

Freesia sneak virus (FreSV) on freesia: a first detection for Virginia and the United States

M. A. Hansen¹, A. M. Vaira^{2,3}, C. Murphy⁴, J. Hammond³, M. Dienelt³, E. Bush^{1*} and C. Sutula⁵

1) Department of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA; 2) CNR Istituto di Virologia Vegetale, 10135 Torino, Italy; 3) USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville, MD; 4) USDA-ARS, Electron Microscopy Unit, Beltsville, MD; 5) Agdia, Inc., Elkhart, IN

ABSTRACT SUMMARY: Samples of freesia cvs. 'Honeymoon' and 'Santana' with unusual symptoms of leaf flecking (Fig. 1) were received from a commercial cut flower grower by the Virginia Tech Plant Disease Clinic in Spring 2008. Symptoms of coalescing, chlorotic, bleached, brown and purple leaf spots were scattered in the planting. After microscopic examination failed to reveal any pathogens or arthropods, images of the unusual symptoms were submitted to the APS Diagnostics Committee's listserv. One responder suggested a newly reported virus, *Freesia sneak virus* (FreSV) might be the cause. Plant samples were forwarded to the USDA-ARS Floral and Nursery Plants Research Unit in Beltsville, MD, for viral analysis. In the Virginia freesia samples, presence of FreSV, but not *Freesia mosaic virus*, was strongly correlated with leaf necrosis symptoms. Use of the diagnostics listserv was instrumental in diagnosing this disease.



Fig. 1. Symptomatic freesia submitted to the Virginia Tech Plant Disease Clinic.

- At the USDA-ARS Floral and Nursery Plants Research Unit nucleic acids were extracted from symptomatic plants (Fig. 1) and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using ophiiovirus-specific degenerate primers.
- Fourteen of 15 plants tested positive for the 136-bp amplification product from the ophiiovirus RdRp gene, which is diagnostic for ophiiovirus (Fig. 2).

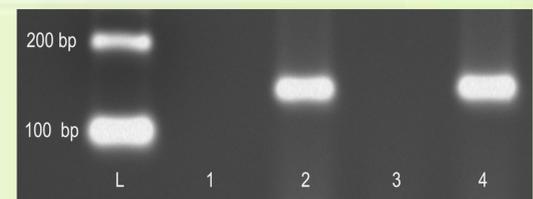


Fig. 2. RT-PCR amplified the ophiiovirus diagnostic band (136 bp) from 14 of 15 freesia plants tested. (L=molecular ladder, 1=healthy leaf tissue, 2=FreSV-infected lachenalia, 3=no template control, 4=symptomatic freesia sample)

- At the USDA-ARS Electron Microscopy Unit partially purified virus preparation was visualized using transmission electron microscopy. Both potyvirus- and ophiiovirus-like particles were observed (Fig. 3).
- Further characterization of the potyvirus revealed a potyvirus-specific amplification product from only 2 of 15 plants.
- Sequences were cloned and the potyvirus was identified as *Freesia mosaic virus*, which is known to cause mosaic symptoms.

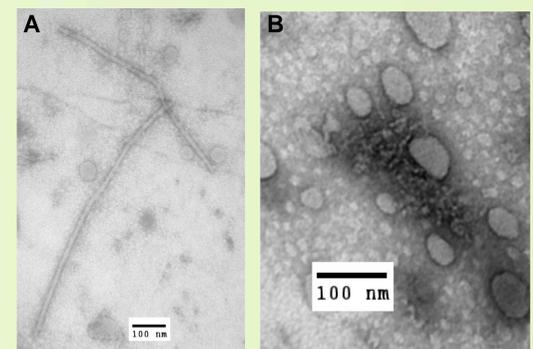


Fig. 3. Potyvirus-like particles (A) and ophiiovirus-like particles (B) were observed in partially purified virus preparation from symptomatic freesia samples using transmission electron microscopy.

- The ophiiovirus coat protein (CP) gene from 2 symptomatic Virginia freesia plants was amplified using RT-PCR and primers based on FreSV sequence information in GenBank.
- Analysis of cloned sequences showed the 2 sequences were identical and 99% identical to a FreSV CP sequence from an Italian freesia sample; this confirmed presence of FreSV in the Virginia symptomatic freesia.
- This is the first report of FreSV from Virginia and the United States.

Ophiiovirus

- proposed family: *Ophioviridae*
- all are plant-infecting (monocots and dicots)
- found in both New and Old World
- viral particles difficult to visualize with electron microscope
- first observed microscopically in 1988
- first characterized in early 1990's

Freesia sneak virus

- transmitted by the soilborne fungus *Olpidium brassicae*
- remains viable for years in vector resting spores in soil
- no effective treatments for vector, so control is difficult

History of

Freesia sneak virus

- necrotic symptoms on freesia described in the Netherlands, England and Germany by early 1970's
- in the late 1980's a similar problem was reported on freesia in Italy and the virus was partially characterized by 2006
- FreSV first reported in *Lachenalia* sp. from South Africa in 2007

Taxonomy

- filamentous particles (ophis=Gr. for snake) form circular structures of various sizes
- primarily negative single-stranded RNA particles
- multipartite (3 to 4 segments)

Diagnosis

- RT-PCR with ophio-specific degenerate primers from the RdRp gene is used for species identification

Current species

- *Citrus psorosis virus*
- *Lettuce ring necrosis virus*
- *Mirafiori lettuce virus*
- *Ranunculus white mottle virus*
- *Tulip mild mottle mosaic virus*
- Proposed: *Freesia sneak virus*

References:

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