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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

The genus *Brevipalpus* Donnadieu (Trombidiformes: Tenuipalpidae), commonly known as flat mites are the only known vector of dichorhaviruses and transmit *Orchid fleck virus* (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 Lilyturf (*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) and High Throughput Sequencing (HTS). Additional samples were taken from other *Liriope* spp., *Ophiopogon* spp., and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) in Leon and Alachua county also tested positive for OFVs. Three mite species were recovered from OFV-infected plants: *Brevipalpus californicus* sensu lato, *B. obovatus* Donnadieu and *B. confusus* Banks. One of these species is presumably responsible for OFV transmission. Florida has various mite species of *Brevipalpus* and a diverse array of susceptible native and introduced plant species in the landscape. We suggest that OFV already has a wide distribution in Florida which will continue to spread if unchecked, representing a potential threat for *Liriope* spp., *Ophiopogon spp.* and *Aspidistra elatior* which are commonly used in landscaping in Florida.

### Keywords:

False spider mite, flat mite, *Brevipalpus*-transmitted viruses, *Liriope*, Rutaceae, Asparagaceae, orchid, Orchidaceae, pests, ornamental plants, orchid fleck virus.

*Orchid fleck virus* (OFV) is the type member for the genus *Dichorhavirus*, family Rhabdoviridae; a bacilliform, nuclear rhabdoviruses composed of two segments of single-stranded, negative-sense RNA infects plan (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* Donnadieu (Trombidiformes: Tenuipalpidae) are the only known vectors for dichorhaviruses (Maeda 1998). These mites transmit OFV in a persistent propagative manner (Kondo et al. 2003).

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).

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 Australia (Lesemann and Begtrup 1971, Lesemann and Doraiswamy 1975, Gibbs 2000), Brazil (Kitajima et al. 1974, Kitajima et al. 2001), China (Peng et al. 2017), Colombia (Kubo et al. 2009), Costa Rica (Freitas-Astúa et al. 2002), Denmark (Begtrup 1972), Fiji (Pearson et al. 1993), France (Sauvêtre et al. 2018), Germany (Petzold 1971, Lesemann and Doraiswamy 1975), Korea Zheng et al. (2013), Paraguay (Ramos-González et al. 2015), South Africa (Blanchfield et al. 2001), the United States (Blanchfield et al. 2001, Bratsch et al. 2015) and Vanuatu (Pearson et al. 1993). The prevalence of OFV and its mite vector is thought to be associated with the importation of infected orchids (Dietzgen, Freitas-Astúa, et al. 2018).

OFV naturally infects more than fifty species of Orchidaceae (Kitajima et al. 2010, Kondo et al; 2006, Peng et al. 2013), some Asparagaceae (Nolinoidaea) Dietzgen, Tassi, et al. (2018), as well as Rutaceae (*Citrus*) where it causes citrus leprosis-like symptoms (Cook et al; 2019, Olmedo-Velarde et al; 2021 and Roy et al., 2015, 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).

During June 2020, chlorotic ringspot symptoms were observed on the liriopogon Giant Lilyturf *Liriope* spp., cv. ‘Gigantea’ in a landscape of Leon County, Florida (Fig. 1). Liropogons belong to the plant family Asparagaceae, subfamily Nolinoidaea, which includes various monocotyledonous lilliod plants (Chase et al. 2009). Liriopogons are native to southeastern Asia, encompassing the closely-related genera *Liriope* and *Ophiopogon*, which are characterized by evergreen, long grass-like leaves, and erect flowers which produce black, purple or blue, berry-like fruits, depending on the cultivar (Lattier et al. 2014, Fantz et al. 2015). These characteristics have given rise to various common names, including: monkey grass, Aztec grass, lilyturf, mondo grass, etc. (*Table 1*). The genus *Liriope* contains eight species, four of which are commonly cultivated, while the genus *Ophiopogon* consists of about 65 species, with only a handful are available for horticulture (Nesom 2010, Lattier et al. 2014). Liripogons are considered the most important ground cover sold by the nursery industry in southeastern US (Mcharo et al. 2003).

Virus infection 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 INSV, TMV and TSWV. 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 a 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 MK522807) (Kondo et al. 2006, 2017). Further samples were taken from various symptomatic cultivars of *Liriope* spp., *Ophiopogon* spp., as well as *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) during subsequent visits to the initial site of collection as well as other locations in Leon county. Samples were tested via RT-PCR at the NFREC (Table 1) to confirm the presence of OFV.

Further surveys of plants belong 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 Leon county which were observed with phase contrast microscopy. We encountered both eriophyoid mites and flat mites which 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) and similar diseases (Kitajima et al. 2010) and are known to feed on a large variety of economically-important plants (Childers et al. 2003, Akyazi et al. 2017). Unfortunately, the *Brevipalpus* mite species complex is known to contain cryptic species (Childers and Rodrigues 2011) whose identification can be improved with molecular methods as well as more advanced microscopy techniques, such as low-temperature scanning electron microscopy (LT-SEM, currently known as Cryo-SEM) (León and Nadler 2010, Skoracka et al. 2015). With that in mind, we sent additional samples of the mites to the USDA-ARS in Beltsville to observe the mites with Cryo -SEM techniques, which agreed with both prior identifications of *B. californicus* s.l. (Fig. 2) but revealed the presence of two other species *B. obovatus* and *B. confusus.*

The earliest mention of a virus which may have been OFV is Ko et al. (1985), who detected nuclear inclusions caused by an undescribed bacilliform rhabdovirus. Ko et al. (1985) described the spoke-wheel configurations typically associated with OFV (Chang et al. 1976), in *Brassia* orchids, but unfortunately 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 United States, using TEM of ultrathin sections of plant tissue as well as molecular sequence analysis and its association with *Brevipalpus* mites. But 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 Nolinoidaea in Australia (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016). Our plants are thought to belong to different species of *Liriope* which are not *L. spicata* and we are not aware of any record other than this manuscript which reports OFV infection in *Ophiopogon* plants. Unfortunately, liriopogons species are very similar in appearance and growth habit, with few useful characters used for their classification (Fantz 2008a). Furthermore, the horticultural industry has created a diverse array of cultivars of these plants, which are often mislabeled (Fantz 2008a). Aside from the taxonomic confusion created by humans, natural hybrids between *Ophiopogon* and *Liriope* have created a natural source of error for reconstructing phylogenies (Zhou et al. 2009). Together, these factors make it difficult to differentiate and identify plants in the landscape by visual inspection alone. These obfuscations of species identity may be accounted for in the future via sequence comparisons of the OFV-infected plants, but these comparisons are beyond the scope of our current report. Nonetheless, we are confident that ours is the first report of OFV infecting *Aspidistra elatior*; although (Zheng et al. 2013) mentions the association of *B. californicus* with *A. elatior* they make no mention of OFV symptoms in this plant.

### Control methods for *Brevipalpus* mites

Chemical control via synthetic acaricides is commonly used to manage *Brevipalpus* spp. (Leeuwen et al. 2015), but acaricides have some drawbacks. Many products control a broad class of arthropods, but often they do so indiscriminately, killing beneficial insects as well as pests (Suckling et al. 2013). Control strategies which rely heavily on chemical control have the potential to encourage pesticide resistance in mite populations and pesticide resistance has been reported in various *Brevipalpus* populations (Alves et al. 2000, Omoto et al. 2000, Campos and Omoto 2002, Rocha et al. 2021). In addition, it is important to consider the interactions which may occur between different chemical applications or tank mixes (Vechia et al. 2018). Lastly, application costs of chemical controls can be large and increase the cost of production (Rodrigues and Machado 2000), nevertheless chemical applications are valuable tools used to consider against *Brevipalpus*.

Various biological control methods have also been studied, but their use has not been widely adopted (Messing and Brodeur 2017). Even so, some studies have found biological control methods which could be used specifically for *Brevipalpus* mites: The predatory mite *Galendromus helveolus* (Chant) (Acari: Phytoseiidae) has been reported to feed on eggs, larvae and nymphs of *B. californicus* (Chen et al. 2006) and studies of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) demonstrated its ability to suppress populations of *B. yothersi* on citrus plants (Argolo et al. 2020). In addition, the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* was found to be pathogenic to *B. phoenicis* (Magalhães et al. 2005). In our own surveys, we have found mites which succumbed to fungal infections (Fig. 3); however the fungal species was not determined.

### 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 and the famous Ghost Orchid, [*Dendrophylax lindenii* (Lindl.) Benth]. ex Rolfe. The first time leprosis has been observed in the Florida in 1860’s and eradicated in the mid-1960s. In fact, Kitajima et al. (2011) found that the Citrus Leprosis virus (CiLV) which previously affected Florida citrus was a nuclear type of citrus leprosis, which are closely related to OFV strains (Roy et al. 2013). Association of a distant relative of OFV which was named as Citrus leprosis dichorhavirus -N0 (CiLV-N0) was confirmed in relation to the leprosis disease outbreak in Florida (Hartung et al; 2015). The recent detection of OFV-Orc1 in South Africa (Cook et al; 2019) in *C. sinensis* (Navel and Valencia orange) and OFV-Orc2 in Hawaii (Olmedo-Velarde et al; 2021) in *C. reticulata* (mandarin) and *C. jambhiri* (rough lemon) highlights the threat of different strains of OFV; which will be a definite concern to the multi-billion dollar citrus industry. Lastly, some OFV isolates are known to be involved with citrus leprosis disease in Mexico (Roy et al. 2015), which may be a cause for concern for the citrus industry. *B. californicus* and *B. yothersi* are both known vectors of Dichorhaviruses (OFV) and Cileviruses (Citrus Leprosis) (Knorr 1968, Kondo et al. 2003, Beltran-Beltran et al. 2020) and *B. obovatus* is a suspected vector as well (Childers et al. 2003). All three mite species/complexes are present in Florida (Childers et al. 2003, Akyazi et al. 2017) (Fig. 4); therefore, it is critical to identify the vector of OFVs in Florida and monitor its spread to determine what risk this virus represents for plants in Florida and the surrounding regions.

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### Table

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| --- | --- | --- |
| Scientific Name | Common Names | Symptoms Observed |
| *Liriope muscari* Bailey | Lilyturf, Orchardgrass, Monkeygrass | Ringspots, Chlorotic Flecking, Necrotic Lesions |
| *Liriope gigantea*\* Hume | Giant Lilyturf | Ringspots, Chlorotic Flecking, Necrotic Lesions |
| *Ophiopogon japonicus* Ker Gawl. | Dwarf Lilyturf, Mondo Grass, Snake’s beard | Ringspots, Chlorotic Flecking, Necrotic Lesions |
| *Ophiopogon intermedius*\*\* Don | Aztec Grass, ‘Argenteomarginatus’ | Ringspots, Chlorotic Flecking, Necrotic Lesions |
| *Aspidistra elatior* Blume | Cast Iron Plant, Bar-room Plant | Chlorosis, Necrotic Lesions |

Table 1: List of plants with symptoms of Orchid fleck dichorhavirus found in northern Florida. \* *L. gigantea* have been traditionally classified as seperate from *L. muscari* by Broussard (2007) and Fantz et al. (2015), although this distinction has been challenged by Wang et al. (2014) and Masiero et al. (2020). \* \* *O. intermedius* is sometimes misclassified as *Liriope muscari* ‘Variegated Evergreen Giant’ Fantz (2009) or ‘Grandiflora White’ (Fantz 2009).

### Figure captions

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

Fig. 2: LT-SEM images of *Brevipalpus californicus* sensu lato displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2012) (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with arrows indicating paired solenidia, characteristic of the genus *Brevipalpus* (e) Enlargement of the microplates of the mite cerotegument (f) Dorsal view of the distal portion of mite abdomen (g) Dorsal view of the mite rostrum (h) Ventral view of mite rostrum, observe 3 distal setae. LT-SEM images provided by Dr. Gary Bauchan, USDA-ARS 2021

Fig. 3: LT-SEM images of unidentified fungus infecting *Brevipalpus californicus* sensu lato: (a) Infested *B. californicus* adult, dorsal (b) Detail of fungal sporangia. LT-SEM images provided by Dr. Gary Bauchan, USDA-ARS 2021

Fig. 4: Florida is home to other common pest species of *Brevipalpus* which are potential vectors of *Orchid fleck dichorhavirus*: (a) *B. yothersi*, dorsal (b) *B. yothersi*, lateral (c) *B. obovatus*, dorsal. SEM images provided by Dr. Ron Ochoa, USDA-ARS 2021

### Figures

   