For submission to the Journal of Integrated Pest Management

Category: ‘Brief Communications’

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

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

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* 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*, Nolinoidaea, *Ophiopogon*, *Aspidistra*, Ruscaceae, Rutaceae, Asparagaceae, orchid, Orchidaceae, pests, ornamental plants, Orchid fleck virus.

*Orchid fleck dichorhavirus*, commonly referred to as Orchid Fleck Virus (OFV), is the type member for the genus *Dichorhavirus*, family *Rhabdoviridae*. OFV is 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 group known to transmit dichorhaviruses (Maeda 1998), and *Brevipalpus californicus* (Banks) sensu lato are the only mites which do so 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, where it causes citrus leprosis-like symptoms (Roy et al. 2015, 2020, Cook et al. 2019, Velarde et al. 2021). Mechanical transmission of OFV is possible under laboratory conditions 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).

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, comprised of grass-like monocotyledonous liliod plants native to southeastern Asia (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* (Asparagaceae: Nolinoidaea) are considered the most important ground cover plant 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 one step conventional RT-PCR, and were found negative for begomovirus, carlavirus, potyvirus, tospovirus, Cucumber mosaic virus and Tobacco mosaic virus.

As initial diagnostics were inconclusive, new samples were collected during July and August of 2020 to collect more of these putatively-infected plants with ringspot symptoms. The plants collected included *Liriope* spp. and *Ophiopogon* spp., as well as *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea). *A. elatior* was suspected to be infected, due to both its proximity to infected *Liriope* and the presence of unusually chlorotic leaves (Fig. 2). Upon collection, the new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification.

The FDACS determined that the pathogen was *Orchid fleck dichorhavirus* using previously published primers and methods to conduct RT-PCR and Sanger sequencing (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). 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).

These samples from FDACS were subsequently retested by the USDA-ARS, in conjunction with tests of fresh samples from both Alachua and Leon counties. The USDA used RT-PCR, RT-qPCR, and High Throughput Sequencing (HTS) in sequence to reconfirm the presence of *Orchid fleck dichorhavirus*. RT-PCR and qPCR with Generic R2-Dicho-GF and R2-Dicho-GR primers amplifed ~800 nt of L-gene (RNA2) amplicon (Roy et al. 2020), and OFV-Orc1 and OFV-Orc2 were detected in both *O. intermedius* and *A. elatior* from Leon County.

HTS reaffirmed the presence of OFV-Orc1 and OFV-Orc2 strains in Leon and Alachua counties (Table 1). HTS results from Leon County revealed that *L. muscari* were coinfected with both strains (OFV-Orc1 and OFV-Orc2), while *A. elatior* were solely infected with OFV-Orc1. HTS of *L. muscari* from Alachua County revealed infections with the OFV-Orc2 strain.

After the initial identification by FDACS of OFV-Orc strains, mite samples were collected from symptomatic Asparagaceae in Leon county. The majority of mites collected were Tenuipalpid mites (flat mites or false spider mites), a known pest of ornamental plants, some of which are known to act as vectors for plant viruses (Childers et al. 2003, Childers and Rodrigues 2011).

Mite taxonomy is complicated by cryptic species complexes which occur in many plant-feeding groups of the Acari (Umina and Hoffmann 1999, Skoracka and Dabert 2010, Arthur et al. 2011, Skoracka et al. 2013), including tenuipalpid mites from the genus *Brevipalpus* (Navia et al. 2013). The commonly used phase-contrast microscopy is insufficient to detect some diagnostic characters for seperation of cryptic species, instead best practices recommend the combination of Differential Interference Contrast (DIC) Microscopy and Scanning Electron Microscopy along with molecular methods to separate cryptic species (Beard et al. 2015).

The flat mites collected were initially suspected to belong to *B. californicus* after inspection with phase contrast microscopy. Subsequent observation via DIC microscopy at FDACS agreed with this tentative identification. Unfortunately, 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). With this in mind, new mite samples were collected from symptomatic liriopogons and *A. elatior* in Leon County 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 description of the spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, this article made no mention of mites or further investigations of the virus. The first report of OFV in the continental US was Bratsch et al. (2015), who confirmed the presence of OFV in *Phalaenopsis* hybrids using Transmission Electron Microscopy of ultrathin sections of plant tissue as well as molecular sequence analysis. They also discuss the association of OFV with *Brevipalpus* mites, but the authors did not make a conclusive species identification beyond suggesting that the mite vector belonged to the *B. californicus* group, referring to Kondo et al. (2003)’s publication (Bratsch et al. 2015).

Later reports of OFV described OFV infecting a previously undescribed Nolinoidaea hosts in Australia (Mei et al. 2016, Dietzgen et al. 2018b), including *Liriope spicata* (Thunb.) Lour, a different species of liriopogon than those identified from the Florida sites. We are not aware of any reports of OFV infecting liriopogons, *A. elatior* nor other Nolinoidaea in the US. Although Zheng et al. (2013) had mentioned an association between *B. californicus* and *A. elatior*, they never reported symptoms of OFV-Orc in this plant. Taking this information into consideration, we believe that our findings indicate the first report of OFV-Orc infecting ornamental Nolinoidaea in Florida, and possibly the US. This publication also marks the first reports of *A. elatior* and *Ophiopogon* spp. as natural hosts of OFV-Orc.

OFV consists of two orchid strains (OFV-Orc1 and OFV-Orc2) and two citrus strains (OFV-Cit1 and OFV-Cit2) (Beltran-Beltran et al. 2020, Roy et al. 2020). The OFV strains detected in Florida are identical in gene order, content, and genome sequence to the orchid strains of OFV infecting citrus in Hawaii, Mexico, Colombia, and South Africa (Beltran-Beltran et al. 2020, Roy et al. 2020). Both OFV-Orc1 and OFV-Orc2 infect citrus (Roy et al. 2020), but none of the citrus strains have been reported from any orchid species. The *Brevipalpus* mites collected from liriopogons and *A. elatior* in Leon county were abundant on OFV-infected plants very near to citrus trees, some plants even surrounding the trunk. *B. californicus* s. l. have been reported as a pest of citrus (Childers et al. 2003) and are often collected from citrus rinds (Baker 1949, Baker and Tuttle 1987), so the proximity of these mite vectors to citrus raises the question: why these trees are not currently infected with OFV-Orc? Each possibility suggest further research, but there are a few possible explanations. It is important to note the uncertainty surrounding the vector for OFV-Orc. There are three mite species which have been recovered from OFV-Orc infected plants: *B. californicus* (the most likely culprit), *B. obovatus*, and *B. confusus*. Each species has its own unique biology and all have been implicated with a variety of different hosts. This suggests that the spread of OFV-Orc would be a function of various combinations of a number of potential factors, possibly including: host preferences, vectorial capacity, viral propagation/circulation in the vector, viral acquisition times, and feeding times required for transmission. Another possiblity is that the *B. californicus* which we find on liriopogons and *A. elatior* are not actually the same species as those found on citrus, and instead represent 2-3 different cryptic 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 cultivated *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 almost eradicated by the mid-1960s (Knorr 1968, Knorr et al. 1968, Childers et al. 2003). An examination of herbarium specimens of Florida citrus found that this historical virus, Citrus leprosis dichorhavirus-N0, is distantly related to the modern strains of OFV (Kitajima et al. 2011, Hartung et al. 2015, Roy et al. 2020). 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), and are difficult to identify by non-experts, or without advanced methodologies. DNA barcoding (Armstrong and Ball 2005) or a similarly simple and accurate method for identification of these mite complexes is vital to determine which of these species are responsible for transmission of OFV-Orc, and therefore which mite populations need to be monitored or controlled. By doing so, we can determine the risk OFV-Orc represents for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

### Acknowledgements

We would like to give 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. 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.

**Armstrong, K. F., and S. L. Ball**. **2005**. DNA barcodes for biosecurity: Invasive species identification. Philosophical Transactions of the Royal Society B: Biological Sciences. 360: 1813–1823.

**Arthur, A. L., A. D. Miller, and A. R. Weeks**. **2011**. Genetic markers indicate a new species complex of emerging pest mites in Australian grains. Annals of the Entomological Society of America. 104: 402–415.

**Baker, E. W.** **1949**. The genus *Brevipalpus* (Acarina: Pseudoleptidae). American Midland Naturalist. 42: 350.

**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, 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.

**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.

**Knorr, L. C.** **1968**. Studies on the etiology of leprosis in citrus. *In* International Organization of Citrus Virologists Conference Proceedings.

**Knorr, L. C., H. A. Denmark, and H. C. Burnett**. **1968**. Occurrence of *Brevipalpus* mites, leprosis, and false leprosis on citrus in Florida. The Florida Entomologist. 51: 11.

**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.

**Navia, D., R. S. Mendonça, F. Ferragut, L. C. Miranda, R. C. Trincado, J. Michaux, and M. Navajas**. **2013**. Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). Zoologica Scripta. 42: 406–426.

**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.

**Skoracka, A., and M. Dabert**. **2010**. The Cereal rust mite *Abacarus hystrix* (Acari: Eriophyoidea) is a complex of species: Evidence from mitochondrial and nuclear DNA sequences. Bulletin of Entomological Research. 100: 263–272.

**Skoracka, A., L. Kuczyński, W. Szydło, and B. Rector**. **2013**. The wheat curl mite *Aceria tosichella* (Acari: Eriophyoidea) is a complex of cryptic lineages with divergent host ranges: Evidence from molecular and plant bioassay data. Biological Journal of the Linnean Society. 109: 165–180.

**Umina, P. A., and A. A. Hoffmann**. **1999**. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) And the redlegged earth mite (*Halotydeus destructor*) to pesticides. Australian Journal of Experimental Agriculture. 39: 621.

**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*, collected from the landscape of northern Florida

|  |  |  |  |
| --- | --- | --- | --- |
| Scientific Name | Common Names | County | Strains |
| *Liriope muscari* cv. ‘Gigantea’\* (Decaisne) Bailey | Lilyturf, Orchardgrass, Monkeygrass | Alachua & Leon | OFV-Orc1 & OFV-Orc2 |
| *Ophiopogon intermedius*\*\* Don | Aztec Grass, ‘Argenteomarginatus’ | Leon | OFV-Orc1 & OFV-Orc2 |
| *Aspidistra elatior* Blume | Cast Iron Plant, Bar-room Plant | Leon | OFV-Orc1 & OFV-Orc2 |

Table 1: \* *Liriope muscari* cv. ‘Gigantea’ has been traditionally classified as *L. gigantea* Hume 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*: (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*: (a) Detail of leaf chlorosis (b) Chlorosis appears similar to sun 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. 2015) (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







