

# First report of *Tomato chlorotic spot virus* on *Mirabilis jalapa*

L. M. L. Duarte<sup>1</sup> · A. L. R. Chaves<sup>1</sup> · E. W. Kitajima<sup>2</sup> · L. K. Rodrigues<sup>1</sup> ·  
R. Harakava<sup>3</sup> · M. A. V. Alexandre<sup>1</sup>

Received: 25 September 2015 / Accepted: 14 March 2016 / Published online: 29 March 2016  
© Australasian Plant Pathology Society Inc. 2016

**Abstract** Mosaic symptoms in *Mirabilis jalapa* plants from a public garden in the city of São Paulo, Brazil, were associated with the presence of *Tomato chlorotic spot virus* (TCSV-Mir-BR07). TCSV-Mir-BR07 was mechanically transmitted to healthy *M. jalapa* plants. Phylogenetic analysis revealed a monophyletic group formed by South American TCSV isolates separate to those from Central and North America. This is the first report of natural TCSV infection in *M. jalapa*.

**Keywords** *Tospovirus* · *Bunyaviridae* · Four-o'clock plant · RT-PCR · Phylogeny

*Mirabilis jalapa* (Nyctaginaceae), also known as the four-o'clock plant, is an ornamental flowering plant from Peru widely used in public and private gardens in Brazil for the beauty of its flowers (Souza and Lorenzi 2012). Moreover,

it is easy to grow from seeds, cuttings and tubers. *M. jalapa* also has medicinal uses, since it contains several active compounds extracted from different organs, including ribosome-inactivating protein (RIP) associated with anti-viral activity, antifungal phenolic compounds, antimicrobial peptides, and natural antioxidant and antimicrobial agents (Hajji et al. 2010).

Only a few natural viral infections of *M. jalapa* have been reported: *Parietaria mottle virus* (*Ilarvirus*) in Italy, *Mirabilis jalapa mottle virus* (*Carlavirus*) in the USA and *Basella rugose mosaic virus* (*Potyvirus*) in China (Parrella 2002; Hatlestad et al. 2011; Wang et al. 2012). A new species of *Caulimovirus* named *Mirabilis mosaic virus* was also described in the United States (Brunt and Kitajima 1973); however, the host was *Mirabilis nyctaginea*, a synonym for *Oxybaphus nyctagineus*. In Brazil, *M. jalapa* has not been described as a natural virus host.

In a survey aimed at detecting viruses in urban ornamental species in the city of São Paulo, Brazil, a garden containing *M. jalapa* plants exhibiting systemic foliar mosaic (Fig. 1) attracted our attention. In order to identify the causal agent, electron microscopy, experimental transmission studies to other host species, RT-PCR and sequencing were carried out.

Several quasi-spherical particles approximately 100 nm in diameter were observed in naturally infected, negatively stained *M. jalapa* leaf extract. In thin sections of the leaf lesions, membrane-bounded particles about the same size of those found in extracts, were found in the lumen of the endoplasmic reticulum (Fig. 2) suggesting that these *M. jalapa* plants were infected with a tospovirus.

<sup>1</sup> Laboratório de Fitovirologia Fisiopatológica, Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, CEP 040140-002, São Paulo, SP, Brazil

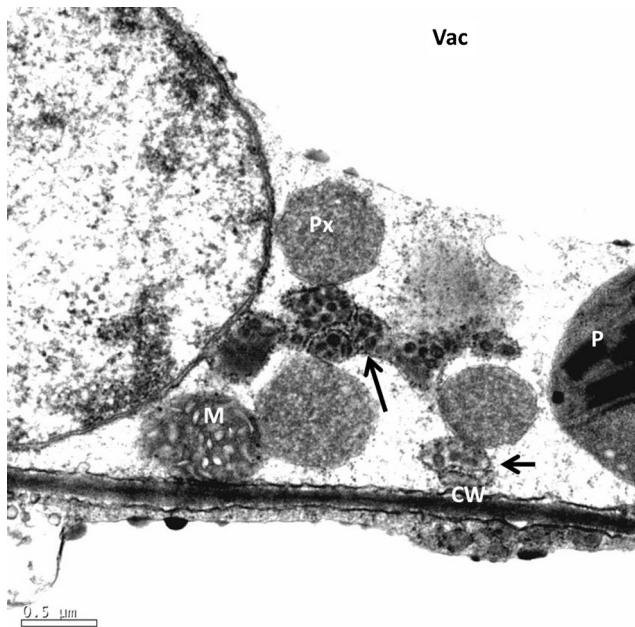
<sup>2</sup> Escola Superior de Agricultura Luiz de Queirós, USP, CP 09, CEP 13418-900, Piracicaba, SP, Brazil

<sup>3</sup> Laboratório de Bioquímica Fitopatológica, Biológico, Av. Conselheiro Rodrigues Alves, 1252, CEP, 040140-002, São Paulo, SP, Brazil



**Fig. 1** Mosaic symptoms on naturally infected *Mirabilis jalapa* leaves

Host plants, including *M. jalapa*, were grown from seeds and kept in insect-proof greenhouses. The sanitary status of *M. jalapa* plants was verified by electron microscope observations. Six plants of each species (*Chenopodium amaranticolor*, *Gomphrena globosa* (Amaranthaceae), *Datura stramonium* and *Nicotiana benthamiana* (Solanaceae) and twenty healthy *M. jalapa* plants were mechanically inoculated with *M. jalapa* infected leaf. One plant of each species was



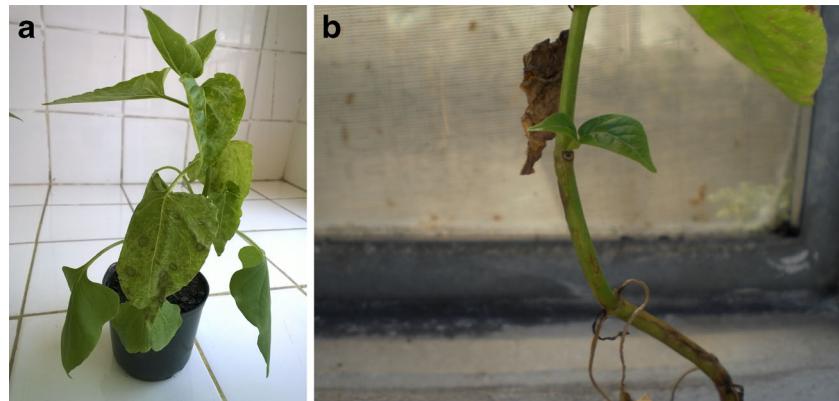
**Fig. 2** In situ transmission electron micrograph of isolate of *Tomato chlorotic spot virus*, in a leaf parenchymal cell of a naturally infected *Mirabilis jalapa* plant. Presumed virions, as judged by morphology, within cisternae of the endoplasmic reticulum (arrows). CW cell wall, M mitochondrion, P chloroplast, Px peroxisome, Vac vacuole

rubbed with inoculation buffer as control. The virus was not transmitted from the donor plant to other hosts, likely due to the presence of potent virus-inhibiting substances common in Caryophyllales species (Chessin 1983). On the other hand, it was transmitted to five of the healthy *M. jalapa* plants, but with more severe symptoms than those seen in the donor plant, including leaf and stem necrosis (Fig. 3). Inoculated plants were kept in a greenhouse at an average temperature of around 30 °C, which could explain the severity of symptoms. According to Llamas-Llamas et al. (1998), a day/night temperature regime of 29/24 °C favoured infection by the tospovirus *Tomato spotted wilt virus* (TSWV) in *D. stramonium*, *N. tabacum* and *Physalis ixocarpa* and resulted in more severe symptoms.

Total RNA from natural and experimentally infected four-o'clock plants was extracted using Trizol LS reagent, according to the manufacturer's instructions (Gibco BRL, USA), precipitated with ethanol and used as a template in RT-PCR with BR60 and UNIV1 primers (Mumford et al. 1996). A ca. 800 bp fragment corresponding to part of the conserved region of *Tospovirus* S RNA was amplified and sequenced using forward and reverse primers, with three replications. After trimming low quality bases at the 5' and 3' ends of the sequenced PCR product, a 678 bp sequence obtained from naturally infected four-o'clock plant was compared to those deposited in GenBank. BlastN analysis confirmed that the sequence of *M. jalapa* tospovirus was similar to *Tomato chlorotic spot virus* (TCSV). The highest nucleotide and amino acid identities were recorded in the TCSV sequences isolated from vegetable and ornamental plants from different geographic regions (Table 1).

Maximum likelihood (ML) analyses (PAUP v. 4.0b10 for Macintosh) were performed using the star-decomposition algorithm and tree bisection and reconnection (TBR) branch-swapping algorithm after estimation of nucleotide substitution and gamma distribution (Modeltest v.3.06). Nucleotide sequences corresponded to the nucleocapsid gene available in GenBank for: i) the *Tospovirus* species, and ii) TCSV isolates. Bootstrap values were calculated using the branch-and-bound method after resampling (1000 replicates). Our results confirmed the findings of Torres et al. (2012), who reported the occurrence of a group of American tospoviruses, based on the formation of a monophyletic group by American *Tospovirus* species, substantiated by a high bootstrap value. *M. jalapa* tospovirus formed a sister group with TCSV, with a 95 % bootstrap value (Fig. 4a).

**Fig. 3** Experimentally infected *Mirabilis jalapa*. **a** Plant showing severe mosaic, leaf deformation, chlorotic and necrotic spot symptoms. **b** Plant showing necrotic lesions on the stem



Phylogenetic analysis of TCSV isolates showed a tendency for clustering according to the geographic region (Fig. 4b). South American isolates formed a separate monophyletic group to those from Central and North America. Webster et al. (2015) reported that the RNAs of TCSV isolates from Florida (USA), Puerto Rico, and the Dominican Republic all share high levels of nucleotide identity with the corresponding RNAs of TCSV

**Table 1** Nucleotide (nt) and amino acid (aa) sequence identities (%) in *Tomato chlorotic spot virus* isolated from *Mirabilis jalapa* (KP276236) as well as different hosts and geographic regions

GenBank number	nt	aa
AF282982 (BR)	99.0	100
KJ399303 (DR)	98.5	98.8
JX244198 (USA)	97.7	97.7
JX 244196 (USA)	98.5	98.8
S54325 (BR)	93.8	91.9
AF521102 (BR)	98.8	100
AF413110 (BR)	93.4	90.7
KP172480 (USA)	98.1	98.8
KJ399304 (DR)	98.5	98.8
HQ634666 (SA)	98.1	98.8
KM610235 (USA)	97.3	96.5
AM887766 (BR)	98.1	100
AY380813 (BR)	98.1	98.8
AF521103 (BR)	98.5	100
U49709 (AR)	96.1	97.7
JQ034525 (BR)	96.1	98.8
AF454913 (BR)	98.5	100

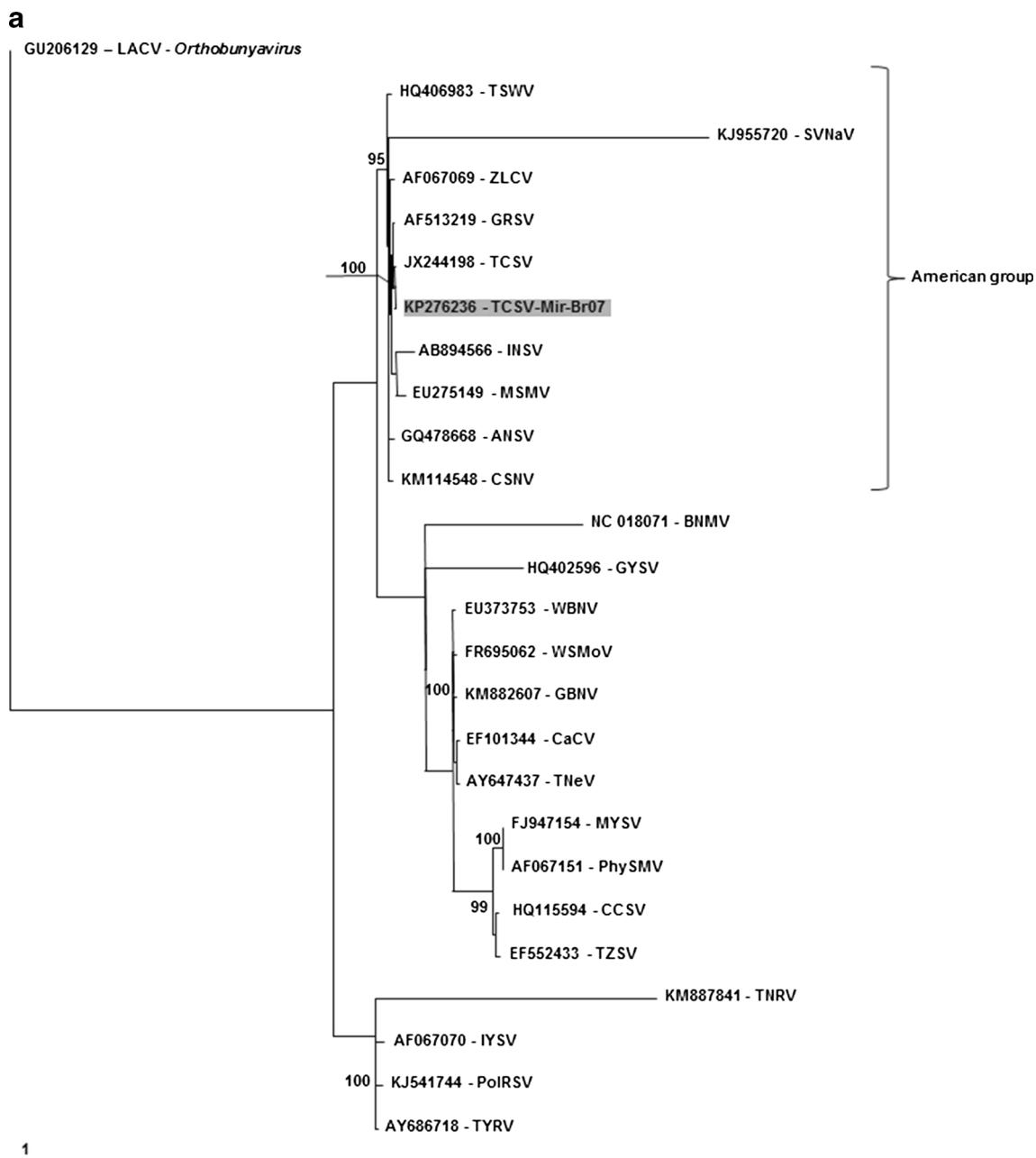
AR = Argentina, BR = Brazil, DR = Dominican Republic, SA = South Africa, USA = United States of America

isolates from South America. With regard to Brazilian TCSV, the isolate from *M. jalapa* (TCSV-Mir-BR07, GenBank number KP276236) formed a monophyletic group with isolates from the states of Rio Grande do Sul (JQ034525) and Minas Gerais (AF282982), as well as Argentina (U49709), in the maximum-likelihood tree. The isolate from *P. peruviana* clustered with Argentinean isolates, corroborating the findings of Eiras et al. (2012). It is important to note that Rio Grande do Sul borders Argentina, possibly facilitating the spread of TCSV, and that the virus is highly prevalent in the border region between Brazil and Argentina (Williams et al. 2001).

Recombination analysis for sequences of TCSV isolates ( $P$ -value = 0.05) conducted using RDP3 software (Martin et al. 2010) was inconclusive because it was not substantiated by at least four of the seven methods implemented in RDP. Furthermore, there are few complete sequences of the TCSV N gene in GenBank, hampering greater robustness of the analysis.

Although tospoviruses can be mechanically transmitted under experimental conditions, their dispersal and survival in nature depends on transmission to plants via thrips vectors (Rotenberg et al. 2015). Approximately 520 thrips species (Thysanoptera) from 139 genera and six families are known in Brazil, with the Terebrantia suborder being the major *Tospovirus* vector species in tropical and subtropical regions (Monteiro 2002). However, there are no reports in the literature of thrips in *M. jalapa* in Brazil.

To date, there is no record of natural tospovirus infection in *M. jalapa*. However, studies conducted in Mexico and Japan found that *M. jalapa* was an experimental host for TSWV (Martinez et al. 1999). On the other hand, it was not experimentally infected by INSV (Okuda et al. 2010). It is noteworthy that neither natural

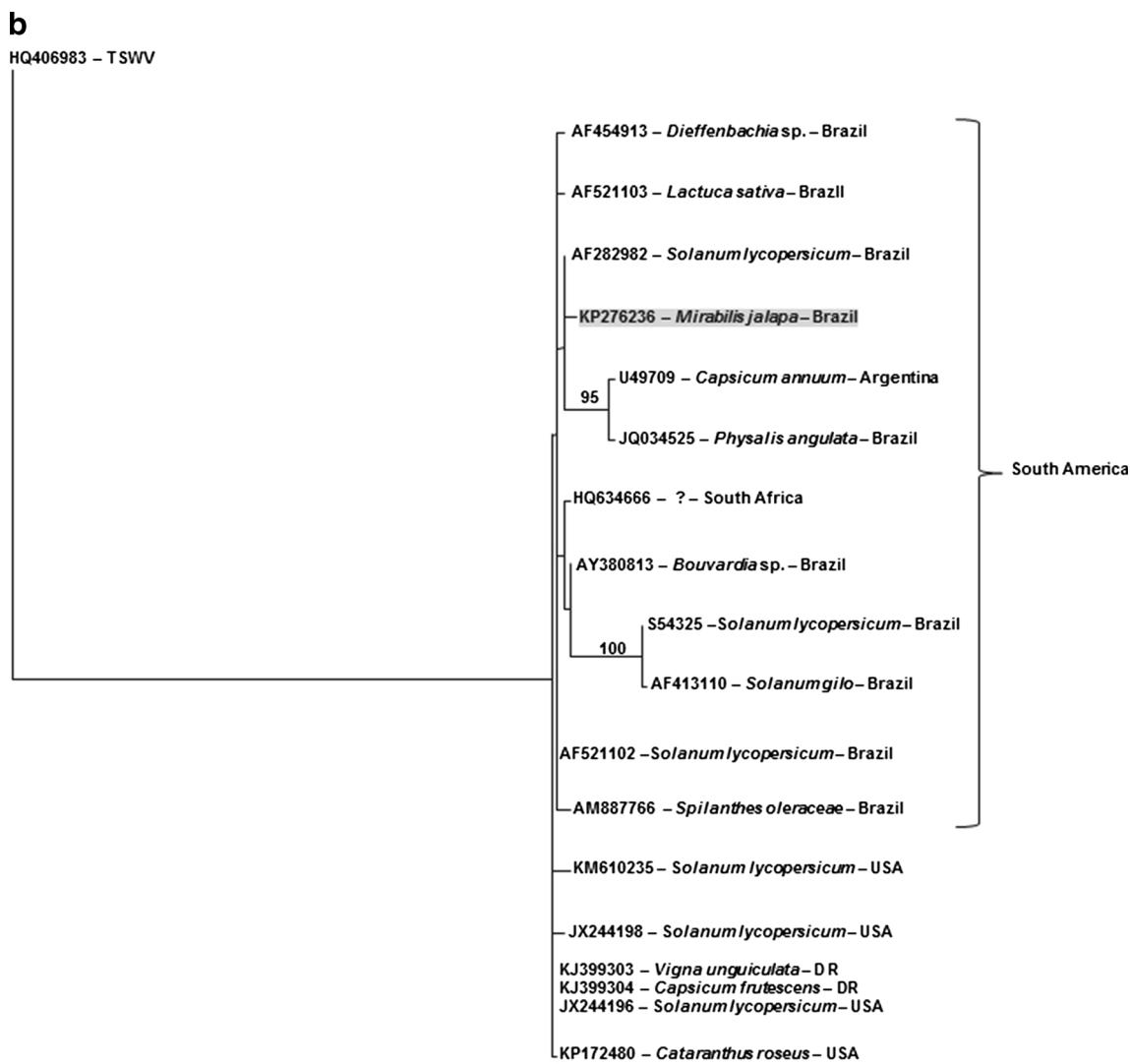


**Fig. 4** A maximum likelihood tree constructed using nucleocapsid (N) partial sequences available in GenBank for (a) The *Tospovirus* species, with the *La Crosse virus* (LACV – *Orthobunyavirus*) as outgroup (TrN nucleotide substitution model + I + G, I = 0.0107, G = 1.1360). (b) The *Tomato chlorotic spot virus* (TCSV) isolates from different hosts and geographic regions, with the Tomato spotted wilt virus (TSWV) as outgroup (HKY nucleotide substitution model). ANSV Alstroemeria necrotic streak virus, BNMV Bean necrotic mosaic virus, CaCV Capsicum chlorosis virus, CCSV Calla lily chlorotic spot virus, CSNV Chrysanthemum stem necrosis virus, GBNV Groundnut bud necrosis

virus, *GRSV* Groundnut ringspot virus, *GYSV* Groundnut yellow spot virus, *INSV* Impatiens necrotic spot virus, *IYSV* Iris yellow spot virus, *MSMV* Melon severe mosaic virus, *MYSV* Melon yellow spot virus, *PhySMV* Physalis severe mottle virus, *PolRSV* Polygonum ringspot virus, *SVNaV* Soybean vein necrosisassociated virus, *TNeV* Tomato necrosis virus, *TNRV* Tomato necrotic ringspot virus, *TZSV* Tomato zonate spot virus, *TYRV* Tomato yellow ring virus, *ZLCV* Zucchini lethal chlorosis virus, *WBNV* Watermelon bud necrosis virus, *WSMoV* Watermelon silver mottle virus. Bootstrap values are indicated near the branches

nor experimental infections by TCSV in *M. jalapa* have been reported.

Although *M. jalapa* plants are also propagated by seeds and TCSV cannot be transmitted in this manner,



**Fig. 4** (continued)

TCSV-infected plants were detected in a public garden in São Paulo, Brazil, suggesting an accidental infection. Thrips may have acquired the virus on colonizing another infected species and transmitted it to the four-o'clock plants. This is the first report of natural TCSV infection in *M. jalapa*.

## References

Brunt AA, Kitajima EW (1973) Intracellular location and some properties of *Mirabilis* mosaic virus, a new member of the Cauliflower mosaic group of viruses. *Phytopath Z* 76:265–275

Chessin M (1983) Is there a plant interferon? *Botanic Rev* 49:1–28

Eiras M, Costa IFD, Chaves ALR, Colaricco A, Harakava R, Tanaka FAO, Garcêz RM, Silva LA (2012) First report of a tospovirus in a commercial crop of Cape gooseberry in Brazil. *New Dis Rep* 25:25

Hajji M, Jarraya R, Lassoued I, Masmoudi O, Damak M, Nasri M (2010) GC/MS and LC/MS analysis, and antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis jalapa* tubers. *Process Biochem* 45:1486–1493

Hatlestad GJ, Elam L, Gonzalez A, Lloyd AM (2011) *Mirabilis jalapa* mottle virus: a new carlavirus infecting four o'clock. *Arch Virol* 156: 2109–2111

Llamas-Llamas ME, Zavaleta-Mejia B, Gonzalez-Hernandez VA, Cervantes-Diaz L, Santizo-Rincon JA, Ochoa-Martinez DL (1998) Effect of temperature on symptom expression and accumulation of tomato spotted wilt virus in different host species. *Plant Pathol* 47: 341–347

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefevre P (2010) RDP 3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* 26:2462–2463

Martinez DLO, Zavaleta-Mejia E, Mora-Aguilera G, Johansen RM (1999) Implications of weed composition and thrips species for the epidemiology of *Tomato spotted wilt virus* in chrysanthemum (*Dendranthema grandiflora*). *Plant Pathol* 48: 707–717

Monteiro CM (2002) The Thysanoptera fauna of Brazil. In: Marullo R, Mound LA (eds) Thrips and tospovirus: proceedings of the 7th International Symposium on Thysanoptera. Australian National Insect Collection, Canberra, pp 325–340

Mumford RA, Barker I, Wood KR (1996) An improved method for detection of Tospoviruses using the polymerase chain reaction. *J Virol Meth* 57:109–115

Okuda M, Fuji S, Okuda O, Sako K, Iwanami T (2010) Evaluation of the potential of thirty-two weed species as infection sources of *Impatiens* necrotic spot virus. *J Plant Pathol* 92(4):357–361

Parrella G (2002) First report of *Parietaria mottle virus* in *Mirabilis jalapa*. *Plant Pathol* 51(3):401

Rotenberg D, Jacobson AL, Schneweis AN, Whitfield AE (2015) Thrips transmission of tospoviruses. *Curr Opin Virol* 15:80–89

Souza VC, Lorenzi H (2012) Botânica sistemática. Instituto Plantarum, Nova Odessa

Torres R, Larenas J, Fribourg C, Romero J (2012) Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. *Arch Virol* 157:609–615

Wang JG, Peng JJ, Chen HR, Chen SY (2012) First report of *Basella rugose mosaic virus* infecting four o'clock (*Mirabilis jalapa*) in China. *Plant Dis* 96:294

Webster CG, Frantz G, Reitz SR, Funderburk JE, Mellinger HC, McAvoy E, Turechek WW, Marshall SH, Tantiwanich Y, McGrath MT, Daughtrey ML, Adkins S (2015) Emergence of *Groundnut ringspot virus* and *Tomato chlorotic spot virus* in vegetables in Florida and the southeastern United States. *Phytopathology* 105:388–398

Williams LV, López Lambertini PM, Shohara K, Biderbost EB (2001) Occurrence and geographical distribution of tospovirus species infecting tomato crops in Argentina. *Plant Dis* 85:1227–1229