First Report of Orchid Fleck Virus in the Orchid Collection of Jardin du Luxembourg, Paris, France

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DISEASE NOTES

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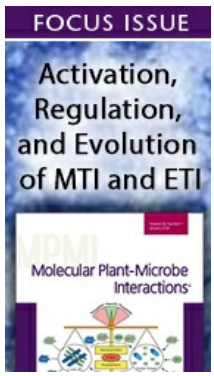

The orchid collection of Jardin du Luxembourg in Paris, France, began about 150 years ago at a botanical garden next to the Medical School of Paris at the southern part of the present Jardin du Luxembourg, organized by Achille Richard, a dedicated orchidologist. Since then, the collection has expanded and is presently maintained by the French Senate and has more than 10,000 plants of 1,300 species (Bertaux et al. 2010). Some plants of this collection exhibited necrotic lesions or ringspots on their leaves similar to those reported associated with orchid fleck virus (OFV, genus *Dichorhavirus*, family *Rhabdoviridae*), known to be distributed worldwide (Kondo et al. 2003). In attempts to detect evidence of the presence of OFV, leaf tissues from the ringspot lesions from seven symptomatic samples (*Maxillaria* [three species], *Phalaenopsis*, *Laeliocattleya*, *Paphiopedilum*, and *Phragmipedium* [one species from each]) were examined by transmission electron microscopy. Tissues from

Maxillaria luteograndiflora and *Paphiopedilum* Germinyanum revealed typical cytopathic effects previously reported for OFV and other dichorhaviruses, including the presence of nuclear electron lucent inclusions (viroplasm) and short, rod-like particles scattered either in the nucleus or in the cytoplasm (Kondo et al. 2003). *Brevipalpus* mites were found and collected from *M. luteograndiflora* and *Phalaenopsis* hybrid and were identified by light (differential interference contrast) and scanning electron microscopy as *B. californicus*, based on morphological characteristics including the reticulation pattern from venter and dorsum, shape of spermatheca vesicle, dorsal opisthosoma chaetotaxy, number of solenidia on tarsus II, and microplates ornamentation (Beard et al. 2012). This mite species is reported as the vector of OFV (Kondo et al. 2003). For molecular detection of OFV in the symptomatic leaves, total RNA was extracted from the lesioned tissues of *M. luteograndiflora* using Trizol Reagent (Life Technologies, Foster City, CA). cDNA was prepared with a RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, Madison, WI), and polymerase chain reaction was performed using GoTaq Master Mix (Promega, Madison, WI) and primers for the detection of OFV *N* (ORF1, RNA1) and *L* (ORF6, RNA2) genes (Kubo et al. 2009; Ramos-González et al. 2016). Single amplicons of approximately 320 and 390 bp were obtained from each reaction, and their sequences (GenBank accessions MG970602 and MG970603, respectively) exhibit 99% nucleotide sequence identity to the OFV *N* and *L* genes of the isolate Cym07 from Japan (LC222629 and LC222630). Our data demonstrated that OFV was detected in orchids from Jardin du Luxembourg: *M. luteograndiflora* by molecular assay and by morphological means in this species and *P.* Germinyanum. This is the first report of the presence of OFV in France; a careful mite control and a survey of symptomatic plants are being undertaken in this collection to eradicate this virus and avoid the spread of the disease.

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