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First report of orchid fleck virus and its mite vector on green cordyline

Ralf G. Dietzgen[1] · Aline D. Tassi[2] · Juliana Freitas-Astúa[3,4] · Elliot W. Kitajima[2]

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Abstract
Orchid fleck virus (OFV) and its mite vector *Brevipalpus californicus* were for the first time identified on green cordyline plants showing distinctive chlorotic and necrotic ringspots. Thin section electron microscopy revealed bacilliform, dichorhavirus-like particles within nuclear viroplasms. RT-PCR using OFV degenerate primers yielded a single amplicon, the nucleotide sequence of which closely matched the nucleoprotein gene of OFV.

Keywords *Cordyline terminalis* · Dichorhavirus · *Brevipalpus* mites · RT-PCR · Nucleoprotein gene sequence · Family *Rhabdoviridae*

The ti plant (*Cordyline terminalis,* syn. *C. fruticosa*) is grown in tropical and subtropical regions as an ornamental, for cultural purposes in Polynesia and as a food crop (Melzer et al. 2011). During a survey of ornamental plants in September 2017, we observed green cordyline plants with distinctive chlorotic and necrotic lesions on their leaves (Fig. 1) growing in the City Botanical Gardens in Brisbane, Queensland, Australia. This type of localized symptom may be caused by negative-sense RNA viruses in the genus *Dichorhavirus*, family *Rhabdoviridae* (Kitajima et al. 2001; Dietzgen et al. 2014, 2017). This prompted us to investigate the potential presence of a dichorhavirus and its vector in/on these diseased plants.

Brevipalpus mites, possible vector of dichorhaviruses, were found on symptomatic plants and fixed in absolute ethanol. For light microscopic identification of these mites, they were mounted in Hoyer medium and examined in a Zeiss Axioskop Imager D2 microscope equipped with differential interference contrast (DIC) system. For scanning electron microscopic observation, specimens were dehydrated in a critical point drier (Leica CPD 300), gold coated in a Baltec SCD 050 sputter coater, mounted on aluminum stubs coated with double stick carbon tape and examined in a JEOL JSM IT300, and images were recorded digitally. The mites collected on green ti plants were identified as *B. californicus* sensu lato, based on morphological characteristics as reticulation pattern from venter and dorsum, microplates ornamentation and shape of spermathecal vesicle (Fig. 2). There were some slight incongruences in the dorsum and venter ornamentation found in these mites (Fig. 2) compared to the original description of *B. californicus* (Beard et al. 2012), therefore these mites found in green ti plants may be a cryptic species within this group.

To observe possible cytopathic effects, small fragments of the leaf lesion tissues were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M, pH 7.2 cacodylate buffer, post-fixed in 1% OsO_4, dehydrated in ethanol and embedded in Spurr's low viscosity epoxy resin. Blocks were sectioned in a Leica EM U6 ultra-microtome equipped with a diamond knife. Sections were contrasted with 3% aqueous uranyl acetate and Reynold's lead citrate, examined in a JEOL JEM 1011 transmission electron microscope and images were recorded using a digital camera. Ultrathin sections from ringspot lesions showed leaf parenchymal cells with nuclei containing electron lucent viroplasms and bacilliform particles associated with the nuclear envelope (Fig. 3). Many dichorhavirus-like particles were seen throughout the viroplasm (Fig. 3d). Some groups of virus particles that formed spoke-wheel configurations were observed in the cytoplasm near nuclei (Fig. 3e).

Total RNA was extracted from both chlorotic and necrotic ringspots using RNeasy Plant Mini kit (Qiagen). Superscript One-Step RT-PCR system with Platinum *Taq* DNA polymerase (Thermo Fisher Scientific, Invitrogen) was used following

✉ Ralf G. Dietzgen
r.dietzgen@uq.edu.au

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, Qld 4072, Australia

[2] Departmento de Fitopatologia e Nematologia, ESALQ/USP, Piracicaba, SP 13418-900, Brazil

[3] Embrapa Cassava and Fruits, Cruz das Almas, BA 44380-000, Brazil

[4] Instituto Biológico, Av. Cons. Rodrigues Alves 1252, São Paulo, SP 04014-900, Brazil

Fig. 1 Images of leaves of green ti plant (*Cordyline terminalis*) collected in the Brisbane City Botanical Garden and analysed in this study

the manufacturer's protocol and primer pair mN2 and polydT/SP6 (Blanchfield et al. 2001). A single amplicon of about 800 bp was obtained (data not shown), similar in size to the product obtained with orchid fleck virus (OFV; species *Orchid fleck dichorhavirus*, genus *Dichorhavirus*, family *Rhabdoviridae*) lilyturf RNA control (Mei et al. 2016), whereas negative control samples contained no detectable bands. Amplified DNA was extracted from the PCR reaction using Wizard SV Gel and PCR clean up kit (Promega), cloned into pGEM-T Easy vector (Promega) and transformed into Omnimax *E. coli* cells (Thermo Fisher Scientific) following the manufacturers' protocols. Following colony PCR, four positive recombinant clones were grown overnight and plasmid DNA was extracted for Sanger sequencing using M13 forward and reverse primers at the Australian Genome Research Facility (Brisbane). The dichorhavirus infecting

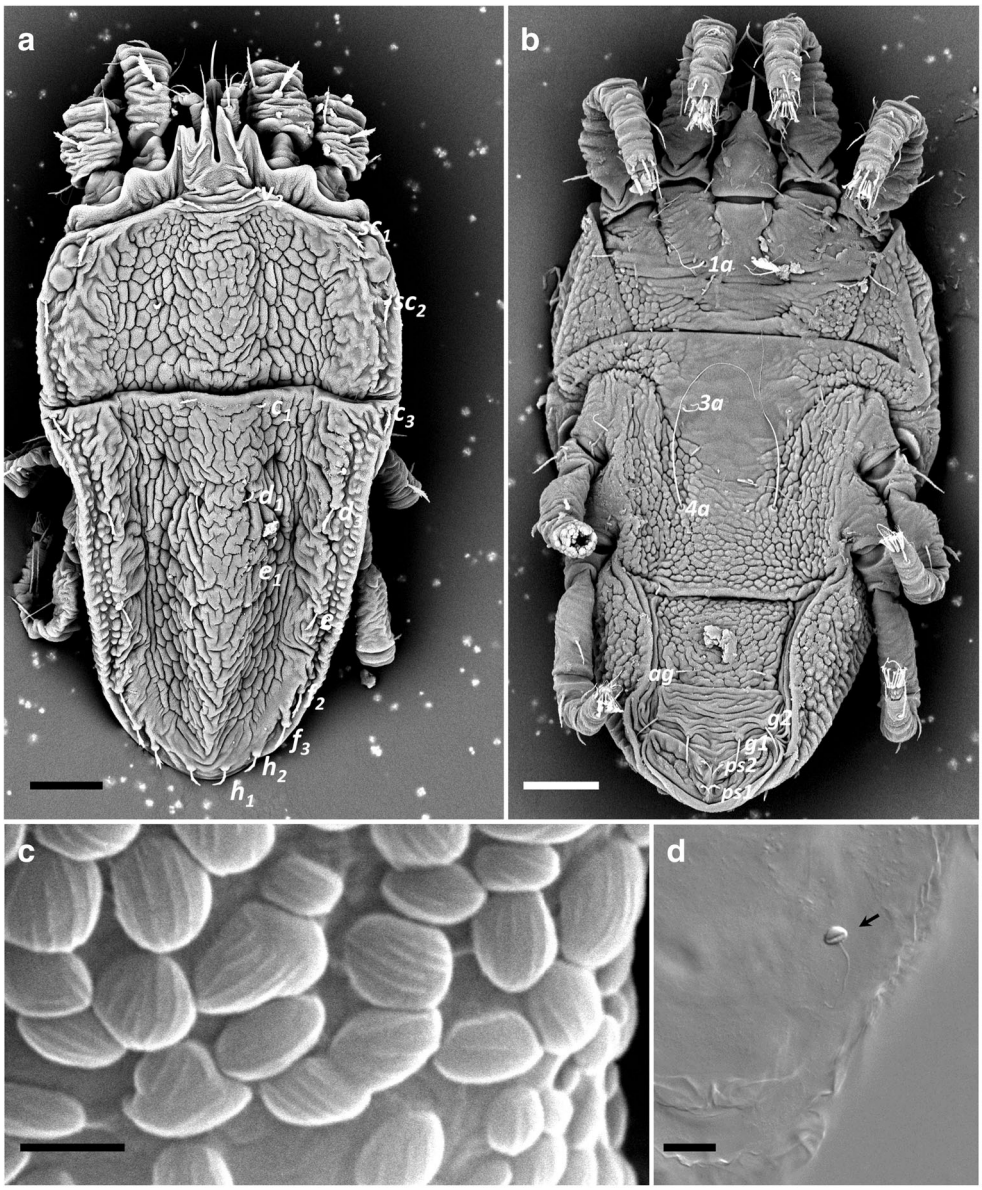

Fig. 2 Morphological characteristics of mites collected on green ti plant showing local lesions, identified as *Brevipalpus californicus* sensu lato. **a**–**c** Scanning electron micrographs. **a** Dorsal view. Cuticle of the propodosoma, showing setae: *v2*, *sc1*, *sc2*, *c1*, *c3*, *d1*, *d3*, *e1*, *e3*, *f2*, *f3*, *h2* and *h1*. **b** Ventral view of the cuticle showing the ventral and genital shield reticulation pattern. **c** Microplates ornamentation pattern. **d** Light micrograph taken using differential interference contrast. Arrow points to spermatheca, with a thick duct terminating in an oval vesicle with a bubble inside. Scale bars: **a** and **b** = 25 μm, **c** = 0.5 μm, and **d** = 20 μm

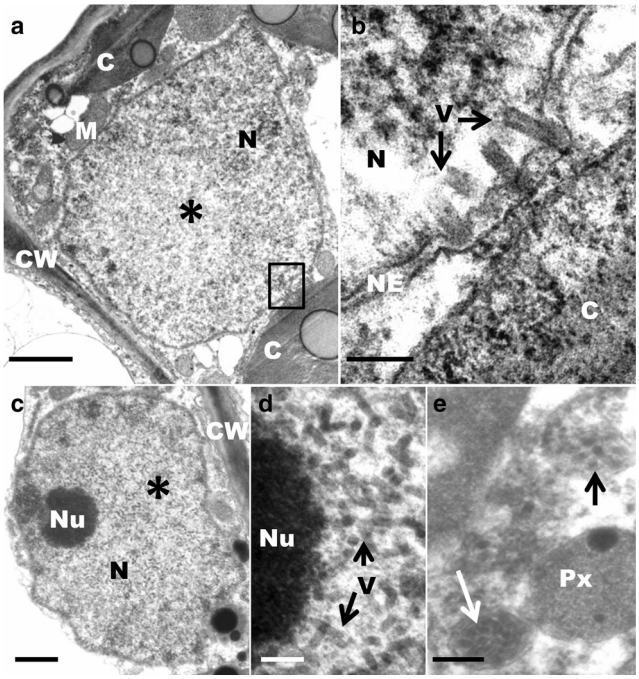

Fig. 3 Transmission electron micrographs of ultrathin sections from ringspot lesions on leaves of green ti plant (*Cordyline terminalis*) in the Brisbane City Botanical Garden. Panels **a**, **b**: Ringspot lesions on a non-senescent leaf. **a** Palisaded parenchymal cell showing a nucleus (N) with electron lucent viroplasm (*). **b** Detail of delimited area in A, showing dichorhavirus-like particles (V) apposed perpendicularly onto the inner membrane of the nuclear envelope (NE). Panels **c**–**e**: Ringspot symptoms on a senescent leaf. **c** Viroplasm (*) in the nucleus of a palisade parenchymal cell, entirely taken up by dichorhavirus-like particles. **d** Detail of C, close to the nucleolus (Nu), showing rod-like particles (V) scattered within the viroplasm. **e** Two groups of dichorhavirus-like particles forming small spokewheel configurations (arrows), in the cytoplasm next to the nucleus. C- chloroplast; CW- cell wall; M- mitochondrion; Px- peroxisome. Scale bars: **a**. 1 μm; **b**. 100 nm; **c**. 0.5 μm; **d**. 100 nm; **e**. 200 nm

green cordyline appears to be OFV, because the nucleotide sequence amplified using dichorhavirus degenerate *N* gene primers (GenBank accession MG812380) was 98% identical to the *N* gene of an OFV isolate infecting cymbidium in Japan (LC222629). The OFV isolate from cordyline was 99% identical in nucleotide sequence to the *N* gene fragment of Australian OFV isolates from lilyturf (KT947974) and cymbidium (KT947975) (Mei et al. 2016), indicating a close relationship between OFV isolates from various plant species in Australia.

This study identified green cordyline as a new host for OFV and for its known vector, *B. californicus* (Kondo et al. 2003). *B. phoenicis* (sensu lato) and *B. obovatus* have been previously found on ti plants (Miranda et al. 2007; Kitajima et al. 2010), but to our knowledge this is the first report of *B. californicus* mites colonizing this species. Since we did not conduct virus transmission assays with the collected *Brevipalpus* mites to reproduce the symptoms, thus far the mites and OFV can only be considered as associated with the disease. Infected cordyline leaf cells displayed nuclear cytopathological effects characteristic of dichorhaviruses (Kitajima et al. 2003) and typical short, bacilliform particles were seen in thin sections in viroplasms in the nucleus. No other virus-like particles were observed. So far, only cytoplasmic-type *Brevipalpus*-transmitted viruses have been reported for this ornamental host, associated with *B. phoenicis* sensu lato mites (Kitajima et al. 2010). Recently, four distinct closteroviruses, although apparently not involved in the etiology of green ti ringspot disease were identified in symptomatic plants in Hawaii (Melzer et al. 2011, 2013), and an emara-like virus has also been reported (Melzer et al. 2014), suggesting that different viruses may be associated with ringspot symptoms of cordyline in different geographical locations.

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Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest.

Human and animal studies This article does not contain any studies with human participants or animals performed by any of the authors.

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