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***Phragmidium violaceum* on *Rubus armeniacus* and *R. laciniatus* in British Columbia**

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Abstract: *Phragmidium violaceum*, an introduced pathogenic rust fungus causing leaf spots and blight is reported for the first time in British Columbia from locations on central and south central Vancouver Island and the lower mainland near Aldergrove. Its hosts are two invasive introduced weed species: *Rubus armeniacus*, Himalayan blackberry, and *R. laciniatus*, evergreen blackberry. The identification of the rust was confirmed by both morphological observations and molecular techniques, and the current known distribution of the pathogen in BC is described.

Key words: *Phragmidium violaceum*, *Rubus*, rust, British Columbia, Canada

Introduction: Himalayan (also known as Armenian) blackberry, *Rubus armeniacus* Focke, native to Armenia, is the most commonly cultivated blackberry in Europe. It was introduced to western North America over one hundred years ago, and in south coastal British Columbia, especially Vancouver Island and the lower mainland, has become well-established on roadsides and wastelands (Ceska, 1999). It is held in conflicted regard as either a thorny, alien and invasive weed, or as a source of abundant fruit for pies, jams and wine. In the autumn of 2008, small patches of Himalayan blackberry near Courtenay, Vancouver Island, BC, were noticeably affected with abundant 1-2 mm diameter purplish spots on adaxial leaf surfaces (Fig. 1) followed by small patches of early -winter defoliation (Fig. 2). Abundant production of hypophyllous blackish rust telia and orange uredinia were also observed on some affected leaves in September and early October (Figs 3, 4). Similar rust collections were made in July, 2007 from evergreen, also known as cut-leaf, blackberry (*R. laciniatus* Willd.) and Himalayan blackberry growing on the mainland near Aldergrove, BC.

Materials and Methods: Compound microscopic observations were made with a Nikon Optiphot II microscope equipped with differential interference contrast optics. Rust spores were measured and photographed at 200-400 x while mounted in distilled water, and photomicrographs were taken with a Nikon Coolpix 4500 digital camera. Dissecting microscope photographs were taken with a Leica EZ4D with integrated CMOS (Complementary metal-oxide-semiconductor) digital camera. Herbarium acronyms, DAVFP and DAOM, are from *Index Herbariorum*, <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>.

Genomic DNA from DAVFP specimens was extracted from pieces of leaf material bearing *Phragmidium* sori, using a PowerPlant DNA

Extraction kit (Mo Bio Laboratories Inc., Carlsbad, California) according to the manufacturer's directions. The DNA concentration was determined using a NanoDrop ND-1000 spectrophotometer prior to performing PCR analysis.

The ITS ribosomal region was amplified with the following primer sets: LR0R (ACCCGCTGAACTTAAGC) - LR7 (TACTACCACCAAGATCT) and a *P. violaceum* sequence (NCBI EF672358) - derived primer set Pv750 F - Pv750R (TAA GAA CTG TTT TCC GCG CT and TTG CAC GTC AGA ATC GCT AC). PCR amplification was performed in 25 µl reactions containing 12.5 µl of 1x Bioline Buffer, 1x Bioline HI-Spec, 1.5 mM magnesium chloride, 5 µM of each appropriate primer, 200 µM dNTPs, 0.3 U Bioline Diamond Taq Polymerase and approximately 10 ng DNA template. PCR cycles were carried in a T-gradient Thermocycler (Biometra, Goettingen, Germany) under the following conditions: 94 C / 1 min denaturing, 47 C / 45 sec annealing, 72 C / 2 min amplification for 37 cycles, followed by 7 min amplification at 72 C. The PCR products were separated by electrophoresis: 1.2% agarose gel containing 0.25 µg/ml ethidium bromide in 1x TAE buffer. The gel, which ran at 80 V for approximately 1 hr, was photographed under UV light.

The 740-788 bp fragment amplified from the *P. violaceum* samples analyzed (DAVFP 29335, 29342, on *R. armeniacus*, and DAVFP 29343, on *R. laciniatus*) was sequenced using Pv750 primers or LR0R-LR7 primers, according to the amplification product sequenced.

Results: The rust was morphologically confirmed as *Phragmidium violaceum* (Schultz) Winter in all of the collections cited herein. All but two of the specimens collected in British Columbia were from *R. armeniacus*. One collection at the southernmost Vancouver Island location (DAVFP 29343), and another from Aldergrove, on the lower mainland (DAOM

240361) were from the less common *R. laciniatus*, another introduced and invasive species. At the collection site of DAVFP 29343, *R. laciniatus* supported more profuse production of telia, in comparison to the adjacent infected foliage of *R. armeniacus* intertwined along the same fence. Observations of rust uredinia, telia, uredinial paraphyses, urediniospores (Fig. 5) and teliospores (Fig. 6) on *R. armeniacus* were consistent with those published by Osterbauer et al. (2005). Teliospores on *R. laciniatus* were only observed in DAVFP 29343, and were similar in appearance to those collected on *R. armeniacus*, except that 2-celled spores were frequently seen and 5-celled spores were not observed. Teliospore size ranges from *R. laciniatus* (DAVFP 29343), excluding stalks and terminal papillae were: (36-) 42-44(-52) x 24-32 µm (2-celled), 60-64 x 32-36 µm (3-celled), and 76-80 x (32-)34-36 µm (4-celled). Teliospore stalks averaged from 64- 80 µm long, with the upper half to two thirds averaging 8-10 µm wide, and the lower swollen base averaging 20-24 µm wide.

The DNA sequence data indicate that the amplified PCR fragments generated by Pv750 and LR0R-LR7 primers were identical for the three DAVFP accessions cited in the Methods section. A BLAST search of the NCBI database was conducted using the two base sequences generated by Pv750 and LR0R-LR7 which resulted in a 98% or 100% homology respectively, with 0% gaps, and corresponded to the following NCBI accession numbers DQ142910 (*P. violaceum* from Oregon) and DQ142909 (*P. violaceum* from France; Osterbauer et al. 2005). Two sequences from DAVFP 29343 generated by each of the primers were submitted to the NCBI database (accession numbers JN790618 for PV750 and JN790619 for LR0R-LR7).

Material examined: *Phragmidium violaceum* (Schultz) Winter on wild *Rubus armeniacus* Focke, **Canada**. British Columbia: Aldergrove, July 8, 2007, Coll. Mark Sweeney, Det. Mark Sweeney and Vippen Joshi. Blackberry (wild)

(Labelled as Variety "Himalayan"). Stage: II and III, DAOM 240362; Courtenay, Vancouver Island: Sept. 2, 2008, West side of estuary near walkway, near Millard Creek, 49°40'11" N, 124°58'30" W, Coll. R. Wall, Det. B. Callan, Stage III, DAVFP 29335; Sept. 2, 2008, north side of Kilpatrick Ave. at Anfield Shopping Centre, 49°40'08" N, 124°58'49" W, Coll. R. Wall, Det. B. Callan, Stages II and III, DAVFP 29357; Sept. 19, 2008, beside estuary near Millard Creek, 49°40'11" N, 124°58'29" W, Coll. R. Wall, Det. B. Callan, Stage II, DAVFP 29358; Sept. 14, 2008, estuary, Dyke Rd., near Portuguese Joe's Fish Market, 49°40'44" N, 124°57'53" W, Coll. R. Wall, Det. B. Callan, Stage III, DAVFP 29359; Sept. 4, 2008, Mitchell Place, Malcolm Morrison Sr. Park, 49°40'58" N, 125°01'15" W, Coll. R. Wall, Det. B. Callan, Stage III, DAVFP 29360; Nanaimo, Vancouver Island, Dec. 5, 2008, near junction of Mt. Sicker Rd. and Trans-Canada Highway, 49°11.26' N, 124°0.15' W, Coll. and Det. B. Callan, Stage III, DAVFP 29342.

Phragmidium violaceum (Schultz) Winter on wild *Rubus laciniatus* Willd., cut-leaf blackberry or evergreen blackberry. **Canada**. British Columbia: Aldergrove, July 8, 2007, Coll. Mark Sweeney, Det. Mark Sweeney and Vippen Joshi (wild) (Labelled as Variety "Evergreen"), Stage II; DAOM 240361; South of Chemainus, Vancouver island, near junction of Mt. Sicker Road and Trans-Canada Highway, Dec. 5, 2008, 48°52'29.46" N, 123°42'20.20" W, Coll. and Det. B. Callan, Stage III, DAVFP 29343.

Discussion: These new Canadian records are not surprising extensions of the range of this rust species considering that Mahaffee et al. (2008) reported that "*P. violaceum* could be found on *Rubus* spp. west of the Sierra Nevada and Cascade mountains from Santa Cruz CA to the Canadian border." Currently the known distribution of the rust in BC consists of localized infections on coastal east central Vancouver Island from Oyster River south to Chemainus, and on the southwest lower mainland near

Aldergrove. The distribution of blackberry rust in southwestern BC does not yet extend to the full range of its host since we have not found *P. violaceum* on the southern tip or on the west coast of Vancouver Island, where large, dense stands of *R. armeniacus* are very common on disturbed areas. Damage to date in affected areas consists of small patches with foliar infections along with even smaller patches of winter defoliation. Widespread establishment of the rust disease could result in loss of the competitively advantageous evergreen status at least during years with moist summer conditions that favour increased production of uredinia.

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Plate 1. Symptoms and signs on *R. armeniacus*. Fig. 1. Purple adaxial leaf spots indicating developing telial sori, Courtenay, Vancouver Island, August, 2009. Fig. 2. Winter defoliation at Oyster River attributed to rust, Vancouver Island, December, 2008. Fig. 3. Telia and uredinia, Courtenay, Vancouver Island, August, 2008. Scale bar = 2 cm. Fig. 4. Enlarged view of telia and uredinia (DAVFP 29335). Scale bar= 10 mm. Fig. 5. Urediniospores (DAVFP 29335) Scale bar = 20 μ m. Fig. 6. Teliospores (DAVFP 29342) Scale bar = 38 μ m.