseries, but none is available from indexed sources under state certification programs. Blueberries are host to seven known viruses and viruslike diseases in the United States, some of which may occur in symptomless plants (4,6,7,9-12). These are tomato ringspot, blueberry shoestring, blueberry leaf mottle, red ringspot, and tobacco ringspot viruses; blueberry stunt mycoplasma; and blueberry mosaic disease.

In anticipation of the development of a

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virus-tested, certified blueberry nursery program in the area, a survey was conducted of highbush blueberry plantings in western Oregon to determine the presence of viruses and viruslike diseases known to infect blueberry that could also be detected by enzyme-linked immunosorbent assay (ELISA) and the occurrence of plant-parasitic nematodes, including those that might be associated with spread of some of these viruses.

MATERIALS AND METHODS

Dormant blueberry canes were submitted for virus testing by 16 growers from five counties in the Willamette Valley in western Oregon. Twenty cultivars were represented, but 72% of the samples came from eight cultivars: Berkeley, Bluecrop, Bluegray, Dixi, Earliblue, Jersey, Olympia, and Pemberton. Sampled plants averaged 16 yr old (range of 1 to 36 yr). Growers were asked to select and pool canes from the weaker plants of each cultivar sampled. These were sent to Oregon State University in the early spring of 1980 and 1981.

Samples of dormant blueberry buds (1980) or leaves from cut, forced canes (1981) were homogenized 1:10 (w:v) in a Polytron homogenizer in a buffer, pH 7.4, consisting of potassium phosphate plus sodium phosphate (0.01 M), sodium chloride (0.14 M), 0.05% Tween 20, 0.2% egg albumin, and 2.0% polyvinylpyrrolidone (mol wt 10,000). ELISA tests were conducted with these homogenates and standard procedures (1) utilizing alkaline phosphatase and *p*-nitrophenyl phosphate.

The antisera used were against tomato

ringspot virus (TomRSV; ATCC PVAS 174, grape strain), tobacco ringspot virus (TobRSV) from blueberry (5), blueberry shoestring virus (BBSSV) (6), and blueberry leaf mottle virus (BBLMV) (7), all prepared by the second author; and *Spiroplasma citri* Saglio (SCM), a mycoplasma-like agent supplied by R. E. Davis (3).

All samples were placed in duplicate wells in Gilford ELISA plates and their absorbance determined in a Gilford EIA-50 Processor-Reader. Reference viruses [TomRSV and BBSSV in blueberry, TobRSV in cucumber (*Cucumis sativus* L.), and BBLMV in *Chenopodium amaranticolor* Coste & Reyn. leaves] and an SCM isolate in *Catharanthus roseus* (L.) G. Don (*Vinca rosea*) and healthy blueberry leaves were used with each serum in the ELISA tests. Absorbance values at A_{405} for known infected sources ranged from 0.31 to 2.7, whereas healthy blueberry leaves had A_{405} readings of 0.0-0.08. Sap from leaves of a blueberry in Michigan known to be infected with blueberry stunt disease did not react with SCM antiserum in ELISA tests. Threshold positive ELISA readings were taken as mean healthy values at A_{405} , plus three standard deviations. Only data from runs having reliable controls are presented, but uniform distribution of the pathogens in the dormant blueberry canes was assumed in the sampling.

In 1981, 1-2 L of soil and accompanying roots dug beneath blueberry plants were collected and sent to us from 10 of the participating growers. We evaluated them for plant-parasitic nematode content by wet sieving and use of the Baermann funnel.

RESULTS

TomRSV was detected by ELISA in 4 of 16 fields tested (Table 1) in the cultivars Atlantic, Dixi, Earliblue, N 51G, Olympia, and Pemberton. TobRSV was detected in 1 of the 16 fields in Atlantic, Dixi, and Pemberton. BBSSV, BBLMV, and SCM were sought but not detected in any of the blueberry samples examined. Shoot samples received from cultivars infected with TomRSV or TobRSV were usually dwarfed and had small, sparse, misshapen, necrotic leaves. Blueberry plants infected with TomRSV in the field were stunted, with few leaves that were often small, necrotic, and distorted (Fig.

1). Dormant budsticks from Pemberton in field 2, Lane County (Table 1), were cleft-grafted into nine symptomless, potted Blueray plants in the greenhouse in April 1979. The Pemberton scions leafed out but their leaves soon turned necrotic, and all nine Blueray plants died within 4 mo; the three ungrafted Blueray plants left as controls remained symptomless.

Blueberry mosaic disease (12) was frequently seen in several blueberry cultivars in the Willamette Valley. We also encountered necrotic and chlorotic, viruslike leaf symptoms in several blueberry cultivars in fields in the Willamette Valley that indexed negative in subsequent ELISA tests with the above antisera. The identity, frequency, and transmissibility of these disorders are unknown.

Plant-parasitic nematodes were found in nine of the 10 blueberry soil samples tested (Table 1). *Pratylenchus* spp. (meadow nematodes) were the most common (in 6/10 samples), followed by *Xiphinema americanum* (Cobb) (dagger nematode; 4/10) and *Trichodorus* spp. (3/10). In fields where they occurred, *Pratylenchus* populations varied from 169 to 1,015/L of soil and *X. americanum* from 42 to 254/L of soil. *X. americanum* was found in two of the four fields where TomRSV was detected and soil samples were taken; it also occurred in two fields where TomRSV and TobRSV were not detected. Soil containing 954 *X. americanum* per liter was dug in August 1981 from the root zone of Earliblue blueberries known by ELISA to be

infected with TomRSV (field code 7, Table 1). The soil was placed in plastic boxes measuring 20 × 35 × 20 cm in a

growth chamber seeded with cucumbers and held at 28 C day, 22 C night (16-hr days) for 30 days. The cucumber roots



Fig. 1. Pemberton highbush blueberry infected with tomato ringspot virus showing stunted growth and sparse, misshapen foliage. Lane County, OR, May 1979.

Table 1. Incidence of four viruses and one mycoplasma-like organism detectable by ELISA and of plant-parasitic nematodes in cultivated highbush blueberries in 16 locations in Oregon

Field code	County	Virus detection ^a			Occurrence ^b of nematodes in pooled soil samples				
		Cultivars		Viruses present	<i>Xiphinema americanum</i>	<i>Pratylenchus</i> spp.	<i>Trichodorus</i> spp.	<i>Paratylenchus</i> spp.	<i>Heterodora trifolii</i>
		Tested (no.)	Infected						
1	Benton	2	0	0 ^c	NT	NT	NT	NT	NT
2	Lane	2	Olympia Pemberton	TomRSV TomRSV	—	+++	+++	—	—
3	Lane	7	N 51-G	TomRSV	++	++	—	—	—
4	Lane	5	0	0 ^c	NT	NT	NT	NT	NT
5	Lane	3	Atlantic Dixi Pemberton	TomRSV, TobRSV TomRSV, TobRSV TomRSV, TobRSV	—	++	—	—	—
6	Lane	4	0	0	—	—	—	++	++
7	Lane	3	Dixi Pemberton Earliblue	TomRSV TomRSV TomRSV	++	++	—	—	—
8	Lane	2	0	0	++	—	—	—	—
9	Marion	4	0	0	—	+++	—	—	—
10	Marion	2	0	0 ^c	NT	NT	NT	NT	NT
11	Marion	4	0	0 ^c	NT	NT	NT	NT	NT
12	Marion	6	0	0 ^c	NT	NT	NT	NT	NT
13	Marion	3	0	0	—	—	+	—	—
14	Multnomah	4	0	0 ^c	NT	NT	NT	NT	NT
15	Washington	5	0	0	—	—	—	—	—
16	Washington	5	0	0	+	+++	+++	—	—

^aEnzyme-linked immunosorbent assay tests of tomato ringspot virus (TomRSV), tobacco ringspot virus (TobRSV), blueberry shoestring virus (BBSSV), blueberry leaf mottle virus (BBLMV), and *Spiroplasma citri* (SCM).

^bNT = not tested, — = none, + = few (1–50/L of soil), ++ = moderate numbers (51–500/L), and +++ = numerous (>500/L).

^cELISA tests of TomRSV, TobRSV, and BBSSV only.

were then dug, pooled in groups of four plants, washed, triturated, and tested for TomRSV by ELISA. Three of seven groups of roots indexed positive for TomRSV ($A_{405} = 0.57$, healthy cucumber roots = 0.008).

DISCUSSION

This is the first report of the occurrence of TomRSV or blueberry necrotic ringspot disease caused by TobRSV in *Vaccinium* spp. in Oregon. TomRSV has previously been reported in one cultivated blueberry field in Washington (4). TobRSV is widespread in cultivated blueberry in the eastern United States, but its occurrence has not previously been authenticated in the western United States (11). Both viruses caused severe damage to most of the blueberry cultivars that they were found infecting. The inclusion of preplant soil testing for dagger nematodes, standard soil fumigation where indicated, and the use of blueberry nursery stock free from these viruses should help to control them in cultivated highbush blueberry.

In the United States, BBSSV has been found in New Jersey, Michigan, and Washington (6), but it was not detected in Oregon in this study or previously. BBLMV was first reported in Michigan (7); however, it remains unreported in Oregon blueberries, as do spiroplasmas having a serological relationship to *S. citri* and a wide host range and distribution in the United States (3).

The nematodes found in this survey parallel, in general, those previously reported in *Vaccinium* in California (8). Little is known about the economic damage caused by plant-parasitic nematodes in cultivated *Vaccinium* in the western United States. The role of *X.*

americanum as a virus vector in blueberry is established, but it is not known to damage blueberries directly (13). Some *Pratylenchus* spp. cause serious damage to several crops in the Pacific Northwest, but their importance or that of *Trichodorus* spp. on *Vaccinium* is unknown. The *Heterodora trifolii* (Goffart) Oostenbrink populations reported in Table 1 were probably associated with leguminous hosts growing in the untilled blueberry fields. *Paratylenchus* spp. have not been linked to a crop disease problem in the Pacific Northwest.

With the exception of blueberry stunt mycoplasma-like agent (9) and blueberry red ringspot virus (10), the presently known viruses infecting cultivated highbush blueberry in North America can either be satisfactorily detected by ELISA, even when they are symptomless (ie, TomRSV, TobRSV, BBSSV, and BBLMV), or they are self-indicating diseases in blueberry cultivars (blueberry mosaic). The high ELISA A_{405} values found for all TomRSV- and TobRSV-infected blueberry samples obtained from infected fields in Oregon, when compared with the low background levels found in all cases, suggest that ELISA is a sensitive and reliable method for detecting these viruses in blueberry plants. Until suitable antisera are developed against all known North American blueberry viruses and viruslike diseases, budding to the susceptible Cabot blueberry to develop characteristic symptomatology can be used to supplement ELISA testing when needed in their detection (2).

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LITERATURE CITED

1. 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.
2. Converse, R. H. 1979. Recommended virus-indexing procedures for new USDA small fruit and grape cultivars. *Plant Dis. Rep.* 63:848-851.
3. Davis, R. E. 1971. Spiroplasmas: Newly recognized arthropod-borne pathogens. Pages 451-484 in: *Leafhopper Vectors and Plant Disease Agents*. K. Maramorosch and K. F. Harris, eds. Academic Press, New York. 654 pp.
4. Johnson, F. 1972. Tomato ringspot virus of blueberries in Washington. (Abstr.) *Phytopathology* 62:1004.
5. Ramsdell, D. C. 1978. A strain of tobacco ringspot virus associated with a decline disease of 'Jersey' highbush blueberry. *Plant Dis. Rep.* 62:1047-1051.
6. Ramsdell, D. C. 1979. Blueberry shoestring virus. *Descriptions of Plant Viruses* No. 204. Commonw. Mycol. Inst./Assoc. Appl. Biol. Kew, Surrey, England.
7. Ramsdell, D. C., and Stace-Smith, R. 1980. Blueberry leaf mottle, a new disease of highbush blueberry. *Acta Hort.* 95:37-48.
8. Siddiqui, I. A., Sher, S. A., and French, A. M. 1973. Distribution of plant parasitic nematodes in California. *Calif. Dep. Food Agric. Div. Plant Ind.* 324 pp.
9. Stretch, A. W., and Varney, E. H. 1970. Blueberry stunt. Pages 175-176 in: *Virus Diseases of Small Fruits and Grapevines*. N. W. Frazier, ed. Univ. Calif. Div. Agric. Sci. 290 pp.
10. Stretch, A. W., and Varney, E. H. 1970. Red ringspot of blueberry. Pages 184-185 in: *Virus Diseases of Small Fruits and Grapevines*. N. W. Frazier, ed. Univ. Calif. Div. Agric. Sci. 290 pp.
11. Varney, E. H. 1970. Necrotic ringspot virus of blueberry. Pages 181-183 in: *Virus Diseases of Small Fruits and Grapevines*. N. W. Frazier, ed. Univ. Calif. Div. Agric. Sci. 290 pp.
12. Varney, E. H. 1970. Blueberry mosaic. Pages 185-186 in: *Virus Diseases of Small Fruits and Grapevines*. N. W. Frazier, ed. Univ. Calif. Div. Agric. Sci. 290 pp.
13. Varney, E. H., and Stretch, A. W. 1966. Diseases and their control. Pages 236-279 in: *Blueberry Culture*. P. Eck and N. F. Childers, eds. Rutgers University Press, New Brunswick, NJ. 378 pp.