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First report of the *Brevipalpus*-transmitted
(Trombidiformes: Tenuipalpidae) virus, *Orchid fleck virus*
(Mononegavirales: Rhabdoviridae) infecting three
ornamentals in Florida

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Abstract

Several flat mite species, all from the genus *Brevipalpus* Donnadiue (Trombidiformes: Tenuipalpidae), are the only known vectors of dichorhaviruses. The *B. californicus* species group exclusively can transmit Orchid fleck dichorhavirus (OFV) in a persistent propagative manner. OFV is the type species for the genus *Dichorhavirus* and infects more than fifty plant species belongs to the family Orchidaceae, Asparagaceae (Nolinoidaea), and Rutaceae (*Citrus*). During June 2020, chlorotic ringspot symptoms on Giant Lilturf (*Liriope* spp., cv. 'Gigantea') were observed in a landscape in Leon County, Florida. Later in the year, the presence of OFV was confirmed using OFV specific conventional reverse transcription polymerase chain assay (RT-PCR) assay and Sanger sequencing. RT-PCR amplicons had a 98% identity with the known OFV sequences available in the Genbank. The identification was also confirmed with the quantitative RT-PCR (RT-qPCR). Additional samples were taken from other Nolinoidaea, including *Liriope muscari*, *Ophiopogon japonicus*, *O. intermedius* and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) in Leon and

Background

Orchid fleck virus (OFV) is the type member for the genus *Dichorhavirus*, family Rhabdoviridae; a bacilliform, nuclear rhabdovirus composed of two segments of single-stranded, negative-sense RNA which infects plants (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Other members of this genus are: *Citrus chlorotic spot virus*, *Citrus leprosis virus N*, *Clerodendrum chlorotic spot virus* and *Coffee ringspot virus* (Dietzgen, Freitas-Astúa, et al. 2018). Flat mites from the genus *Brevipalpus* Donnadiieu (Trombidiformes: Tenuipalpidae) are the only known vector for dichorhaviruses (Maeda 1998), and *Brevipalpus californicus* (Banks) mites are the only mite known to transmit OFV in a persistent propagative manner (Kondo et al. 2003).

Symptoms

OFV-infected plants exhibit various symptoms dependent on the infected plant species as well as the strain of the OFV (Kubo et al. 2009), but symptoms typically appear as chlorotic flecks, which ultimately coalesce into larger spots or ringspot patterns (Fig. 1).

Distribution

OFV was first described infecting *Cymbidium* orchids in Japan (Doi et al. 1977). Many countries have reported OFV and OFV-like rhabdoviruses infecting orchids worldwide (Kondo et al. 2003), including Asia: [China (Peng et al. 2017), Korea Zheng et al. (2013)], Africa: [South Africa (Blanchfield et al. 2001)], North America: [The United States (Blanchfield et al. 2001, Bratsch et al. 2015)], South America: [Brazil (Kitajima et al. 1974, Kitajima et al. 2001), Colombia (Kubo et al. 2009), Costa Rica (Freitas-Astúa et al. 2002), Paraguay (Ramos-González et al. 2015)], Europe: [Denmark (Begtrup 1972), France (Sauvêtre et al. 2018), Germany (Petzold 1971, Lesemann and Doraiswamy 1975)] and Oceania: [Australia (Lesemann and Begtrup 1971, Lesemann and Doraiswamy 1975, Gibbs 2000), Fiji (Pearson et al. 1993), Vanuatu (Pearson et al. 1993)]. The prevalence of OFV and its mite vector is thought to be associated with the movement of infected orchids (Dietzgen, Freitas-Astúa, et al. 2018).

Infectivity

OFV naturally infects more than fifty species of Orchidaceae (Kitajima et al. 2010, Peng et al. 2013), some Asparagaceae (Nolinoidaea) (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), as well as Rutaceae: *Citrus* where it causes citrus leprosis-like symptoms (García-Escamilla et al. 2018, Beltran-Beltran et al. 2020, Roy et al. 2020). Mechanical transmission of OFV is possible under lab conditions to various Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013).

Detection

During June 2020, chlorotic ringspot symptoms were observed on Giant Liliy turf *Liriope* spp., cv. 'Gigantea' in a landscape of Leon County, Florida (Fig. 1). *Liriope* belong to a group of plants in the family Asparagaceae, subfamily Nolinoidae, which includes a diverse array of various monocotyledonous lilioid plants which are native to southeastern Asia (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* Ker Gawler (Asparagaceae: Nolinoidae) are considered the most important ground cover sold by the nursery industry in southeastern US (Mcharo et al. 2003).

Testing

Viral infections of suspected plant leaf samples were initially tested at the Plant Disease Diagnostic Clinic at the North Florida Research and Education Center (NFREC) in Quincy, FL. All the samples tested negative for begomovirus, potyvirus, tospovirus as well as for Impatiens necrotic spot virus, Tobacco mosaic virus and Tomato spotted wilt virus. The infected materials were subsequently sent to the Florida Department of Agriculture and Consumer Services (FDACS). The presence of OFV was confirmed using OFV generic R2-Dicho-GF and R2-Dicho-GR primers (Roy et al. 2020) by one step conventional RT-PCR, amplifying ~800 nt of L-gene (RNA2) amplicon from an infected *Liriope* leaf sample. The RT-PCR amplicon was sequenced using Sanger sequencing and confirmed the presence of OFV in the tested samples. Sanger sequencing of RT-PCR amplicons shared 98% nucleotide identity with orchid strains of OFV (GenBank Accession numbers: AB244418 and LC222630) (Kondo et al. 2006, 2017). Further surveys of putatively OFV-infected plants were taken during subsequent visits to the initial site of collection. Plant samples

Map

Further surveys of plants belonging to the subfamily Nolinoidaea in Florida have revealed more sites with symptomatic plants in both Leon and Alachua counties. In addition to the discovery of the OFV-infected plants, we collected mites from symptomatic plants in both Leon and Alachua counties. In addition to the discovery of the OFV-infected plants, we collected mites from symptomatic plants in Leon county which were observed with phase contrast microscopy. We encountered both an unidentified eriophyoid mite species on the *Liriope* spp., along with flat mites on all species tested (*Liriope* spp., *Ophiopogon* spp., and *A. elatior*). The flat mites were originally identified as *Brevipalpus californicus* (Banks) sensu lato, an identity which was confirmed by the FDACS via Differential Interference Contrast (DIC) microscopy. *Brevipalpus* mites have been previously associated with OFV (Dietzgen, Tassi, et al. 2018, García-Escamilla et al. 2018, Beltran-Beltran et al. 2020) as well as similar diseases (Kitajima et al. 2010) and are known to feed on a large variety of economically important plants (Childers, Rodrigues, and Welbourn 2003, Akyazi et al. 2017).

Previous Reports

The first report of OFV in the United States is thought to be Ko et al. (1985), who describes nuclear inclusions caused by an undescribed bacilliform rhabdovirus in *Brassia* orchids. The significance of this report is their reference to spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, Ko et al. (1985) made no mention of mites or further investigations of this virus. The first certain report of OFV in the US was made by Bratsch et al. (2015), who confirmed the presence of OFV in *Phalaenopsis* hybrids in the US, using TEM of ultrathin sections of plant tissue as well as molecular sequence analysis and its association with *Brevipalpus* mites. The authors did not make a conclusive species identification but suggested the mite species was *B. californicus* group as the vector as referred by Kondo et al. (2003).

OFV has been reported in other Nolinoidae in Australia (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016). The Florida collected plants of

Conclusion

OFV is the type member of the genus *Dichorhavirus* (family *Rhabdoviridae*, order *Mononegavirales*) contain a bipartite, single-stranded and negative sense RNA genome. The dichorhavirus that infects citrus in Hawaii, Mexico, Colombia, and South Africa are identical to the OFV in gene order, content, and the genome sequence. According to the International committee on Taxonomy of Viruses (ICTV) classification, OFV consist of two orchid strains (OFV-Orc1 and OFV-Orc2) and two citrus strains (OFV-Cit1 and OFV-Cit2). Both the orchid strains of OFV infects citrus but none of the citrus strain have been reported from any orchid species. Detecting OFV in Florida represents a concern for horticulturists who grow orchids, *Liriope*, *Ophiopogon*, or other susceptible Asparagaceae species which are commonly used in landscaping. Florida is also home to a plethora of native and naturalized orchid species, many of which are threatened, including cultivating *Vanilla* in southern Florida (Chambers et al. 2019) and the famous Ghost Orchid, [*Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe]. The first time leprosis has been observed in the Florida

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Figure captions

Fig. 1: Variety of symptoms expressed by plants infected with Orchid fleack dichorhavirus: (a) ringspot symptoms on *Liriope gigantea* (b) chlorotic flecking on *Aspidistra elatior*.

Figures



a



b