



First report of the identification of a '*Candidatus* Phytoplasma pruni'-related strain in *Trillium* species in Canada

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Received: 26 Aug 2016. Published: 20 Oct 2016.

Trillium (family Melanthiaceae) is a genus of perennial flowering plants native to temperate regions of North America and Asia, and traditionally used as medicinal plants (Case, 2000). There are 49 species within the genus *Trillium* amongst which the white trillium, *T. grandiflorum* serves as the emblem and official flower of the Canadian province of Ontario. Other species include *T. erectum*, also known as red trillium. Many of these plants are adapted to conditions found in suburban or rural gardens. Compared with symptomless plants (Fig. 1), plants of *T. grandiflorum* and *T. erectum* with symptoms of virescence typical of phytoplasma infection (Figs. 2-3) were observed growing as weeds on a private property in the Sparta area of London, Ontario in May 2016.

Plants were removed by the owner, placed in plastic bags and transported to the laboratory in ice coolers. Leaves, petioles and flowers were excised from eight symptom-bearing and two symptomless plants of each species. Total DNA was extracted and used as a template for a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene, R16mF2/R1 for the first PCR reaction, and R16F2n/R2 for the nested reaction (Gundersen & Lee, 1996). All samples from symptom-bearing plants yielded R16F2n/R2 amplicons. No PCR amplicons were obtained from the symptomless plants. One R16F2n/R2 amplicon from *T. grandiflorum* and three from *T. erectum* were purified (EZNA Cycle Pure Kit, Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega) and sequenced (University of Toronto, Canada).

Sequences were compared with those of phytoplasmas in NCBI. Sequences from *T. grandiflorum* (Genbank Accession No. KX470428) and *T. erectum* (KX470427, KX470429 and KX470430) shared over 99.7% sequence identity and were 99% similar to those of phytoplasma members of the group 16SrIII (Western X-disease). Phylogenetic analysis (using MEGA v4.0) of the 16S rDNA sequence (Fig. 4) supported the grouping of the phytoplasma isolated from both *T. grandiflorum* and *T. erectum* plants as a '*Candidatus* Phytoplasma pruni'-related strain, with 99% sequence identity

to the reference strain for the species (Milkweed yellows phytoplasma, 16SrIII-F, HQ589200).

No phytoplasmas have been reported from *Trillium* plants in Canada. The X-disease (group 16SrIII) was first reported in 1941 in Ontario in the Niagara peninsula (Stevens & Stevens, 1947). In 1971, peach X-disease was detected in Essex County (Dhanvantari & Kappel, 1978) affecting more than 50% of peach orchards. In North America, phytoplasmas of the 16SrIII group are associated with several diseases in cherry and peach (Scott & Zimmerman, 2001). The presence of a '*Ca. P. pruni*'-related strain in *Trillium* not only poses a threat for *T. grandiflorum* as a protected species, and other *Trillium* species in Ontario, but also for other plant species grown nearby. To our knowledge, this is the first detection of a phytoplasma in *Trillium* species in Canada and worldwide.

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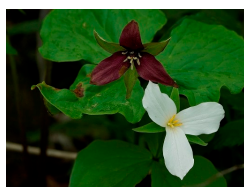


Figure 1



Figure 2



Figure 3

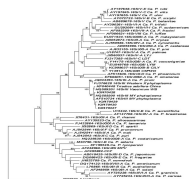


Figure 4

To cite this report: Arocha-Rosete Y, Morales-Lizcano NP, Hasan A, Yoshioka K, Moeder W, Michelutti R, Satta E, Bertaccini A, Scott J, 2016. First report of the identification of a '*Candidatus* Phytoplasma pruni'-related strain in *Trillium* species in Canada. *New Disease Reports* **34**, 19. <http://dx.doi.org/10.5197/j.2044-0588.2016.034.019>
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