Identification of *Taeniothrips eucharii* (Thysanoptera: Thripidae) as a Vector of Hippeastrum chlorotic ringspot virus in Southern China

Ye Xu, College of Plant Protection, Yunnan Agricultural University, Kunming 650201, P.R. China; Xue Gao, Zhiqiang Jia, and Wengui Li, Center of Crop Science Experimental Teaching, College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming 650201, P.R. China; John Hu, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, 96822; Yajin Li, College of Plant Protection, Yunnan Agricultural University, Kunming 650201, P.R. China; Yongzhong Li,[†] Yunnan Plateau Characteristic Agricultural Industry Research Institute, Kunming 650201, P.R. China; and Yating Liu,[†] Center of Crop Science Experimental Teaching, College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming 650201, P.R. China

Abstract

Hippeastrum chlorotic ringspot virus (HCRV) is a putative new member of the genus *Tospovirus*, which was first identified infecting *Hymenocallis littoralis* in Yunnan Province, southwestern China. *Taeniothrips eucharii*, the dominant thrips species found on *H. littoralis*, was tested for its efficiency as a vector of the virus. We used Western blot analysis

of adult thrips to detect the N protein. Transmission experiments demonstrated an average acquisition efficiency of $43.7 \pm 3.4\%$ (AAP = 24 h) for the first larval stage and a transmission efficiency of $19.1 \pm 2.4\%$ (IAP = 24 h) for adult thrips. This study reports *T. eucharii* as a new genus and species of thrips transmitting a *Tospovirus*, HCRV.

Thrips are insects belonging to the order Thysanoptera and over 6,000 species have been recorded worldwide (Mound and Morris 2004, 2007). Thrips transmit plant viruses classified in five genera (Jones 2005): *Tospovirus*, *Ilarvirus* (Sdoodee and Teakle 1993), *Carmovirus* (Krczal et al. 1995), *Sobemovirus* (Hardy and Teakle 1992), and *Machlomovirus* (Cabanias et al. 2013).

The *Tospovirus* genus is the sole genus in the family *Bunyaviridae*, which contains plant-infecting species (Whitfield et al. 2005). The typical *Tospovirus* genome contains three single-stranded RNAs that encode six viral proteins. The type species, *Tomato spotted wilt virus* (TSWV), infects more than 900 plant species. Currently 15 species of thrips are known to be vectors of *Tospovirus* species (Riley et al. 2011). They belong to six genera: *Frankliniella* (van de Wetering et al. 1996), *Thrips* (Wijkamp et al. 1995), *Scirtothrips* (Gopal et al. 2010), *Ceratothripoides* (Premachandra et al. 2005), *Neohydatothrips* (Keough et al. 2016), and *Dicthyothrips* (Ciuffo et al. 2010).

Since 1930, nine thrips species have been reported as vectors of TSWV (De Borbón et al. 1999; Ohnishi et al. 2006; Whetzel 1923b). A study of TSWV revealed that thrips could only acquire the virus in the first and early second larval stages (Moritz et al. 2004). After acquisition, the virus persists in the thrips tissues throughout its life.

Hippeastrum chlorotic ringspot virus (HCRV), a putative new member of the *Tospovirus* genus, was recently identified and its genome sequenced (Dong et al. 2013; Xu et al. 2013, 2014). Virus protein sequence analysis revealed that HCRV is closely related to *Polygonum ringspot virus* (PolRSV) (Ciuffo et al. 2008), *Tomato yellow ring virus* (TYRV) (Hassani-Mehraban et al. 2005), and *Iris yellow spot virus* (IYSV) (Cortês et al. 1998). Laboratory and field tests of the host range of HCRV suggest that this virus can infect many vegetables and flowers, including *Cucumis sativus*, *Cucurbita moschata*, *Emilia sonchifolia*, *Hippeastrum striatum*, *Hymenocallis littoralis*, *Lactuca sativa*, *Solanum lycopersicum*, *Phaseolus vulgaris*, *Tropaeolum majus*, and *Vigna unguiculata* (Xu et al. 2013).

Symptoms expressed by HCRV-infected plants include concentric chlorotic rings, leaf malformation, and local chlorosis and necrosis. Field surveys showed that HCRV was epidemic in landscape plantings of *H. littoralis* in the south of China (Dong et al. 2013; Xu et al. 2013, 2014) and *T. eucharii* was also common (O'Neill 1963); however, whether *T. eucharii* could transmit HCRV or other tospoviruses was unknown. The main objective of this study was to confirm the competence of *T. eucharii* as a vector of HCRV by measuring acquisition and transmission efficiency.

Materials and Methods

Virus isolation and maintenance. HCRV HLS1-2, an isolate from *H. littoralis* in Kunming City, Yunnan Province (Dong et al. 2013; Xu et al. 2013, 2014), was used in this study. It was identified by indirect enzyme-linked immunosorbent assay (ID-ELISA) using specific polyclonal antibodies. The virus was maintained by mechanically inoculating *E. sonchifolia* and *Nicotiana benthamiana* with the virus in a buffer of 0.01 M Tris-HCl, pH 7.8, 0.01 M Na₂SO₃, and 0.006 M cysteine HCl with plants maintained at $25 \pm 2^\circ\text{C}$ in an insect-proof glasshouse. Symptom development was monitored weekly. Leaflets were selected for the acquisition tests after the first symptoms of disease appeared.

Thrips identification. Specimens of *T. eucharii* were identified by Prof. H. R. Zhang of Yunnan Agricultural University based on morphological characteristics following published descriptions (Hodder et al. 2012). Slides with voucher specimens were deposited at the Department of Entomology, College of Plant Pathology, Yunnan Agricultural University. *T. eucharii* were reared and maintained on *Allium porrum* and the pods of *P. vulgaris* at $25 \pm 2^\circ\text{C}$ and a 16-h light and 8-h dark cycle.

Western blot analysis of field-collected *T. eucharii*. The recombinant *E. coli*-expressed NP of HCRV as an immunogen was utilized to induce rabbit polyclonal antibodies. The resulting antibodies were used for the development of a Western blot assay that indicated the antibodies were specific for HCRV (data not presented). Five adult *T. eucharii* individuals were collected from HCRV-infected plants. All thrips were fed on noninfected *A. porrum* and the pods of *P. vulgaris* at $25 \pm 2^\circ\text{C}$ and a 16-h light and 8-h dark cycle for 3 days to purge their guts of potentially virus-containing plant tissue. Adults were analyzed for virus using a modified method of van den Heuvel et al. (1994). Adults were macerated in 100 μl SDS-PAGE sample buffer (62 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol) and incubated at 90°C for 5 min. The extract was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 12% acrylamide). The separated

[†]Corresponding authors: Yongzhong Li, E-mail: liyongzhong168@163.com; and Yating Liu, E-mail: liuyating66@163.com

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is published online.

proteins were transferred to a 0.22- μ m PVDF membrane (Merck Millipore, Darmstadt, Germany), and HCRV-specific proteins were identified by Western blot analysis. Purified immunoglobulin G (IgG) was used as a primary antibody (dilution 1:5,000) for identifying HCRV N proteins and an HRP-conjugated goat anti-rabbit IgG (H+L) was used as a secondary antibody (Vazyme, Nanjing, China). Bands were detected with Immobilon Western chemiluminescent HRP substrate (Millipore Corporation, Billerica, U.S.A.). Five adult *T. eucharii* were collected from HCRV-infected plants and analyzed by Western blotting. Total protein extracts from HCRV-infected *E. sonchifolia* were used as positive controls. Total protein extracts from healthy *E. sonchifolia* and from *F. occidentalis* individuals raised in the laboratory were used as negative controls.

Virus acquisition and transmission experiments with *T. eucharii*. Virus-free *T. eucharii* colonies were obtained after being reared on healthy *A. porrum* and green bean pods. For virus acquisition, approximately 100 first-stage larval instars were placed on the detached systemically infected leaves of *E. sonchifolia* in a closed cage for a 24-h acquisition access period (AAP) (Chatzivassiliou et al. 1999). The assay was repeated three times. The larval instars were then transferred to noninfected *A. porrum* and reared to adults for the transmission experiment. After rearing, 56, 72, and 46 individual adult thrips were collected respectively from the three assays.

Virus transmissions were performed in 1.5-ml Eppendorf tubes with each tube containing a single *E. sonchifolia* seedling (Supplementary Fig. S1). One adult thrips from the acquisition study was introduced into each Eppendorf tube for a 24-h inoculation access period (IAP). Each thrips was then carefully removed and tested individually for HCRV using ID-ELISA. The *E. sonchifolia* seedlings were transplanted and moved to an insect-proof greenhouse and grown at $25 \pm 2^\circ\text{C}$. Symptom development was monitored weekly and mature leaves collected about 3 weeks after the IAP for detection of HCRV by ID-ELISA. To ensure that all thrips used in the

transmission assays were viruliferous, thrips that fed on *E. sonchifolia* testing negative for HCRV following the ID-ELISA assays were excluded from transmission rate assays. The efficiency of virus acquisition and of virus transmission was calculated respectively as the percentage of thrips and seedlings that were infected. The experiment was repeated three times.

Acquisition and transmission efficiency of male and female *T. eucharii* were also determined. Fifty first-stage larval instars were placed on HCRV-infected leaves of *E. sonchifolia* in a closed cage for a 24-h AAP and then reared to adults on noninfected *A. porrum* where only 27, 32, and 40 individuals were reared to adults. The adults were then separated according to gender. The percentage of virus acquisition and transmission for each gender was determined by ID-ELISA. This experiment was repeated three times.

Virus detection by ID-ELISA. ID-ELISA was used to detect HCRV N protein in plant extracts and extracts of individual *T. eucharii*. Adult thrips from each of the transmission experiments were collected and homogenized individually with mortar and pestle in 100 μ l of sample buffer (0.1 M phosphate buffer [pH 7.0], 0.1% [v/v] 2-mercaptoethanol, 0.1% [w/v] bovine serum albumin [BSA], 10 mM EDTA, and 0.15% [w/v] polyvinylpyrrolidone-40). The 100 μ l samples were then added to separate wells of a microtiter plate and incubated overnight at 4°C . Each well was washed three times with 200 μ l of a phosphate buffered Tween solution (PBST), and then blocked with 100 μ l PBST containing 5% BSA and incubated for 2 h at 37°C . Purified IgG against HCRV N protein was diluted (1:5,000) with PBST containing 3% BSA, and then 100 μ l of antibody solution was added to each well and incubated at 37°C for 2 h, or 4°C overnight. HRP-conjugated goat anti-rabbit IgG (H+L) (Vazyme, Nanjing, China) was diluted (1:5,000) and used as a secondary antibody. Freshly prepared substrate solution was then added to each well after incubation with the secondary antibody at 37°C for 2 h. Absorbance in each well was measured at 405 nm (iMark Microplate Absorbance Reader; Bio-Rad) 30 min after incubation at room temperature. Nonviruliferous thrips were used as negative controls. Samples were considered positive when the absorbance was greater than two times the average value of the fresh negative control. The background absorbance was automatically subtracted using buffer wells before positive and negative threshold values were calculated. To detect HCRV in the plants, about 100 μ g of *E. sonchifolia* leaves were homogenized individually with mortar and pestle in 1 ml of PBST and 100 μ l of supernatant was analyzed as described above.

Field survey for *T. eucharii* and *F. occidentalis*. Thrips species on HCRV-positive *H. littoralis* were collected every week in Kunming, China ($25^\circ07'48.25''\text{N}$, $102^\circ44'35.23''\text{E}$) from June to September 2016. Plants were assayed by ID-ELISA as previously described. Adult thrips samples were collected weekly between 8:00 and 10:00 a.m. Adult thrips were taken from all flowers, leaves, and the base of each of 203 *H. littoralis* plants. All adult thrips were identified and assayed for HCRV using ID-ELISA as described above.

Results

Detection of N protein in adult *T. eucharii*. Five *T. eucharii* adults from the same sample were analyzed by Western blot and a 30-kDa band corresponding to the size of the N protein of HCRV was detected in extracts from each adult (Fig. 1, lanes 4 through 8). A similarly sized protein was found in extracts from an HCRV-infected *E. sonchifolia* plant (Fig. 1, lane 2) but not in an extract from a virus-free plant (Fig. 1, lane 1) nor in extracts from individuals of nonviruliferous *F. occidentalis* (Fig. 1, lane 3).

Virus acquisition and transmission by *T. eucharii*. For the three virus acquisition assays, 27/56 (48.2%), 33/72 (45.8%), and 17/46 (37.0%) individual first instar larvae tested positive for HCRV by ID-ELISA. The mean virus acquisition efficiency for first instar larvae after 24 h was $43.7 \pm 3.4\%$ (mean $\pm$ SE). Acquisition efficiency (AAP = 24 h) of HCRV by male thrips from *E. sonchifolia* was 17/37 (45.9%), 15/34 (44.1%), and 13/29 (44.8%) for three trials, with a mean of $44.9 \pm 0.5\%$ (Table 1). For females, the results of three trials yielded 9/27 (33.3%), 11/32 (34.4%), and 17/40 (42.5%), with a mean of $36.7 \pm 2.9\%$ (Table 1). The difference in acquisition

Fig. 1. Western blot analysis of total protein extracted from field-collected *Taeniothrips eucharii* for detection of HCRV N protein. M, molecular weight marker; 1, noninfected *Emilia sonchifolia*; 2, HCRV-infected *E. sonchifolia*, used as a positive control; 3, a laboratory colony of *Frankliniella occidentalis* used as a negative control; 4-8, total protein from field-collected *T. eucharii* adults.

Table 1. Acquisition and transmission efficiency of HCRV HLS1-2 by *Taeniothrips eucharii*

	Efficiency (%) ^a						P
	Male	SE	Female	SE	Total	SE	
Acquisition	44.9	± 0.5	36.7	± 0.5	43.7	± 0.5	>0.05
Transmission	19.9	± 3.4	15.6	± 2.3	19.1	± 2.4	>0.05

^a Cumulative mean percentages of three trials. There is no significant difference between acquisition or transmission efficiency by males and females based on a χ^2 analysis.

efficiency between males and females was not significant ($\chi^2 = 1.19$, $n = 1$, $P > 0.05$). Three transmission assays with adult thrips yielded cumulatively for both male and female thrips 5/27 (18.5%), 5/33 (15.2%), and 4/17 (23.5%) seedlings testing positive for HCRV by ID-ELISA. The mean transmission efficiency of *T. eucharii* adults averaged $19.1 \pm 2.4\%$ (IAP = 24 h) (Table 1). Transmission efficiency of males over three trials was $19.9 \pm 3.4\%$, with 3/17 (17.6%), 4/15 (26.7%), and 2/13 (15.4%) *E. sonchifolia* seedlings infected and $15.6 \pm 2.3\%$ for females with 1/9 (11.1%), 2/11 (18.2%), and 3/17 (17.6%) seedlings infected. Transmission efficiency was not significantly different between males and females ($\chi^2 = 0.19$, $n = 1$, $P > 0.05$).

Field survey of *T. eucharii* and *F. occidentalis*. There were 2,339 thrips collected from *H. littoralis* plants from June to September 2016 in Kunming, Yunnan Province. Within this group, 1,998 (85.4%) were *T. eucharii* and 341 (14.6%) were *F. occidentalis*. Individuals of both species were tested for HCRV with ID-ELISA. Results showed that 466/1,998 (23.3%) of *T. eucharii* individuals tested positive for HCRV. No *F. occidentalis* thrips tested positive for the virus.

Discussion

HCRV was first identified and its whole genome sequenced in 2013 (Dong et al. 2013; Xu et al. 2013). A field survey and laboratory host range test determined that many crops in the agricultural and floral industries were susceptible to the virus (Xu et al. 2013). HCRV is widespread in southern China, especially in *H. littoralis*, a common landscape plant. We found two lines of evidence indicating *T. eucharii* is a vector of HCRV on *H. littoralis*: (i) the first larval stage acquired HCRV from test plants, and (ii) adult *T. eucharii* could transmit HCRV in whole-plant assays. This is the first report of a species in the genus *Taeniothrips* as a tospovirus vector and of *T. eucharii* as the only confirmed vector of HCRV.

The leaf disk assay is a classic method of testing viral transmission by thrips (Groves et al. 2001; Wijkamp and Peters 1993). In this study, however, we used an improved whole plant assay system. By using living plants to test for virus transmission, we could monitor symptom development over an extended length of time and use various assays to test for the presence of the virus.

Transmission efficiency of tospoviruses by thrips species varies. *F. occidentalis* is a highly efficient transmitter of TSWV (30 to 43%) and INSV (79 to 90%) (Nagata et al. 2004). Adult *Ceratothripoides claratrix* transmits Capsicum chlorosis virus with 69% efficiency (Premachandra et al. 2005). These indicated that transmission efficiency may vary among virus isolates and among thrips populations (Nagata et al. 2007). In this study, *T. eucharii* transmitted HCRV with an average transmission efficiency of $19.1 \pm 2.4\%$ (IAP = 24 h). This lower efficiency could be due to the interactions between viral proteins and *T. eucharii* proteins, or to the several tissue barriers associated with the midgut and the salivary glands in *T. eucharii*.

The distribution of certain virus diseases is related to the distribution of their virus vectors. For example, the worldwide expansion of *F. occidentalis* was consistent with the spread of TSWV (Hobbs et al. 1993). Similarly, an epidemic of groundnut ringspot disease, caused by *Groundnut ringspot virus*, was associated with the distribution of *F. schultzei* (Nagata et al. 1999). The results of our study indicated that *T. eucharii* is a vector of HCRV on *H. littoralis*. We did not detect HCRV in *F. occidentalis* individuals we collected from *H. littoralis*. However, the ability of *F. occidentalis* or other thrips species to acquire and transmit HCRV was not tested experimentally in this study. It is possible that other thrips species in addition to *T. eucharii* might be involved in the spread of HCRV in this area.

Currently, 15 thrips species in six genera have been reported as vectors of tospoviruses including *Frankliniella*, *Thrips*, *Ceratothripoides*, *Dictyothrips*, *Neohydatothrips*, and *Scirtothrips* (Riley et al. 2011). *T. eucharii* was discovered on *Eucharis lily* during the study of a mosaic disease in Bermuda in 1922 (Whetzel 1923a). Steinweden and Moulton (1930) recorded it on *Lamium* and *Tucurium* spp., and on the flowers of broadbean (*Vicia faba*), in Kashing, Zhejiang Province, China. In 1933, *T. eucharii* was recorded on

Hymenocallis spp. from Hawaii, where it was widespread (O'Neill 1963). *T. eucharii* has been intercepted numerous times by quarantine procedures in Japan, Hong Kong, and Formosa (O'Neill 1963). The main host plants of *T. eucharii* are in the Liliaceae and Amaryllidaceae, including species of *Crinum*, *Eucharis*, *Hymenocallis*, *Liriope*, *Lycoris*, *Narcissus*, and *Zephyranthes* (O'Neill 1963). *T. eucharii* is widespread in Bermuda (Nakahara and Hilburn 1989), Korea, China, Taiwan, Malaysia, Japan, Hawaii, the Netherlands, and northern Australia (Mound and Tree 2008).

The wide host range of HCRV and the wide geographic and host range of its vector, *T. eucharii*, present a threat to agriculture and landscapes in and around Kunming City, China, and likely in other regions where the virus, the appropriate hosts, and the vector are found.

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Literature Cited

- Cabanas, D., Watanabe, S., Higashi, C., and Bressan, A. 2013. Dissecting the mode of Maize chlorotic mottle virus transmission (Tombusviridae: *Machlomovirus*) by *Frankliniella williamsi* (Thysanoptera: Thripidae). J. Econ. Entomol. 106: 16-24.
- Chatzivassiliou, E., Nagata, T., Katis, N., and Peters, D. 1999. Transmission of tomato spotted wilt tospovirus by *Thrips tabaci* populations originating from leek. Plant Pathol. 48:700-706.
- Ciuffo, M., Mautino, G., Bosco, L., Turina, M., and Tavella, L. 2010. Identification of *Dictyothrips betae* as the vector of Polygonum ring spot virus. Ann. Appl. Biol. 157:299-307.
- Ciuffo, M., Tavella, L., Pacifico, D., Masenga, V., and Turina, M. 2008. A member of a new *Tospovirus* species isolated in Italy from wild buckwheat (*Polygonum convolvulus*). Arch. Virol. 153:2059-2068.
- Cortès, I., Livieratos, I., Derks, A., Peters, D., and Kormelink, R. 1998. Molecular and serological characterization of *Iris yellow spot virus*, a new and distinct tospovirus species. Phytopathology 88:1276-1282.
- De Borbón, C., Gracia, O., and De Santis, L. 1999. Survey of Thysanoptera occurring on vegetable crops as potential *Tospovirus* vectors in Mendoza, Argentina. Rev. Soc. Entomol. Argent. 58:59-66.
- Dong, J. H., Yin, Y. Y., Fang, Q., McBeath, J. H., and Zhang, Z. K. 2013. A new tospovirus causing chlorotic ringspot on *Hippeastrum* sp. in China. Virus Genes 46:567-570.
- Gopal, K., Krishna Reddy, M., Reddy, D., and Muniyappa, V. 2010. Transmission of peanut yellow spot virus (PYSV) by Thrips, *Scirtothrips dorsalis* Hood in groundnut. Arch. Phytopathol. Plant Prot. 43:421-429.
- Groves, R. L., Walgenbach, J. F., Moyer, J. W., and Kennedy, G. G. 2001. Overwintering of *Frankliniella fusca* (Thysanoptera: Thripidae) on winter annual weeds infected with *Tomato spotted wilt virus* and patterns of virus movement between susceptible weed hosts. Phytopathology 91:891-899.
- Hardy, V., and Teakle, D. 1992. Transmission of sowbane mosaic virus by *Thrips tabaci* in the presence and absence of virus-carrying pollen. Ann. Appl. Biol. 121:315-320.
- Hassani-Mehraban, A., Saaier, J., Peters, D., Goldbach, R., and Kormelink, R. 2005. A new tomato-infecting tospovirus from Iran. Phytopathology 95: 852-858.
- Hobbs, H., Black, L., Story, R., Valverde, R., Bond, W., Gatti, J., Schaeffer, D., and Johnson, R. 1993. Transmission of tomato spotted wilt virus from pepper and three weed hosts by *Frankliniella fusca*. Plant Dis. 77:797-799.
- Hoddle, M., Mound, L., and Paris, D. 2012. Thrips of California. CBIT Publishing, Queensland.
- Jones, D. R. 2005. Plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113: 119-157.
- Keough, S., Han, J., Shuman, T., Wise, K., and Nachappa, P. 2016. Effects of Soybean Vein Necrosis Virus on life history and host preference of its vector, *Neohydatothrips variabilis*, and evaluation of vector status of *Frankliniella tritici* and *Frankliniella fusca*. J. Econ. Entomol. 109:1979-1987.
- Krczal, G., Albouy, J., Damy, I., Kusiak, C., Moreau, J. P., Berkelmann, B., and Wohanka, W. 1995. Transmission of Pelargonium flower break virus (PFBV) in irrigation systems and by thrips. Plant Dis. 79:163-166.
- Moritz, G., Kumm, S., and Mound, L. 2004. *Tospovirus* transmission depends on thrips ontogeny. Virus Res. 100:143-149.
- Mound, L., and Morris, D. 2004. Thysanoptera phylogeny-the morphological background. Acta Phytopathol. Entomol. Hung. 39:101-113.

- Mound, L. A., and Morris, D. C. 2007. The insect order Thysanoptera: classification versus systematics. *Zootaxa* 1668:395-411.
- Mound, L. A., and Tree, D. J. 2008. The oriental lily-flower thrips *Taeniothrips eucharis* (Whetzel)(Thysanoptera: Thripidae) new to Australia. *Aust. Entomol.* 35:159-160.
- Nagata, T., Almeida, A., Resende, R. O., and DeAvila, A. 2004. The competence of four thrips species to transmit and replicate four tospoviruses. *Plant Pathol.* 53:136-140.
- Nagata, T., Mound, L. A., França, F. H., and de Ávila, A. C. 1999. Identification and rearing of four thrips species vectors of Tospovirus in the Federal District, Brazil. *An. Soc. Entomol. Brasil* 28:535-539.
- Nagata, T., Resende, R. O., Inoue-Nagata, A. K., and Ávila, A. C. d. 2007. The fluctuation of transmission specificity and efficiency of *Tomato spotted wilt virus* by *Frankliniella schultzei*. *Fitopatol. Bras.* 32:439.
- Nakahara, S., and Hilburn, D. J. 1989. Annotated checklist of the Thysanoptera of Bermuda. *J. N.Y. Entomol. Soc.* 97:251-260.
- O'Neill, K. 1963. An oriental *Taeniothrips* (Thysanoptera: Thripidae) infesting certain Amaryllidaceae. *Ann. Entomol. Soc. Am.* 56:399-401.
- Ohnishi, J., Katsuzaki, H., Tsuda, S., Sakurai, T., Akutsu, K., and Murai, T. 2006. *Frankliniella cephalica*, a new vector for *Tomato spotted wilt virus*. *Plant Dis.* 90:685.
- Premachandra, W., Borgemeister, C., Maiss, E., Knierim, D., and Poehling, H. M. 2005. *Ceratothripoides claratris*, a new vector of a Capsicum chlorosis virus isolate infecting tomato in Thailand. *Phytopathology* 95:659-663.
- Riley, D. G., Joseph, S. V., Srinivasan, R., and Diffie, S. 2011. Thrips vectors of tospoviruses. *J. Integr. Pest Manag.* 2:11-110.
- Sdoodee, R., and Teakle, D. 1993. Studies on the mechanism of transmission of pollen-associated tobacco streak ilarvirus virus by *Thrips tabaci*. *Plant Pathol.* 42:88-92.
- Steinweden, J. B., and Moulton, D. 1930. Thysanoptera from China. *Proc. Nat. Hist. Soc. Fukien Christin Univ.* 3:1-12.
- van de Wetering, F., Goldbach, R., and Peters, D. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology* 86:900-905.
- van den Heuvel, J. F., Verbeek, M., and van der Wilk, F. 1994. Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. *J. Gen. Virol.* 75:2559-2565.
- Whetzel, H. 1923a. Report of the plant pathologist for the period January 1st to May 31st, 1922. Pages 28-32 in: Reports Board & Dept. Agric. Bermuda for the year 1922.
- Whetzel, H. H. 1923b. Report of the plant pathologist for the period January 1st to May 31st, 1922.
- Whitfield, A. E., Ullman, D. E., and German, T. L. 2005. *Tospovirus*-thrips interactions. *Annu. Rev. Phytopathol.* 43:459-489.
- Wijkamp, I., Almaraz, N., Goldbach, R., and Peters, D. 1995. Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology* 85:1069-1074.
- Wijkamp, I., and Peters, D. 1993. Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis*, using a novel leaf disk assay. *Phytopathology* 83:986-991.
- Xu, Y., Lou, S. G., Li, Q. F., Hu, Q., Sun, L. P., Li, Z. Y., Li, Y. Z., and Liu, Y. T. 2014. Molecular characterization of the hippeastrum chlorotic ringspot virus L segment and its protein. *Arch. Virol.* 159:2805-2807.
- Xu, Y., Lou, S. G., Li, X. L., Zheng, Y. X., Wang, W. C., and Liu, Y. T. 2013. The complete S RNA and M RNA nucleotide sequences of a hippeastrum chlorotic ringspot virus (HCRV) isolate from *Hymenocallis littoralis* (Jacq.) Salisb in China. *Arch. Virol.* 158:2597-2601.