# Brevipalpus yothersi (ACARI: TENUIPALPIDAE) AS VIRUSES AND FUNGI DISSEMINATOR TO CULTIVATED PLANTS IN BAHIA, BRAZIL

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Brevipalpus yothersi has received attention worldwide as a vector of Brevipalpus-transmitted viruses (BTVs) to plants. However, there are still few studies on it as a putative fungal disperser. Considering the limited knowledge on this mite as viruses and fungi disseminator in Bahia, this study aimed to identify possible associations of these organisms with the mite on four plant species cultivated regionally. BTVs from vegetal parts containing chlorosis and/or leprosis were identified by transmission electron microscopy and RT-PCR. Fungi isolated from individual mites in culture mediums were identified by microscopy. Two BTVs and seven of the fungi taxa were identified: the citrus leprosis virus C (CiLV-C, Cilevirus leprosis) in Citrus sinensis, the clerodendrum chlorotic spot virus (CICSV, Dichorhavirus clerodendri) in Clerodendrum x speciosum, Aspergillus sp. in C. x speciosum, Cladosporium cladosporioides in C. sinensis, Colletotrichum gloeosporioides s.l. in C. sinensis and Theobroma cacao, Curvularia lunata in C. x speciosum, Diaporthe sp. in C. sinensis and Passiflora edulis, Gilmaniella sp. in C. sinensis, and a possible new taxon of Hyphomycetes. Considering the potential of B. yothersi as a disseminator of phytopathogenic viruses and fungi, further studies are needed for management/control of plant diseases in Bahia.

Key words: Flat mites, plant protection, BTVs, spores, dispersion.

Brevipalpus yothersi (Acari: Tenuipalpidae) como disseminador de vírus e fungos em plantas cultivadas na Bahia, Brasil. Brevipalpus yothersi tem recebido atenção mundial como vetor de vírus transmitidos por Brevipalpus (VTBs) em plantas. No entanto, ainda existem poucos estudos sobre ele como suposto dispersor de fungos. Considerando o conhecimento limitado sobre este ácaro como disseminador de vírus e fungos na Bahia, este estudo objetivou identificar possíveis associações desses organismos com o ácaro em quatro espécies de plantas cultivadas regionalmente. Os VTBs de partes vegetais contendo clorose e/ou leprose foram identificados por microscopia eletrônica de transmissão e RT-PCR. Os fungos isolados de indivíduos de ácaros em meios de cultura foram identificados por microscopia. Dois VTBs e sete táxons de fungos foram identificados: o vírus C da leprose dos citros (CiLV-C, Cilevirus leprosis) em Citrus sinensis, o vírus da mancha clorótica de clerodendrum (ClCSV, Dichorhavirus clerodendri) em Clerodendrum x speciosum, Aspergillus sp. em C. x speciosum, Cladosporium cladosporioides em C. sinensis, Colletotrichum gloeosporioides s.l. em C. sinensis e Theobroma cacao, Curvularia lunata em C. x speciosum, Diaporthe sp. em C. sinensis e Passiflora edulis, Gilmaniella sp. em C. sinensis, e um possível novo táxon de Hyphomycetes. Considerando o potencial de B. yothersi como disseminador de vírus e fungos fitopatogênicos, futuros estudos são necessários para o manejo/controle de doenças de plantas na Bahia.

Palavras-chave: Ácaros planos, proteção de plantas, VTBs, esporos, dispersão.

# Introduction

Some Brevipalpus species (Acari: Tenuipalpidae), especially those belonging to the Brevipalpus phoenicis (Geijskes) species complex, have received significant attention worldwide as vectors of Brevipalpus-transmitted viruses (BTVs) (Beard et al., 2015; Kitajima et al., 2020; Ramos-González et al., 2023). Brevipalpus yothersi Baker, also identified as B. phoenicis before Beard et al. (2015), is the most important BTV vector in Brazil (Kitajima, 2020). In the state of Bahia, there are reports of B. yothersi transmitting citrus leprosis virus C (CiLV-C, Cilevirus leprosis) (Bastianel et al., 2010; Ramos-González et al., 2016), and passion fruit green spot virus (PfGSV, Cilevirus passiflorae) (Santos Filho et al., 1999; Ramos-González et al., 2020).

About 40 species of fungi in 25 genera, including several belonging to phytoparasitic and saprophytic groups, have been identified either internally or externally from species of the *B. phoenicis* complex (Evans, Cromroy & Ochoa, 1993, 1998; Quirós et al., 2014; Rodrigues et al., 2019). Although it is expected that these mites may play a relevant role in the dispersion of fungi to plants while carrying spores adhered to their cuticle (Evans, Cromroy & Ochoa, 1998), little attention has been given to them as potential fungi disseminators, with no records of the association of *Brevipalpus* species with fungi in Bahia.

Considering that a large number of unidentified fungal structures have been found on individuals of *B. yothersi* (Figure 1) in previous studies conducted in Bahia (Souza, 2019; Nascimento et al., 2023), and the limited knowledge on the role of these mites as disseminators of viruses and fungi locally, the aim of this study was to identify potential associations of those microorganisms with *B. yothersi* in four cultivated plants in the region.

#### **Materials and Methods**

Leaves, branches and/or fruits of *Citrus sinensis* (L.) Osbeck (Rutaceae), *Clerodendrum x speciosum* (Lamiaceae), *Passiflora edulis* Sims (Passifloraceae), and *Theobroma cacao* L. (Malvaceae) were collected once with a pruning shear from July 2022 to March 2023 from three localities (Table 1) in the municipality

of Ilhéus, BA, Brazil (coordinates ranging from 14°47'53" to 14°78'75" S and 39°05'00" to 39°21'97" W). The samples from each plant were labeled, packaged in polyethylene bags and taken to laboratory, after a maximum of one hour, where processing took place. Samples were analyzed under a stereomicroscope for the collection of *B. yothersi*, with at least 10 adult mites from each plant mounted in slides with Hoyer's medium (Moraes, Castilho & Flechtmann, 2024). Species identification was possible with a phase contrast microscope following the parameters described by Beard et al. (2015). Vouchers specimens were deposited in the scientific collections of UESC.

Plant material (leaves, branches and/or fruits) exhibiting localized chlorotic or necrotic lesions, resembling symptoms induced by BTVs, were investigated under Transmission Electronic Microscopy (TEM) and RT-PCR. For microscopic analysis, small fragments of leaf tissue containing lesions were fixed in an aldehyde mixture (formaldehyde and glutaraldehyde) and post-fixed in OsO<sup>4</sup>. After dehydration, fragments were embedded in epoxy resin, sectioned on an ultra-microtome, and examined under TEM for ultrastructural detection of BTVs (Alberti; Kitajima, 2014). RT-PCR assays were carried out for the molecular identification of BTVs with ca. 100 mg per plant of fresh leaf and/or fruit tissues containing lesions treated with Trizol for total RNA extraction. About 500 ng of RNA was used for cDNA production, with random hexamer primers and GoScript<sup>TM</sup> Reverse Transcriptase Kit. For PCR assays of citrus samples, 1 µL of cDNA was tested using primer pairs that specifically detect the two prevalent strains of CiLV-C, SJP and CRD. The primer pairs amplify regions within the ORFs p29 (RNA1, 456 bp and 330 bp for SJP and CRD, respectively) and p24 (RNA2, 393 bp and 522 bp for SJP and CRD, respectively) of CiLV-C (Ramos-González et al., 2016; Chabi-Jesus et al., 2021). For the PCR assays of clerodendrum samples, a primer pair that specifically amplifies a 577 bp region within the G gene of ClCSV was used (Ramos González et al., 2018). Amplicons of the expected sizes were visualized in 1% agarose gels, sequenced, and the sequences were compared with those available at the GenBank.

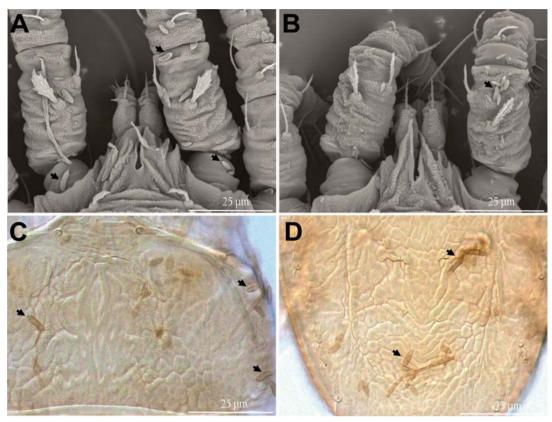


Figure 1. Unidentified fungal structures (black arrows) attached to the cuticle of *Brevipalpus yothersi* collected from A. *Passiflora edulis* and B–D. *Citrus* sp. during the studies conducted by Souza (2019) and Nascimento et al. (2023) in the municipalities of Ibirapitanga and Uruçuca, Bahia, Brazil.

Table 1- Fungi isolates identified from *Brevipalpus yothersi* (12 mites/plant) collected from leaves and/or branches of cultivated plants in the municipality of Ilhéus, Bahia, Brazil, from July 2022 to March 2023.

Original host plant	Fungal isolate	N. of mites	Locality	Coordinates
Citrus sinensis (Rutaceae)	Cladosporium cladosporioides Colletotrichum gloeosporioides s.l. Diaporthe sp. Gilmaniella sp. Unidentified No fungal growth	1 2 2 1 4 2	Rodovia BR-415	14°78'75" S 39°21'97" W
Clerodendrum x speciosum (Lamiaceae)	Aspergillus sp. Curvularia lunata Unidentified No fungal growth	3 3 4 2	Sapetinga (urban area)	14°49'33" S 39°05'00" W
Passiflora edulis (Passifloraceae)	Diaporthe sp. Unidentified No fungal growth	5 3 4	Campus of UESC	14°47'53" S 39°10'20" W
Theobroma cacao (Malvaceae)	Colletotrichum gloeosporioides s.l. Hyphomycetes sp. Unidentified No fungal growth	1 1 7 3		

For the isolation of fungi associated with B. yothersi, 12 adult mites collected from the leaves and/ or branches of each plant species in the same locality were placed in a disinfected leaf using a fine, sterilized needle. The same transference procedure was used to stick each mite alive in the center of a 90 mm diameter sterilized Petri dish containing a 2% Agar-Agar (AA) substrate. The Petri dishes were kept at about 25 °C and 12/12 light cycle for eight days before inspections for the presence of mycelial growth from the mites. Each group of mycelia produced was transferred to a Petri dish containing Potato-Dextrose-Agar (PDA) supplemented with the antibiotic chloramphenicol for sporulation. Dishes incubated at the same conditions above were analyzed for the presence of spores after eight to fifteen days. In the absence of sporulation, an additional plating of the inoculum on corn meal agar or mineral liquid culture meal with agar toinduce spore growth was provided. Fungal isolates were identified with the help of taxonomic keys and specific descriptions (Ellis, 1971; Seifert et al., 2011), based on macro and microscopic characteristics of the colonies, hyphae and spores slide-mounted on a drop of lactophenol (Dhingra; Sinclair, 1995).

# Results

Two BTVs were identified in the samples: citrus leprosis virus C, CiLV-C, in C. sinensis (Figure 2), and clerodendrum chlorotic spot virus (ClCSV) in C. x speciosum (Figure 3). Typical symptoms of localized infection caused by BTVs were observed in sweet orange leaves, fruits, and branches, such as ringspots, chlorotic and necrotic lesions (Figures 2A, B). The presence of short bacilliform particles, characteristic of CiLV-C, and electron-dense, vacuolated inclusions (viroplasms) were visualized by TEM in the cytoplasm of infected cells (Figure 2C). The presence of CiLV-C (strain CRD) was confirmed in symptomatic samples of C. sinensis, whereas viruses of the CiLV-C SJP strain were not detected (Figure 2D). Leaves of C. x speciosum affected by ClCSV exhibited chlorotic spots that sometimes showed necrotic central areas (Figure 3A). The presence of ClCSV was confirmed by primers that amplified a 577-bp fragment of the G gene (RNA1) of the virus (Figure 3B).

Approximately 80% of the Petri dishes displayed any mycelial growth from their mites (Table 1, Figure 4), with reproductive structures obtained from about half of them, allowing the identification of the following fungal isolates: Aspergillus sp. (3 mites in C. x speciosum), Cladosporium cladosporioides (Fresen) G.A. de Vries (1 in C. sinensis), Colletotrichum gloeosporioides s.l. (Penzig) Saccardo (2 in C. sinensis and 1 in T. cacao), Curvularia lunata (Wakker) Boedijn (3 in C. x speciosum), Diaporthe sp. (2 in C. sinensis and 5 in P. edulis), Gilmaniella sp. (1 in C. sinensis), and an unidentified Hyphomycetes (1 in T. cacao). The fungi most commonly found in the samples were C. gloeosporioides s.l. from C. sinensis and T. cacao, and Diaporthe sp. from C. sinensis and P. edulis.

#### **Discussion**

Although some reports of B. yothersi as vectors of BTVs to citrus and passion fruit plants have been published in Bahia (Santos Filho et al., 1999; Bastianel et al., 2010; Ramos-González et al., 2016, 2020; Kitajima, 2020), this is the first record of the dichorhavirus ClCSV in C. x speciosum in the state. On the other hand, the detection of viruses solely of the CRD strain of CiLV-C in C. sinensis was expected, since they are found endemically in citrus orchards throughout Brazil and other American countries, whereas those of the SJP strain have never been detected outside the citrus belt of São Paulo and Minas Gerais (Ramos-González et al., 2016; Chabi-Jesus et al., 2021). Although all the fungal taxa identified, except Gilmaniella sp., have already been reported in Bahia (Rubini et al., 2005; Hanada et al., 2010; Oliveira et al., 2012; Cerqueira et al., 2013; Mattos Sobrinho et al., 2015; Santos et al., 2018; Oliveira et al., 2020) and/or previously isolated from B. yothersi (Quirós et al., 2014; Rodrigues et al., 2019), this study presented the first local records of their association to the mite. Another interesting finding of this study was the unidentified Hyphomycetes isolated from B. vothersi collected in T. cacao, which is possibly an undescribed taxon, with brown, septate, branched, smooth-walled hyphae, conidiomata consisting of a dark stromal base that serves as support for unicellular, subcylindrical, parallel, smooth-walled

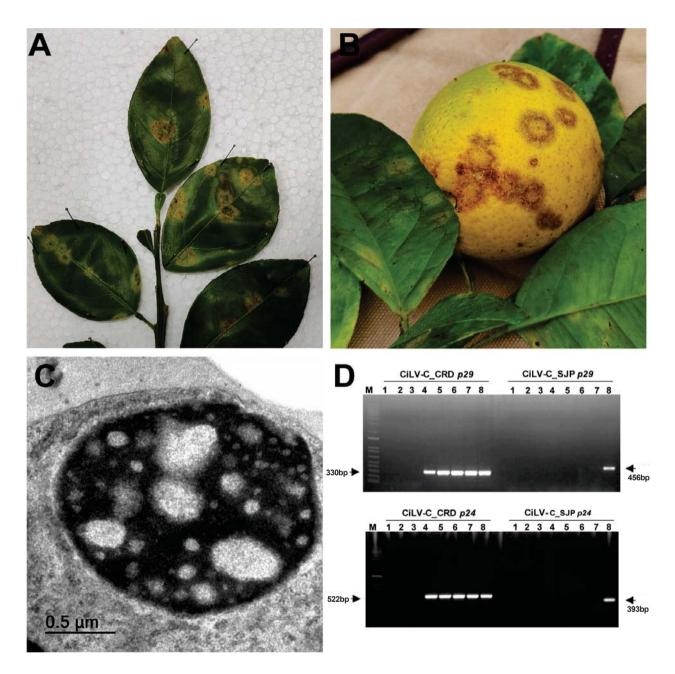


Figure 2. Detection of the cilevirus CiLV-C (*Cilevirus leprosis*) in *Citrus x sinensis* samples from Ilhéus, Bahia, Brazil. A and B. Chlorotic and necrotic lesions typical of citrus leprosis in citrus leaves and fruit, respectively; C. Micrograph of cytoplasm inclusion (viroplasm) typical of CiLV-C infection; D. 1% agarose gel electrophoresis of RT-PCR products obtained with primers that amplify fragments of the genes *p29* (RNA1) or *p24* (RNA2) from the CRD and SJP strains of CiLV-C, respectively: M- Molecular marker 1 Kb Plus DNA Ladder Invitrogen (Thermo Fisher Scientific); 1- Blank; 2- Negative control (healthy orange fruit); 3- Negative control (healthy orange leaf); 4 to 7- Samples with typical citrus leprosis symptoms (A and B); 4 and 5- Isolated lesions collected from sweet orange fruit (B); 6 and 7- Isolated lesions collected from sweet orange leaves (A); 8- Positive controls consisting of samples of sweet orange tissues infected with CiLV-C\_CRD or SJP.

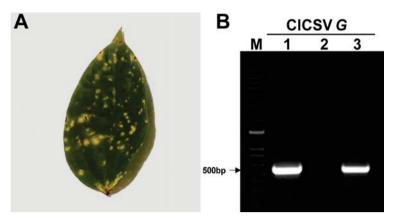


Figure 3. Detection of the dichorhavirus ClCSV (*Dichorhavirus clerodendri*) in *Clerodendrum x speciosum* samples from Ilhéus, Bahia, Brazil. A. Leaf with typical chlorotic spots associated with ClCSV; B. 1% agarose gel electrophoresis of RT-PCR products obtained with primers that amplify a fragment of the gene G (RNA1) of ClCSV: M-Molecular marker 1 Kb Plus DNA Ladder Invitrogen (Thermo Fisher Scientific); 1- Sample collected from the symptomatic clerodendrum leaf (A); 2- Negative control (blank); 3- Positive control consisting of a sample of *Clerodendrum* sp. infected with ClCSV.

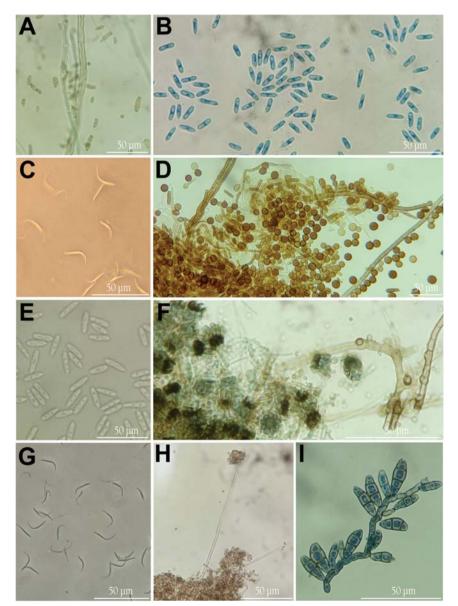


Figure 4. Micrographs of fungi isolated from Brevipalpus yothersi in cultivated plants from Ilhéus, Bahia, Brazil. A. Cladosporium cladosporioides, B. Colletotrichum gloeosporioides s.l., C. Diaporthe sp. and D. Gilmaniella sp. in Citrus sinensis; E. Colletotrichum gloeosporioides s.l. and F. Hyphomycetes sp. in Theobroma cacao; G. Diaporthe sp. in Passiflora edulis; H. Aspergillus sp. and I. Curvularia lunata in Clerodendrum x speciosum.

phialides, with subglobose to angular, olivaceous, unicellular and smooth conidia formed at the ends (Figure 4F). Molecular analyses are yet necessary to confirm its status as a new taxon.

Isolates of all the fungi found in this study have been previously reported as endophytes, saprobes or pathogens depending on the host plant (Hyde et al., 2009; Aly, Debbab & Proksch, 2011; Gomes et al., 2013; Yuan et al., 2019; Wang et al., 2022). Species of Colletotrichum, for example, have already been found causing diseases such as anthracnose in citrus (Phoulivong, Mckenzie & Hyde, 2012; Wang et al., 2021), and cacao (Rojas et al., 2010; Nascimento et al., 2019; Mohali-Castillo & Stewart, 2022), while Diaporthe spp. was reported causing rot in different plant parts of passion fruit (Moreira et al., 2020) and melanosis in citrus (Melo; Andrade, 2006; Chaisiri et al., 2020). Considering that B. yothersi is a possible disseminator of pathogenic fungi to crops, further studies are needed to investigate the importance of such associations for the management/control of plant diseases in Bahia.

# Conclusion

The following BTVs and fungi were found or isolated from *B. yothersi* in the present study: citrus leprosis virus C (CiLV-C) in *C. sinensis*, clerodendrum chlorotic spot virus (CICSV) in *C. x speciosum*, *Aspergillus* sp. in *C. x speciosum*, *C. cladosporioides* in *C. sinensis*, *C. gloeosporioides* s.l. in *C. sinensis* and *T. cacao*, *C. lunata* in *C. x speciosum*, *Diaporthe* sp. in *C. sinensis* and *P. edulis*, *Gilmaniella* sp. in *C. sinensis*, and a possible new genus and/or species of Hyphomycetes in *T. cacao*. Considering that *B. yothersi* is a possible disseminator of pathogenic viruses and fungi to crops in Bahia, further studies are needed to support management/control of plant diseases locally.

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