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### First report of the *Brevipalpus*-transmitted (Trombidiformes: Tenuipalpidae) *Orchid fleck dichorhavirus* infecting three ornamentals in Florida

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### Abstract

We describe the first outbreaks of *Orchid fleck dichorhavirus*, belonging to the orchid-infecting subgroup (OFV-Orc), from three unreported hosts: *Liriope muscari*, cv. ’Gigantea (Decaisne) Bailey, *Ophiopogon intermedius* Don and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) in Leon and Alachua Counties, FL. Strains of OFV-Orc infect over 50 plant species belonging to the plant families Orchidaceae, Asparagaceae (Nolinoidaea), and infects *Citrus* (Rutaceae) as citrus leprosis disease. The only known vectors of OFV-Orc are flat mites, genus *Brevipalpus* (Trombidiformes: Tenuipalpidae). Florida has various plants in the landscape which *Brevipalpus* spp. feed on, which are susceptible to infection by OFV-Orc.

Chlorotic ringspots and flecking was seen affecting Liriopogons (*Liriope* spp. and *Ophiopogon* spp.) in Leon County, FL. Nearby *A. elatior* also appeared chlorotic. Local diagnostics returned negative for common plant pathogens, therefore new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) and USDA-ARS for identification.

Two orchid-infecting strains of Orchid fleck virus were detected via combinations of conventional RT-PCR, RT-qPCR, Sanger sequencing and High Throughput Sequencing. Amplicons shared 98% nucleotide identity with OFV-Orc1 and OFV-Orc2 available in NCBI GenBank. Coinfections were seen in each county, but single strains of OFV-Orc were seen in *L. muscari* (Alachua, OFV-Orc2) and *A. elatior* (Leon, OFV-Orc1).

Three potential mite vectors were identified via cryo-scanning electron microscopy (Cryo-SEM): *Brevipalpus californicus* (Banks) sensu lato, *B. obovatus* Donnadieu, and *B. confusus* Baker.

In conclusion, *Orchid fleck dichorhavirus* is present in northern Florida, representing a risk for susceptible plants in the southeastern US.

### Keywords:

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

*Orchid fleck dichorhavirus* (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). Flat mites from the genus *Brevipalpus* (Trombidiformes: Tenuipalpidae) are the only known vectors of dichorhaviruses (Maeda 1998). *Brevipalpus californicus* (Banks) sensu lato mites are the only group known to transmit OFV in a persistent propagative manner (Kondo et al. 2003).

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

OFV was first described as infecting *Cymbidium* orchids in Japan (Doi et al. 1977). OFV and OFV-like rhabdoviruses have been reported infecting orchids in Asia, Africa, North America, South America, Europe, and Oceania. The prevalence of OFV and its mite vector is thought to be associated with the movement of infected orchids (Dietzgen et al. 2018a).

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 et al. 2018b), and Rutaceae: (*Citrus*), where it causes citrus leprosis-like symptoms (Roy et al. 2015, 2020, Cook et al. 2019, Olmedo-Velarde et al. 2019). Mechanical transmission of OFV is possible to some plants belonging to the plant families Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013), under laboratory conditions.

During June 2020, chlorotic ringspot symptoms were observed on Giant Lilyturf *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 Nolinoidaea, which includes a diverse array of various monocotyledonous southeastern Asia native liliod plants (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* Ker Gawler (Asparagaceae: Nolinoidaea) are considered the most important ground cover nursery plants in the southeastern United States (Mcharo et al. 2003).

Viral infections of suspected 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 were tested with RT-PCR and were found negative for begomovirus, carlavirus, potyvirus, tospovirus, and *Cucumber mosaic virus*, *Impatiens necrotic spot virus*, *Tobacco mosaic virus*, and *Tomato spotted wilt virus*.

The initial site of collection was visited two more times during 2020 to gather plants for identification of the unidentified plant pathogen. These surveys were conducted during July and August to collect more putatively infected plants, including more *Liriope* spp. as well as a new species which also belongs to the family Asparagaceae; *Aspidistra elatior* Blume, which was suspected to be infected due to unusually chlorotic leaves (Fig. 2). Upon collection, these new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification. The FDACS determined that the virus was *Orchid fleck dichorhavirus* using previously published primers and methods (Kubo 2006a, 2006b, Kubo et al. 2009b, Kubo et al. 2009a, Ramos-González et al. 2015). Orchid subgroup 1, OFV-Orc was identified following the methods described in Kondo et al. (2017).

Samples sent to the USDA confirmed the presence of OFV via one step conventional RT-PCR and RT-qPCR, using generic R2-Dicho-GF and R2-Dicho-GR primers, amplifying ~800 nt of L-gene (RNA2) amplicon (Roy et al. 2020). Sanger sequencing demonstrated a shared 98% nucleotide identity with the orchid strain subgroup, OFV-Orc (isolates So and Br with GenBank Accession numbers: AB244418 and MK522807, respectively) (Kondo et al. 2006, 2017). High Throughput sequencing results confirmed the presence of OFV-Orc2 strain in the *Liriope* sample.

Subsequent surveys of plants belonging to the subfamily Nolinoidaea in Florida have revealed more sites with Asparagaceae putatively infected with OFV in Leon and Alachua counties (Table 1). Mites were collected from symptomatic plants in Leon county and observed with phase contrast microscopy. Tenuipalpid mites (flat mites or false spider mites) were commonly found in abundance on the Asparagaceae, which tested positive for OFV. These flat mites were initially identified as *B. californicus* s. l. and later confirmed by the FDACS via Differential Interference Contrast (DIC) microscopy. The *B. californicus* s. l. species group, sensu Baker and Tuttle (1987) is suspected to contain cryptic species (Childers and Rodrigues 2011, Rodrigues and Childers 2013), as seen in other *Brevipalpus* species, such as *B. phoenicis* (Geijskes) (Beard et al. 2015).

New mite samples were collected from the site where OFV was first confirmed and sent to USDA-ARS’s Electron and Confocal Microscopy Unit for analysis. Three mite species were recovered and examined under cryo scanning electron microscopy (Cryo-SEM): *B. californicus* s.l. (Fig. 3), *B. obovatus* Baker and *B. confusus* Donnadieu (Fig. 4).

The first report of OFV in the US 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 was from Hawaii in 2001 (Blanchfield et al. 2001), while the first report from the continental US was by Bratsch et al. (2015). In that publication, the authors 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 (Bratsch et al. 2015). The authors did not make a conclusive species identification but suggested the mite vector was within the *B. californicus* group, as referred by Kondo et al. (2003).

OFV has been reported in other Nolinoidaea in Australia (Mei et al. 2016, Dietzgen et al. 2018b), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016) but not in the US. The *Liriope* spp., cv ‘Gigantea’ is considered a synonym of *Liriope muscari* (Wang et al. 2014, Masiero et al. 2020), but some consider it a separate species: *Liriope gigantea* Hume (Fantz et al. 2015). We are not aware of any previous report of OFV infection in *Ophiopogon* plants. Although Zheng et al. (2013) mention the association of *B. californicus* with *A. elatior*, but never reported OFV symptoms in this plant. However, our finding will be notified as the first report of OFV in Florida, in the US on ornamentals and among them, *A. elatior* is a new natural host of OFV.

### Conclusion

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 (Roy et al. 2020), but none of the citrus strains 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]. Citrus leprosis was present in Florida during the 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 closely related to OFV strains (Hartung et al. 2015, Roy et al. 2020). Association of a distant relative of OFV named 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 (Velarde et al. 2021) in *C. reticulata* (mandarin) and *C. jambhiri* (rough lemon) associated with leprosis-like symptoms highlights the threat of different strains of OFV on citrus; which will be a definite concern to the US multi-billion dollar citrus industry. *B. californicus*, as well as *B. yothersi* (Baker), are both known vectors of Dichorhaviruses (OFV) (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 these strains of OFV in Florida to monitor their spread, and to determine the risk they represent for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

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### References

**Akyazi, R., E. A. Ueckermann, and O. E. Liburd**. **2017**. New report of *Brevipalpus yothersi* (Prostigmata: Tenuipalpidae) on blueberry in Florida. Florida Entomologist. 100: 731–739.

**Amarasinghe, G. K., M. A. Ayllón, Y. Bào, C. F. Basler, S. Bavari, K. R. Blasdell, T. Briese, P. A. Brown, A. Bukreyev, A. Balkema-Buschmann, U. J. Buchholz, C. Chabi-Jesus, K. Chandran, C. Chiapponi, I. Crozier, R. L. de Swart, R. G. Dietzgen, O. Dolnik, J. F. Drexler, R. Dürrwald, W. G. Dundon, W. P. Duprex, J. M. Dye, A. J. Easton, A. R. Fooks, P. B. H. Formenty, R. A. M. Fouchier, J. Freitas-Astúa, A. Griffiths, R. Hewson, M. Horie, T. H. Hyndman, D. Jiāng, E. W. Kitajima, G. P. Kobinger, H. Kondō, G. Kurath, I. V. Kuzmin, R. A. Lamb, A. Lavazza, B. Lee, D. Lelli, E. M. Leroy, J. Lǐ, P. Maes, S.-Y. L. Marzano, A. Moreno, E. Mühlberger, S. V. Netesov, N. Nowotny, A. Nylund, A. L. Økland, G. Palacios, B. Pályi, J. T. Pawęska, S. L. Payne, A. Prosperi, P. L. Ramos-González, B. K. Rima, P. Rota, D. Rubbenstroth, M. Shı̄, P. Simmonds, S. J. Smither, E. Sozzi, K. Spann, M. D. Stenglein, D. M. Stone, A. Takada, R. B. Tesh, K. Tomonaga, N. Tordo, J. S. Towner, B. van den Hoogen, N. Vasilakis, V. Wahl, P. J. Walker, L.-F. Wang, A. E. Whitfield, J. V. Williams, F. M. Zerbini, T. Zhāng, Y.-Z. Zhang, and J. H. Kuhn**. **2019**. Taxonomy of the order Mononegavirales: Update 2019. Archives of Virology. 164: 1967–1980.

**Baker, E. W., and D. M. Tuttle**. **1987**. The false spider mites of Mexico (Tenuipalpidae: Acari). (technical report No. 1706). The United States Department of Agriculture - Agricultural Research Service.

**Beard, J. J., R. Ochoa, R. Bauchan G., D. Trice M., J. Redford A., W. Walters T., and C. Mitter**. **2012**. Flat mites of the world edition 2. (<http://idtools.org/id/mites/flatmites/>).

**Beard, J. J., R. Ochoa, W. E. Braswell, and G. R. Bauchan**. **2015**. *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae) a closer look. Zootaxa. 3944: 1.

**Beltran-Beltran, A. K., M. T. Santillán-Galicia, A. W. Guzmán-Franco, D. Teliz-Ortiz, M. A. Gutiérrez-Espinoza, F. Romero-Rosales, and P. L. Robles-Garcı́a**. **2020**. Incidence of Citrus leprosis virus C and *Orchid fleck dichorhavirus* citrus strain in mites of the genus *Brevipalpus* in Mexico. Journal of Economic Entomology. 113: 1576–1581.

**Blanchfield, A. L., A. M. Mackenzie, A. Gibbs, H. Kondo, T. Tamada, and C. R. Wilson**. **2001**. Identification of Orchid fleck virus by reverse transcriptase-polymerase chain reaction and analysis of isolate relationships. Journal of Phytopathology. 149: 713–718.

**Bratsch, S. A., B. E. Lockhart, and C. Ishimaru**. **2015**. Confirmation of first report of Orchid fleck virus in *Phalaenopsis* hybrid orchids in the USA. Plant Health Progress. 16: 146–148.

**Broussard, M. C.** **2007**. A horticultural study of *Liriope* and *Ophiopogon*: Nomenclature, morphology, and culture (PhD thesis). Louisiana State University, Department of Horticulture.

**Chambers, A. H., P. Moon, V. Edmond, and E. Bassil**. **2019**. Vanilla cultivation in southern Florida. EDIS. 2019: 7.

**Chang, M. U., Arai. Kei, Doi. Yoji, and Yora. Kiyoshi**. **1976**. Morphology and intracellular appearance of Orchid fleck virus. Japanese Journal of Phytopathology. 42: 156–157.

**Chase, Mark. W., James. L. Reveal, and M. F. Fay**. **2009**. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. Botanical Journal of the Linnean Society. 161: 132–136.

**Childers, C. C., and J. C. V. Rodrigues**. **2011**. An overview of *Brevipalpus* (Acari: Tenuipalpidae) and the plant viruses they transmit. Zoosymposia. 6: 180–192.

**Childers, C. C., J. C. V. Rodrigues, K. S. Derrick, D. S. Achor, J. V. French, W. C. Welbourn, R. Ochoa, and E. W. Kitajima**. **2003**. Citrus leprosis and its status in Florida and Texas: Past and present. Experimental and Applied Acarology. 30: 181–202.

**Cook, G., W. Kirkman, R. Clase, C. Steyn, E. Basson, P. H. Fourie, S. D. Moore, T. G. Grout, E. Carstens, and V. Hattingh**. **2019**. Orchid fleck virus associated with the first case of Citrus leprosis-N in South Africa. European Journal of Plant Pathology. 155: 1373–1379.

**Dietzgen, R. G., J. Freitas-Astúa, C. Chabi-Jesus, P. L. Ramos-González, M. M. Goodin, H. Kondo, A. D. Tassi, and E. W. Kitajima**. **2018a**. Dichorhaviruses in their host plants and mite vectors. Elsevier.

**Dietzgen, R. G., J. H. Kuhn, A. N. Clawson, J. Freitas-Astúa, M. M. Goodin, E. W. Kitajima, H. Kondo, T. Wetzel, and A. E. Whitfield**. **2014**. *Dichorhavirus*: A proposed new genus for *Brevipalpus* mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant viruses. Archives of Virology. 159: 607–619.

**Dietzgen, R. G., A. D. Tassi, J. Freitas-Astúa, and E. W. Kitajima**. **2018b**. First report of Orchid fleck virus and its mite vector on Green cordyline. Australasian Plant Disease Notes. 13.

**Doi, Y., M. U. Chang, and K. Yora**. **1977**. Orchid fleck virus. CMI/AAB descriptions of plant viruses.

**Fantz, P. R.** **2008**. Species of *Liriope* cultivated in the southeastern United States. HortTechnology. 18: 343–348.

**Fantz, P. R.** **2009**. Names and species of *Ophiopogon* cultivated in the southeastern United States. HortTechnology. 19: 385–394.

**Fantz, P. R., D. Carey, T. Avent, and J. Lattier**. **2015**. Inventory, descriptions, and keys to segregation and identification of liriopogons cultivated in the southeastern United States. HortScience. 50: 957–993.

**Hartung, J. S., A. Roy, S. Fu, J. Shao, W. L. Schneider, and R. H. Brlansky**. **2015**. History and diversity of Citrus leprosis virus recorded in herbarium specimens. Phytopathology. 105: 1277–1284.

**Kitajima, E. W., C. M. Chagas, R. Harakava, R. F. Calegario, J. Freitas-Astúa, J. C. V. Rodrigues, and C. C. Childers**. **2011**. Citrus leprosis in Florida, USA, appears to have been caused by the nuclear type of Citrus leprosis virus (CilLV-N). Virus Reviews & Research. 16.

**Kitajima, E. W., J. C. V. Rodrigues, and J. Freitas-Astua**. **2010**. An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. Scientia Agricola. 67: 348–371.

**Ko, N.-J., F. W. Zettler, J. R. Edwardson, and R. G. Christie**. **1985**. Light microscopic techniques for detecting orchid viruses. Acta Horticulturae. 241–254.

**Kondo, H., K. Hirota, K. Maruyama, I. B. Andika, and N. Suzuki**. **2017**. A possible occurrence of genome reassortment among bipartite rhabdoviruses. Virology. 508: 18–25.

**Kondo, H., T. Maeda, Y. Shirako, and T. Tamada**. **2006**. Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. Journal of General Virology. 87: 2413–2421.

**Kondo, H., T. Maeda, and T. Tamada**. **2003**. Orchid fleck virus: *Brevipalpus californicus* mite transmission, biological properties and genome structure. Experimental and Applied Acarology. 30: 215–223.

**Kubo, K. S.** **2006a**. Estudo da variabilidade genetica do orchid fleck virus (OFV) por SSCP. Summa Phytopathol. 32: S29.

**Kubo, K. S.** **2006b**. Otimizacao da diagnose molecular da mancha anular da orquidea. Summa Phytopathol. 32: S30.

**Kubo, K. S., J. Freitas-Astúa, M. A. Machado, and E. W. Kitajima**. **2009a**. Orchid fleck symptoms may be caused naturally by two different viruses transmitted by *Brevipalpus*. Journal of General Plant Pathology. 75: 250–255.

**Kubo, K. S., R. M. Stuart, J. Freitas-Astúa, R. Antonioli-Luizon, E. C. Locali-Fabris, H. D. Coletta-Filho, M. A. Machado, and E. W. Kitajima**. **2009b**. Evaluation of the genetic variability of Orchid fleck virus by single-strand conformational polymorphism analysis and nucleotide sequencing of a fragment from the nucleocapsid gene. Archives of Virology. 154: 1009–1014.

**Maeda, T.** **1998**. Evidence that Orchid fleck virus is efficiently transmitted in a persistent manner by the mite *Brevipalpus californicus*. Abstr., 7th Int. Cong. Plant Pathol. 3.

**Masiero, E., D. Banik, J. Abson, P. Greene, A. Slater, and T. Sgamma**. **2020**. Molecular verification of the UK national collection of cultivated *Liriope* and *Ophiopogon* plants. Plants. 9: 558.

**Mcharo, M., E. Bush, D. L. Bonte, C. Broussard, and L. Urbatsch**. **2003**. Molecular and morphological investigation of ornamental liriopogons. Journal of the American Society for Horticultural Science. 128: 575–577.

**Mei, Y., N. Bejerman, K. S. Crew, N. McCaffrey, and R. G. Dietzgen**. **2016**. First report of Orchid fleck virus in lilyturf (*Liriope spicata*) in Australia. Plant Disease. 100: 1028–1028.

**Meng, R., L.-Y. Luo, J.-Y. Zhang, D.-G. Zhang, Z.-L. Nie, and Y. Meng**. **2021**. The deep evolutionary relationships of the morphologically heterogeneous Nolinoideae (Asparagaceae) revealed by transcriptome data. Frontiers in Plant Science. 11.

**Olmedo-Velarde, A., A. C. Park, J. Sugano, J. Y. Uchida, M. Kawate, W. B. Borth, J. S. Hu, and M. J. Melzer**. **2019**. Characterization of Ti ringspot-associated virus, a novel emaravirus associated with an emerging ringspot disease of *Cordyline fruticosa*. Plant Disease. 103: 2345–2352.

**Peng, D. W., G. H. Zheng, Z. Z. Zheng, Q. X. Tong, and Y. L. Ming**. **2013**. Orchid fleck virus: An unclassified bipartite, negative-sense RNA plant virus. Archives of Virology. 158: 313–323.

**Ramos-González, P. L., H. Sarubbi-Orue, L. Gonzales-Segnana, C. Chabi-Jesus, J. Freitas-Astúa, and E. W. Kitajima**. **2015**. Orchid fleck virus infecting orchids in Paraguay: First report and use of degenerate primers for its detection. Journal of Phytopathology. 164: 342–347.

**Rodrigues, J. C. V., and C. C. Childers**. **2013**. *Brevipalpus* mites (Acari: Tenuipalpidae): Vectors of invasive, non-systemic cytoplasmic and nuclear viruses in plants. Experimental and Applied Acarology. 59: 165–175.

**Roy, A., A. L. Stone, G. Otero-Colina, G. Wei, R. H. Brlansky, R. Ochoa, G. Bauchan, W. L. Schneider, M. K. Nakhla, and J. S. Hartung**. **2020**. Reassortment of genome segments creates stable lineages among strains of Orchid fleck virus infecting citrus in Mexico. Phytopathology. 110: 106–120.

**Roy, A., A. L. Stone, J. Shao, G. Otero-Colina, G. Wei, N. Choudhary, D. Achor, L. Levy, M. K. Nakhla, J. S. Hartung, W. L. Schneider, and R. H. Brlansky**. **2015**. Identification and molecular characterization of nuclear Citrus leprosis virus, a member of the proposed dichorhavirus genus infecting multiple citrus species in Mexico. Phytopathology. 105: 564–575.

**Velarde, A. O., A. Roy, C. Padmanabhan, S. Nunziata, M. K. Nakhla, and M. Melzer**. **2021**. First report of Orchid fleck virus associated with Citrus leprosis symptoms in rough lemon (*Citrus jambhiri*) and mandarin (*C. reticulata*) the United States. Plant Disease.

**Walker, P. J., K. R. Blasdell, C. H. Calisher, R. G. Dietzgen, H. Kondo, G. Kurath, B. Longdon, D. M. Stone, R. B. Tesh, N. Tordo, N. Vasilakis, and A. E. Whitfield**. **2018**. ICTV virus taxonomy profile: *Rhabdoviridae*. Journal of General Virology. 99: 447–448.

**Wang, G.-Y., Y. Meng, J.-L. Huang, and Y.-P. Yang**. **2014**. Molecular phylogeny of *Ophiopogon* (Asparagaceae) inferred from nuclear and plastid DNA sequences. Systematic Botany. 39: 776–784.

**Zheng, G. H., Z. Z. Zheng, Q. X. Tong, Y. L. Ming, and others**. **2013**. Orchid fleck virus: An unclassified bipartite, negative-sense RNA plant virus. Archives of virology. 158: 313–323.

### Table 1: List of Asparagaceae (Nolinoidaea) species with verified cases of *Orchid fleck dichorhavirus*, along with basic descriptions of foliar symptoms seen in the landscape of northern Florida

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

Table 1: \* *L. gigantea* has 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 *Liriope* spp. infected with Orchid fleck dichorhavirus (OFV): (a) ringspot symptoms on *Liriope gigantea* (b-c) Details of ringspot symptoms on *Liriope gigantea* (d) chlorotic ringspot *Liriope muscari* cv. ‘Silvery Sunproof’

Fig. 2: Symptoms expressed by *Aspidistra elatior* infected with Orchid fleck dichorhavirus (OFV): (a) Detail of leaf chlorosis (b) Chlorosis caused by OFV appears similar to sunburn damage (c-d) Chlorotic ringspot may indicate early symptoms of OFV

Fig. 3: Cryo-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.

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

### Figures







