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Infection by the semi-persistently transmitted *Tomato chlorosis virus* alters the biology and behaviour of *Bemisia tabaci* on two potato clones

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Abstract

Insect-borne plant viruses usually alter the interactions between host plant and insect vector in ways conducive to their transmission ('host manipulation hypothesis'). Most studies have tested this hypothesis with persistently and non-persistently transmitted viruses, while few have examined semi-persistently transmitted viruses. The crinivirus *Tomato chlorosis virus* (ToCV) is semi-persistently transmitted virus by whiteflies, and has been recently reported infecting potato plants in Brazil, where *Bemisia tabaci* Middle East Asia Minor 1 (MEAM1) is a competent vector. We investigated how ToCV infection modifies the interaction between potato plants and *B. tabaci* in ways that increase the likelihood of ToCV transmission, in two clones, one susceptible ('Agata') and the other moderately resistant (Bach-4) to *B. tabaci*. Whiteflies alighted and laid more eggs on ToCV-infected plants than mock-inoculated plants of Bach-4. When non-viruliferous whiteflies were released on ToCV-infected plants near mock-inoculated plants, adults moved more intensely towards non-infected plants than in the reverse condition for both clones. Feeding on ToCV-infected plants reduced egg-incubation period in both clones, but the egg–adult cycle was similar for whiteflies fed on ToCV-infected and mock-inoculated plants. Our results demonstrated that ToCV infection in potato plants alters *B. tabaci* behaviour and development in distinct ways depending on the host clone, with potential implications for ToCV spread.

Keywords: host manipulation hypothesis, plant–pathogen–vector interaction, silverleaf whitefly, ToCV, vector-borne plant viruses

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Introduction

Many plant viruses depend on the movement of insect vectors for their dissemination. As a result, viruses have evolved ways to manipulate their vectors by directly modifying their behaviour and biology (Ingwell *et al*., 2012; Rajabaskar *et al*., 2014; Su *et al*., 2015), or indirectly through changes in the host plant (Mauck *et al*., 2010; Bosque-Peréz & Eigenbrode, 2011; Luan *et al*., 2013). Both direct and indirect effects of insect-borne plant viruses on vectors are usually conducive to enhancing virus transmission and spread, as proposed by the 'host manipulation hypothesis' (Poulin, 1998).

These virus-induced changes in vector behaviour are generally compatible with the mode of virus transmission (Mauck *et al*., 2012). Virions of non-persistently transmitted plant viruses, for example, are quickly acquired by vectors during probes of epidermal cells, but they are retained only for a short period of time, i.e., minutes (Ng & Perry, 2004). Therefore, infection by non-persistently transmitted viruses usually makes plants more attractive to vectors, but at the same time less palatable to them (Mauck *et al*., 2012). In this way, vectors land on infected plants, acquire virions during host quality assessment, and rapidly move to neighbouring plants, favouring the virus spread. On the other hand, acquisition of persistently transmitted plant viruses requires longer periods of vector feeding, but vectors remain viruliferous for hours, days, or even the insect's entire lifespan (Hogenhout *et al*., 2008). Plants infected by a persistently transmitted virus, similarly to non-persistently transmitted viruses, also become more attractive to insect vectors (Jiménez-Martínez *et al*., 2004a; Ngumbi *et al*., 2007), but they are usually superior hosts for vectors than healthy hosts (Jiménez-Martínez *et al*., 2004b; Mauck *et al*., 2012). Hence, vectors are often attracted to and settle on plants infected by persistently transmitted viruses, favouring the acquisition of virions (Alvarez *et al*., 2007). The transmission of persistently transmitted viruses likely occurs when individuals migrate to neighbour plants, motivated by crowding (Mauck *et al*., 2012).

Most of the studies that have tested the 'host manipulation hypothesis' for plant viruses have used persistently and non-persistently transmitted viruses, while semi-persistently transmitted viruses have been far less studied (Macias & Mink, 1969; Musser *et al*., 2003; Fereres *et al*., 2016; Peñaflor *et al*., 2016; Shrestha *et al*., 2017). Virions of semi-persistently transmitted viruses are quickly acquired by insect vectors (i.e., after several minutes to hours of feeding) but, up to a certain point, prolonged feeding increases transmission rates (Ng & Perry, 2004; Webb *et al*., 2012). Semi-persistently transmitted viruses likely benefit from vectors being attracted to and settling on infected plants, which are generally palatable and suitable hosts (Mauck *et al*., 2012).

Tomato chlorosis virus (ToCV, *Closteroviridae*) is transmitted by whiteflies, such as *Bemisia tabaci* (Gennadius) New World 1, Middle East Asia Minor 1 (MEAM1) and Mediterranean (MED) (former biotypes A, B and Q, respectively), *Trialeurodes abutilonea* Haldeman, and *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) (Wisler, 1998; Navas-Castillo *et al*., 2000; Wintermantel & Wisler, 2006), in a semi-persistent manner. This virus infects several plant species from different families (Wintermantel & Wisler, 2006), but the viral disease has been an especially serious problem in tomato crops worldwide (Wisler *et al*., 1998; Navas-Castillo *et al*., 2000; Dovas *et al*., 2002). ToCV infection symptoms (yellowing and necrotic flecking) in tomato plants cause significant loss of photosynthetic area, leading to reduction in the number and size of fruits (Wisler *et al*., 1998). More recently, ToCV has been found infecting potato plants, which exhibit symptoms resembling those caused by *Potato leaf roll virus*, such as leaf roll and interveinal chlorosis on older leaves (Fortes & Navas-Castillo, 2012; Freitas *et al*., 2012). In potato, *B. tabaci* MEAM1 and MED are efficient at transmitting ToCV (Fortes & Navas-Castillo, 2012; Freitas, 2012).

Recently, Fereres *et al*. (2016) have shown that ToCV infection up-regulates some terpenes in the volatile blend released by tomato plants, but this virus-induced change does not make plants more attractive to the vector *B. tabaci* MEAM1. Indeed, non-viruliferous whiteflies were found to avoid alighting and settling on ToCV-infected tomato plants, indicating that the behaviour of this whitefly does not favour the acquisition of ToCV from tomato (Maluta *et al*., 2017). As host-mediated effects of plant viruses on vectors vary across different host species (Shrestha *et al*., 2017), it is important to compare virus effects on transmission among different host plants (e.g., ToCV in potato) to gain insight into virus evolution (Mauck, 2016) as well as plant phenotypes that are conducive, or not, to virus transmission by whiteflies.

Here, we investigated whether the behaviour and biology of *B. tabaci* MEAM1 increases the likelihood of ToCV acquisition, transmission and spread in potato. We selected two potato clones, one susceptible ('Agata') and the other moderately resistant (Bach-4) to *B. tabaci* MEAM1 (Silva *et al*., 2008; Rocha *et al*., 2012). We specifically tested the following hypotheses for both clones: (*i*) whiteflies orient preferentially to ToCV-infected potato clones compared with non-infected clones; (*ii*) whitefly females prefer to oviposit on ToCV-infected potato clones compared with non-infected clones; (*iii*) whiteflies feeding on the ToCV-infected clone migrate preferentially to the non-infected clone, optimizing the spread of the virus; (*iv*) the life cycle of whiteflies that have been fed on the ToCV-infected potato plants is shorter compared with individuals that have been fed on the non-infected plants; and (*v*) virus manipulation of the plant for enhancing transmission is more pronounced in the susceptible than in the moderately resistant clone, as proposed by Mauck (2016). To address these hypotheses, we conducted a series of behavioural assays with non-viruliferous whiteflies in a greenhouse.

Material and methods

Potato clones, insect vectors and viral isolate

Potato plants with susceptibility ('Agata') and moderate resistance (Bach-4) clones to *B. tabaci* MEAM1 had two fully expanded true leaves when used in the assays (approximately 15–20 days after sowing). 'Agata' is a Dutch clone of the original cross between 'Bohm' and 'Sirco', and Bach-4 is a clone from the cross between 'Bannock Russet' and *Solanum chacoense* subsp. *muelleri* (Hawkes & Hjerting) (Rocha *et al*., 2012). Potato tubers were grown in 3 litres-capacity pots (one tuber/pot) containing a mixture of soil and organic matter (Raij *et al*., 1997). Irrigation and fertilization of potato tubers were done according to recommendations for potato growers described by Raij *et al*. (1997). Plants were kept in an insect-proof greenhouse without control of temperature, light or humidity (from August 2014 to May 2015, Campinas, SP, Brazil).

Whiteflies used in the experiments were from a rearing colony kept in a separate greenhouse (3 m × 5 m) with whitefly-proof screen walls and glass roof under natural and oscillating

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temperature, humidity and light (Campinas, SP, Brazil). Whiteflies were fed on cabbage plants (*Brassica oleracea* var. *acephala*), which were replaced weekly by new ones. This whitefly population has been molecularly characterized as *B. tabaci* MEAM1 (De Barro *et al.*, 2003; 2011).

The ToCV isolate was obtained from the Departamento de Fitopatologia e Nematologia (ESALQ/USP, Piracicaba, SP, Brazil) from infected tomato plants, collected from Sumaré, SP, Brazil, and showing characteristic symptoms, i.e., interveinal chlorosis of the lower leaves, evolving to the tips, with necrotic reddish spots and curling of the leaves (Wisler *et al.*, 1998). The isolate was maintained in tomato and potato plants in greenhouses, and ToCV infections were confirmed by a reverse transcription-polymerase chain reaction (RT-PCR), as described below.

To obtain ToCV-infected potato plants, 50 adults of *B. tabaci* MEAM1 were enclosed in a clip-cage on infected tomato plants for an acquisition access period (AAP) of 24 h. Afterwards, tomato leaves with insects confined in the clip-cages were excised and kept on ice for 5 min, to render the insects inactive; subsequently, clip-cages containing viruliferous insects were attached to potato leaves for an inoculation access period of 7 days. The same method was employed on mock-inoculated potato plants (control), but using non-viruliferous whiteflies. To avoid contamination, mock-inoculated and ToCV-infected plants were kept in different sections of the same greenhouse described above for plant cultivation.

ToCV infection of all plants tested in the assays was confirmed after 25–30 days from inoculation by nested-RT-PCR, from total RNA extracted from the leaf tissue, following the protocol described by Dovas *et al.* (2002). Total RNA extracts from non-infected and ToCV-infected tomatoes were used as positive and negative controls, respectively. Thermal cycler conditions were one cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 49°C for 40 s, 72°C for 50 s and a final extension at 72°C for 10 min. The amplified DNA was stained with SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific Inc., Waltham, MA, USA) and separated using 1% agarose gel electrophoresis for about 40 min. The amplicons were visualized in a UV-light transilluminator.

Host and oviposition preference in free-choice tests

The experiments for host and oviposition preferences of *B. tabaci* MEAM1 for mock-inoculated or ToCV-infected potato clones ('Agata' and 'Bach-4') were conducted in the greenhouse. A randomized-block design was used, with the four treatments and 15 blocks. Soybean seedlings, cultivated and infested with about 600 non-viruliferous *B. tabaci* MEAM1 adults/plant as in Prado *et al.* (2016), served as a source for whiteflies in this test. A potted soybean seedling was positioned in the centre of each block and equidistant (50 cm) from the potato plants. Each block containing four potato plants and one soybean seedling was covered by a whitefly-proof screen cage (1.0 m width and 0.6 m high). The numbers of adults on the abaxial surface of the two completely developed apical leaves of potato plants were recorded at 30 min, 2, 4, 6, 12, 24, 48 and 72 h. Insects were observed with the help of a mirror to avoid touching the leaf and disturbing the insects. One week after the beginning of the experiment, the number of eggs laid on leaves of potato plants were counted using a stereomicroscope. Then, leaf areas were measured using the software ImageJ® 1.47v to estimate the number of eggs per cm^2.

Oviposition preference in no-choice tests

The no-choice oviposition assay was conducted in the greenhouse in a randomized-block design with four treatments (mock-inoculated and ToCV-infected plants of the two potato clones) and 15 replicates. Each pot, containing a single plant, was infested with approximately 150 non-viruliferous *B. tabaci* MEAM1 adults, from the insect rearing without discrimination by age or sex, and covered with a cage of fine-mesh fabric. The number of eggs laid on each plant by the whiteflies was counted after 1 week, as described above.

Settling assays

Whitefly settlement was determined by assessing the movement of *B. tabaci* MEAM1 adults from mock-inoculated to ToCV-infected potato clones, and *vice-versa*. The experiments were conducted in cages (wire structure covered with whitefly-proof screen fabric) (1.0 m width and 0.6 m high) kept in the greenhouse. Fifteen replicates were performed; each experimental unit contained one mock-inoculated and one ToCV-infected potato plant of either clone. Prior to the experiment, whitefly adults collected from the rearing underwent a 24 h acclimation period on healthy potato plants of the 'Baraka' clone (a different clone to prevent conditioning), and were then starved for 1 h. Twenty non-viruliferous whitefly adults were released either on mock-inoculated or on ToCV-infected plants. The number of whiteflies on the abaxial leaf surface of the neighbour plant (ToCV-infected or mock-inoculated) was recorded at 5, 15, 30 and 60 min, and 6 and 24 h after release. A mirror was used to avoid touching the leaf and disturbing whiteflies on leaves while counting.

Egg–adult development

To obtain eggs on mock-inoculated and ToCV-infected plants of the two potato clones, plants were exposed to a colony of non-viruliferous *B. tabaci* MEAM1 for 4 h in the greenhouse. Afterward, the adults were aspirated and the plants transferred to the laboratory. Areas containing 20 eggs on the foliole were marked using a red pen (ø 1 mm) under a stereomicroscope (40×). Two fully developed folioles from the middle third of the plant were selected per plant, totalling 40 eggs. The clones were kept in individual cages in an insect-free greenhouse, and the numbers of eggs, nymphs and empty pupae (which indicated emergence of adults) were monitored daily. Based on these data, the periods (number of days) for the egg–adult development and the percentage adult emergence were estimated. Six replicates were performed in a randomized-block design.

Statistical analysis

Log-transformed data on whitefly host preference and settling assays over time were analysed by a general linear mixed model (glmm) (treatment as fixed effect, and time and block as random effects) and means compared by Tukey's test. Data from the whitefly settling experiment were also analysed by linear correlation obtained as a function. Numbers of eggs laid on the plants in the choice test were analysed by two-way analysis of variance (ANOVA) (treatment as fixed effect and blocks as random effect) and means compared by Tukey's test. In the no-choice oviposition assay, numbers of eggs laid by whiteflies on the treatments were analysed by one-way

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ANOVA. The duration of each whitefly developmental stage for insects fed in each treatment was analysed by the non-parametric test Kruskal–Wallis, and means compared by Dunn's test. Normality of the data was tested by Kolmorogov–Smirnov test. All statistical tests were performed in the software Minitab (Minitab Inc., State College, PA, USA).

Results

Host selection and oviposition preference in free- and no-choice tests

Non-viruliferous whitefly adults preferred ToCV-infected potato plants of the two clones and mock-inoculated plants of 'Agata' over mock-inoculated plants of Bach-4 (fig. 1, glmm, treatment effect $F = 5.85$, $P = 0.001$, Tukey's test $P < 0.05$). Whiteflies laid more eggs on ToCV-infected plants than mock-inoculated plants of the moderately resistant Bach-4 clone in choice tests (table 1, two-way ANOVA, treatment effect $F = 3.45$, $P = 0.025$, Tukey's test $P < 0.05$). In contrast, whiteflies laid similar numbers of eggs on ToCV-infected and mock-inoculated plants of both clones in no-choice tests (table 1, one-way ANOVA, $F = 0.28$, $P = 0.840$).

Whitefly settling

Non-viruliferous whitefly adults exhibited higher rates of movement from ToCV-infected plants to mock-inoculated plants of both 'Agata' and Bach-4 compared with the reverse (from mock-inoculated to ToCV-infected plants) (fig. 2, two-way ANOVA, 'Agata': treatment effect $F = 70.95$, $P < 0.001$, time $F = 7.35$, $P < 0.001$, treatment × time $F = 2.53$, $P = 0.030$; Bach-4: treatment effect $F = 39.45$, $P < 0.001$, time $F = 3.22$, $P < 0.01$, treatment × time $F = 4.48$, $P = 0.001$). A positive correlation was found for the number of adults moving from mock-inoculated to ToCV-infected plants of the susceptible clone 'Agata' over time (Supplementary fig. S1, linear correlation, from mock to ToCV: $y = 0.184x + 0.607$, $R = 0.985$). Similar correlation was found for the reverse direction, i.e., movement of whiteflies from ToCV-infected to mock-inoculated plants of the susceptible clone 'Agata' (Supplementary fig. S1, from ToCV to mock: $y = 0.684x + 0.968$, $R = 0.944$). In contrast, no linear correlation was found for the movement of *B. tabaci* MEAM1 from ToCV-infected plants to mock-inoculated plants of Bach-4 (from ToCV to mock: $y = 0.055x + 1.992$, $R = 0.008$), while a slightly negative linear correlation was observed for the reverse movement (mock to ToCV: $y = -0.092x + 0.969$, $R = 0.570$).

Egg–adult development

The embryonic phase of non-viruliferous *B. tabaci* MEAM1 was shorter on the ToCV-infected plants compared with the mock-inoculated plants for the two clones (table 2, Kruskal–Wallis, Dunn's test, $P < 0.05$). Durations of the first, second and third nymphal instars were similar between mock-inoculated and ToCV-infected potato plants of both clones. In contrast, fourth-instar nymphs showed a shorter development period when fed on mock-inoculated and ToCV-infected plants of 'Agata' compared with the mock-inoculated plants of the moderately resistant clone Bach-4. The egg–adult *B. tabaci* MEAM1 cycle was similar for whiteflies fed on ToCV-infected and mock-inoculated plants of both clones.

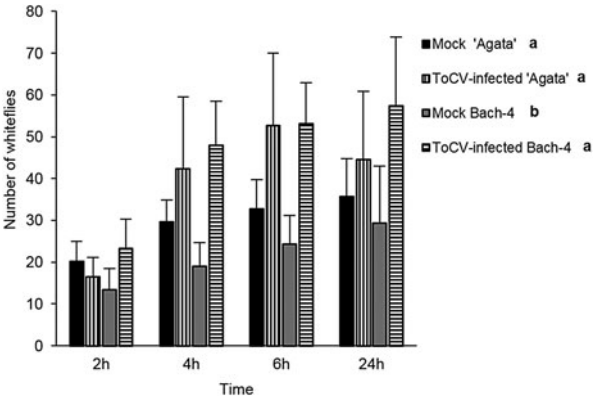

Fig. 1. Host preference of non-viruliferous *Bemisia tabaci* MEAM1 (mean number of adults ± SE) for mock-inoculated (mock) and *Tomato chlorosis virus*-infected potato plants (ToCV) of the clones 'Agata' and Bach-4, over time. Different letters in bold on the right side of the legend indicate significant differences between treatments (Tukey's test, $P < 0.05$).

Table 1. Oviposition (eggs 10 cm^{-2}) (mean ± SE) of non-viruliferous *Bemisia tabaci* MEAM1 on mock-inoculated (mock) and *Tomato chlorosis virus* (ToCV)-infected potato plants of 'Agata' and Bach-4 clones, in choice- and no-choice tests.

Treatment	*Bemisia tabaci* oviposition	
	Choice[1]	No-choice[ns]
Mock 'Agata'	23.5 ± 3.3 ab	9.4 ± 0.9
ToCV-infected 'Agata'	24.2 ± 6.4 ab	9.3 ± 0.7
Mock Bach-4	23.3 ± 6.1 a	10.2 ± 0.8
ToCV-infected Bach-4	28.8 ± 3.7 b	9.8 ± 0.6

[1]Values followed by different letters differ according to Tukey's test ($P < 0.05$).
[ns]No significant difference among treatments according to one-way ANOVA.

Discussion

The attraction of the insect vector to the infected plant, irrespective of the virus transmission mode, is a behaviour believed to be conducive to virus acquisition (Mauck *et al.*, 2012). Our study showed that adults of *B. tabaci* MEAM1 preferred ToCV-infected potato plants of both clones as a host relative to mock-inoculated plants of Bach-4, but not compared with mock-inoculated plants of 'Agata'. This difference in the whitefly response to mock-inoculated plants may be due to the natural susceptibility of 'Agata' to *B. tabaci* MEAM1 compared with Bach-4 (Rocha *et al.*, 2012). Even though adults of *B. tabaci* MEAM1 did not discriminate ToCV-infected plants from mock-inoculated plants of 'Agata' clone, whitefly adults alighted and settled on ToCV-infected plants of both clones equally, indicating that the virus induces a phenotype that optimizes its acquisition by *B. tabaci* MEAM1 in both clones.

Yellowing caused by viral infections can play an important role in attracting whitefly vectors to infected plants, but plant volatile emissions are also known to influence the whitefly preferential alighting on infected plants (Fang *et al.*, 2013; Fereres *et al.*, 2016). In a separate experiment (Supplementary information 1), we observed that composition of the volatile

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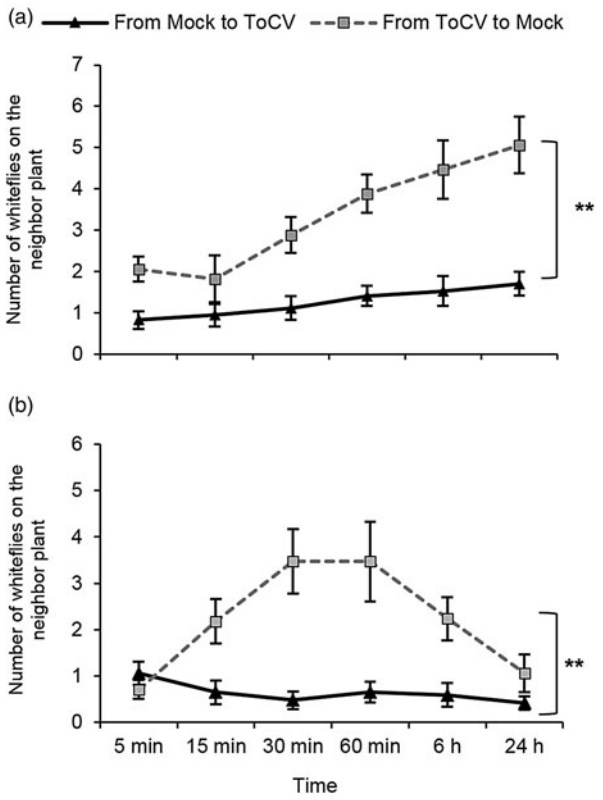

Fig. 2. Movement of non-viruliferous *Bemisia tabaci* MEAM1 (mean number of adults ± SE) released on either mock-inoculated (mock) or *Tomato chlorosis virus*-infected potato plants (ToCV) to the neighbour plant (mock or ToCV) of clones 'Agata' (a) and Bach-4 (b), over time. $^{**}P < 0.01$ (two-way ANOVA).

profiles emitted by the two clones were qualitatively different, but ToCV infection mainly reduced the concentration of terpenes in the blend of both potato clones (Supplementary table S1). The Bach-4 clone released a more complex volatile blend, and the suppression of terpenes caused by ToCV infection was more evident compared with the 'Agata' volatile profile. In contrast to the virus effect on plant volatile emissions in potato, ToCV infection in tomato plants increased the emission of most terpenes (Fereres *et al.*, 2016).

Reduced amounts of terpenes in plant volatiles can increase the attraction of *B. tabaci* to plants, because they are repellent (Bleeker *et al.*, 2009, 2011; Li *et al.*, 2014). For example, infection by the begomovirus *Tomato yellow leaf curl virus* in tomato down-regulates the emission of terpenes, making infected plants more attractive to whiteflies (Fang *et al.*, 2013), while increased terpene emissions of ToCV-infected tomato plants likely reduce the attractiveness to non-viruliferous whiteflies (Fereres *et al.*, 2016). The initial preference of *B. tabaci* MEAM 1 for odours of uninfected over those of ToCV-infected tomato reported by the latter study corresponded well to settling preference after 24 h (Maluta *et al.*, 2017). Although our study did not conclusively demonstrate that non-viruliferous whiteflies are guided by ToCV-infected potato plant volatiles, composition of blend volatiles may have influenced whitefly alighting on ToCV-infected plants of the moderately resistant clone Bach-4 in arena assays.

The time that the vector spends feeding on the virus-infected plant before moving to a new host is crucial for the virus transmission. Semi-persistently transmitted viruses require minutes to hours of feeding by the insect vector to be acquired, and an increased feeding time generally increases the likelihood of sufficient virus acquisition to enable transmission (Navas-Castillo *et al.*, 2011). ToCV, as a semi-persistently transmitted virus, follows this pattern. The transmission efficiency of the Brazilian ToCV isolate by *B. tabaci* MEAM1 is only 10% after 5–20 min of AAP. After 1 h of vector feeding on ToCV-infected plants, the transmission rises to 40%, reaching 100% efficiency in an AAP of 24 h (Freitas, 2012). In our study, whiteflies preferentially settle on mock-inoculated plants rather than ToCV-infected plants. We observed higher rates of insect movement when whiteflies were released on ToCV-infected plants and allowed to migrate to mock-inoculated plants, than in the reverse direction, for both clones. However, this migration from ToCV-infected to mock-inoculated plant occurred mostly within 15–60 min for both potato clones, indicating that, although the movement from infected to mock favours the virus spread, the timing is not optimal for ToCV transmission. Comparing the potato clones, movement of whiteflies from ToCV-infected to mock-inoculated seems to be more favourable for virus transmission in 'Agata' than in Bach-4. An increasing number of whiteflies kept moving to mock-inoculated plants from 15 min to 24 h after release on ToCV-infected plants of the 'Agata' clone, in contrast to Bach-4, in which whiteflies apparently left mock-infected plants between 60 min and 24 h. Although whitefly alighting preference to ToCV-infected potato contrasts with results found in tomato system, the movement pattern of whiteflies from ToCV-infected plant towards mock-inoculated plant seems to be similar in the two solanaceous crops (Maluta *et al.*, 2017).

The concentrations of free amino acids and carbohydrates in the phloem as well as plant defence levels of infected plants can influence the migration of insect vectors to neighbouring healthy plants (Mauck *et al.*, 2014). Nevertheless, ToCV infection apparently increased the host quality in potato, as whitefly development was accelerated in ToCV-infected potato plants of both clones, suggesting that plant suitability is not a driving factor for whitefly migration from ToCV-infected to healthy potato plants.

The embryonic phase of *B. tabaci* MEAM1, for example, was shorter on ToCV-infected plants compared with mock-inoculated plants of both clones. Eggs of *B. tabaci* have a pedicel for attachment on the leaf (Buckner *et al.*, 2002) and for the passage of water and solutes from the leaf epidermal cells (Walker *et al.*, 2010). ToCV infection likely changes chemical and/or physical aspects of the leaves that favour whitefly egg nutrition and/or attachment in both clones. Nevertheless, only in Bach-4 did ToCV infection positively influence the whitefly oviposition preference relative to the mock-inoculated plant.

Fourth-instar nymphal stage of *B. tabaci* MEAM1 had a shorter duration of development on ToCV-infected and mock-inoculated plants of 'Agata' compared with mock-inoculated plants of Bach-4. Despite the reduced duration of eggs and fourth-instar nymphal stage, total development time (egg to adult) of the whitefly vector was unaltered by ToCV infection on both clones, indicating that feeding on ToCV-infected plants does not result in higher population levels of the whitefly. In contrast, Maluta *et al.* (2018) found some negative effects of ToCV infection in tomato on *B. tabaci* MEAM1 biology, such

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Table 2. Duration (days; mean ± SE) of immature stages and egg–adult cycle of non-viruliferous *Bemisia tabaci* MEAM1 on mock-inoculated and *Tomato chlorosis virus* (ToCV)-infected potato plants of 'Agata' and Bach-4 clones.

Treatments	Developmental stages					
	egg	First instar	Second instar	Third instar	Fourth instar	Egg–adult cycle
Mock 'Agata'	7.16 ± 0.01 a	6.33 ± 0.06	4.55 ± 0.02	6.33 ± 0.02	4.00 ± 0.02 b	28.33 ± 0.65
ToCV 'Agata'	5.83 ± 0.01 b	6.16 ± 0.06	4.83 ± 0.05	5.83 ± 0.04	4.16 ± 0.03 b	26.67 ± 0.87
Mock Bach-4	7.00 ± 0.01 a	4.83 ± 0.04	4.50 ± 0.04	5.33 ± 0.03	5.66 ± 0.02 a	27.33 ± 0.40
ToCV Bach-4	5.83 ± 0.01 b	6.16 ± 0.02	4.66 ± 0.04	5.16 ± 0.03	4.83 ± 0.05 ab	26.67 ± 0.40
P-value	0.002*	0.264^{ns}	0.936^{ns}	0.214^{ns}	0.027*	0.247

Values followed by different letter in the column do not differ according to Dunn's test ($P < 0.05$).
^{ns}Not significant ($P > 0.05$).
*Indicates significant difference according to according to Kruskal−Wall ($P < 0.05$).

as prolonged duration of nymphal stage and reduced nymphal viability.

As ToCV isolate tested in our study is the same that of Fereres *et al.* (2016), we observe that infection differently affects the vector *B. tabaci* MEAM1 in ways that its behaviour and biology is more conducive for ToCV transmission in potato than tomato (Fereres *et al.*, 2016; Maluta *et al.*, 2017; 2018). Indeed, a recent study demonstrated *B. tabaci* MED transmits ToCV in tomato more efficiently than *B. tabaci* MEAM 1 and is likely responsible for ToCV spread in tomato in China (Shi *et al.*, 2018).

Varying indirect effects of virus infection on whitefly behaviour and biology depending on the host species have been previously reported in the literature. For example, *Squash vein yellowing virus*, another whitefly-semi-persistently transmitted virus, also shows different effects on *B. tabaci* MEAM1 behaviour depending on the host cucurbit species (Shrestha *et al.*, 2017). As pointed out by Mauck (2016), a complex set of factors play a role in shaping the ability of plant viruses to manipulate plant phenotypes of multiple plant species, making it even more difficult to understand the natural selection of vector-borne plant viruses in agricultural systems dominated by a single plant species (monocultures) of low genetic diversity.

Overall, our results suggest that ToCV manipulates the host phenotype on potato in ways that likely enhance its spread by *B. tabaci* MEAM1 in potato fields. However, it is unclear whether ToCV transmission by *B. tabaci* MEAM1 is enhanced to a greater extent in plantings of 'Agata', which is susceptible to both the whitefly and ToCV (Silva *et al.*, 2008; Freitas *et al.*, 2012) and therefore may serve as a better reservoir than the moderately whitefly-resistant Bach-4. On one hand, we did not find that infection by ToCV in 'Agata' increases the chances of virus acquisition by *B. tabaci* MEAM1, as the whiteflies did not discriminate ToCV-infected from mock-inoculated plants of 'Agata'. On the other hand, the likelihood of ToCV transmission seems to be increased in 'Agata' more than in Bach-4, as increasing numbers of whiteflies continuously move from ToCV-infected to mock-inoculated plants over time in 'Agata'. Although we did not find the same benefit of ToCV infection for *B. tabaci* MEAM1 in Bach-4, ToCV not only makes plants more attractive to whiteflies, but also for ovipositing. As a result, it is expected that a whitefly population would be higher on ToCV-infected plants than on healthy plants of Bach-4. This study system deserves further use in investigations on the role of the suppression of volatile terpenes and gustatory cues of ToCV-infected plants in whitefly alighting as well as migration of whiteflies from ToCV-infected to healthy plants, including plant metabolite profiling and electrical penetration graphs. Moreover, the recent report of *B. tabaci* MED in Brazilian potato fields (Barbosa *et al.*, 2015) calls attention to monitoring ToCV spread as the virus can be more efficiently acquired and transmitted by the MED species than the MEAM1 (Shi *et al.*, 2018).

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485318000974

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