Occurrence and Some Properties of Raspberry Bushy Dwarf Virus in Rubus Species in The United States

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ABSTRACT

Raspberry bushy dwarf virus (RBDV) has been identified serologically in the United States in Boysen, 'Canby' red raspberry, and in three black-raspberry cultivars in four states. The production of symptoms typical of RBDV in the test host, *Chenopodium quinoa*, used to index 31 *Rubus* cultivars from 89 fields in 12 states, indicates that RBDV is probably widespread in the United States in cultivated *Rubus*. 'Munger' black raspberry, freed of RBDV by heat treatment and then inoculated with RBDV by grafting or naturally in the field, failed to develop symptoms. In a quantitative field comparison of Munger with and without RBDV, there were no differences in fruit yield or quality over a 2-year period, although vegetative growth was significantly less in RBDV-infected plants in some measurements. RBDV was seed-borne (22%) in open-pollinated Canby red raspberry. Pollen germination was not depressed in Munger black raspberry naturally infected with RBDV, compared with RBDV-free Munger. Properties of the virus preparation from an Oregon source of Munger agreed with those of the original isolate of the isometric virus, raspberry bushy dwarf, described in Scotland.

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Raspberry bushy dwarf virus (RBDV) was first reported in cultivated red raspberry (*Rubus idaeus* L.) in Scotland (1, 3) and in loganberry (*Rubus ursinus* Cham. & Schlecht. var. *loganobaccus* Bailey) in England (1, 12). It was also found in 'Canby', 'Indian Summer', and 'Trent', three North American red-raspberry cultivars being grown in Scotland during the period of this investigation (1). The virus was purified from *Chenopodium quinoa* Willd., in which it produced local and systemic chlorotic spots, mottle, and ring and line patterns (1). Barnett & Murant (1) found RBDV to be an isometric particle,

33-nm diam, with two major components, ($s^{o}_{20,w}$) of 111 and 116 S. An antiserum prepared against RBDV had an endpoint of 1/512 in agar-gel diffusion tests. RBDV failed to react serologically with antisera against 24 other isometric viruses or with antiserum against apple chlorotic leaf spot virus (1), to which raspberry bushy dwarf virus was earlier reported to be related (4). Barnett & Murant (1, 2) recovered RBDV from red-raspberry seedlings mechanically inoculated with RBDV from *C. quinoa*, but the infected raspberry seedlings were symptomless. They found RBDV to be seed-borne in red raspberry, and that the infected seedlings were also symptomless (1).

This paper reports the occurrence of RBDV in cultivated *Rubus* in the United States and compares some of the properties of the virus with those reported from Scotland (1). An abstract dealing with this work has appeared (8).

MATERIALS AND METHODS.—*Chenopodium quinoa* was used as the test host throughout these studies. During the winter months it was grown on the greenhouse bench at 18-24 C under natural light supplemented at midnight with 3 hr of artificial light (2,100 lx at leaf surface), and at other times of the year in growth chambers programmed for 21 C, and a 16-hr photoperiod of 22,500 lx (incandescent plus fluorescent light).

Rubus plants to be indexed for RBDV were sampled in the spring when leaves were growing rapidly, or dormant canes were cut and forced in the greenhouse. Young, vigorously growing shoots were ground with mortar and pestle with an equal volume of 2% nicotine alkaloid and a little Celite. The resulting sap was rubbed on fully expanded leaves of *C. quinoa* plants about 8-15 cm high.

Rate-zonal sucrose density-gradient centrifugation was conducted in a Spinco L2-65B ultracentrifuge and an SW-27 rotor. Sucrose gradients of 5-30% were prepared, using an MSE 80-ml capacity gradient former. Density gradients were eluted, and absorbance (A_{254}) was recorded with an ISCO density-gradient fractionator. The sedimentation coefficient of a partly purified preparation of RBDV was determined with a Spinco Model E analytical ultracentrifuge with ultraviolet optics and an electronic scanner.

Agar-gel diffusion serological tests were made in 9-cm diam petri plates, using 7 ml of 0.7% Ionagar No. 2 (Consolidated Laboratories, Inc., Chicago, Ill.), with 0.05% sodium azide added as preservative. Well spacing was 2 mm between 3-mm diam wells.

Pollen viability was determined on a 10% sucrose, yeast extract, salts agar (11) by direct microscopic examination of pollen grains after 7 hr of incubation at 25 C.

RESULTS.—*Occurrence in Rubus cultivars.*—Preliminary surveys (6, 7) of cultivated *Rubus* cultivars in the eastern and western United States showed that many plants were infected with a virus (or viruses) that caused chlorotic spots and ring and line patterns, but no necrosis, on *C. quinoa*. This symptomatology on *C. quinoa* was quite unlike the symptoms caused on this host by the other sap-transmissible viruses commonly found on *Rubus* in the United States. For example, black-raspberry latent virus (BRLV) (10), tobacco streak virus (TSV-R) (9), or tomato ringspot virus (TmRSV) (13) all cause necrotic local lesions and systemic necrosis when sap-transmitted to *C. quinoa*.

In the United States, virus isolates which caused local chlorotic spots and/or systemic ring and oakleaf patterns, distortion, and systemic chlorotic spots in *C. quinoa* were found in 31 *Rubus* cultivars (black and red raspberries, blackberries, and Boysen) from a total of 89 fields in 12 states. Frequently, the initial local chlorotic leaf spots were followed by systemic shoot necrosis in inoculated *C. quinoa*. TSV-R and TmRSV, as well as RBDV, were identified serologically in some of these cases, indicating multiple virus infections.

Some *Rubus* virus isolates were tested for their serological relationship to the Scottish source of RBDV antiserum obtained from A. F. Murant. Healthy *C. quinoa* sap failed to react with the RBDV antiserum under the conditions of the tests (Table 1). Ten of the isolates in Table 1 caused only yellow leaf spots and ring and line patterns on *C. quinoa*, whereas two isolates first produced yellow leaf spots and subsequently systemic necrosis of this host, and four isolates caused only systemic necrosis. BRLV, TSV-R, and TmRSV were also detected serologically in some of the virus cultures that caused systemic necrosis in *C. quinoa*, but not in those that failed to cause systemic necrosis in this host.

Development of black-raspberry cultures free from RBDV.—A clone of black raspberry, *Rubus occidentalis* L. 'Munger', carrying only RBDV, was placed in a growth chamber programmed for 37 (± 1)C, a 16-hr day-length, and 22,500 lx incandescent and fluorescent light. Small tip cuttings were rooted in sand in a mist bed in the greenhouse at intervals thereafter. The number of resulting plants found to be free from RBDV after indicated periods at 37 C were: 38 days, 2/11 (the numerator is the number of plants indexing negative on *C. quinoa* for 2 years after the heat treatment, and the denominator is the number of plants indexed); 48 days, 2/4; and 88 days, 1/3. When the black-raspberry cultivar 'Plum Farmer', infected with RBDV, was heat-treated under

TABLE 1. Virus isolates from *Rubus* cultivars, from several locations in the United States, that tested positive for raspberry bushy dwarf virus in agar-gel serological tests

Rubus type	Cultivar	State of origin	No. of isolates examined
Black raspberry	'Munger'	Oregon	7
	'New Logan'	Maryland	1
	'Plum Farmer'	Oregon	1
Red raspberry	'Canby'	Oregon	1
		Washington	1
Boysen		Oregon	1
		California	4
		Total	16

similar conditions for 207 days, 4/5 of the resulting plants indexed free of RBDV. At present, the following black-raspberry cultivars have been indexed and found free from RBDV and other known viruses: 'Black Hawk-64', 'Bristol-69', 'Cumberland-69', 'Munger-70', 'New Logan-69', and Plum Farmer-70. Numbers after the cultivars indicate the year of release of these indexed stocks by the USDA.

Influence of RBDV on growth and yield of Munger black raspberry.—Because Munger is so generally infected with RBDV and often has poor yields of crumbly or unevenly ripening fruit, a comparative-yield plot of Munger-70 and commercial Munger, known to be infected with RBDV, was planted in Corvallis, Oregon, in April 1970. The planting was made in dichloropropene-fumigated soil (Telone, Dow Chemical Co., Midland, Mich.) at the rate of 50 gal/acre, with 30 pairs of RBDV-free (Mu-70) and RBDV-infected (Mu–BD) Munger plants. The entire test was planted in a second adjoining planting in April 1971. The effects of RBDV on growth and yield were measured in 1971 and 1972 (Table 2). There were no visible symptoms on foliage or fruit of RBDV-infected plants. Only two quantitative differences were significant statistically, the number of primocanes per plant in June 1971 in the 1970 planting and the weight of floricanes pruned from the 1971 planting in January 1972. In both cases, growth of the Mu-70 plants exceeded that of the Mu-BD plants at the 1% probability level. During these tests all Mu-70 plants were indexed yearly on *C. quinoa*, and plants found infected with RBDV or other viruses like TSV-R were removed from consideration in the statistical analysis.

In June 1971, the germination rate of Mu-BD pollen was 49%, compared to 54% for Mu-70 (900 pollen grains of each source were streaked on sucrose-yeast extract agar and checked for germination), a statistically nonsignificant difference.

Field infection of Rubus cultivars by RBDV.—The rate of infection of Munger-70 was observed from 1970 to 1972 at Corvallis, Oregon, and elsewhere in Oregon and Washington. More than 50 plants in 10 fields were indexed yearly. When the nearest unindexed cultivated *Rubus* were 3-20 miles distant, no RBDV infections were detected in 1971 or 1972. When the nearest indexed RBDV-infected Munger plants were 5-10 ft away from Mu-70 on unfumigated land, 26 and 22% in 1971 and 1972, respectively, of the Munger-70 plants became infected with viruses that caused symptoms on *C. quinoa* typical of RBDV. Five representative isolates were serologically related to RBDV. In 1971, 21 'Boysen-72' plants (free from known viruses, including RBDV) were planted on fumigated land (methyl bromide + chloropicrin 57:43% under tarpaulin, 425 lb/acre) near Roseburg, Oregon, on a tree-fruit farm located 3 miles from cultivated unindexed *Rubus*. In 1972, none of the Boysen plants showed symptoms, but 3/21 were found by indexing to be infected with a virus that produced symptoms on *C. quinoa* that were identical to those produced by RBDV.

Seed transmission of RBDV.—Open-pollinated seed was collected from Canby red raspberry, known to be infected with RBDV. Eighteen of the resulting seedlings were grown in the greenhouse and were indexed for RBDV on *C. quinoa*. Four seedlings (22%) were infected, although all were symptomless. Sample virus isolates from these seedlings were identified serologically as RBDV.

Graft transmission of RBDV.—Buds from Munger black raspberry infected with RBDV were grafted onto 13 Munger-70 black-raspberry plants free from known viruses in February 1970 in the greenhouse at Oregon State University. Six unbudded Munger-70 plants were held on the same greenhouse bench. In May 1971, all plants were indexed by sap inoculation to *C. quinoa*. In 9/13 cases, virus isolates were recovered on *C. quinoa* from Munger-70 plants budded with RBDV-infected buds. These isolates all produced symptoms characteristic of RBDV on *C. quinoa*. Budded Munger-70 plants did not develop

TABLE 2. Influence of raspberry bushy dwarf virus on growth and yield of 'Munger' black raspberry in field plots in Corvallis, Oregon, 1971-1972

Planting date	Treatment	No. rooted tips/ plant Feb. 1971	No. primocanes pruned/plant		Wt (gm) old canes pruned/plant		Fruit yield (gm)/plant	
			June 1971	June 1972	Mar. 1972	Jan. 1972	July 1971	July 1972
1970	Free from known viruses	14.5	5.2		3,686		683	554
1970	Raspberry bushy dwarf-infected	12.1	3.3		3,446		729	580
	t-value and probability level of difference	1.03, NS	3.54, 1%		0.55, NS		0.35, NS	0.27, NS
1971	Free from known viruses			5.7		267		683
	Raspberry bushy dwarf-infected			6.1		177		774
	t-value and probability level of difference			0.58, NS		4.06, 1%		1.55, NS

symptoms during the course of the experiment. The unbudded Munger-70 plants all indexed negative for RBDV in May 1971.

Purification of RBDV.—An isolate of RBDV from Munger black raspberry was increased by sap inoculation of *C. quinoa* grown in growth chambers. Purification studies were made, using the procedures of Barnett & Murant (1), which involved buffer extraction, acid precipitation of the virus, and differential and rate-zonal density-gradient centrifugation. In repeated tests, one peak (A_{254}) appeared 9-13 ml below the meniscus. The preparation from this peak infected *C. quinoa*, producing symptoms typical of RBDV, and also reacted positively with RBDV antiserum from Scotland.

An isolate of RBDV from *R. occidentalis* 'Munger' from Mulino, Oregon, maintained in *C. quinoa*, has been deposited with the American Type Culture Collection as the type culture of RBDV in the United States (ATCC PV-179).

DISCUSSION.—Raspberry bushy dwarf virus clearly is widespread in cultivated *Rubus* in many parts of the United States. In some cultivars, like Munger black raspberry, half of the plantings examined were infected with RBDV. It is likely that the entire stock of the red-raspberry cultivar Canby is infected with RBDV. We found no specific disease symptoms that could be associated with RBDV in any cultivar even when newly infected. In a well-managed, irrigated Munger black-raspberry planting, RBDV was demonstrated to depress vegetative vigor significantly in some measurements but not in others, yet neither fruit yield nor quality was depressed. Barnett & Murant (1) were also unable to detect any characteristic symptomatology associated with RBDV infection in red raspberry. It seems likely that if severe damage is caused by RBDV in *Rubus*, it will be in association with some of the numerous other viruses already reported to infect this genus, or under other stress-inducing conditions.

RBDV (incorrectly identified at the time as apple chlorotic leaf spot virus) was demonstrated to be pollen-transmitted in red raspberry, causing back-infection of the mother plant (5), and was also 30-40% seed-borne in red raspberry (1). We found 22% seed transmission of RBDV in Canby red raspberry, and that RBDV did not significantly depress pollen germination in Munger. We observed that about 25% of virust-tested young Munger plants interplanted among RBDV-infected Munger stock in the field became infected yearly in a 2-year test. These virus-tested Munger plants were allowed to flower, but we have no direct evidence that RBDV was transmitted to these plants by infected pollen. More puzzling was the infection of 14% of virus-tested Boysen plants in 1 year in a planting at Roseburg, Oregon, which was 3 miles from the nearest commercial unindexed *Rubus* stock. An experimental planting of indexed Munger adjoining the Boysen plants remained free from all viruses we could detect for a 2-year period.

LITERATURE CITED

1. BARNETT, O. W., & A. F. MURANT. 1970. Host range, properties, and purification of raspberry bushy dwarf virus. Ann. Appl. Biol. 65:435-449.
2. BARNETT, O. W., & A. F. MURANT. 1971. Differential hosts and some properties of raspberry bushy dwarf virus. Ann. Phytopathol. 3:129-139.
3. CADMAN, C. H. 1961. Raspberry viruses and virus diseases in Britain. Hort. Res. 1:47-61.
4. CADMAN, C. H. 1963. Affinities of viruses infecting fruit trees and raspberry. Plant Dis. Reptr. 47:459-462.
5. CADMAN, C. H. 1965. Filamentous viruses infecting fruit trees and raspberry and their possible mode of spread. Plant Dis. Reptr. 49:230-232.
6. CONVERSE, R. H. 1967. Pollen- and seed-borne raspberry viruses. Phytopathology 57:97-98 (Abstr.).
7. CONVERSE, R. H. 1970. Survey of viruses in red and black raspberries in Oregon. Oregon Hort. Soc. Proc. 61:104-105.
8. CONVERSE, R. H. 1972. Occurrence of raspberry bushy dwarf virus in Rubus species in the United States. Phytopathology 62:752 (Abstr.).
9. CONVERSE, R. H. 1972. Tobacco streak virus in black raspberry. Phytopathology 62:1001-1004.
10. CONVERSE, R. H., & R. M. LISTER. 1969. The occurrence and some properties of black raspberry latent virus. Phytopathology 59:325-333.
11. KLEIN, R. M., & D. T. KLEIN. 1970. Research Methods in Plant Science. Natural History Press, Garden City, N.Y. 756 p.
12. LEGG, J. T. 1960. A virus-degeneration of loganberry. Rept. E. Malling Res. Stn. 1959, 102-103.
13. STACE-SMITH, R. 1970. Tomato ringspot virus. No. 18. *In* Descriptions of Plant Viruses. Commonwealth Mycological Institute and Association of Applied Biologists (Ferry Lane, Kew, Surrey, England).