First Report of *Rose rosette virus* Associated with Rose Rosette Disease in *Rosa hybrida* in Minnesota

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Accepted for publication 10 May 2017.

Garden or landscape roses, *Rosa* spp. (family *Rosaceae*), are woody perennials frequently planted in gardens and commercial landscapes for their flowers. Recent breeding efforts have produced cold-hardy, colorful, disease- and heat-tolerant plants that have greatly increased the popularity of roses (Pemberton and Karlik 2013).

A single *R. hybrida* Above and Beyond ('ZLEEltonStrack') plant was identified in a public garden in Carver Co., MN, in September 2015 with rose rosette disease (RRD) like symptoms. The plant exhibited abnormal, late-season, lateral shoot growth, abundant red pigmentation, excessive thorniness, and witches'-broom (Fig. 1). RRD symptoms are characteristic of *Rose rosette virus* (RRV) infected plants (Laney et al. 2011).

RRD causing witches'-broom of rose was first noted in 1940–1941 in Manitoba, California, Nebraska, and Wyoming (Connors 1941). RRD is widespread throughout the South, the Midwest, and east of the Rocky Mountains (Martin 2014), and is the most destructive rose virus in the United States (Dobhal et al. 2016). RRV, the causal agent of RRD, from Arkansas, was sequenced in 2011 and a molecular diagnostic test was created (Laney et al. 2011).

Total RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Frederick, MD) from symptomatic tissue and tested by reverse-transcription polymerase chain reaction (RT-PCR) (illustra Ready-to-Go beads; GE Healthcare Life Sciences, Marlborough, MA) using RRV detection primers (Laney et al. 2011) designed to amplify a region of RNA1. No amplification products were observed, which is consistent with a report by Dobhal et al. (2016) of difficulty detecting all RRV positive samples using those primers.

New primers were designed (Primer-BLAST) based on conserved RRV sequences from RNA3 and RNA4 (15 sequences for both) available from NCBI and amplify partial coding regions of the putative nucleocapsid and movement proteins (Table 1). They were used in RT-PCR with reaction conditions of 42°C for 45 min, and 94°C for 5 min; and 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by an extension of 10 min at 72°C. The reactions yielded 411-nt and 475-nt products for the RNA3 and RNA4 targets, respectively (Fig. 2).

The amplicons were purified, ligated, and cloned (pGEM-T Easy Vector System; Promega, Madison, WI). Four clones were sequenced in both directions for both amplicons (MCLAB; South San Francisco, CA). The consensus sequences (Sequencher 5.1; Gene Codes Corp., Ann Arbor, MI) were deposited in GenBank (Accession Nos. KU195474 and KU195475). The RNA3 product had 100% query coverage and 99% nucleotide identity (E value = 0.0)

to deposited RRV RNA3 sequences (KT835651.1, HQ891903.1, and 15 more). The RNA4 product had 100% query coverage and 99% nucleotide identity (E value = 0.0) to RRV RNA4 sequences (HQ89189.1, HQ871945.1, and 12 more), thus confirming the symptomatic rose is infected with RRV.

It is likely the rose, which was planted in spring 2015, was infected naturally. This cultivar was bred in Minnesota and the commercial supply chain had not yet left Minnesota. It was clonally propagated by cuttings (own-root) and not grafting onto rootstock. Additional Above and Beyond plants, from the commercial nursery licensed to produce these roses, were screened for symptoms but none were observed. Five random rose samples were tested as above and yielded negative reactions. The public garden from which the symptomatic plant originated was surveyed for roses with RRD symptoms. None were observed in the fall of 2015 or the summer of 2016.

RRV has been found in the bordering states of Wisconsin and Iowa and in the Canadian province Manitoba. The native Woods rose (*R. woodsii*) and invasive *R. multiflora* are found throughout Minnesota and neighboring states, and are thought to be a reservoir of RRV and the eriophyid mite vector (*Phyllocoptes fructiphilus*) (Martin 2014; Amrine 1996). *Phyllocoptes fructiphilus* is easily disseminated by wind and has been found throughout the United States (Amrine 1996).

FIGURE 1

Rosa hybrida Above and Beyond ('ZLEEltonStrack') exhibiting symptoms of abnormal late season lateral shoot growth, abundant red pigmentation, excessive thorniness (inset, B), and witches'-broom.

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TABLE 1
Primers designed for use in detecting RNA3 and RNA4 of *Rose rosette virus* (RRV), their binding site, encoded protein product, and the expected product size

Primer name	Sequence	Binding site	Gene product	Product size
RRV3F2	5'-AGACTTGAATGAGTCTGATGTTCT-3'	375-398	Partial coat protein	411 bp
RRV3R2	5'-GCATCATCGAAAGGAGTAGTCTC-3'	785-763		
RRV4F1	5'-CGTGACAGGCTCACTTGAYT-3'	465-484	Partial movement protein	475 bp
RRV4R1	5'-CCTGACAGTGCAGAGCTTAAT-3'	959-939

FIGURE 2

Reverse transcription polymerase chain reaction result: Lane 1, 100 base pair ladder; Lane 2 and 3, amplicons from symptomatic *Rosa hybrida* Above and Beyond ('ZLEEltonStrack') using primers RRV3F2+R2 (411-bp product) and RRV4F1+R1 (475-bp product), respectively.

The wide distribution of introduced or native roses in Minnesota, the presence of both RRV and its vector *P. fructiphilus* in adjacent areas, and our inability to trace the infection back to infected source plants all supports the conclusion this rose was infected naturally. To our knowledge, this is the first report of RRV infecting rose in Minnesota.

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