

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

Austin **Fife**¹, Daniel **Carrillo**², Gary **Knox**³, Fanny **Iriarte**⁴, Kishore **Dey**⁵, Avijit **Roy**⁶, Ronald **Ochoa**⁷, Gary **Bauchan**⁸, Mathews **Paret**^{4,9}, Xavier **Martini**^{1,*}

¹ University of Florida, Department of Entomology and Nematology, North Florida Research and Education Center, Quincy FL 32351

² University of Florida, Department of Entomology and Nematology, Tropical Research and Education Center, Homestead FL 33031

³ University of Florida, Department of Environmental Horticulture, North Florida Research and Education Center, Quincy FL 32351

⁴ University of Florida, Department of Plant Pathology, North Florida Research and Education Center, Quincy FL 32351

⁵ The Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Section of Plant Pathology, Doyle Conner Building, 1911 SW 34th street, Gainesville, FL 32608

⁶ United States Department of Agriculture – Agriculture Research Service, Molecular Plant Pathology Laboratory, 10300 Baltimore Ave, Bldg. 4 BARC-West, Beltsville, MD 20705

⁷ United States Department of Agriculture - Agriculture Research Service, Systematic Entomology Laboratory 10300 Baltimore Ave, Bldg. 5 BARC-West, Beltsville, MD 20705

⁸ United States Department of Agriculture - Animal and Plant Health Inspection Service, Electron and Confocal Microscopy Unit, Bldg. 12 BARC-West, 10300 Baltimore Ave, Beltsville, MD 20705

⁹ Plant Pathology Department, University of Florida, Gainesville, FL 32611

*Corresponding author; E-mail: xmartini@ufl.edu Phone: 850-875-7160 Fax: 352-846-6617

Abstract

Orchid Fleck Virus (OFV) infects over fifty plant species belonging to the family Orchidaceae, Asparagaceae (Nolinoidae), and Rutaceae (Citrus). The sole vectors for dichorhavirus are flat mites from the genus *Brevipalpus* (Trombidiformes: Tenuipalpidae), which are also known to spread cileviruses. OFV was found infecting liriopogons *Liriope* spp. and *Ophiopogon* spp. and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidae) in the landscape in Leon County, Florida, during the summer of 2020. ~~The presence~~ **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 NCBI GenBank. Virus identity was also confirmed via quantitative RT-PCR (RT-qPCR). Additional leaf samples were collected from other possibly infected Asparagaceae from Leon and Alachua counties. Identification of partial genome sequence confirmed the presence of both OFV orchid strains (OFV-Orc1 and OFV-Orc2) in Florida. These strains of OFV are known to infect citrus and cause citrus leprosis disease. Three potential mite vectors were identified from OFV-infected plants using cryo-scanning electron microscopy (Cryo-SEM): *Brevipalpus*

Commented [JCR1]: Suggestion: Use the virus species name:
Orchid fleck dichorhavirus

californicus s.l., *B. obovatus* and *B. confusus*. Florida has various native and introduced plants in the landscape that host *Brevipalpus* spp. and are potentially susceptible to OFV. In this study, we report three new hosts from the family Asparagaceae from multiple locations. Our data suggested that OFV is widely distributed in Florida and might be a threat for various plant species and possibly citrus growing in the southeastern US.

Keywords:

False spider mite, flat mite, *Brevipalpus*-transmitted viruses, *Liriope*, Nolinoideae, *Ophiopogon*, 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 sole vectors for cileviruses and dichorhaviruses (Maeda 1998), and *B. californicus* (Banks) group of mites are the only 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. 2009), but symptoms typically appear as chlorotic flecks, which ultimately coalesce into larger spots or ringspot patterns ([Fig. 1](#), [Fig. 2](#)).

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, Freitas-Astúa, et al. 2018).

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), 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 lilioid 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 as well as for~~ *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 presence of OFV was confirmed using OFV generic R2-Dicho-GF and R2-Dicho-GR primers by one step conventional RT-PCR (Roy et al. 2020), amplifying ~800 nt of L-gene (RNA2) amplicon from an infected *Liriope* leaf sample. Sanger sequencing of RT-PCR amplicons shared 98% nucleotide identity with orchid strains of OFV: OFV-Orc1 and OFV-Orc2 (GenBank Accession numbers: AB244418 and LC222630) (Kondo et al. 2006, 2017).

Subsequent surveys of plants belonging to the subfamily Nolinoideae 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~~~~originally identified~~ as *Brevipalpus californicus* (Banks) sensu lato and later confirmed by the FDACS via Differential Interference Contrast (DIC) microscopy. The *Brevipalpus* mite species complex is known to contain cryptic species (Childers and Rodrigues 2011) that require advanced microscopy techniques, such as cryo scanning electron microscopy (Cryo-SEM) for species identification (León and Nadler 2010, Beard et al. 2015, Skoracka et al. 2015). Additional mite samples were collected from the original OFV detection site, and examined under Cryo-SEM (Fig. 3). The determinations approved prior identifications

of *B. californicus* s.l. but revealed the presence of two other species *B. obovatus* Donnadieu and *B. confusus* Banks (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 Nolinoideae in Australia (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016) but not in the US. The Florida collected plants of *Liriope* spp., cv 'Gigantea' are thought to belong to either *Liriope muscari* or *Liriope gigantea*. We are not aware of any previous report of OFV infection in *Ophiopogon* plants. Although Zheng et al. (2013) mentions the association of *B. californicus* with *A. elatior*, but they did not mention reported the presence of 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

Commented [JCR2]: repeating the word "mention"

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-No (CiLV-No) was confirmed in relation to the leprosis disease outbreak in Florida (Hartung et al. 2015). The recent detection of OFV-Orc1 in South Africa (Cok 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-revipalpus californicus*, as well as *B. yothersi* (Baker), 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

Commented [JCR3]: The reference listed is not the correct. Use: Kitajima E W, Chagas CM, Harakava R, Calegario RF, Freitas-Astúa J, Rodrigues JCV, Childers, CC. 2011. Citrus Leprosis in Florida, USA, appears to have been caused by the Nuclear Type of Citrus Leprosis Virus (CiLV-N). Virus Reviews & Research 16:1-5.

vector of OFVs in Florida and monitor its spread to determine the risk this virus represents for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

Acknowledgements

We would like to give a special thanks to the Tallahassee Museum for their patience, cooperation, and support with collecting plant samples. We also want to thank Drs. Sam Bolton, FDACS and Aline Tassi, Univ. of Sao Paulo, Brazil for checking the mites we have sent for species validation. Furthermore, we are grateful for Dr. Marc S. Frank's identification of the Liriopogons collected. We are especially indebted to the late Dr. Gary Bauchan for his contributions to this study and the field of acarology, he will be greatly missed. This research was partly funded by the USDA National Institute of Food and Agriculture, Hatch project FLA-NFC-005607. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

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. Blasdel, T. Bries, 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. Dürwald, 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. Li, 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. Shi, 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-García. 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 *Asparagaceae* ~~asparagalean~~ families *Amaryllidaceae* ~~amaryllidaceae~~, *Asparagaceae* ~~asparagaceae~~ and *Xanthorrhoeaceae* ~~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. 2018. Dichorhavirus in their host plants and mite vectors, pp. 119–148. *In* *Advances in Virus Research*. Elsevier.
- Dietzgen, R. G., J. H. Kuhn, A. N. Clawson, J. Freitas-Astúa, M. M. Goodin, E. W. Kitajima, H. Kondo, T. Wetzels, 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. 2018. 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. Bransky. 2015. History and diversity of citrus leprosis virus recorded in herbarium specimens. *Phytopathology*. 105: 1277–1284.
- Kitajima, E. W., C. M. Chagas, M. T. Braghini, L. C. Fazuoli, E. C. Locali-Fabris, and R. B. Salaroli. 2011. Natural infection of several *Coffea* species and hybrids and ~~*psilanthus*~~ *Psilanthus ebracteolatus* by the coffee ringspot virus (CoRSV). *Scientia Agricola*. 68: 503–507.
- 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.

Commented [JCR4]: See comment on the text and replace by the correct reference

- Knorr, L. C. 1968.** Studies on the etiology of leprosis in citrus. *In* International Organization of Citrus Virologists Conference Proceedings.
- 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., J. Freitas-Astúa, M. A. Machado, and E. W. Kitajima. 2009.** Orchid fleck symptoms may be caused naturally by two different viruses transmitted by *Brevipalpus*. *Journal of General Plant Pathology*. 75: 250–255.
- León, G. P.-P. de, and S. A. Nadler. 2010.** What we don't recognize can hurt us: A plea for awareness about cryptic species. *Journal of Parasitology*. 96: 453–464.
- 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.

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.

Skoracka, A., S. Magalhães, B. G. Rector, and L. Kuczyński. 2015. Cryptic speciation in the acari: A function of species lifestyles or our ability to separate species? Experimental and Applied Acarology. 67: 165–182.

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. Blasdel, 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*~~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*~~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*~~rna~~ plant virus. Archives of virology. 158: 313–323.

Table

Table 1: List of plants with symptoms of Orchid fleck dichorhavirus found in northern Florida.

Scientific Name	Common Names	Symptoms Observed	County
-----------------	--------------	-------------------	--------

<i>Liriope muscari</i> Bailey	Lilyturf, Orchardgrass, Monkeygrass	Ringspots, Chlorotic Flecking, Necrotic Lesions	Alachua & Leon
<i>Liriope gigantea</i> * Hume	Giant Lilyturf	Ringspots, Chlorotic Flecking, Necrotic Lesions	Alachua & Leon
<i>Ophiopogon japonicus</i> Ker Gawl.	Dwarf Lilyturf, Mondo Grass, Snake's beard	Ringspots, Chlorotic Flecking, Necrotic Lesions	Leon
<i>Ophiopogon intermedius</i> ** Don	Aztec Grass, 'Argenteomarginatus'	Ringspots, Chlorotic Flecking, Necrotic Lesions	Alachua & Leon
<i>Aspidistra elatior</i> Blume	Cast Iron Plant, Bar-room Plant	Chlorosis, Chlorotic Flecking, Necrotic Lesions	Leon

* *L. gigantea* ~~has~~ have been traditionally classified as separate 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 ringspots *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 ringspots 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. yothersi*, dorsal (b) *B. yothersi*, lateral (c) *B. obovatus*, dorsal.

Figures