

Characterization of Aphid-Transmitted Virus Associated with Black Raspberry Decline in Oregon

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Abstract

A serious disease of black raspberry (*Rubus occidentalis*) was observed in Oregon in the last decade. Plants showing mosaic symptoms declined rapidly and in many cases died over a period of several years. Double-stranded RNA extraction from symptomatic black raspberry revealed the presence of two high molecular weight bands, which were cloned and partially sequenced. Sequence analysis disclosed the presence of Black raspberry virus (BRV). A newly planted field of black raspberries in Oregon was studied to assess the rate of spread of BRV. The timing of BRV infection as it relates to aphid populations and flights was also determined. Testing of nearby vegetation identified several symptomless *Rubus* hosts of BRV. It was determined that BRV spreads rapidly with a low aphid threshold and was associated with symptoms of decline in black raspberries in Oregon.

INTRODUCTION

Black raspberry decline has been an increasing problem on black raspberries in Oregon and has a significant impact on yield and vigor. Leaves may become puckered and exhibit symptoms of chlorosis and mosaic in the cooler spring and fall (Fig. 1), followed by cane dieback resulting in reduced yield. Affected fields are replaced every 3–4 years. Associated with these symptoms.

Component of raspberry mosaic disease described previously and caused by a complex of *Rubus yellow net virus* (RYNV) and *Black raspberry necrosis virus* (BRNV) in North America and *Raspberry leaf mottle* and *Raspberry leaf spot viruses* in Europe (Converse et al., 1987). Other than RYNV (Jones et al., 2002), viruses of the mosaic disease complex characterized in terms of particle morphology, serology and nucleotide sequence. This paper describes the partial sequence of BRV, its distribution and incidence, alternate hosts and transmission properties.

MATERIALS AND METHODS

Double stranded RNA (dsRNA) was purified from diseased black raspberry tissue using a modified Yoshikawa and Converse method (Halgren, 2006). Synthesis of cDNA was performed using dsRNA as a template as described previously (Tzanetakis et al., 2005). All clones and PCR products were sequenced and identified using BLAST (Altschul et al., 1997) and analyzed using CAP3 software (Huang and Madan, 1999). GenBank accessions of related viruses were obtained using BLAST and multiple alignments and phylogenies were constructed with the CLUSTALW program (Thompson et al., 1994) with its default parameters, after bootstrapping with 1000 pseudoreplicates. Cluster algorithm phylogenetic trees in Phylip format of conserved RNA-dependent RNA polymerase (RdRp) motifs were visualized with TreeView (Page, 1996).

The timing and extent of BRV infection and the testing of alternate hosts was conducted via reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from plant samples for use in RT-PCR detection tests as described elsewhere (Susaimuthu et al., 2006). RNA constituted 1–5% of the RT reaction, which was performed according to enzyme manufacturer's recommendations (Invitrogen, Carlsbad, CA). The RT product represent less than 4% of the total PCR reaction volume to prevent

inhibition from plant secondary metabolites carried over from the RNA. PCR reactions were carried out according to the polymerase manufacturer's instructions (Genscript, Piscataway, NJ). Two sets of primers were designed and used in tandem for detection. Primer set 1 amplified a 417 basepairs (bp) fragment of the RNA dependent RNA polymerase (RdRp) region of RNA 1, and consisted of forward primer 5'ATGCTGAGCCACTTGTGA3' and reverse primer 5'ATCTGGTGTGTTCCGCAT3'. Primer set 2, forward primer 5'CAATGTCTTGGGAAGCCAC3' and reverse primer 5'AGCATGGTTCGTCATCTG3', amplified a 350 bp fragment further downstream at the 3' end of the RdRp region. The PCR program for detection consisted of initial denaturation for 5 min at 94°C followed by 40 cycles with denaturation for 30 sec at 94°C, annealing for 45 sec at 55°C and extension for 30 sec at 72°C, with a final 10 minute extension step at 72°C. Amplicons were visualized with UV light after separation by electrophoresis in agarose gels. Amplification of the highly conserved plant gene NADH dehydrogenase ND2 subunit (*ndhB* gene) was used as an internal control to verify the quality of the total RNA extraction and effectiveness of the RT-PCR (Martin et al., 2006).

Samples were collected from plants growing near fields of BRV-infected black raspberry. Two plant categories were considered: commercial *Rubus* fields (i.e., red raspberry, blackberry or hybrid berries) adjacent to infected black raspberry and volunteer and native *Rubus* growing in woody areas and in fence rows surrounding infected fields. Samples were collected at several times throughout the growing season.

A series of field experiments was carried out in a field near Sandy, Ore., southeast of Portland. This 12-acre field was planted with cuttings of BRV-infected plants. In the spring and summer of 2003, 2004 and spring, summer, and fall of 2005, 16 virus-tested, tissue-culture-derived, potted black raspberry plants were placed in this field and replaced with new plants every two weeks. The test plants were placed within the rows of the permanent plants, in a 4×4-grid like formation. Aphid populations in the field were monitored using 12 sticky traps that were placed within the field and collected with each set of plants. After two weeks in the field, plants were returned to the lab, where they were treated with either granular or foliar Marathon™ insecticide and stored outside. Seven sets of plants from the 2003 season, including one set which overwintered in the field, and 12 sets of plants from 2004, including one field overwintering set, were tested in May and October 2005. Fourteen sets of plants from 2005 were tested in November 2005. Average daily temperature for the field was compiled from Weather Underground, Inc., for the city of Boring, Ore.

Regression analysis was used to test the following relationships: number of plants testing BRV-positive versus aphid numbers, and maximum daily temperature versus aphid numbers. For all analyses, S-Plus version 7.0 (Insightful Corporation) was used.

RESULTS

dsRNA extraction from declining black raspberry revealed two major bands of about 8 and 7 Kbp based on agarose gel electrophoresis (data not shown). Analysis of the sequence obtained from the cloning of dsRNA showed the virus to be related to *Strawberry mottle virus* (SMoV) (Thompson et al., 2002). The conserved domains of the RNA dependent RNA polymerase (RdRp) were aligned with those of related viruses to generate a phylogenetic tree (Fig. 2). Two distinct clades emerged, one containing branches for the *Comoviridae* and the other containing members of the *Sequiviridae* and *Sadwavirus* genus.

BRV was found to be symptomless in several commercial *Rubus* species commonly grown alongside black raspberry, including red raspberry (*R. idaeus*), Evergreen blackberry (*R. laciniatus*) and 'Marion' blackberry (Fig. 3), as well as in Himalaya blackberry (*R. armeniacus*) and Pacific blackberry (*R. ursinus*).

During the summer of 2003, aphid numbers on traps peaked in late-May, then declined (Fig. 4). Regardless of this decline, infection of experimental plants continued throughout the season. Alate aphids were present on the traps throughout the testing

season but apterous aphids were observed on the plants only on June 25. Collection dates of each rotation and number of plants (out of 16) testing positive were as follows: May 16: 6, May 30: 8, June 13: 6, June 25: 11, July 11: 5, and July 28: 1 (Fig. 4). Similar results were obtained in 2004, but few positives were detected in the mid-summer of 2005.

There was no evidence of a linear correlation between maximum daily temperature and aphid number ($R^2=0.008$, $y=0.0289x + 0.542$, p -value = 0.3926 on 82 D.F.) There was a statistically significant relationship between aphid number and number of BRV-positive plants for the 2003 and 2004 trap plants ($R^2=0.4338$, $y=1.7587x + 2.6198$, p -value=0.004 on 15 D.F.). There was no evidence the field's mean aphid number over the course of one growing season differed from its mean aphid number over other growing seasons.

DISCUSSION

BRV has sequence and genome organization closest to that of SMoV (Thompson et al., 2002), a new member of the genus *Sadwavirus* (Mayo, 2005). Until recently, this unclassified genus had been termed "SDV-like viruses", consisting of members with genomic similarity to *Satsuma dwarf virus* yet with no consistency of vector. Like the nepoviruses, sadwaviruses have a single-stranded, positive-sense RNA, bipartite genome; however, sadwaviruses produce two distinct coat proteins compared to the single coat protein produced by nepoviruses.

There was a correlation between BRV incidence and peak number of colonizing aphids ($R^2=0.4338$), though the highest disease incidence did not consistently occur when aphid numbers were highest. This suggests that either aphid threshold for infection is extremely low, or other variables relating to plant physiology or environmental conditions play a role in disease development. It is likely there is a lag time between inoculation and detection time that is dependent on an array of factors that affect plant physiological status, including temperature, precipitation, nutrition, and pre-existing disease. Perhaps there is also an overwintering component to this equation, such that a virus is capable of reaching higher levels or is more evenly distributed after a plant has been through dormancy. Such a phenomenon has been observed with *Blueberry shock virus* (MacDonald et al., 1991). This could explain the reason for the few positives detected in 2005. These plants were tested before they had a chance to overwinter, possibly before BRV could reach a detectable titer or spread systemically throughout the plant. Further testing of these plants will reveal the significance of the overwintering component in virus detection.

ACKNOWLEDGEMENTS

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Figures

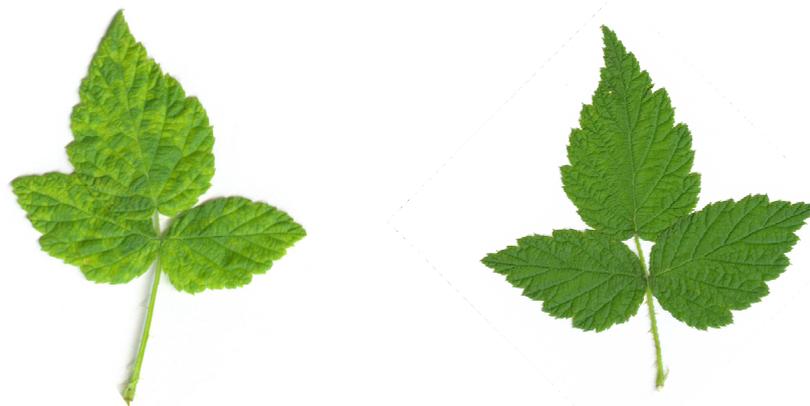


Fig. 1. Left: Symptomatic leaf affected with *Black raspberry virus*, showing chlorosis, mottling, and puckering. Right: Healthy black raspberry leaf.

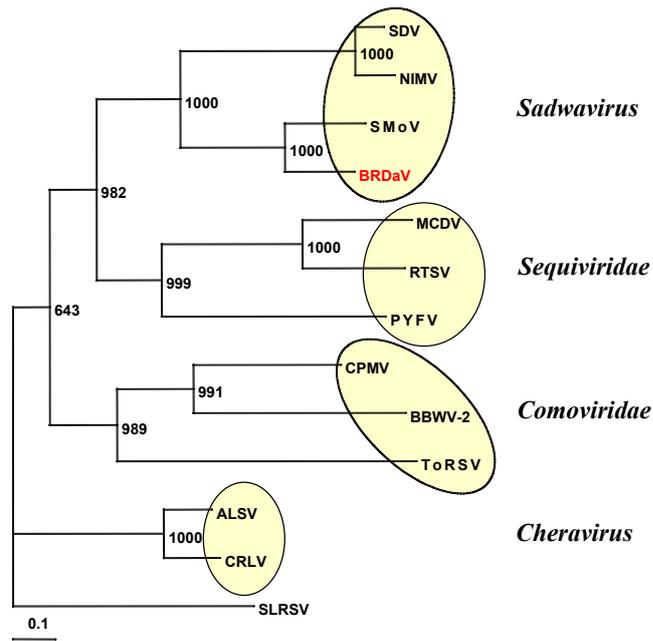


Fig. 2. Unrooted tree of the conserved regions of the RdRp of BRDaV and homologous regions of related viruses.

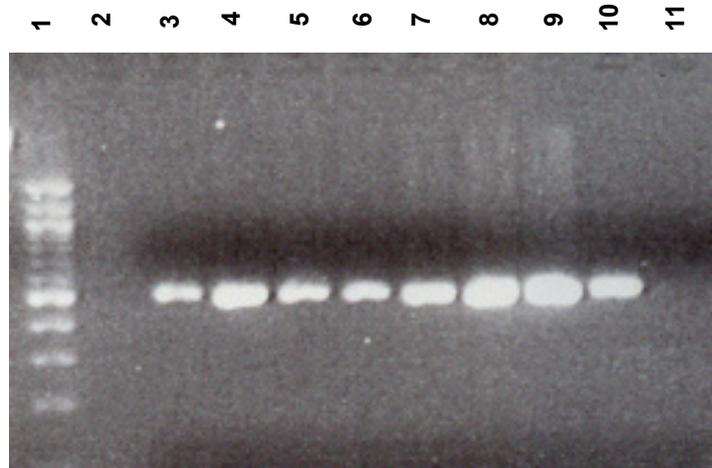


Fig. 3. RT-PCR detection of a region of the RdRp from BRV. Lane 1: 100bp ladder (NEB); Lane 2: Blank; Lane 3, 7, 8, 9, 10: Symptomatic black raspberry; Lane 4: Red raspberry; Lane 5: 'Evergreen' blackberry; Lane 6: 'Marion' blackberry; Lane 11: Healthy black raspberry control.

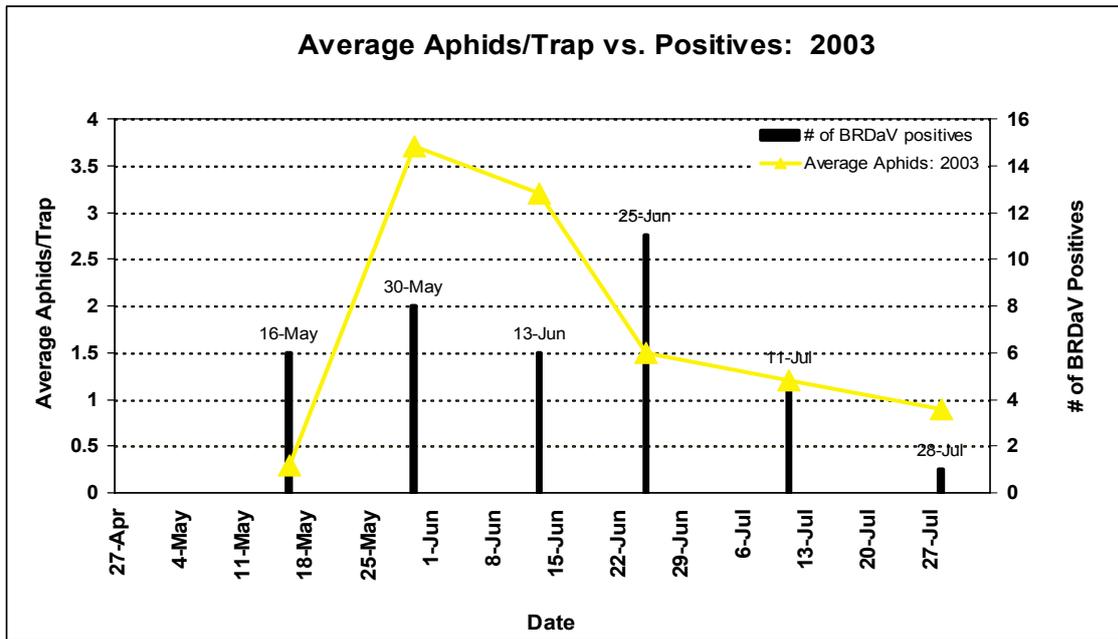


Fig. 4. 2003 average aphids caught per trap (line graph, plotted on the 1^o y-axis) vs. # of plants (out of 16) for each rotation testing positive for BRV (histogram, plotted on the 2^o y-axis).