First Report of *Lilac ring mottle virus* Infecting Lilac in the United States

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In May 2015, the Oregon Department of Agriculture investigated a report of potential virus symptoms being observed on lilac plants (*Syringa vulgaris* L.) produced by a nursery in Marion County. Simultaneously, symptomatic lilac plant samples were received by the Oregon State University Plant Clinic from a customer of the same nursery. Lilac ‘President Grevy’ plants inspected at the nursery showed symptoms of leaf deformation, reduction in leaf size, ring spots, and line patterns (Fig. 1). Two months later, similar foliar symptoms were observed on lilac ‘Krasavitsa Moskvyy’ plants at the same nursery (Fig. 2). Symptoms on both cultivars resembled those reported for *Lilac ring mottle virus* (LiRMoV) (Van Der Meer et al. 1976). LiRMoV is an isometric RNA virus within the family *Bromoviridae* and genus *Ilarvirus* (Scott and Ge 1995; Scott and Zimmerman 2008) and has only been reported from the Netherlands. The virus was sap transmissible to herbaceous hosts under experimental conditions and was seed transmissible in the experimental hosts *Chenopodium quinoa*, *C. amaranticolor*, and *Celosia argentea* (Van der Meer et al. 1976). Symptom expression in infected lilac is influenced by environmental conditions and may be erratic; thus, infections may remain cryptic for years (Van der Meer et al. 1976).

To determine if LiRMoV was present in the symptomatic lilacs, total nucleic acids (TNA) were extracted from symptomatic leaf tissues using a procedure modified from Hughes and Galau (1988). The TNA was used in ONE-STEP PCR reactions (Qiagen, Germantown, MD) at a melting temperature of 55°C and with primers (downstream: 5'-GAGACCGAAGTCTTCTTCC-3' and upstream: 5'-CCACGTGCTTCTCACCC-3') specific for the movement protein of the RNA3 of LiRMoV (GenBank Accession No. U17391) (Scott and Zimmerman 2008). In addition to the TNA from the samples, a positive control (plasmid pLRMV-7) (Scott and Zimmerman 2008) and negative controls (healthy plant tissue and water) were analyzed in concurrent PCR reactions. The anticipated 649-bp amplicon was produced in the lilac samples and in the positive control, but not in the negative controls. The amplicons from seven lilac samples were cloned using the pGem-T Easy Vector (Promega, Madison, WI), selected by blue-white screening, and then sequenced using the primer M13F. The contiguous sequence generated was deposited into GenBank (Accession No. KX090269). Six of the seven clones contained an

FIGURE 1

Foliar symptoms caused by *Lilac ring mottle virus* on lilac ‘President Grevy.’

FIGURE 2

Foliar symptoms caused by *Lilac ring mottle virus* on lilac ‘Krasavitsa Moskvyy.’

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insert that showed >99% homology with the published sequence for the RNA3 of LiRMoV (GenBank Accession No. U17391).

Tomato mosaic virus (ToMV), *Arabidopsis mosaic virus* (ArMV), and *Lilac leaf chlorosis virus* (LLVC) have also been described as infecting lilac (Cooper 1993). Additional PCR testing of the lilac samples' TNA for these three viruses was completed. Amplicons were produced from the lilac samples of the appropriate sizes for LLCV (271 bp) (James et al. 2010) and for ToMV (318 bp, using the primers 1180, 5'-CGAGAGGGGCAACAAACAT-3', and 1181, 5'-ACCTGTCTCCATCTCTTTGG-3' [S. Scott unpublished]); ArMV was not detected. Identity of the amplicons produced was confirmed by sequencing.

Samples received by the OSU Plant Clinic were examined using transmission electron microscopy (TEM) at the OSU Electron Microscopy Facility, which used two methods to examine symptomatic leaves. In the first, the leaf tissue was cut into 1-mm strips and put in fixative (2.5% glutaraldehyde plus 1% paraformaldehyde in sodium cacodylate buffer, 0.1M at 7.4 pH) overnight. The following day the tissue was crushed to express sap, which was collected on a copper grid, stained with 2% aqueous ammonium phosphotungstic acid, and examined. The second method was a simple expression of sap from unfixed tissue, which was then stained with 2% uranyl acetate in 50% ethanol. Both preparations were examined using a FEI Titan 80-200 TEM/STEM electron microscope. No virus particles were observed in either preparation.

This is the first report of LiRMoV from lilac cultivars President Grevy and Krasavitsa Moskvya in the United States. These cultivars were introduced into North America before 1950 and are grown in landscapes in 31 states and four Canadian provinces (Favorite 2006). LLCV was recently detected and described from Canada (James et al. 2010) and ToMV has previously been reported infecting a lilac hybrid in New York (Castello et al.

1992). Fruit trees infected by *Ilarvirus* and other viruses often exhibit an acute phase, which occurs when the plant is initially infected and symptoms are obvious, and a chronic phase, which occurs in subsequent growing seasons when systemic infection has been established and plants may appear asymptomatic (Nemeth 1986). It is possible LiRMoV, ToMV, and LLCV have been present in these cultivars for many years as cryptic infections. The infected plants at the Marion County nursery were destroyed.

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