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**First report of *Orchid fleck dichorhavirus* (Mononegavirales: Rhabdoviridae) infecting various Ruscaceae in the Florida panhandle**

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*Orchid fleck dichorhavirus* (OFV) is the type species for the genus *Dichorhavirus*, a group of plant-infecting, bacilliform, nuclear rhabdoviruses composed of two segments of single-stranded, negative-sense RNA (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Other members of this genus are: *Citrus chlorotic spot dichorhavirus*, *Citrus leprosis N dichorhavirus*, *Clerodendrum chlorotic spot dichorhavirus* and *Coffee ringspot dichorhavirus* (Dietzgen, Freitas-Astúa, et al. 2018). Flat mites from the genus *Brevipalpus* Donnadieu (Acari: Tenuipalpidae) are the only known vector for dichorhaviruses (Maeda 1998), and transmit OFV in a persistent propagative manner (Kondo et al. 2003).

OFV-infected plants exhibit various symptoms dependent on the variety of plant infected as well as the strain of the virus (Kubo et al. 2009). Symptoms initially appear as chlorotic flecks or necrotic lesions, which may 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, 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 (Chang 1991), 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 movement of infected orchids (Dietzgen, Freitas-Astúa, et al. 2018).

OFV naturally infects 50+ spp of Orchidaceae (Kitajima et al. 2010, Peng et al. 2013), some Ruscaceae (Mei et al. 2016), and *Citrus* (García-Escamilla et al. 2018, Beltran-Beltran 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).

On June 2020, chlorotic ringspot symptoms were observed on the liriopogon Giant Lilyturf *Liriope* spp., cv. ‘Gigantea’ (‘Giant Lilyturf’ have been traditionally classified as *Liriope gigantea* (Broussard 2007, Fantz et al. 2015), but Wang et al. (2014) and Masiero et al. (2020) consider Giant Lilyturf to be a cultivar of *Liriope muscari*) in the landscape of Leon County, Florida. Liriopogons encompasses the genera *Liriope* sp*.* and *Ophiopogon* sp*.* and are often referred to the common names: monkey grasses, Aztec grasses, lilyturf etc. Native to southeast Asia, *Liriope* sp. contains eight species of which four are commonly cultivated, and *Ophiopogon* sp. consists of about 65 species but only a handful are commonly cultivated (Lattier et al., 2014; Nesom 2010). Liripogons are the most important ground cover sold by the nursery industry in southeastern US (Mcharo et al., 2003). They are characterized by evergreen long grass-like leaves, erected flowers and berry like fruits.

Plant samples were initially tested at the Plant Disease Diagnostic Clinic at the North Florida Research and Education Center (NFREC) in Quincy, FL. The plant samples were tested for a wide range of pathogens including XXXX, but the results were inconclusive. The infected materials were subsequently sent to the Florida Department of Agriculture and Consumer Services (FDACS) that determined the pathogen to be *Orchid fleck dichorhavirus* OFV-2 via RT-qPCR with a partial sequence match, following the procedures described by Kubo et al. (2009). Further samples were taken from other *Liriope* spp., *Ophiopogon* spp., as well as *Aspidistra elatior* Blume (Asparagales: Ruscaceae) in Leon county. All symptomatic plants were tested positive for OFV.

More cases of OFV-infected ruscaceae were observed in subsequent visits to the initial site of collection (Fig. 2). After a brief search, it appears that the disease is widely distributed in Florida, with symptomatic plants found in Leon, Alachua and Duval counties.

In addition, flat mites were collected from symptomatic plants and initially identified as *Brevipalpus californicus* (Banks) *sensu lato* with phase contrast microscopy at the NFREC. *Brevipalpus* mites are widespread and known to feed on a large variety of economically-important plants (Childers et al. 2003, Akyazi et al. 2017). Identification confirmation was made by Dr. Sam Bolton at the FDACS, who verified the *B. californicus* group using Differential Interference Contrast (DIC) microscopy. Light microscopy is not considered sufficient to verify species identity in this group, so additional samples of the mites were sent to the Electron & Confocal Microscopy Unit at the United States Department of Agriculture in Beltsville, Maryland. The mites were observed with cryo-Scanning Electron Microscopy techniques. Cryo-SEM revealed that these mites may be a new subspecies in the *Brevipalpus* group, associated with ruscaceae (Fig. 3).

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 (see (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 US, using TEM of ultrathin sections of plant tissue as well as molecular sequence analysis. In addition, Bratsch et al. (2015) detected *Brevipalpus* mites and their exoskeletons associated with OFV-infected plants. It appears that Bratsch et al. (2015) did not make a conclusive species identification, but did cite Kondo et al. (2003), suggesting that they suspected *B. californicus* as their vector.

OFV has been reported in other ruscaceae in Australia (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016). We are not aware of any record 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 horticultural confusion created by humans, natural hybrids between *Ophiopogon* and *Liriope* have created another source of confusion for phylogeny (Zhou et al. 2009). Together, these factors make it difficult to differentiate and identify species in the landscape. These sources of error 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.

Detecting OFV in Florida represents a concern for horticulturalist which grow susceptible plants of economic importance such as *Liriope*, *Ophiopogon*, and other susceptible *Asparagales* spp. 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 the famous Ghost Orchid, *Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe. Furthermore, OFV represents an obstacle to overcome for the burgeoning interest in cultivating Vanilla in southern Florida (Chambers et al. 2019). Lastly, some OFV isolates, including OFV-2, are known to be involved with nuclear types of Citrus Leprosis (Roy et al. 2015), which may be a cause for concern for the citrus industry. In fact, Kitajima et al. (2011) found that the Citrus Leprosis virus which previously affected Florida citrus was a nuclear type of citrus leprosis, which are closely related to OFV strains (Roy et al. 2013). *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) (see Fig. 4), therefore it is critical to monitor the spread of Orchid Fleck Virus and its mite vector(s) to determine what risk this virus represents for plants in Florida and the surrounding regions.

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Figure Captions

Figure 1: Variety of symptoms expressed by plants infected with *Orchid fleck dichorhavirus*: (a) ringspot symptoms on *Liriope gigantea* (b)

chlorosis on *Aspidistra elatior*.

Figure2: XXXXXXX

Fig. 3: Cryo-SEM images of *Brevipalpus californicus* sensu lato used for identification (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with paired solenidia (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 Cryo-SEM images provided by Dr. Gary Bauchan, USDA-ARS 2021

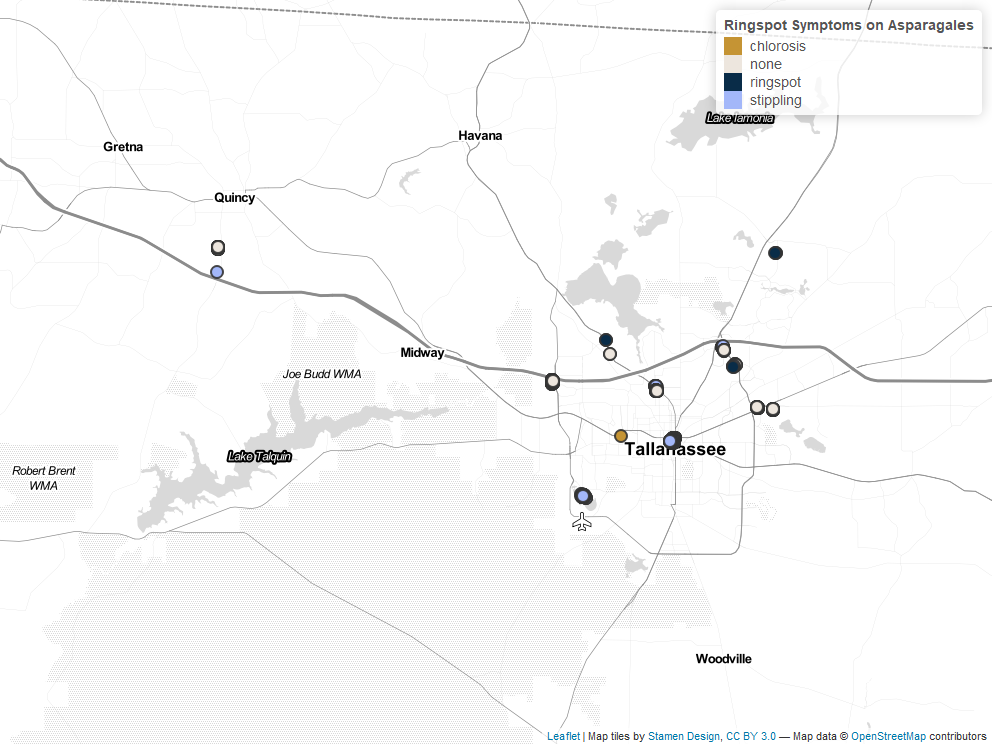
Figure 4: Florida is home to other common pest species of *Brevipalpus* which are potential vectors of *Orchid fleck dichorhavirus*: (a) *B. yothersi*, dorsal (b) *B. yothersi*, lateral (c) *B. obovatus*, dorsal

|  |  |  |
| --- | --- | --- |
| 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 2008b) or ‘Grandiflora White’ (Fantz 2009).



Figure 1



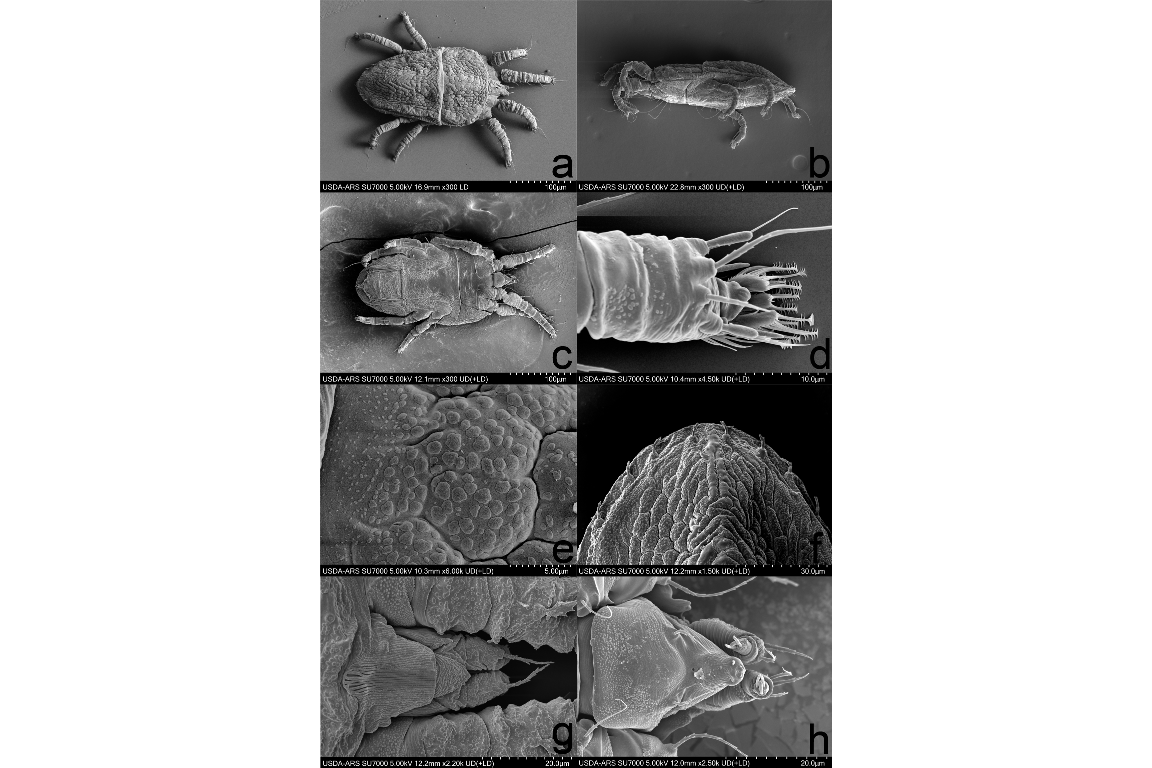


Figure 4