Effect of Host Plant Resistance to *Tomato yellow leaf curl virus* (TYLCV) on Virus Acquisition and Transmission by Its Whitefly Vector

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ABSTRACT

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The effect that *Tomato yellow leaf curl virus* (TYLCV)-infected resistant tomato plants may have on virus epidemiology was studied. Four tomato genotypes that exhibit different levels of viral resistance, ranging from fully susceptible to highly resistant, served as TYLCV-infected source plants. Viral acquisition and transmission rates by whiteflies following feeding on the different source plants were evaluated. TYLCV transmission rate by whiteflies that had fed on infected source plants 21 days postinoculation (DPI), shortly after the appearance of TYLCV symptoms, was negatively correlated with the level of resistance displayed by the source plant. Therefore, the higher the resistance, the lower the transmission rate. In addition, TYLCV DNA accumulation was shown to be lower in the resistant source plants compared with the susceptible plants. Whitefly survival rate, following feeding on source plants 21 DPI, was similar for all the cultivars tested. Significant differences in whitefly survival were found, however, following feeding on the infected source plants at 35 DPI; here, whitefly survival rate increased with higher levels of resistance displayed by the source plant. At 35 DPI, the susceptible plants had developed severe TYLCV disease symptoms, and transmission rates from these plants were the lowest, presumably due to the poor condition of these plants. Transmission rates from source plants displaying a medium level of resistance level were highest, with rates declining following feeding on source plants displaying higher levels of TYLCV resistance. TYLCV DNA accumulation in whiteflies following feeding on infected source plants at both 21 and 35 DPI was directly correlated with viral DNA accumulation in source plants. Results show that, in essence, the higher the resistance expressed, the less suitable the plant was as a viral source. Consequently, following acquisition from a highly resistant plant, TYLCV transmission by whiteflies will be less efficient.

Tomato yellow leaf curl virus (TYLCV) is one of the most devastating begomoviruses of cultivated tomatoes in tropical and subtropical regions. Tomato leaf curl disease has long been known in the Middle East, North and Central Africa, and Southeast Asia, and it has spread to southern Europe, where severe outbreaks of TYLCV have been reported recently (7,11,13). TYLCV has also been identified in the Caribbean region (12,19), Mexico (3) and in the United States, initially in Florida (20), soon after in Georgia (10), and most recently in Louisiana (22).

TYLCV is a monopartite begomovirus transmitted by the whitefly, *Bemisia tabaci* (Genn.). TYLCV epidemics tend to be associated with high populations of the whitefly vector (4). The virus infects tomato in the Mediterranean Basin mainly during the summer and autumn, and can cause up to 100% yield loss. In many tomato-growing areas, TYLCV has become the limiting factor for production, both in the field and in protected screen houses (15).

Control measures traditionally have emphasized reducing vector populations (4). Chemical control methods have been only partially effective. Furthermore, there are concerns about chemical control due to the potential for the vector to develop pesticide resistance and the deleterious effect on the environment (15). Fine-mesh screens have been used in the Mediterranean Basin as a means of protecting the crop (4). More recently, UV-absorbing plastic sheets and screens have been used to inhibit penetration of whiteflies into covered greenhouses (2). However, these screens create problems of shading, overheating, and poor ventilation (4). Thus, one of the best ways to reduce losses due to TYLCV is to develop tomatoes that are resistant or tolerant to the virus (4).

Over the last 20 years, considerable efforts have been devoted to the development of TYLCV-resistant cultivars. Because all *Lycopersicum esculentum* tomatoes tested have been susceptible to TYLCV, wild *Lycopersicon* spp. have been screened for their response to the virus (8,17,18,23,24). The first commercially available tolerant cultivar, 'TY20', carrying resistance derived from *L. peruvianum*, showed delayed symptoms and reduced accumulation of viral DNA (18,21). Recently, advanced breeding lines with high levels of resistance derived from various wild *Lycopersicon* spp. have been developed and are being extensively utilized in the breeding of high-quality F1 hybrids (8,9,23,24).

Whereas disease resistant phenotypes reduce the deleterious effects of the virus, the potential role of resistant cultivars as virus reservoirs is unknown. In this study, the potential role of resistant tomato plants on TYLCV spread was investigated. The specific objectives studied were to assess: (i) the effect of tomato plants displaying different levels of TYLCV resistance on whitefly survival and TYLCV transmission following feeding on these plants, and (ii) the effect of TYLCV infection on whitefly reproduction on tomato plants displaying different levels of TYLCV resistance.

MATERIALS AND METHODS

Virus. The TYLCV used in this study was derived from the original culture described by Cohen and Nitzany (6). It originated in the field and is a mixture of two TYLCV isolates described by Navot et al. (14) and Antignus and Cohen (1). The virus was maintained (in tomato) in an insect-proof greenhouse.

Plant material. Four tomato cultivars that exhibit different levels of viral resistance, ranging from fully susceptible to highly

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resistant, were used as TYLCV-infected source plants for virus acquisition. These cultivars were the highly TYLCV-resistant breeding line TY172 (Volcani Center line) (8,9), the TYLCV-resistant commercial cvs. 8484 (Hazera Genetics Ltd., Brurim, Israel) and Fiona (S & G, Enkhuizen, Netherlands), and the highly susceptible tomato breeding line L27. The TYLCV resistance level of the different cultivars has been determined previously, following inoculation with TYLCV at first-leaf stage (9). The inoculated plants of each cultivar were compared with their respective control noninoculated plants (of the same cultivar) in terms of total yield, fruit weight and number, and plant fresh weight. Disease symptom development and virus accumulation in the inoculated plants were monitored. Resistance levels were as follows: TY172 expressed the highest level of resistance (less than 20% yield loss, no symptoms), followed by Fiona (56% yield loss, mild symptoms) and 8484 (67% yield loss, pronounced symptoms but milder than symptoms displayed by susceptible plants). Under the same trial conditions, TYLCV-susceptible plants gave no yield and developed severe symptoms following inoculation with TYLCV (9).

TYLCV symptom severity rating. Symptom development was evaluated according to the following scale: 0 = no visible symptoms, inoculated plants show similar growth and development as noninoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling, and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling; plants stop growth.

Whitefly maintenance and inoculation of source plants. Whitefly (*Bemisia tabaci*) colonies were reared on cotton plants grown in muslin-covered cages maintained within an insect-proof greenhouse. Adult whiteflies were given a 48-h acquisition access period (AAP) on TYLCV-infected tomato source plants, after which they were provided with a 48-h inoculation access period (IAP) on 21-day resistant and susceptible tomato plants, at a density of 50 whiteflies per plant. Following the IAP, whiteflies were removed by treating plants with a pyrethroid insecticide (Smash; Aagan Chemical Manufactures, Ltd, Ashdod, Israel) and plants were maintained in separate cages in an insect-proof greenhouse.

TYLCV acquisition and transmission. TYLCV-infected tomato plants of each cultivar, obtained as described above, were used for the following experiments. Nonviruliferous whiteflies were given a 48-h AAP on TYLCV-infected source plants. During the AAP, plants were kept in different cages isolated from one another. Exposure of whiteflies to infected source plants was performed twice, first at 21 days postinoculation (DPI) of the source plants, and a second exposure at 35 DPI. At 21 DPI, under greenhouse conditions, the disease symptoms are pronounced but are not expressed completely yet; whereas, at 35 DPI, the symptoms are at full severity. At least 10 plants of each source plant cultivar were used in each of three independent experiments. Following acquisition, 15 whiteflies were collected from each source plant and individually transferred to young (first-leaf stage) susceptible 'Marmande' tomato host plants (a single whitefly per host plant). Thus, for each of the 10 infected plants of each cultivar, 15 whiteflies were transferred to 15 separate test plants (a total of 150 whiteflies transferred from each cultivar). Inoculated tomato seedlings were covered with ventilated plastic cups, and the whiteflies were allowed a 24-h IAP. Host plants that had a live whitefly at the end of the transmission period were treated with imidacloprid (Confidor; Bayer, Leverkusen, Germany), kept in an insect-proof greenhouse for 4 weeks, and monitored for TYLCV symptom development. Plants without a live whitefly at the end of the 24-h IAP were discarded. The above procedure (experiment) was repeated on three different occasions covering different seasons of the year. The results of the three independent experiments were of the same trend. Results presented here are from one representative experiment.

Whitefly survival rate. At the end of the 24-h IAP, the plastic cups covering the plants were removed, the plants were inspected visually, and the number of live whiteflies was recorded. Whitefly survival was determined as the percentage of whiteflies that were alive at the end of the 24-h IAP. In order to avoid accumulation of high humidity under the plastic cups, the IAP was kept as short as possible without affecting transmission efficiency. It has been previously shown that a 24-hr IAP is sufficient in order to attain optimal transmission rates of TYLCV by whiteflies (1,11).

Viral DNA detection. Viral DNA accumulation in the uppermost leaf of infected source plants of each cultivar as well as in whiteflies following AAP on these plants was estimated by dot blot hybridization (9,16). Samples were taken from each of the 10 different plants of each of the infected cultivars and from whiteflies exposed to these plants at 21 and 35 DPI.

Following virus acquisition by whiteflies, leaf tissue (0.1 g) from the uppermost fully expanded leaf from the plant apex was ground in 0.5 ml of 0.4 M NaOH, and 10-µl aliquots were dotted on a nylon membrane (Hybond N+; Amersham Pharmacia, Freiburg, Germany) as described (9). Negative controls consisted of control, noninoculated, healthy plants of each cultivar.

TABLE 1. Rates of whitefly survival and *Tomato yellow leaf curl virus* (TYLCV) transmission following a 24-h inoculation access period on tomato plants having different levels of TYLCV resistance and TYLCV DNA accumulation in plants and whiteflies[w]

		Average (±SE) amount of TYLCV DNA (ng)[x]		Rate (%)	
Cultivar[y]	Symptom severity[z]	In plants	In whiteflies	Whitefly survival	TYLCV transmission
21 DPI					
L27	3	30.2 ± 3.3 a	3.7 ± 0.4 a	54 a	59 a
8484	2	16.0 ± 1.3 b	2.4 ± 0.3 b	43 a	52 a
Fiona	1	4.8 ± 0.2 c	1.6 ± 0.1 c	44 a	34 b
TY172	0	5.8 ± 0.7 c	1.3 ± 0.03 c	46 a	17 c
35 DPI					
L27	4	18.4 ± 1.5 a	2.1 ± 0.1 a	36 a	12 a
8484	3	16.9 ± 1.1 a	2.2 ± 0.1 a	36 a	48 b
Fiona	1.5	4.1 ± 0.3 b	1.6 ± 0.1 b	58 b	18 a
TY172	0	3.4 ± 0.3 b	1.5 ± 0.03 b	60 b	25 a

[w] Number of experimental units was 10 for all source plants in the two dates. Within columns, different letters denote means that significantly differ, $P < 0.05$.

[x] TYLCV DNA content in the plants and whiteflies was determined by dot-blot hybridization. Plant samples were taken from 10 individual plants of each of the infected source plants. Whiteflies were collected following a 48-h acquisition access period on the infected source plants. Ten whiteflies were sampled together from individual plants; samples included 10 individual plants from each source plant cultivar.

[y] Cultivars listed for two access periods; DPI = days postinoculation.

[z] Severity levels: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling, and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling; plants stop growth.

Whiteflies were collected separately from 10 individual plants per source plant cultivar. Ten whiteflies from each source plant were collected and placed at –70°C for 24 h. The 10 frozen whiteflies from each plant were ground in 20 µl of 0.4 M NaOH, and 7.5-µl aliquots were dotted on a nylon membrane. Negative controls consisted of nonviruliferous whiteflies, which were reared on healthy cotton plants.

TYLCV cDNA in pBluescript (Stratagene, La Jolla, CA) served as a template for the production of an in vitro synthesized ^{32}P-labeled viral riboprobe, corresponding to the full-length viral genome. Membranes were hybridized with labeled viral riboprobe as described (9), washed, and exposed to a phosphorimager screen (Bio-imaging analyzer, BAS-1500; FUJIFILM, Tokyo). The amount of viral DNA in each spot was quantified and the background level was subtracted from each measurement. The amount of TYLCV DNA in each sample was calculated according to a standard curve of TYLCV cDNA (ranging from 0.5 to 50 ng of TYLCV cDNA), which was dotted on a nylon membrane and hybridized with labeled viral riboprobe (9).

Whitefly reproduction. The influence of TYLCV-infected plants on whitefly reproduction was examined following the method of Cohen et al. (5). Healthy 9-day-old female whiteflies from the same cohort were maintained on healthy and TYLCV-infected plants of each cultivar for 5 days in leaf cages. Afterward, adult females were removed and eggs that were oviposited during the 5-day period were allowed to mature. We used 10 whiteflies per plant, from each of 10 plants of each different tomato cultivar (100 whiteflies/cultivar). Test plants were 25 days old, and infected plants were used at 10 DPI. After adult female whiteflies were removed from the plants, plants were kept for an additional 15 days in separate cages and the number of third and fourth (pupa) nymphal instars per leaf was counted. The above experiment was repeated on three different occasions. The results of the three independent experiments were of the same trend. Statistical analysis was carried out by means of one-way analysis of variance (ANOVA) (SAS Institute, Cary, NC).

RESULTS

TYLCV acquisition and transmission by whiteflies from resistant source plants 21 DPI. When TYLCV-infected resistant and susceptible plants were used as source plants at 21 DPI, whitefly survival, as measured by how many individual whiteflies survived on the test plants at the end of the 24-h IAP, was similar for all four cultivars tested (Table 1). However, TYLCV transmission frequency differed significantly among the plant cultivars (Table 1), with the highest rate of transmission (59%), achieved with whiteflies acquiring the virus from susceptible cv. L27 (Table 1). A similar level of transmission was obtained when the source plant was 8484, despite the large difference between TYLCV DNA accumulation by the two plant cultivars (Fig. 1 and Table 1). Susceptible cv. L27 accumulated 30 ng of viral DNA, 8484 accumulated 16 ng, Fiona accumulated 4.8 ng, and TY172 accumulated 5.8 ng (Table 1). A lower rate of transmission (34%), was achieved with whiteflies from resistant cv. Fiona (Table 1), which was consistent with the lower level of TYLCV accumulation in these plants (Fig. 1 and Table 1). The lowest level of transmission (17%) was by whiteflies that had acquired the virus from TY172 plants (Table 1), even though TYLCV DNA accumulation was similar to that of Fiona plants (Fig. 1 and Table 1).

The above experiment (Table 1) was conducted in June and July (i.e., midsummer, with elevated temperatures and long days). The experiment was repeated in different seasons and the results were of the same trend. When the experiment was conducted in February and March (end of winter), transmission rates following whiteflies feeding on 21-DPI source plants were: 33% (a) from L27, 37% (a) from 8484, 24% (b) from Fiona, and 21% (b) from 172. When the experiment was conducted in October and November (autumn to winter) transmission rates following whiteflies feeding on 21-DPI source plants were 46.5% (a) from L27, 42.4% (a) from 8484, 29.6% (b) from Fiona, and 16% (c) from 172 (different letters denote means that significantly differ, $P < 0.05$.).

TYLCV-DNA level was also assayed in the whiteflies following virus acquisition from the various source plants. A good correlation was found between the TYLCV-DNA level in the whiteflies and in the infected source plants from which the virus had been acquired (Table 1). The highest level of TYLCV-DNA was detected in whiteflies that fed on the susceptible L27 plants (3.7 ng), with less TYLCV-DNA detected in those that fed on 8484 (2.4 ng) and the least TYLCV DNA detected in those that fed on Fiona (1.6 ng) and TY172 (1.3 ng) (Table 1). There were no significant differences between the amounts of TYLCV DNA detected in whiteflies exposed to Fiona or TY172 plants (Table 1).

TYLCV acquisition and transmission by whiteflies from resistant source plants 35 DPI. Transmission rates of whiteflies exposed to TYLCV-infected resistant and susceptible source plants at 35 DPI, when disease symptoms had become more pronounced, were also assessed. At 35 DPI, L27 plant growth was stunted and the plants exhibited severe TYLCV symptoms. The growth of 8484 was not stunted, but plants exhibited pronounced TYLCV symptoms (though milder than L27 plants). Plants of cv. Fiona exhibited very mild symptoms, whereas TY172 plants showed no symptoms at all (Table 1). Whiteflies that fed on plants of susceptible cv. L27, and on moderately resistant 8484 plants had only a 36% survival rate, whereas those that fed on cvs. Fiona and TY172 had a survival rate of ≈60% (Table 1). Despite the relatively high level of TYLCV accumulation in L27 plants at 35 DPI (18.4 ng) (Table 1), TYLCV transmission rates of whiteflies that fed on infected L27 plants were the lowest of the cultivars tested (12%) (Table 1). Interestingly, although the TYLCV accumulation level (16.9 ng) and the whitefly survival rate was similar for both 8484 and L27, the transmission rate of whiteflies that fed on 8484 plants were the highest (48%) (Table 1). Despite

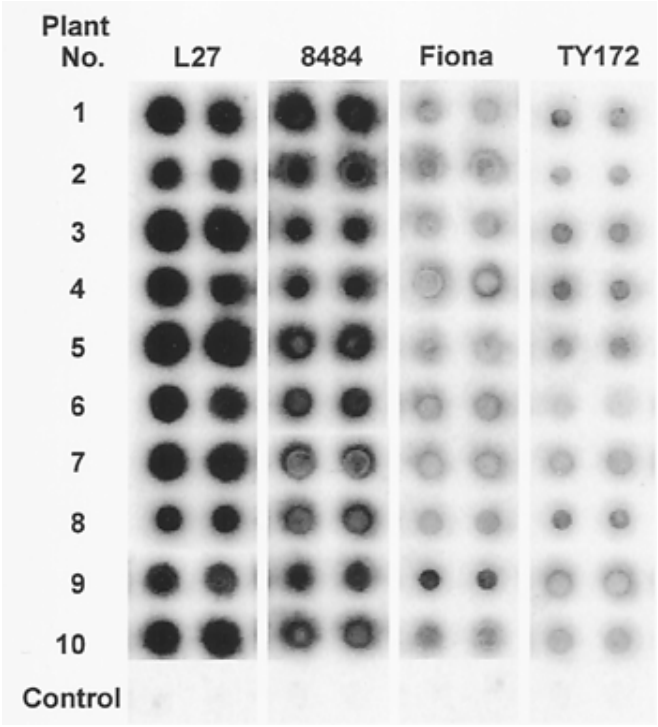

Fig. 1. Dot-blot hybridization of *Tomato yellow leaf curl virus* DNA extracted from infected source plants. Samples were taken from 10 individual plants of each of the infected source plants at 21 days postinoculation. Controls consisted of samples taken from noninoculated plants of each line. Each sample was dotted twice on the hybridized membrane. Numbers from 1 to 10 represent different individual plants.

the high survival rates for whiteflies that fed on infected Fiona and TY172 plants, transmission rates were low (Table 1), consistent with the low levels of TYLCV accumulation in these plants (4.1 and 3.4 ng, respectively; Table 1). TYLCV accumulation levels in the whiteflies having fed on infected source plants at 35 DPI were positively correlated with the levels of virus accumulation in the plants (Table 1).

TYLCV effect on whitefly reproduction. The effect that TYLCV infection has on whitefly reproduction on the various tomato cultivars was then assessed. Healthy, nonviruliferous 9-day-old female whiteflies were cultured on healthy and on TYLCV-infected plants for 5 days. At the end of the 5-day period, female whiteflies were removed, the plants were kept in insect-proof cages, and the number of third and fourth whitefly nymphal instars was counted 15 days later (Table 2). Virus infection had a deleterious effect on whitefly reproduction on L27 and 8484 plants. Significantly fewer third and fourth whitefly instars were observed on the TYLCV-infected plants, compared with healthy plants of the same cultivar (Table 2). In contrast, when whitefly fecundity was assayed on highly TYLCV-resistant plants (i.e., Fiona and TY172), there was no significant difference in the number of third and fourth whitefly instars on TYLCV-infected plants when compared with noninoculated plants (Table 2).

DISCUSSION

We examined the survival and TYLCV transmission rates for whiteflies having fed on TYLCV-infected tomato plants that varied in their level of TYLCV resistance. Four different tomato cultivars that exhibit different levels of viral resistance, ranging from fully susceptible to highly resistant, served as TYLCV-infected source plants. Survival rates following feeding on the different source plants at 21 DPI, shortly after appearance of TYLCV symptoms, were similar regardless of the cultivar. Significant differences in whitefly survival rates were found after whiteflies had fed on the infected source plants at 35 DPI, with the whitefly survival rate increasing with higher levels of resistance displayed by the source plant. This may be due to the deleterious effect of TYLCV on the infected plant. At 35 DPI, susceptible L27 and moderately resistant 8484 plants exhibited pronounced TYLCV disease symptoms, including smaller, thicker and up-curled leaves, presumably making the plant less suitable for feeding by whiteflies. In contrast, highly resistant plants (Fiona and TY172) were much healthier, which may favor whitefly survival. This is supported by results obtained from whitefly reproduction studies, in which reproduction on highly resistant plants was similar on TYLCV-infected and uninfected plants.

The TYLCV level in the whiteflies following feeding was found to be in direct correlation with the virus level in the source plant. Thus, the higher the level in the source plant, the higher the TYLCV level in the whitefly. This correlation was the same, regardless of the time of feeding (21 or 35 DPI) and regardless of the state of the source plants. The severity of disease symptoms exhibited by the source plants did not seem to affect TYLCV acquisition by the whiteflies.

TYLCV transmission was affected by virus level in the source plants. At 21 DPI, transmission rates of whiteflies having fed on TYLCV-infected Fiona and TY172 plants were much lower than rates for whiteflies having fed on infected 8484 and L27 plants. This suggests a positive correlation between TYLCV level in the plant and whitefly transmission rate. However, transmission efficiency following feeding on TY172 was lower than following feeding on Fiona, despite the same level of TYLCV in both plants. Moreover, at 21 DPI, transmission rates by whiteflies that had fed on 8484 and L27 was the same, despite the significant differences in TYLCV level in these source plants (i.e., susceptible L27 plants had nearly twice the level of TYLCV as resistant 8484 plants). Furthermore, at 35 DPI, transmission rates by whiteflies that had fed on 8484 were higher than by whiteflies that had fed on L27 plants, although both cultivars accumulated similar amounts of virus. It is possible that there are certain threshold levels of TYLCV accumulation in the source plants or in the whitefly that determine the transmission rate. Thus, it is possible that the TYLCV level in 8484 (at 21 DPI), despite being half that in L27, was above the threshold required for maximum transmission. The higher transmission rate following feeding on 8484 plants at 35 DPI was probably due to the TYLCV resistance expressed by this plant. Although the deleterious effects of the virus were very pronounced on the susceptible L27 plants, the moderately resistant 8484 plants expressed milder symptoms. Thus, following feeding on both plants, the whiteflies acquired the same amount of virus but, probably due to difficulties in terms of whitefly feeding on the badly diseased L27 plants, transmission rate by whiteflies following feeding on L27 plants dropped sharply.

Another possibility is that not all TYLCV detected in the plant was accessible to the feeding whiteflies. This could explain the lack of direct correlation between virus level in the plant and transmission efficiency by whiteflies. However, the positive correlation between virus level in the plant and virus level in the whitefly (following feeding) does not support this hypothesis.

Our results suggest at least two major factors that affect the efficiency of TYLCV transmission by whiteflies: (i) virus accumulation in the source plants and (ii) the fitness of the whiteflies, which is affected by the physiological condition of the source plants. Based on our results, it can be postulated that a TYLCV-infected field of susceptible tomato plants may serve as a high-risk virus reservoir soon after infection. However, as the plants deteriorate due to expression of TYLCV disease symptoms, the potential of these plants to serve as a source of virus declines. In contrast, a field of moderately resistant plants, such as 8484, will serve as an effective reservoir of virus throughout the season, because plants do not deteriorate as badly as highly susceptible plants. Tomato plants expressing a high level of resistance to TYLCV pose the lowest risk to the surrounding plants in terms of outbreaks of viral epidemics. Hence, the greater the virus resistance level expressed by the infected plant, the less suitable it is as a viral source plant.

However, it also is clear from our results that even highly TYLCV-resistant tomato cultivars can serve as a source of TYLCV inoculum. Thus, despite being symptomless following in-

TABLE 2. Whitefly reproduction on *Tomato yellow leaf curl virus*-inoculated versus noninoculated plants

Cultivar[z]	No. of instars per leaf[y]		
	Experiment I	Experiment II	Experiment III
L27			
Noninoculated	130.8	178	259.2
Inoculated	90.7	80.1	99.8
P	<0.01	<0.02	<0.01
8484			
Noninoculated	104.3	189	223.2
Inoculated	75	99	139.8
P	<0.04	<0.01	<0.05
Fiona			
Noninoculated	141.9	195	185.7
Inoculated	109.3	139	129.7
P	>0.1	>0.07	>0.08
TY172			
Noninoculated	119	168.7	145.7
Inoculated	90.8	124.6	99.7
P	>0.1	>0.07	>0.1

[y] Number of whitefly third and fourth instars per leaf, 15 days following culturing for 5 days of 10 adult female whiteflies. The number of instars is an average of 10 replications. Experiment I was conducted in March and April, experiment II June and July, and experiment III in September and October.

[z] *P* = significance by unpaired *t* test.

fection, these resistant cultivars are not suitable for planting in a host-free period used for TYLCV management.

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