Tropical Plant Pathology, vol. 33, 1, 012-019 (2008)

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RESEARCH ARTICLE / *ARTIGO*

Brevipalpus-associated viruses in the central Amazon Basin

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ABSTRACT

A survey of *Brevipalpus*-transmitted plant viruses (BTrV) was carried out in the cities of Manaus and Presidente Figueiredo, and in the oil exploratory base of Urucu, Amazonas State, Central Amazon Basin, Brazil. The main characteristics considered during the survey were the occurrence of localized symptoms (chlorotic or brown spots, ringspots, green spots in senescent leaves) similar to those previously described for BTrV and infestation by *Brevipalpus* mites. For the evaluation of the infection by putative BTrV, small fragments of the leaf lesions were fixed *in loco* and later processed for ultrastructural electron microscopy at Piracicaba, SP. Thirteen plant hosts of presumed BTrV were found. Three of them probably represent previously described BTrV infecting *Hibiscus rosa sinensis, H. syriacus* and *Clerodendrum* x *speciosum*. For the first time we report cases of infection by putative BTrV of nuclear type in chlorotic spot in *Piper callosum* (Piperaceae), chlorotic ringspot in *Monstera deliciosa* (Araceae), chlorotic spot in *Ruellia chartacea* (Acanthaceae), chlorotic spot in *Bidens* sp. (Asteraceae), green spot in *Allamanda chatartica* (Apocynaceae), chlorotic spot in *Gardenia* sp. (Rubiaceae), chlorotic ringspot and green spot in *Mussaenda erythrophylla*. (Rubiaceae) and of cytoplasmic type in *Clerodendrum speciosissimum* (Lamiaceaae) and ringspots in *Arundina graminifolia* (Orchidaceae). All these plants were infested by *Brevipalpu. phoenicis.*
Keywords: cytopathology, *Acari, Tenuipalpidae*, *Brevipalpus phoenicis,* flat mite, invasive species.

RESUMO

Viroses associadas a *Brevipalpus* na Amazônia Central

Foi feito um levantamento no estado do Amazonas, na região da capital Manaus, na base de exploração de petróleo de Urucu (700 km a sudoeste de Manaus) e em Presidente Figueiredo (100 km ao norte de Manaus) à procura de vírus transmitidos por ácaros *Brevipalpus* (VTrB) em diferentes espécies de plantas. Foram coletadas 33 espécies de plantas caracterizadas por lesões localizadas em folhas (manchas cloróticas, manchas anelares, manchas verdes em folhas senescentes) e pela presença de ácaros *Brevipalpus* (os quais foram coletados para identificação e avaliação de sua diversidade) em plantas cultivadas, ornamentais e silvestres. Fragmentos das lesões foram fixados no local e processados para microscopia eletrônica ultraestrutural e examinados em Piracicaba, SP. Foram constatados treze casos positivos de detecção de efeitos citopáticos do tipo citoplasmático ou nuclear de VTrB nas plantas coletadas em Manaus. Três deles parecem representar casos anteriormente descritos de VTrB em *Hibiscus rosa sinensis, H. syriacus* e *Clerodendrum x speciosum*. Presumíveis VTrB do tipo nuclear foram constatados em mancha clorótica em *Piper callosum* (Piperaceae), mancha clorótica anelar em *Monstera deliciosa* (Araceae), mancha clorótica em *Ruellia chartacea* (Acanthaceae), manchas cloróticas em *Bidens* sp. (Asteraceae), mancha verde em *Allamanda chatartica* (Apocynaceae), mancha clorótica em *Gardenia* sp. (Rubiaceae), mancha verde em *Mussaenda erythrophylla* (Rubiacea) e do tipo citoplasmático em mancha clorótica em *Clerodendrum speciosissimum* (Lamiaceae) e mancha anular em *Arundina graminifolia* (Orchidaceae). Todas estas plantas achavam-se naturalmente infestadas por *Brevipalpus phoenicis.*
Palavras-chaves: citopatologia, *Acari, Tenuipalpidae*, *Brevipalpus phoenicis,* ácaros planos, espécies invasivas.

INTRODUCTION

Brevipalpus-transmitted viruses (BTrV) or putative BTrV have been described in more than 40 plant species (Kitajima *et al.*, 2003; 2006, Nogueira *et al.*, 2003). A common feature of diseases associated with these viruses is that they usually induce consistently localized symptoms in the leaves, stems, fruit and sometimes in the flower of the host plants. BTrV or putative BTrV have been grouped in two types based upon comparative cytopathology of the lesions (Kitajima *et al.*, 2003). The first type is characterized by the accumulations of short, bacilliform particles in the cisternae of the endoplasmatic reticulum of the infected cells and the presence of large electron-dense, vacuolated inclusions (viroplasms) in the cytoplasm. This type has been called **cytoplasmatic type**, with the Citrus leprosis virus cytoplasmatic type (CiLV-C) being the prototype virus in this group, whose genome has been completely sequenced (Pascon *et al.*, 2006). Because the genome organization of CiLV-C is different from other known viruses, the genus,

Cilevirus, has been proposed (Locali-Fabris *et al.*, 2006). The second BTrV group, called **nuclear type**, is characterized by the occurrence of short, rod-like particles in the nucleus and cytoplasm, and an electron lucent viroplasm in the nucleus of the infected host cell. Orchid fleck virus (OFV) may be considered the type species of the nuclear type of BTrV. Its genome was sequenced and presents similarities to that of rhabdoviruses. Because of the bipartite genome, a new genus, *Dichorhabdovirus*, was proposed within the family *Rhabdoviridae* to accommodate OFV (Kondo *et al.*, 2006). In addition to citrus, other plants such as *Clerodendrum* spp., orchid species and *Hibiscus* spp., *Salvia leucantha* Cav. and *Spathiphyllum wallisi* Regel have been reported as hosts for both types of BTrV (Kitajima *et al.*, 2006).

CiLV-C is one of the most significant economic pest problems in the Brazilian citrus industry and the most important of the *Brevipalpus*-transmitted disease agents. Control of citrus leprosis is expensive, being based on chemical control of the mite vector, whose populations are monitored through bi-weekly inspections and reduced with frequent acaricide sprays. Other practices, like removal of infected branches and the removal of alternative virus and vector hosts, have been added to the traditional chemical control (Rodrigues, 2002; Rodrigues *et al.*, 2003). The fact that non-citrus plants can also naturally host CiLV-C (Rodrigues *et al.*, 2005; Nunes *et al.*, 2006; Groot *et al.*, 2006) could make the control strategies more complex. It also raises the importance of properly identifying all the other potential BTrV hosts as well as the places where they occur. Beyond the concerns over these viruses in citrus, the growing ornamental industry based on tropical and subtropical plants could be negatively affected by some of the BTrV.

This paper reports thirteen plant hosts collected in the central Amazon Basin that had infestations of *Brevipalpus* mites, showed localized symptoms, and harbored virus-like particles typical of BTrV in their leaf lesions.

MATERIALS AND METHODS

Plants were inspected between October 2004 and December 2005 in the region around Manaus (S 03°04' / W 59°57'), Urucu (S 04°53' / W 65°18', 700 km Southwest of Manaus) and Presidente Figueiredo (S 02°03 / W 60°03'), Amazonas State, Brazil. The surveys were conducted in natural forest areas, parks, yards, and plant nurseries. Thirty-three plants (Table 1) showing symptoms similar to those described for BTrV's (Kitajima *et al.*, 2003) were sampled for electron microscopy analysis. Small sections of the lesions were fixed immediately after collection in plastic tubes with a modified Karnovsky solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2), and sent by mail to Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, at Piracicaba, SP, for further processing, dehydration and embedding in low viscosity Spurr resin. Embedded tissues were cut with a Leica UC6 microtome equipped with a diamond knife and the sections stained with uranyl acetate and Reynold's lead citrate (Kitajima & Nome, 1999). Sections were examined in a Zeiss EM 900 transmission electron microscope.

In the field, symptomatic plants were inspected for the presence of *Brevipalpus* mites using a 10-20 X-magnification hand lens, and plant twigs and leaves were washed in an ethanol and water (80:20) solution. The solution was taken to the laboratory and inspected for false spider mites. When present, they were slide-mounted in Hoyer's medium and identified by light microscopy (Jeppson *et al.*, 1975). Mite voucher specimens were deposited at "Coleção de Ácaros de Referência para a Segurança Biológica", Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF.

RESULTS AND DISCUSSION

Out of thirty-three plant species exhibiting local lesion symptoms on the leaves and analyzed for cytopathology by transmission electron microscopy, thirteen (Figures 1 and 2) showed BTrV-like particles and characteristic inclusions in the parenchymal cells from the leaf lesions (Figures 3 and 4). All of these plant species were infested by *Brevipalpus* mites, as were most of the plants sampled during this survey (Table 1). These mites were identified as *B. phoenicis* Geijskes based on Baker (1949) and Baker and Tuttle (1987) keys.

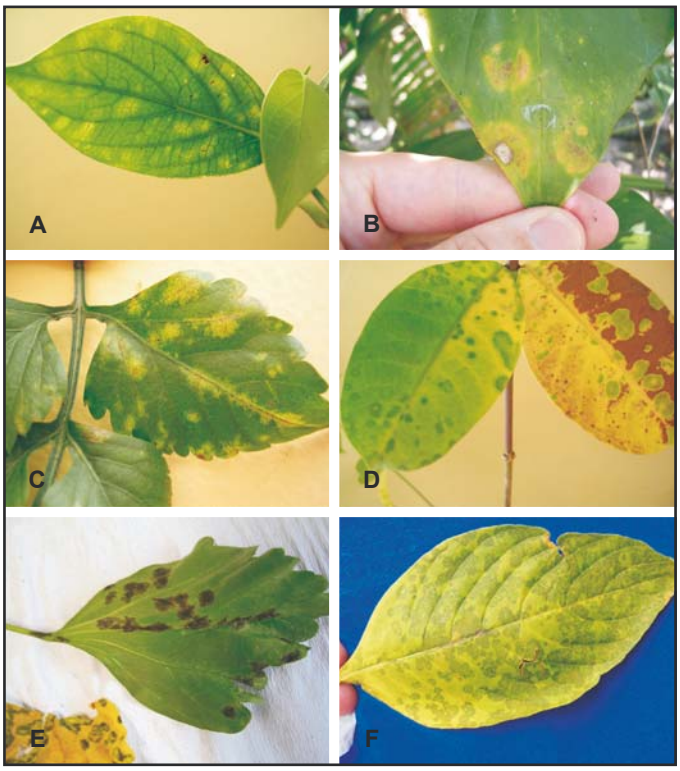
Hibiscus spp. were the plants with the highest occurrence and abundance of green spot symptoms on their leaves throughout the Manaus region. This is widely used as an ornamental plant in gardens and public parks, and because of its vegetative multiplication and evergreen habits, it could be a very effective vehicle for spread of both viruses and vectors to new areas. The green spot symptoms (Figure 2B) were similar to those caused by the previously reported *Hibiscus* green spot virus (HGSV), a cytoplasmic type of BTrV (Kitajima *et al.*, 1999). Hibiscus (*H. rosa sinensis* L.*, H. schizopetalus* Hook.f. and *H. syriacus* L.) is one of the BTrV hosts with the widest geographical distribution. Affected plants were found in several parts of Brazil, in Panama City (Panama), and Havana (Cuba) (Kitajima *et al.*, 2004), and more recently in Bolivia (Kitajima, E.W., unpublished data) and Argentina (Dal Bo *et al.*, 2007). A typical cytoplasmic type of cell alteration was found in the tissues of these green spots (Figure 3 D). Chlorotic spots on *H. rosa sinensis* leaves (image not shown) and brown spots on *H. syriacus* leaves (Figure 1 E) exhibited a cytopathic effect characteristic of the nuclear type of BTrV (Figures 3 C and 4 G). These cases may represent infection by the previously described *Hibiscus* chlorotic spot virus (HibCSV). Cases of the same host plant being susceptible to both types of BTrV or putative BTrV are not uncommon. *Citrus* spp., *Solanum violaefolium* Schott., *Clerodendrum* spp., *Salvia leucantha, Pelargnoium x hortorum* Hort. and some species of orchids are examples, and in a few instances, co-infection of a single cell by the

TABLE 1 - List of plants inspected for *Brevipalpus* and possible infection by *Brevipalpus*-transmitted viruses in Amazon region

Local	Family	Species	*Brevipalpus* and symptoms[1]	[2]Cytopathic effects
Manaus	Acanthaceae	*Ruellia chartacea* (T. Anderson) Wash.	+ / Green spots	+ / N
		Hemigraphis sp.	+ / Dark spots	-
	Anonacea	*Rollinia orthopetala* A.DC.	+ / Green spots	-
	Apocynaceae	*Allamanda cathartica* L.	+ / Green spots	+ / N
	Arecaceae	*Rhapis excelsa* (Thunb.) Henry ex Rehder	+ / Chlorotic symptoms	-
	Araceae	*Monstera deliciosa* Liebm.	+ / Chlorotic ringspots	+ / N
		Aglaonema costatum N.E.Br.	+ / Chlorotic spots	-
		Aglaonema commutatum Schott	+ / Chlorotic spots	-
		Syngonium angustatum Schott	+ / Chlorotic spots Symptoms - fungi	-
	Asteraceae	*Bidens* sp.	+ / Vein clearing and ringspots	+ / N
	Liliaceae	*Dracaena marginata* Hort.	+ / Chlorotic spots	-
	Saxifragaceae	*Hydrangea macrophylla* (Thunb.) Ser.	+ / Brown spots	-
	Leguminosae	*Mimosa caesalpiniifolia* Benth.	+ / Mite feeding damage	-
	Malvaceae	*Hibiscus rosa-sinensis* L.	+ / Green spots	+ / C
			+/ Chlorotic spots	+/ N
		Hibiscus syriacus L.	+/ Brown spots	+ / N
	Rubiaceae	*Mussaenda erythrophylla* Schumach. & Thonn.	+ / Green spots	+ / N
	Myrtaceae	*Eugenia uniflora* L.	+ / Chlorotic spots	-
	Orchidaceae	*Arundina graminifolia* (D. Don) Hochr.	+ / Dark spots	+ / C
	Piperaceae	*Piper callosum Ruiz et Pav.*	+ / Chlorotic spots	+ / N
	Polypodiaceae	*Platycerium bifurcatum* (Cav.) C.Chr	*Tenuipalpus* / chlorotic feeding damage	-
	Polypodiaceae	*Polypodium* sp.	+ and Tenuipalpus / feeding damage	-
	Rubiaceae	*Ixora* sp.	+ / Chlorotic ringspot and oak leaf pattern	-
	Solanaceae	*Brugmansia suaveolens* (Willd.) Bercht. & J. Presl.	- / Chlorotic spots	-
	Lamiaceae	*Clerodendrum x speciosum* Tiejsm. & Binn	+ / Chlorotic spots	+ / N
		Clerodendrum speciosissimum Paxt.	+ / Green spots on senescent leaves	- + / C
	Zingiberaceae	*Alpinia* sp.	+ / Chlorotic spots	-
Presidente Figueiredo	Malvaceae	*Hibiscus rosa-sinensis* L.	+ / Chorotic spots	-
	Rutaceae	*Citrus sinensis* Osbeck	+ / Yellow spots	-
Urucu	Anacardiaceae	*Anacardium occidentale* L.	- / Chlorotic spots	-
	Malvaceae	*Hibiscus rosa-sinensis* L.	+ / Chlorotic spots	-
	Orchidaceae	*Maxillaria* sp.	+ / Chlorotic spots	-
		Stanhopea candida Barb. Rodr.	- / Chlorotic spots	-
	Rubiaceae	*Gardenia* sp.	+ / Chlorotic spots	+ / N

[1] + Presence of *Brevipalpus* mites ; - absence.

[2] + Presence and – absence of cytopathic effects in the tissues of the lesion. C = Cytopathic effect of the cytoplasmatic type of *Brevipalpus* transmitted viruses; N = idem, of nuclear type.



FIGS. 1 and 2 - Leaf lesions in different plant specieAs associated with the infection of presumed viruses transmitted by *Brevipalpus* (Tenuipalpidae) mites found in the Amazon basin.

FIG. 1 - A. Chlorotic lesions - *Piper callosum*; **B**. Chlorotic ringspot - *Monstera deliciosa;* **C**. Chlorotic spots/ *Bidens* sp.; **D**. Green spots on senescent leaves - *Allamanda cathartica*; **E**. Brown spots - *Hibiscus syriacus*; **F**. Green spots on senescent leaves - *Mussaenda erythrophylla*.

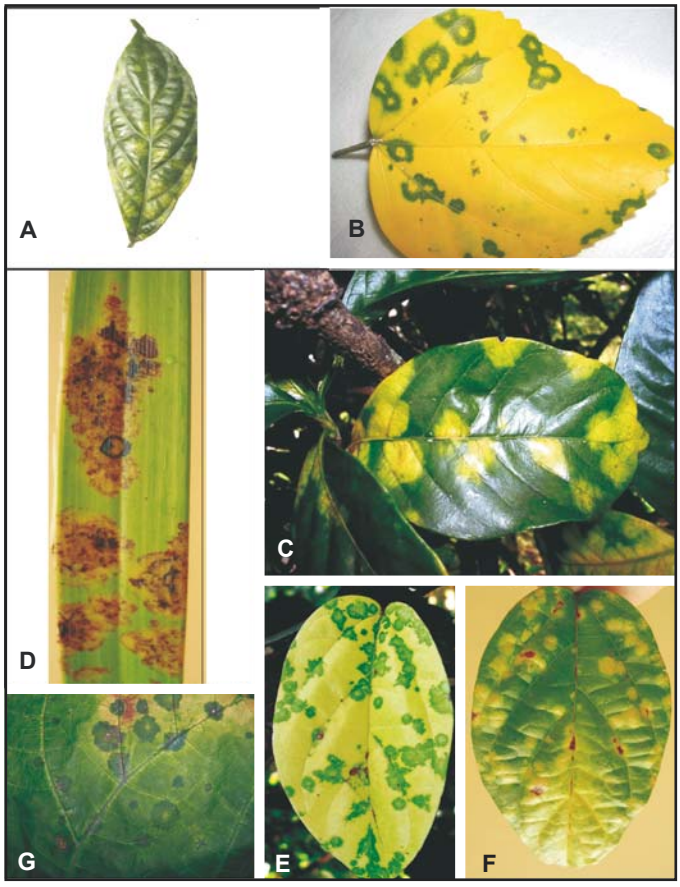
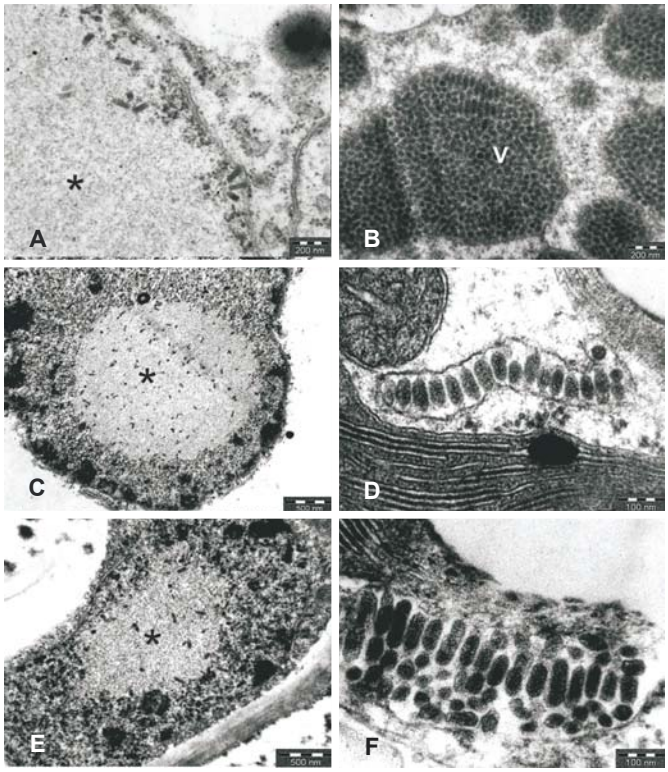


FIG. 2 – **A.** Chlorotic spots - *Ruellia chartacea;* **B**. Green spots - *Hibiscus rosa sinensis*; **C**. Chlorotic spots - *Gardenia* sp. **D**. Brown ringspots - *Arundina graminifolia*; **E.** Green spots on senescent leaves and chloroctis spots; **F**. *Clerodendrum* x *speciosum;* **G**. Green spots - *C. speciosissimum.*



FIGS 3 and 4 - Transmission electron micrographs of thin sections of the leaf lesions from different plant species, showing either the nuclear (N) or cytoplasmic (C) type of cytopathology, caused by viruses transmitted by *Brevipalpus* mites (BrTV) observed in specimens collected in the Amazon basin**.**

FIG. 3 - **A**. Nuclear type of BrTV cytopathology (electron lucent viroplasm [*] in the nucleus and short rod-like particles in the nucleus and cytoplasm) in the chlorotic spots on *Clerodendrum x speciosum;* **B**. A large aggregate of short, bacilliform particles (v) in the cisternae of the endoplasmic reticulum, characteristic of the cytoplasmic type of cytopathology in the parenchymal cell of the green spots on leaves of *C. speciosissimum;* **C**. Nuclear type of cytopathology in a parenchymal cell of chlorotic spots on the leaves of *Hibiscus rosa sinensis*. A viroplasm (*) is clearly seen in the nucleus, in which rod-like particles are interspersed; **D.** Section through green spots on senescent leaves of *H. rosa sinensis*. A row of short, bacilliform particles can be seen within cisternae of the endoplasmic reticulum; **E.** Electron lucent viroplasm (*) in the nucleus of a parenchymal cell from a chlorotic lesion on the leaf of *Piper callosum;* **F.** Group of short bacilliform particles contained in the endoplasmic reticulum in a parenchymal cell from the brown spot on the leaf of *Arundina gaminifolia.*

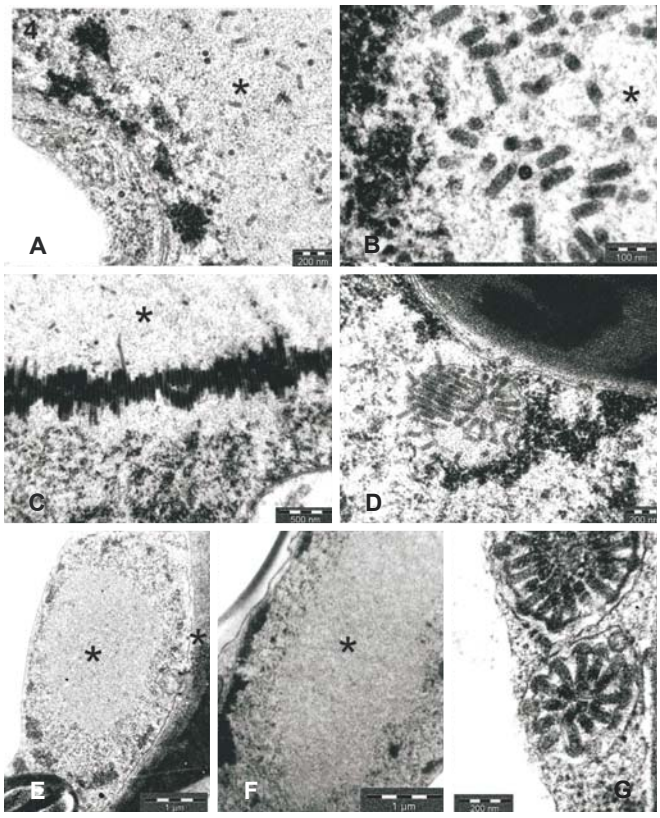


FIG. 4 - **A**. Part of the nucleus of leaf parenchymal cells from the chlorotic lesions in *Bidens sp.* Viroplasm (*) and short rod-like particles can be seen; **B**. Detail of a group of rod-like particles in the nucleus of parenchymal cells in the green spots on the leaves of *Allamanda cathartica;* **C**. A lamellar aggregate of rod-like particles immersed in the viroplasm of a parenchymal cell nucleus in the chlorotic ringspot of *Monstera deliciosa* leaf; **D**. Group of rod-like particles producing the so-called "spokewheel" pattern in the cytoplasm of a chlorotic lesion on *Ruellia chartacea* leaf; **E and F.** Nuclear viroplasm (*) in parenchymal cells from chlorotic lesions on the leaves of *Gardenia sp.;* **E.** and *Mussaenda erythrophylla;* **F.** respectively; **G.** Detail of spokewheel configurations of viral particles in a parenchymal cell of a leaf lesion of *Hibiscus syriacus.*

two types has been observed (Kitajima *et al.*, 2003; 2006).

Bleeding heart (*Clerodendrum x speciosum* Tiejsm. & Binn.) plants with chlorotic spots and green spots in senescent leaves (Figures 2 E and F) were found in a residential garden in Manaus, associated with *B. phoenicis* infestation. Electron microscopic examination of the lesions revealed that chlorotic spots (Figure 2 F) showed cytopathic effect of the nuclear type of BTrV (Figure 3A), while in the tissues of the green spot lesions, cell alterations were of the cytoplasmic type of BTrV. As in the case of *Hibiscus* spp., bleeding heart is susceptible to both types of BTrV (Kitajima *et al.*, 2003). The putative nuclear type of BTrV found in bleeding heart is probably the previously described *Clerodendrum* chlorotic spot virus (ClCSV) (Kitajima & Moraes, 2000; Kitajima *et al.*, 2007), which was recently purified and had its genome partially sequenced (Boari *et al.*, 2006; Kubo, K.S., unpublished data). Another species of *Clerodendrum* found in Manaus, *C. speciosissimum* Paxt., with green spot symptoms (Figure 2 G), showed a cytoplasmic type cytopathology in the cells of the lesion (Figure 3 B). This is a new species of the genus *Clerodendrum* (Lamiaceae) found to be infected with a putative BTrV, besides *C. x speciosum, C. thomsonae* Bauf. and *C. splendens* Don. (Kitajima *et al.*, 2003; 2006).

This survey revealed other previously unreported host plant species for putative BTrV (Table 1) such as *Ruellia chartacea* (T. Anderson) Wash. (Acanthaceae) (Figure 2 A), *Allamanda cathartica* L. (Apocynaceae) (Figure 1 D), *Monstera deliciosa* Liebm. (Araceae) (Figure 1 B), *Bidens sp.* (Asteraceae) (Figure 1 C), *Mussaenda erythrophylla* Schumach. & Thonn. (Figure 1 F) and *Gardenia sp.* (Figure 2 C) (Rubiaceae) and *Piper callosum* Ruiz et Pav. (Piperaceae) (Figure 1A). They exhibited symptoms and cytopathological features consistent with those of BTrV's (Figures 4 A/*Bidens sp.*; 4 B/ *Allamanda cathartica*; 4 C/*Monstera deliciosa*; 4 D/*Ruellia chartacea*; 4 E/*Gardenia sp.*; 4 F/*Mussaenda erythrophylla*).

Gardenia chlorotic spot (Figure 2 C) was associated with the presence of a putative nuclear type BTrV (Figure 4 E). The symptomatic plants were located in Urucu, which is an isolated oil and gas exploration facility located in the forest about 700 km southwest of Manaus. The infected plants were observed in a garden around the dormitories within this oil exploration complex. No other BTrV's were found in native or ornamental plants in the vicinity, which suggests that the virus and vector were introduced with the *Gardenia sp.* plants brought in for the garden. This reinforces the concern that ornamental plants could act as pathways for the introduction of these viruses into new areas. Most of the virus hosts found in this study were plants that are not native to the Amazon.

Ixora sp. (image not shown), *Piper callosum* (Figure 1A) and *Ruellia chartacea* (Figure 2A) with chlorotic spots, *Monstera deliciosa* (Figure 1B) with ringspots, and *Mussaenda erythrophylla* (Figure 1 F) and *Allamanda chartacea* (Figure 1D) with green spots, (Figures 3 C, 4 D, 4 C, 4 F and 4 B, respectively) showed cell changes of the nuclear type of BTrV. However, there is no information about whether or not these putative nuclear types of BTrV found in these plants are distinct from other viruses previously reported. There is one previous description in the Amazon basin (Tome Açú, State of Pará) of a putative nuclear type of BTrV in black pepper (*Piper nigrum* L.) (Yamashita *et al.*, 2004).

Bidens sp., a common Asteraceae weed, was found with chlorotic spots on the leaves (Figure 1 C), associated with infestation by *B. phoenicis*, and cytopathology typical of the nuclear type of BTrV (Figure 4 A) in the tissues of the lesions. In Gainesville, Florida and in San Juan, Puerto Rico, the senior author observed high infestations of *B. phoenicis* mites on *Bidens pilosa* L. plants, although neither symptoms nor virus particles were detected. Also in Florida, Knorr (1968) reported that a year after *Brevipalpus* mites were transferred from non-symptomatic *Bidens* plants to sweet orange seedlings, leprosis-like symptoms appeared on the sweet orange. Because assays were conducted with citrus-to-citrus transmission at the same time, cross-contamination could have occurred. However, these results, along with our observations in the field and the growing list of *Brevipalpus*-transmitted virus hosts, strongly suggest that the BTrV can move between different plant species, genera, or families (Rodrigues *et al.*, 2005).

The only example, in this survey, of the putative cytoplasmic type BTrV in the Amazon basin, besides HGSV and the green spots on *C. x speciosum* leaves, was found in leaf tissues of *Arundina graminifolia* (D. Don) Hochr., an orchid found growing in Manaus. RT-PCR conducted using primers designed for Orchid fleck virus (OFV-nuclear type) (Blanchfield *et al.*, 2001), did not generate amplification products from *A. graminifolia* samples (Karen S. Kubo, unpublished data). This putative cytoplasmic type BTrV found in *Arundina* may be similar to that previously found in São Paulo State (Freitas-Astua *et al.*, 1999). Like most of the putative BTrV-positive plants reported in this work, the terrestrial bamboo orchid, *A. graminifolia*, is an exotic species in the Amazon region, introduced from Southeast Asia, which has been heavily cultivated and naturalized in some tropical areas (Smith, 1991). This plant could represent an additional source for the introduction of plant pests and viruses, as well as being a reservoir for them.

Crops such as coffee (*Coffea arabica* L.), sweet orange (*Citrus sinensis* (L.) Osbeck) and passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) are economically important host plants for BTrV, and these plants were inspected for possible infection by these viruses during the current study. Despite the occurrence of infestation by *Brevipalpus* mites, no symptoms attributable to BTrV were observed. There are recent reports of the detection of CiLV-C in sweet orange plantations in Itacotiara and Manaus, AM (Freitas-Astua *et al.*, 2006) and Capitão Poço, state of Pará (Boari *et al.*, 2007).

During the development of this work, a few BTrV

genomes were partially or completely sequenced: OFV (Kondo *et al.*, 2006), Coffee ringspot virus (CoRSV) (Locali *et al.*, 2005), ClCSV (Karen S. Kubo, unpublished data), citrus leprosis of the cytoplasmic type (CiLV-C) (Pascon *et al.*, 2006; Locali-Fabris *et al.*, 2006) and *Solanum violaefolium* ringspot virus (SvRSV) (Ferreira *et al.*, 2004). Antibodies are also now available for OFV, CoRSV (Boari *et al.*, 2004) and ClCSV (Boari *et al.*, 2007). This information will be useful in the further characterization of the putative BTrV reported here using molecular and immunological tools to confirm their relationship with better-characterized BTrV.

Haramoto (1969) suggested that the genus *Brevipalpus* originated in the tropics although it was first described in Northern Europe. This assumption could be extended to the viruses vectored by *Brevipalpus*, based on the growing number of plants reported to be susceptible to BTrV in tropical regions. (Childers *et al.*, 2003). If these viruses did not originate in the tropics then these regions are probably new centers of diversification, perhaps because of the favorable conditions for spread and perseverance. It should be mentioned that so far, BTrV's and putative BTrV's have been found only in the American continent (Kitajima *et al.*, 2003; 2006), except OFV, which has a worldwide distribution due to the intense exchange of orchids by collectors and global trade. The same fate may occur with other ornamentals, as many of them seem to be susceptible to BTrV's and putative BTrV's. An introduction of the *Brevipalpus* mite vector and the virus may result in their spread to other ornamentals and cultivated plants, further stressing the potential importance of this virus group.

ACKNOWLEDGEMENTS

Thanks are due to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for providing two visiting fellowships (October, 2004 and January, 2007); CT-Petro Amazônia Network; Finep for partial support for the project "Artificial regeneration technology applied to forest gaps resulting from oil and gas exploration"; Petrobrás for financial and logistic support; Amazon Biotechnology Center (CBA)/Biochemistry and Molecular Biology Laboratory and Fapeam, Manaus for a Fix-1A fellowship (2005) to the senior author. This work received financial support from Fundação de Amparo do Estado de São Paulo FAPESP (Proc. 2000/11805-0). Thanks are due to Priscilla Antony (INPA) for help with identification of some symptomatic plant species, to Dra. Denise Navia, Embrapa/Cenargen, for receiving the voucher specimens, and to S. C. Garrett-Rodrigues for reviewing a draft version of the manuscript.

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Received 8 August 2007 - Accepted 28 January 2008 - TPP 7086
Associate Editor: Álvaro M.R. Rodrigues