Expanding Knowledge of the Host Range of *Tomato chlorosis virus* and Host Plant Preference of *Bemisia tabaci* MEAM1

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Abstract

The crinivirus *Tomato chlorosis virus* (ToCV) is often found infecting tomato crops in Brazil, with variable incidence, but associated with prevalence of its primary vector, *Bemisia tabaci* MEAM1. ToCV control is difficult because there are no resistant commercial tomato varieties or hybrids available and chemical spray for control of the whitefly vector has not been effective. The present study evaluated the partial host range of a Brazilian isolate of ToCV and the preference of *B. tabaci* MEAM1 for oviposition on those species identified as susceptible to the virus. Subsequently, transmission tests were performed using plants of each ToCV host species as sources of inoculum to elucidate the epidemiological importance of nontomato sources of inoculum for infection of tomato. Among 80 species experimentally inoculated, 25 were susceptible, including 6 previously not known to be hosts (*Jalomatia procumbens*, *Physalis pruinosa*, *Solanum aculeatissimum*, *S. vilarum*,

Beta vulgaris var. *cicla*, and *Chenopodium quinoa*). Preference of whitefly for oviposition and infection by ToCV under free-choice transmission tests varied among the susceptible species. When ToCV-infected tomato, eggplant, and *C. quinoa* were used separately as sources of inoculum for virus transmission to tomato plants, mean percentages of infected plants were 76.6, 3, and 0%, respectively. Average oviposition of *Bemisia tabaci* on these three hosts were 2.7, 10.6, and 0.0 eggs/cm², respectively. Additional studies will be necessary to evaluate the importance of ToCV host plants under field conditions and their efficiency as sources of inoculum for virus acquisition and transmission to tomato crops.

Keywords: crinivirus, epidemiology, *Solanum lycopersicum*, tomato, whitefly.

Tomato chlorosis virus (ToCV), genus *Crinivirus*, family *Closteroviridae*, was first described causing a disease named “yellow leaf disorder” in tomato (*Solanum lycopersicum*) plants cultivated in Florida, United States, in 1989 (Wisler et al. 1998). Since then, ToCV has been reported in many countries throughout the Americas, Europe, Africa, and Asia (Navas-Castillo et al. 2011). In Brazil, where its presence was first found in 2006 (Barbosa et al. 2008), ToCV occurrence has been confirmed in at least five states (Barbosa et al. 2011). In addition to tomato, the virus has been detected in Brazil infecting potato (*S. tuberosum*), pepper (*Capsicum annuum*), eggplant (*S. melongena*), and scarlet eggplant (*S. aethiopicum*) (Barbosa et al. 2010; Fonseca et al. 2016; Freitas et al. 2012). Recently, eight additional *Solanum* spp. with long vegetative cycles were found naturally infected with ToCV in the regions of Pernambuco State and Brasília, DF, Brazil (Boiteux et al. 2018).

Tomato plants infected by ToCV develop interveinal chlorotic areas, especially on older leaves, which become brittle and may exhibit necrotic spots (Wintermantel and Wisler 2006). In addition, tomato plants infected at early stage of development may have reductions in yield of 0.2 to 51.8%, depending on the cultivar (Mansilla-Córdova et al. 2018). ToCV has a bipartite genome of positive-sense single-stranded RNA encapsidated in flexuous virions of 800 to 850 nm in length (Liu et al. 2000). The virus is transmitted in a semipersistent manner by several cryptic species of the *Bemisia tabaci* complex, including New World 1 (NW1) and New World 2

(NW2; formerly known as biotype A and NW) (Marubayashi et al. 2012), Middle East-Asia Minor 1 (MEAM1; formerly biotype B), and Mediterranean (MED; formerly biotype Q), as well as by *Trialeurodes abutiloneus* and *T. vaporariorum* (DeMarchi et al. 2017; Navas-Castillo et al. 2000; Shi et al. 2018; Wintermantel and Wisler 2006). All of these vector species have now been reported in Brazil (DeMarchi et al. 2017; Lourenção et al. 2008), except for *T. abutiloneus* (A. L. Lourenção, personal information). *B. tabaci* MEAM1 is the most prevalent and widely disseminated whitefly species in the country (Inoue-Nagata et al. 2016).

Studies on the spatial dynamics of ToCV in tomato crops, with intensive chemical control of *B. tabaci* MEAM1 as the only measure to manage the disease, showed that the incidence and spread of the virus are mainly associated with the permanent influx of viruliferous whiteflies from areas surrounding tomato fields, with very little secondary spread within the field (Macedo et al. 2019). Whiteflies are polyphagous and are known to feed and reproduce on a wide array of cultivated plant species, weeds, or ornamentals (Shah et al. 2015), some of which may be infected by ToCV. Until now, this crinivirus has been reported naturally or experimentally infecting approximately 60 species of cultivated and weed host plants belonging to 29 families (Boiteux et al. 2018; Fiallo-Olivé et al. 2014; Kil et al. 2015; Morris et al. 2006; Shakeel et al. 2017; Wintermantel and Wisler 2006; Zhou et al. 2015). Despite such knowledge, little is known about the agricultural importance of these hosts with regard to *B. tabaci* MEAM1 preference, or as sources or reservoir hosts of ToCV for virus acquisition by the whitefly and subsequent transmission to cultivated solanaceous plants.

The present study evaluated the preference of *B. tabaci* MEAM1 for oviposition on different ToCV host species, as well as acquisition and subsequent transmission of the virus to tomato plants. This information can be used to develop strategies for management of ToCV inoculum sources external to fields, and to minimize the primary spread of the virus associated with the influx of viruliferous whiteflies into tomato fields.

Materials and Methods

Virus isolate and whitefly colony. An isolate of ToCV (GenBank EU868927), originally collected from a field-infected tomato plant in

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the Sumaré region, São Paulo State, Brazil (Barbosa et al. 2008), was used for all assays. The virus was maintained on Kada tomato plants through transmission by *B. tabaci* MEAM1. Plants were maintained in a greenhouse at the Department of Plant Pathology, ESALQ/USP, Piracicaba, São Paulo State, Brazil. The presence of ToCV in source plants was confirmed by partial nucleotide sequencing of the Hsp70h gene amplified by reverse-transcription polymerase chain reaction (RT-PCR). Randomly selected amplicons were purified with Wizard SV Gel and the PCR Clean-Up System (Promega Corp.) or the PureLink PCR Purification Kit (Invitrogen), following the protocol of each manufacturer, and sent for direct nucleotide sequencing at Macrogen. The consensus nucleotide sequences were compared with corresponding nucleotide sequences of ToCV available in GenBank using the BLASTn program, available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>.

A colony of virus-free *B. tabaci* MEAM1 whiteflies was used for virus transmission assays. Whiteflies were reared on collard (*Brassica oleracea* L.) plants, which are immune to ToCV, and maintained in insect-proof cages in a greenhouse. The identity of *B. tabaci* MEAM1 was periodically checked by PCR using the primers Bem23F (5'-CGGAGCTTGCGCCTTAGTC-3') and Bem23R (5'-CGGCTTATCATAGCTCTCT-3'), which differentiate *B. tabaci* MEAM1 and MED, amplifying a microsatellite locus of approximately 200 and 400 bp for MEAM1 and MED, respectively (De Barro et al. 2003).

ToCV detection. Total RNA was extracted from leaf tissue using Trizol LS (Invitrogen), following the manufacturer's protocol, and detection of ToCV in test plants was conducted on extracts using RT-PCR. Total RNA extracted from ToCV-infected tomato was used as a positive control for RT-PCR, whereas total RNA from virus-free plants was used as a negative control. RT was carried out with M-MLV RT (Promega Corp.) at 37°C for 50 min. RT-PCR was performed with primer pair HS11/HS12 followed by nested PCR with primers TOC5 and TOC6 (Dovas et al. 2002) to allow detection of lower levels of the virus that may occur in some host plants. Conditions for RT-PCR consisted of 1 cycle at 95°C for 1 min; followed by 40 cycles at 95°C for 20 s, 60°C for 15 s, and 72°C for 10 s; with a final extension at 72°C for 2 min. PCR products were separated by electrophoresis in 1% agarose gels and stained with SYBR safe DNA gel stain (Invitrogen).

No-choice transmission tests with *B. tabaci* MEAM1. Plants of 80 species, belonging to 13 families (Supplementary Table S1), were grown in 1.5-liter pots under greenhouse conditions and inoculated with ToCV by *B. tabaci* MEAM1. For each transmission test, virus-free whiteflies were allowed a 24-h acquisition access period (AAP) on ToCV-infected tomato leaves confined within 50-ml Falcon tubes. Approximately 300 adult whiteflies were confined in each tube. Viruliferous insects were then transferred to five plants of each species, confined within individual cages to guarantee *B. tabaci* a no-choice inoculation access period (IAP) of 24 h. In all, 200 viruliferous whiteflies were released into each cage, providing an average of 40 whiteflies/plant. After the IAP, plants were sprayed with the systemic insecticide Thiamethoxam (Actara at 2 g per 10 liters) and maintained in the greenhouse. Plants were evaluated for symptom expression at 30 to 45 days after inoculation, with confirmation of virus infection by nested RT-PCR (Dovas et al. 2002) on total RNA extracts of each plant. ToCV-infected tomato cultivar Kada was used as a positive control.

Transmission tests with free choice for *B. tabaci* MEAM1. Plant species that were susceptible to ToCV infection (Table 1) were further evaluated in free-choice transmission tests with *B. tabaci* MEAM1. For these experiments, 25 ToCV-infected tomato and three collard plants heavily colonized by virus-free whiteflies were placed in an insect-proof cage (2 by 1.70 by 0.80 m) in the greenhouse. Five healthy plants of each of the ToCV host species to be evaluated were randomly placed among ToCV-infected tomato plants for a period of 7 days. Five healthy tomato plants were included as control. Once a day, the plants were gently shaken to displace the insects and facilitate virus acquisition or transmission. Plants were subsequently removed from the cage, sprayed with systemic insecticide

Thiamethoxam (Actara), and transferred to another greenhouse to monitor symptom development. At 30 days following removal from cages, total nucleic acid was extracted from leaves newly developed since exposure to whiteflies, and evaluated for ToCV infection by nested-PCR. The experiment was repeated three times. In total, 15 plants of each species were evaluated.

Preference of *B. tabaci* MEAM1 for oviposition. The preference of *B. tabaci* MEAM1 for oviposition on plants of ToCV host species used in the free-choice experiments was evaluated 7 days after exposure to the insects, and just prior to spraying the plants with insecticide. Egg counting was performed on three leaves located at the lower, middle, and upper region of each plant. For each leaf, eggs were counted on three sections, selected at random, using a 1-cm² grid. A 16× magnifying glass with a diameter of 60 mm was used for identification of eggs. Total numbers of eggs on the three leaves of each plant were used to calculate the average number of eggs per plant. The average number of eggs per plant (total of 15 plants per species) was compared using a Tukey test at 5% probability in the Past 3 statistical program.

Evaluation of ToCV host species as sources for virus acquisition by *B. tabaci* MEAM1. All ToCV host species were separately evaluated as sources for acquisition by *B. tabaci* MEAM1 as well as for subsequent transmission of the virus to tomato plants in no-choice transmission tests. Virus-free whiteflies were allowed a 24-h AAP on detached leaves of each ToCV host plant species separately confined in 50-ml Falcon tubes. From each source of inoculum, insects were transferred to cages containing five healthy tomato plants each. In

Table 1. Susceptibility of different plant species to infection with *Tomato chlorosis virus*, transmitted by *Bemisia tabaci* MEAM1, in experiments with no choice and free choice of host plant

Family, species ^y	No-choice test		Free-choice test ^x	
	Inf/Inoc (n) ^z	Infected (%)	Inf/Inoc (n) ^z	Infected (%)
Amaranthaceae				
<i>Beta vulgaris</i> var. cicla*	3/5	60	–	–
<i>Chenopodium album</i>	5/5	100	4/15	26.6
<i>C. quinoa</i> *	2/2	100	1/15	6.6
<i>Gomphrena globosa</i>	4/5	80	9/15	60.0
<i>Spinacia oleracea</i>	5/5	100	5/15	33.3
Solanaceae				
<i>Capsicum annuum</i>	3/5	60	3/15	20.0
<i>Datura stramonium</i>	5/5	100	15/15	100
<i>Jaltomata procumbens</i> *	5/5	100	–	–
<i>Nicotiana benthamiana</i>	5/5	100	14/15	93.3
<i>N. clevelandii</i>	5/5	100	14/15	93.3
<i>N. edwardsonii</i>	5/5	100	14/15	93.3
<i>N. glutinosa</i>	5/5	100	7/15	46.6
<i>N. tabacum</i>	5/5	100	14/15	93.3
<i>Physalis angulata</i>	5/5	100	15/15	100
<i>P. peruviana</i>	5/5	100	15/15	100
<i>P. pruinosa</i> *	5/5	100	–	–
<i>Solanum aculeatissimum</i> *	3/3	100	–	–
<i>S. americanum</i>	5/5	100	15/15	100
<i>S. lycopersicum</i>	5/5	100	15/15	100
<i>S. melongena</i> var. Napoli	4/5	80	15/15	100
<i>S. pimpinellifolium</i>	4/5	80	12/15	80.0
<i>S. sessiliflorum</i>	5/5	100	15/15	100
<i>S. sisymbriifolium</i>	4/4	80	–	–
<i>S. tuberosum</i> var. Asterix	4/5	80	11/15	73.3
<i>S. viarum</i> *	3/4	75	–	–

^x Results of three independent experiments; – indicates not tested.

^y An asterisk (*) indicates first documented case of the host.

^z Number of infected plants/number of inoculated plants.

all, 200 viruliferous whiteflies (an average of 40 insects/plant) were released into each cage for an IAP of 24 h. Following the IAP, the plants were sprayed with systemic insecticide Thiamethoxam (Actara) and maintained in the greenhouse for subsequent analysis as previously described.

Subsequently, free-choice tests for acquisition and transmission were performed using only the ToCV host species *S. melongena*, *S. lycopersicum*, and *C. quinoa* as sources of inoculum, based on determination of the relative preference of *B. tabaci* MEAM1 for oviposition on these species. The three species were selected because they showed high (*S. melongena*), medium (*S. lycopersicum*), and low (*C. quinoa*) relative preferences for *B. tabaci* oviposition, respectively (Table 2). Three different mesh cages were used, each containing a single plant of one of the three ToCV host species confirmed to be infected by RT-PCR, along with 10 healthy tomato seedlings. Approximately 350 virus-free whiteflies were released into each cage for ToCV acquisition and transmission. Test plants were not shaken to displace the insects. Seven days later, tomato plants were sprayed with systemic insecticide Thiamethoxam (Actara) and transferred to individual cages in order to avoid secondary transmission of the virus. Detection of ToCV by RT-PCR was performed on tomato plant extracts 37 days after inoculation.

Results

Transmission tests with *B. tabaci* MEAM1. Plants of 80 species, representing 13 families, were inoculated with ToCV in no-choice transmission tests with *B. tabaci* MEAM1, and plants of 24 species became infected (Table 1). The majority ($n = 19$) of the infected species were in the family Solanaceae, whereas the remaining ($n = 5$) were in the family Amaranthaceae. The rate of infection of the susceptible species under no-choice inoculation conditions varied from 60 to 100%. ToCV infection in all plants was confirmed by nested RT-PCR, which generated specific amplicons of 463 bp. The nucleotide sequences from 10 randomly chosen amplicons shared 99% identity with corresponding nucleotide sequences of different ToCV isolates available in GenBank: accessions KP217197 (China), AY903448 (USA), DQ136143 (Spain), KJ433489 (Saudi Arabia), and 100% identity with a potato-infecting Brazilian isolate (GenBank accession JQ288897). Symptoms exhibited by most of the susceptible species consisted primarily of interveinal chlorosis (Fig. 1). Infected plants of *C. album*, *C. quinoa* (Amaranthaceae), and *S.*

melongena var. Napoli (Solanaceae) were asymptomatic. When plants of 18 of 24 species previously shown to become infected with ToCV were used in free-choice transmission tests with *B. tabaci* MEAM1, ToCV infection was confirmed for all 18 species. However, variation in the percentage of infected plants was wider among host plant species, varying from 6.6 to 100% (Table 1). The lowest percentages of infected plants in free-choice tests were found with plants of three Amaranthaceae species (*C. quinoa*, 6.6%; *C. album*, 26.6%; and *S. oleracea*, 33.3%) and one member of the family Solanaceae (*C. annuum*, 20%).

Preference of *B. tabaci* MEAM1 for oviposition. The oviposition results of *B. tabaci* MEAM1 in the plants of the 19 species used in the ToCV transmission tests in which the vector had free-choice of plants on which to feed are shown in Table 2. Leaves of eggplant (*S. melongena*) were most preferred for oviposition by *B. tabaci* MEAM1, with a mean of 10.61 eggs/cm². No eggs were found on the leaves of the amaranthaceous hosts *S. oleracea*, *C. album*, and *C. quinoa*.

Efficiency of ToCV host plant species as sources of inoculum for *B. tabaci* MEAM1. When ToCV host species were separately evaluated as sources of inoculum in no-choice transmission tests with 24-h AAP by *B. tabaci* MEAM1 and a subsequent 24-IAP on tomato plants, transmission varied from 80%, when *Capsicum annuum*, *Chenopodium album*, *Gomphrena globosa*, *Nicotiana edwardsonii*, *Solanum pimpinellifolium*, and *S. tuberosum* were the sources of ToCV inoculum for whitefly acquisition, to 100%, when *C. quinoa*, *Datura stramonium*, *N. benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. tabacum*, *Physalis angulata*, *P. peruviana*, *S. americanum*, *S. lycopersicum*, *S. melongena*, *S. sessiliflorum*, and *S. oleracea* were the sources of ToCV (data not shown).

The results of three independent experiments to evaluate the efficiency of ToCV-infected *S. melongena*, *S. lycopersicum*, and *C. quinoa* as sources of inoculum for *B. tabaci* MEAM1 and subsequent transmission to tomato plants in free-choice acquisition or transmission tests are presented in Table 3. ToCV was transmitted with greater efficiency (76.6%) when adults of the whitefly acquired the virus from *S. lycopersicum* plants. When the vector acquired the crinivirus from infected *S. melongena*, the transmission rate for tomato plants was reduced to 3.0%. No tomato plants became infected when fed on by adults of *B. tabaci* MEAM1 allowed to acquire the virus from infected plants of *C. quinoa*.

Discussion

Until recently, species from 29 botanic families had been identified as ToCV hosts, including the cultivated hosts tomato, potato, sweet pepper, tobacco, eggplant, scarlet eggplant, zinnia, pumpkin, cowpea, and other *Solanum* spp. within the subgenus *Leptostemonum* (Barbosa et al. 2010; Boiteux et al. 2018; Fiallo-Olivé et al. 2014; Fonseca et al. 2016; Kil et al. 2015; Orfanidou et al. 2016; Shakeel et al. 2017; Sun et al. 2016; Wang et al. 2018; Wintermantel and Wisler 2006; Wisler et al. 1998; Zhou et al. 2015). In the present work, plants of 18 species within the family Solanaceae and 5 within the family Amaranthaceae inoculated with ToCV by *B. tabaci* MEAM1 under no-choice whitefly feeding experiments became infected (Table 1). Among these there are six species identified for the first time as susceptible to ToCV: two members of the family Amaranthaceae (*B. vulgaris* var. *cicla* and *C. quinoa*) and four species of Solanaceae (*J. procumbens*, *P. pruinosa*, *S. aculeatissimum*, and *S. viarum*). When plants of 19 of 25 ToCV host species were provided inoculation access under free-choice tests with *Bemisia tabaci* MEAM1, using infected tomato plants as sources of inoculum, susceptibility was confirmed for all of these species (Table 1).

A few contradictory results are found among the reported hosts of ToCV in different countries. For example, Morris et al. (2006) found that eggplant (*S. melongena*), potato (*S. tuberosum*), and pepper (*C. annuum*) were not susceptible to ToCV. Wintermantel and Wisler (2006) reported that *D. stramonium* and pepper were not susceptible to ToCV. Other studies have shown that some of these hosts can, indeed, be infected by some ToCV isolates under some conditions (Fortes and Navas-Castillo 2012; Fortes et al. 2012). In fact, all four

Table 2. Preference of *Bemisia tabaci* MEAM1 for oviposition on *Tomato chlorosis virus* host plant species, in three independent experiments with free choice of host plants^z

Family, susceptible species	Eggs/cm ²	Standard deviation
Solanaceae		
<i>Solanum melongena</i>	10.61 a	5.6
<i>Nicotiana tabacum</i>	6.84 b	2.0
<i>Physalis peruviana</i>	6.54 bc	1.6
<i>N. glutinosa</i>	6.06 bc	3.2
<i>N. clevelandii</i>	5.54 bcd	2.0
<i>N. edwardsonii</i>	5.50 bcd	2.3
<i>Datura stramonium</i>	4.10 bcde	4.1
<i>N. benthamiana</i>	3.48 bcdef	2.0
<i>S. americanum</i>	3.46 bcdef	1.7
<i>S. sessiliflorum</i>	3.92 cdef	2.1
<i>S. pimpinellifolium</i>	2.82 cdef	2.1
<i>S. lycopersicum</i>	2.67 def	1.1
<i>P. angulata</i>	1.90 def	1.5
<i>S. tuberosum</i>	1.77 ef	0.9
<i>Capsicum annuum</i>	0.83f	0.7
Amaranthaceae		
<i>Gomphrena globosa</i>	0.20 f	0.3
<i>Spinacia oleracea</i>	0.0	-
<i>Chenopodium album</i>	0.0	-
<i>C. quinoa</i>	0.0	-

^z Means followed by the same letter do not differ significantly by the Tukey test at 5% probability.

of these species were susceptible to the Brazilian isolate of ToCV used in the present work. Orfanidou et al. (2016) and Tsai et al. (2004) demonstrated that *Amaranthus retroflexus* and *Zinnia elegans* were susceptible to the virus whereas, in the present work, they were unable to be infected by the Brazilian isolate. Such differences may be related to the small number of replications used in the present study, minor genetic differences among ToCV isolates (Kang et al. 2018), variation among plant species or cultivars, or variation in the efficiency of transmission to these hosts by the different whitefly species that transmit ToCV. The number of ToCV host species, although extensive, cannot be considered definitive because the potential for identification of additional new host plants or cultivars remains. Despite the large number of cultivated and weed species susceptible to natural or experimental infection with ToCV around the world, only 19 species have been found to be naturally infected in South America: tomato, potato, pepper, eggplant, scarlet eggplant (Solanaceae), *Commelina benghalensis* (Commelinaceae), *Eruca sativa*, *Raphanus* sp. (Brassicaceae), *Sida* sp. (Malvaceae), *S. americanum*, *S. jamaicense*, *S. mammosum*, *S. sessiliflorum*, *S. sisymbriifolium*, *S. scuticum*, *S. stramonifolium*, *S. subnerme*, *S. velleum*, and *P. angulata* (Solanaceae) (Arruabarrena et al. 2015; Boiteux et al. 2016, 2018; Fonseca et al. 2013, 2016; Freitas et al. 2012;).

It has long been known that plant disease epidemics begin with the introduction of the inoculum from external sources (primary spread) and that further development of the epidemic is governed by secondary spread within fields. As mentioned before, studies of the spatial dynamics of the disease in tomato crops with intensive chemical control of *B. tabaci* MEAM1 demonstrated that the incidence and spread of the ToCV into tomato fields in Brazil were mainly associated with several movements of viruliferous whiteflies into fields; specifically, a predominance of primary spread with very limited secondary spread within fields (Macedo et al. 2019). The importance of primary spread in the epidemiology of tomato virus diseases transmitted by whiteflies was also shown for the begomoviruses *Tomato mottle*

virus (Polston et al. 1996), *Tomato leaf curl virus* (Holt et al. 1999), *Tomato yellow leaf curl virus* (Cohen et al. 1988; Ioannou 1987; Sawalha 2013), *Tomato yellow vein streak virus* (Della Vecchia et al. 2007), and *Tomato severe rugose virus* (ToSRV) (Barbosa et al. 2016; Macedo et al. 2016, 2019). Therefore, a broad knowledge of alternative virus hosts, relevant vector populations, and their importance in the epidemiology of the disease (primary spread) is of fundamental importance for the establishment of appropriate cultural practices for the management of the disease in and around fields.

The role of the ToCV host plant species as a source of inoculum is governed by several factors, including prevalence in the region, season of occurrence relative to vector prevalence and abundance, susceptibility to natural infection with the virus, preference for vector colonization or feeding, and virus titer in the plant for vector acquisition, among others (Kil et al. 2015; Shi et al. 2018; Wintermantel and Wisler 2006). The results of the present work showed that all 25 species identified as hosts to the Brazilian isolate of ToCV proved to be good sources of inoculum in no-choice transmission tests. In particular, when ToCV-infected tomato (*S. lycopersicum*), eggplant (*S. melongena*), and *C. quinoa* were the sources of inoculum,

Table 3. Evaluation of *Tomato chlorosis virus* (ToCV) host species as potential sources for virus acquisition by *Bemisia tabaci* MEAM1 and subsequent transmission to tomato plants

Species source of inoculum	Number of infected plants/ number of tested plants			Total infected plants (%)
	First assay	Second assay	Third assay	
<i>Solanum melongena</i>	0/10	1/10	2/10	3.0
<i>S. lycopersicum</i>	7/10	8/10	8/10	76.6
<i>Chenopodium quinoa</i>	0/10	0/10	0/10	0.0

Fig. 1. Symptoms of *Tomato chlorosis virus* on a range host plant species: **A**, *Physalis peruviana*; **B**, *P. angulata*; **C**, *P. pruinosa*; **D**, *Solanum americanum*; **E**, *Datura stramonium*; **F**, *S. sisymbriifolium*; **G**, *S. aculeatissimum*; and **H**, *Gomphrena globosa*.

transmission efficiencies to tomato were 100%. However, when these three species were used as sources for acquisition of ToCV by *B. tabaci* MEAM1 in free-choice acquisition or transmission assays, only tomato yielded high rates of transmission to new plants at 76%. The other two hosts had low (3%) or no transmission. (Table 3). The results of these experiments do not allow us to identify the reasons for the differences. However, it can be speculated that, in the case of *S. melongena*, which exhibited the highest preference for whitefly oviposition, the insect may remain longer on the plant, with fewer insects moving to neighboring plants for virus transmission due to preference of the vector for this species. *C. quinoa*, on the other hand, showed the lowest preference for whitefly oviposition. It is likely that the whitefly vector may remain on the plant for only a short period if given an opportunity to move to more favored hosts and, therefore, feeding patterns and duration may be insufficient to efficiently acquire ToCV for subsequent transmission to neighboring plants. Further studies to better understand the behavior of *B. tabaci* MEAM1 on different host plants that vary for virus accumulation or whitefly feeding behavior will be necessary to more fully understand the differences between reservoir hosts from which ToCV can be efficiently transmitted to crops, and dead-end hosts, which become infected but are not epidemiologically important. Orfanidou et al. (2016) found significant differences on ToCV transmission by *B. tabaci* MED when it used *A. retroflexus*, *C. album*, *S. lycopersicum*, *S. nigrum*, and *Sonchus oleraceus* as sources of inoculum, having similar virus titer. *C. album*, a close relative of *C. quinoa*, was the least efficient source of inoculum, although whitefly oviposition was never observed on this *C. album*. The authors of that study believed that transmission efficiency was related to host preference of *B. tabaci* MED, regardless of the concentration of the virus.

The effective control of the disease caused by ToCV on tomato requires the establishment of an efficient integrated management approach, like that proposed for the disease associated with the begomovirus ToSRV on a landscape scale by Macedo et al. (2016). There is a steady migration of viruliferous whiteflies carrying ToCV from areas near tomato fields, and insecticide treatment of tomato plants has not been effective for controlling the primary infection of the crop, as recently demonstrated experimentally by Macedo et al. (2019). Because there are no ToCV-resistant varieties available commercially to tomato growers (González-Arcos et al. 2018; Mansilla-Córdova et al. 2018), strategies for reducing ToCV spread should include the management of ToCV host species within and near fields, and should consider variation in factors related to transmission in the different regions of the country where these host plant species are found. Furthermore, the elimination of alternative hosts, infected crop residues, and volunteer plants in areas near tomato production fields will contribute toward reducing sources of inoculum and, consequently, the primary spread of ToCV into tomato crops. It is critical that external sources of ToCV be reduced and managed effectively prior to new plantings in order to avoid infection of young plants and to prevent chronic yield losses. Chemical control of the whitefly vector, a common practice among tomato growers, should be rational, involve a rotation of chemicals with different active ingredients to prevent development of insecticide-resistant whitefly, and be used only to prevent the secondary spread of the virus. Due to the importance of primary inoculum in ToCV epidemiology in Brazilian tomato production areas (Macedo et al. 2019), the implementation of effective management for weed reservoir hosts may greatly improve disease control.

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