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Effect of plant age at inoculation on expression of genetic resistance to tomato yellow leaf curl virus

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Summary

To determine the effects of plant age on the expression of genetic resistance to tomato yellow leaf curl virus (TYLCV), six TYLCV-resistant and two susceptible tomato varieties were inoculated at 14, 28 or 45 days after sowing (DAS). Inoculation at 14 and 28 DAS was performed in the greenhouse, and the plants were transplanted to the field at 30 DAS. Inoculation at 45 DAS was performed in the field, by covering the target plants with polypropylene (''Agril'') sheets and releasing viruliferous whiteflies under them. Resistance was assayed mainly by comparing yield components of inoculated plants to those of control, non-inoculated plants of the same variety. Symptom severity and plant height were also followed. Plant age at inoculation had no effect on disease-severity scores of the susceptible varieties, and little or no effect on those of the resistant varieties. In contrast, plant age at inoculation had a significant effect on the yield of all varieties tested. All varieties suffered a significant yield reduction due to inoculation with TYLCV; the lowest yield was produced by plants inoculated at 14 DAS. A smaller TYLCV-induced yield reduction (yield increase of 50 to 100%, depending on the variety's resistance level), was achieved following inoculation at 28 DAS. A further reduction in yield loss (yield increase of 30 to 40%) was achieved following inoculation at 45 DAS. Our results clearly demonstrate the occurrence of age-related (or mature-plant) resistance in tomato plants to TYLCV.

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Introduction

Tomato yellow leaf curl virus (TYLCV) is currently one of the most devastating viruses of cultivated tomatoes in tropical and subtropical regions. Although originally found in the eastern Mediterranean [3], it is now a worldwide problem in tomato cultivation [14, 21]. The virus is a monopartite begomovirus, transmitted by the whitefly *Bemisia tabaci* (Gennadius), whose severe population outbreaks are usually associated with high incidence of the disease. Control measures in infected regions are traditionally based on limiting vector populations. Chemical control has been only par-

tially effective, especially under high disease pressure, and in addition to its deleterious effects on the environment, the vector has been shown to develop pesticide resistance [6, 17]. The use of physical barriers such as fine-mesh screens and UV-absorbing plastic sheets and screens has become widespread in the Mediterranean basin as a means of crop protection [1, 2]. However, these screens create problems of overheating and poor ventilation. Genetic resistance in the host plant is the best defense against whitefly-transmitted viruses, since it requires no chemical input and/or plant seclusion and may be stable and long-lasting. Thus, the best way to reduce the spread of TYLCV is by breeding tomatoes that are resistant or tolerant to the virus [8].

Over the last 25 years, extensive effort has been invested in breeding tomato cultivars resistant to TYLCV. Since all cultivars of tomato (*Solanum lycopersicum*) are extremely susceptible to TYLCV, wild *Solanum* species were screened for their response to the virus to identify genes for resistance [8, 11, 15, 19, 20, 24]. Breeding programs have been based on the transfer of resistance genes from accessions of wild origin into the cultivated tomato. However, progress in breeding for TYLCV resistance has been slow, due in part to the complex genetics of the resistance and the presence of interspecific barriers between the wild and domesticated tomato species [8].

Another setback in the development of TYLCV resistance is that while most screening assays rely on severity of TYLCV-induced disease symptoms, the most relevant evaluation of resistance level is TYLCV-induced yield reduction [7, 9]. Thus it is recommended that in addition to monitoring symptoms, the effect of infection on total yield and yield components be tested and compared to that in equivalent, non-infected plants. Usually, tests comparing different varieties are carried out under field inoculation, and no comparison is made to the full yield potential of uninfected plants, which has a direct bearing on the yields of the infected plants. Nevertheless, such expensive and time-consuming tests can only be carried out on the most promising resistant varieties, and not on segregating populations.

Another obstacle in the development of TYLCV resistance has been the lack of a standard method for the assessment of resistance [7]. Variability in assay conditions has led to contradictory results, where different resistance levels have been attributed to the same genetic sources [10, 18, 24]. The response of a plant to infection by a pathogen may be affected by test conditions such as temperature, light, growth conditions, inoculation pressure and plant age (or developmental stage) at the time of infection. The last of these phenomena has been referred to as age-related or mature-plant resistance [12]. In some instances, it has been shown that mature plants resist or tolerate virus infection much better than plants infected at an early stage of development, leading to what appears to be increased viral resistance [5, 13, 22, 23].

In this study, we tested for the possible effects of plant age on the expression of genetic resistance to TYLCV. Tomato plants expressing different levels of resistance to TYLCV were inoculated at three different ages-14, 28 and 45 days after sowing (DAS). Resistance was assayed mainly by comparing yield components of inoculated plants to those of control, non-inoculated plants of the same line or variety.

Materials and methods

Virus and whitefly maintenance

Cultures of the Israeli isolate of TYLCV [16] (GenBank Acc. No. X15656) were maintained in tomato (*Lycopersicon esculentum* L.) in an insect-proof greenhouse. Whitefly (*Bemisia tabaci*, biotype B) colonies were reared on cotton (*Gossypium hirsutum* L.) plants grown in muslin-covered cages maintained inside an insect-proof greenhouse.

Plant material

Lines: A TYLCV-susceptible 'Marmande' type tomato line, Rehovot-13 (R-13; Hazera Genetics Ltd., Brurim, Israel), and a highly TYLCV-resistant tomato line, TY-199 (Volcani Center), were used. F1 hybrids: The TYLCV-susceptible tomato, 144, and TYLCV-resistant tomatoes-3193, 3205 and 3209 (Hazera Genetics Ltd.), Tyjoco (Sluis & Groot/Syngenta, Enkhuizen, The Netherlands) and Anastasia (Bruinsma Seeds, Enkhuizen, The Netherlands), were used.

Test plants were sown in 128-cell Todd Planter Flats (also known as "speedling" trays) and kept in the trays for 30

days until transplanted to the field or to large buckets in the greenhouse.

TYLCV inoculation

Adult whiteflies were provided a 48-h acquisition access period (AAP) on TYLCV-infected tomato source plants. Following the AAP, whiteflies were allowed a 24-h inoculation access period (IAP) on tomato test plants by two methods, by clip cage and 'free choice'.

Free choice inoculation

Tomato plants inoculated at 14 and 28 DAS were inoculated in the greenhouse, whereas plants inoculated at 45 DAS were inoculated in the field.

Greenhouse inoculation

To ensure 100% infection, inoculation was performed at a density of about 50 whiteflies per plant. The different tomato varieties were inoculated at 14 or 28 DAS. Control, non-inoculated plants of the same varieties were exposed to virus-free whiteflies for 24 h. Following the IAP, whiteflies were removed by treating plants with imidacloprid (Confidor, Bayer, Leverkusen, Germany). The plants were maintained in an insect-proof greenhouse at 26–32 °C prior to transplant to the field at 30 DAS.

Inoculation in the field: Adult whiteflies were provided a 48-h AAP on TYLCV-infected tomato source plants, after which the source plants containing the whiteflies were moved into sealed buckets. In the field, the target plants for inoculation were covered with non-woven polypropylene (Agril) sheets (Sodoca, Biesheim, France) mounted on a wooden frame. The buckets were taken to the field, positioned under the Agril sheets and then opened to release the whiteflies. The whiteflies were allowed a 24-h IAP on the test tomato plants followed by application of imidacloprid. The Agril sheets were removed 24 h after imidacloprid application.

Inoculation using clip cages: following the AAP, 50 whiteflies were placed in each clip cage, and then one clip cage was attached onto the uppermost leaf of each tomato test plant. Whiteflies were allowed a 24-h IAP on the test plants, followed by removal of the clip cages and application of imidacloprid. The plants were maintained in an insect-proof greenhouse at 26–32 °C prior to transplant to large pots in an insect-proof glasshouse equipped for plant growth at 30 DAS. Plants that were inoculated at 45 DAS were inoculated (using clip cages) after transplanting to large pots.

TYLCV symptom-severity rating

Symptom development was evaluated according to the symptom-severity scale described by Friedmann et al. [4] as follows: 0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated 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, but plants continue to develop; 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops. Symptom severity was evaluated in the field, 5 weeks after transplanting. Plant height was measured a month later.

Field trial

Following controlled inoculation in the greenhouse, the plants were transplanted to the field in April and grown through the spring and summer seasons. Prior to transplanting, the plants were treated with imidacloprid. Plants of each variety were planted in paired rows-inoculated and non-inoculated (control), on 1-m-wide beds, five plants per row. The within-row and between-row spacings were 0.5 and 1.2 m, respectively. Each pair of rows served as a replicate for the experiment, and a total of 10 randomly distributed replicates were planted in the field. Imidacloprid was applied through the drip-irrigation system at 4 and 8 weeks after transplanting. Fruits were picked in three harvests: in the first and second harvests, only mature-red fruits were collected; in the third harvest, all mature-red and immature green fruits were collected. Culls were discarded. The following parameters were assayed: total yield, total number of fruits and average fruit weight. Data were taken per row and were averaged for all rows.

Glasshouse trial

Following inoculation (using clip cages) in the greenhouse, the inoculated and non-inoculated control plants were transplanted to an insect-proof glasshouse in 10 liters of Lava soil grade 0–4. The plants were transplanted in April and grown through the spring and summer seasons. The plants were trained on a single stem. Once the plants reached to the upper level of the pruning string (about 2 m), the tips of the plants were removed. Plants of each variety were planted in paired rows – inoculated and non-inoculated (control), each plant in a in 10-liter pot. A single plant served as a replica, and 12 plants from each treatment were randomly distributed in the glasshouse. Imidacloprid was applied through the drip-irrigation system at 4 and 8 weeks after transplanting. Fruits were picked in two harvests as described for the field trial.

Viral DNA detection

Viral DNA accumulation level in the plant apex of infected plants in the glasshouse trial was determined by dot blot hybridization. Leaf tissue (0.1 g) from the second leaf from the top was ground in 0.5 ml 0.4 M NaOH, and 10-µl sam-

ples were dotted on a nylon membrane as described previously [9]. For background determination, samples were taken from a healthy control non-inoculated plant of each line. An 856-nucleotide-long TYLCV DNA fragment, corresponding to the 5′ half of the C1 gene of TYLCV (nucleotides 2087 to 171) served as a template for the production of an *in vitro*-synthesized ^{32}P-labeled viral riboprobe. The membrane was reacted with the labeled viral riboprobe, rinsed under high stringency conditions and exposed to a phosphorimager screen (Bio-imaging analyzer, FLA 5000, FUJIFILM, Japan). The amount of viral DNA in each spot was quantitated 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 1 to 50 ng of TYLCV cDNA), which was dotted on a nylon membrane and reacted with the labeled viral riboprobe.

Statistical analysis was performed by means of one-way analysis of variance (ANOVA) test (SAS Institute, Cary, NC).

Results

Effect of age on TYLCV-induced yield reduction – field experiment

To test the effect of plant age on genetic resistance to TYLCV, plants were inoculated at 14, 28 or 45 days after sowing (DAS). At 14 and 28 DAS the plants were inoculated in the greenhouse, and at 30 DAS the plants were transplanted to the field and grown for yield. Inoculation at 45 DAS was done in the field, following transplanting. The highest level of resistance, as reflected by the lowest yield reduction induced by TYLCV, was expressed by the resistant line TY-199 (Table 1). Following inoculation at 14 DAS-the first true-leaf stage, TY-199 plants showed no disease symptoms but nonetheless produced only 45.5% of the yield of the non-inoculated control plants. TY-199 was followed by the F1 hybrid 3205, which produced 42% of the yield of its non-inoculated control. The resistant F1 hybrids 3193, 3209 and Anastasia expressed practically the same level of resistance, which was much lower than that expressed by TY-199 and 3205, producing 27.4%, 26.4% and 25.4%, respectively, of the yield of their non-inoculated counterparts. Of all the resistant varieties tested, Tyjoco showed the lowest level of resistance following inoculation, producing only 18% of the yield of its non-inoculated control (Table 1). However, all the resistant varieties performed much better than the two susceptible controls, R-13 and 144, both of which barely produced any fruit following inoculation-0.0% and 2.6%, respectively, compared to the yield of their non-inoculated counterparts. The TYLCV-induced yield reduction was mainly due to the strong reduction in the number of fruits per plant, although in the case of the susceptible varieties, there was also a strong reduction in fruit size-ranging from 57% of the size of the control fruits for 144 to 66.5% of the size of the control fruits for R-13. Only two of the resistant varieties, Tyjoco and 3209, suffered a significant reduction in fruit size following inoculation: Tyjoco's fruit size was 69% of that of its non-inoculated controls, and 3209 fruit size was 77% of that of its non-inoculated controls (Table 1). The other resistant varieties suffered minor reductions in fruit size due to TYLCV inoculation, ranging from Anastasia, which lost only 14% of its fruit size, to TY-199, 3205 and 3193, which showed no significant reduction in fruit size at all (Table 1).

Disease-severity score was in essence correlated to yield reduction: the higher the score, the higher the yield reduction. Both susceptible varieties had the highest disease severity score of 4, followed by Anastasia and Tyjoco (2.4 and 2.3, respectively), 3209 and 3193 (1.3 and 1.1, respectively), and finally 3205 with 0.7 and TY-199, which showed practically no disease symptoms (Table 1).

In terms of plant height, the susceptible plants were the most affected, both showing a severe reduction in plant height due to the virus, i.e. only 35 to 36% of the height of their control counterparts. The most affected resistant variety was Anastasia, which reached 68% of the height of its control, while the other resistant varieties ranged from 74% for Tyjoco to 96% (not statistically significant) for 3209, 3205 and TY-199 (Table 1).

When the different varieties (resistant and susceptible) were inoculated with TYLCV at 28 DAS, all produced higher yields compared to inoculation at 14 DAS. The yield increase (or actually smaller TYLCV-induced yield reduction) was substantial, ranging from 50% for TY-199 and 3205, to 100% or more for 3193 and Tyjoco (Table 1). In all

Table 1. The effect of plant age at the time of inoculation with tomato yellow leaf curl virus on yield components

Cultivar	Plant age at inoculation (DAS)[a]	Symptom-severity score[b]	Av. plant height (cm)[c]	Av. yield (kg/plant)	Av. fruit no. per plant	Av. fruit size per plant (g)
R-13	non-inoculated	0.0	131.0[a]	4.2[a]	32.4[a]	128.5[a]
	14	4.0	47.8[b]	0.0[b]	0.2[b]	86.3[b]
	28	4.0	73.5[c]	0.1[c]	1.6[b]	70.3[c]
	45	4.0	75.6[c]	0.9[d]	10.3[c]	85.4[c]
144	non-inoculated	0.0	150.0[a]	6.7[a]	71.1[a]	94.7[a]
	14	4.0	52.5[b]	0.2[b]	3.4[b]	52.2[b]
	28	4.0	85.5[c]	0.6[c]	10.6[b]	60.0[b]
	45	4.0	92.7[c]	1.4[d]	20.9[c]	68.2[c]
TY-199	non-inoculated	0.0	164.4[a]	4.4[a]	55.2[a]	80.1[a]
	14	0.1	144.5[b]	2.0[b]	25.9[b]	77.6[a]
	28	0.1	160.0[a]	3.1[c]	41.7[c]	74.8[a]
	45	0.0	157.8[a]	4.0[a]	52.0[a]	76.7[a]
3205	non-inoculated	0.0	159.4[a]	6.7[a]	101.6[a]	65.6[a]
	14	0.7	128.6[b]	2.8[b]	46.9[b]	60.0[a]
	28	0.3	149.4[c]	4.3[c]	70.0[c]	62.0[a]
	45	0.0	153.8[a,c]	5.5[d]	90.2[a]	61.7[a]
3193	non-inoculated	0.0	161.9[a]	6.2[a]	58.3[a]	106.0[a]
	14	1.1	121.9[b]	1.7[b]	17.9[b]	96.7[a]
	28	1.0	145.0[c]	3.4[c]	32.8[c]	103.8[a]
	45	0.4	142.5[c]	4.4[d]	45.0[d]	98.4[a]
3209	non-inoculated	0.0	157.5[a]	7.2[a]	46.6[a]	155.9[a]
	14	1.3	131.3[b]	1.9[b]	16.1[b]	120.2[b]
	28	1.3	146.3[a,b]	3.3[c]	28.2[c]	118.1[b]
	45	0.3	151.3[a]	5.5[d]	37.3[d]	146.2[a]
Tyjoco	non-inoculated	0.0	154.4[a]	6.1[a]	64.2[a]	95.8[a]
	14	2.3	113.8[b]	1.1[b]	17.4[b]	62.7[b]
	28	1.9	131.1[c]	2.7[c]	36.0[c]	75.5[c]
	45	1.4	142.9[d]	3.9[d]	52.5[d]	74.3[c]
Anastasia	non-inoculated	0.0	161.9[a]	5.9[a]	56.7[a]	103.6[a]
	14	2.4	111.3[b]	1.5[b]	17.0[b]	88.9[b]
	28	2.2	127.5[c]	2.3[c]	26.0[c]	91.4[b]
	45	1.9	140.0[d]	3.5[d]	39.6[d]	89.3[b]

[a] *DAS* Days after sowing; [b] symptom severity was evaluated in the field, 5 weeks after transplanting; [c] plant height was measured a month later.
Means with different letters differ significantly at $P < 0.05$ when analyzed by one-way ANOVA.

cases, the plants that were inoculated later were also taller. However, regardless of the large increase in yield, the symptom-severity score barely changed between plants inoculated at 14 DAS and those inoculated 2 weeks later (Table 1). A further substantial decrease in TYLCV-induced yield reduction (a yield increase) was achieved by all of the tested varieties (resistant and susceptible alike) following inoculation at 45 DAS. The yield increase for the variety expressing the highest level of TYLCV resistance, TY-199, was from 2 kg/plant following inoculation at 14 DAS, 3.1 kg/plant plant following inoculation at 28 DAS, to 4 kg/plant following inoculation at 45 DAS (Table 1). For varieties expressing a lower level of TYLCV resistance, the yield increase was even more dramatic: Tyjoco's yield, which was 1.1 kg/plant following inoculation at 14 DAS, more than doubled to 2.7 kg/plant following inoculation at 28 DAS and reached 3.9 kg/plant following inoculation at 45 DAS

(Table 1). The susceptible varieties showed a more marked increase in yield due to the effect of plant age at the time of infection. R-13, which produced 0.01 kg/plant following inoculation at 14 DAS, reached 0.9 kg/plant following inoculation at 45 DAS-quite a substantial increase. The same was true for 144-its yield increased from 0.2 kg/plant following inoculation at 14 DAS to 1.4 kg/plant following inoculation at 45 DAS (Table 1).

Assessing TYLCV accumulation in plant apex

To follow TYLCV accumulation in the inoculated plants, plants had to be inoculated with the same inoculum level – the same number of viruliferous whiteflies per plant. Thus, plants were inoculated (at 14, 28 and 45 DAS) using clip cages, 50 whiteflies per clip cage, a single clip cage per plant. Following inoculation, the plants were transplanted to 10-liter pots and grown in an insect-proof glasshouse protected from other insects which may be encountered in the field, and from further inoculation by whiteflies. Following inoculation of the susceptible variety 144, samples were taken from plant apex at 7, 14, 21 and 28 days after inoculation (DAI) and assayed for TYLCV level using dot-blot hybridization (Fig. 1). Until 21 DAI, the virus level was nearly the same (not statistically significant) regardless of plant age at the time of inoculation – the virus level increased with time between 7 and 21 DAI, but at a moderate rate (slope was 0.25 for 14 DAS, 0.23 for 28 DAS and 0.21 for 45 DAS). However, after 21 DAI the virus level increased sharply when plants were inoculated at 14 DAS (slope of 1.3), in contrast to plants inoculated at 28 and 45 DAS, where the virus level continued to increase at a moderate rate (slope of 0.4 for both 28 and 45 DAS) (Fig. 1). We have also assayed the TYLCV level in three resistant varieties, TY199, 3193 and Anastasia, but due to the TYLCV-resistance displayed by these varieties, the virus level was too low to follow accurately using dot-blot hybridization (data not shown). This is in agreement with previous results showing a low TYLCV accumulation level in resistant plants [4, 9, 18, 19]. We were able to measure the virus level in all of the resistant plants inoculated at 14 DAS, but we were not able to detect virus in plants inoculated at 28 and 45 DAS (not shown). In order to test whether indeed the virus accumulation level was too low for detection by dot-blot hybridization, and to make sure that all the plants were indeed infected, we have assayed these plants for TYLCV presence using PCR. Indeed, all the inoculated plants were found to be positive for TYLCV when tested by PCR (not shown).

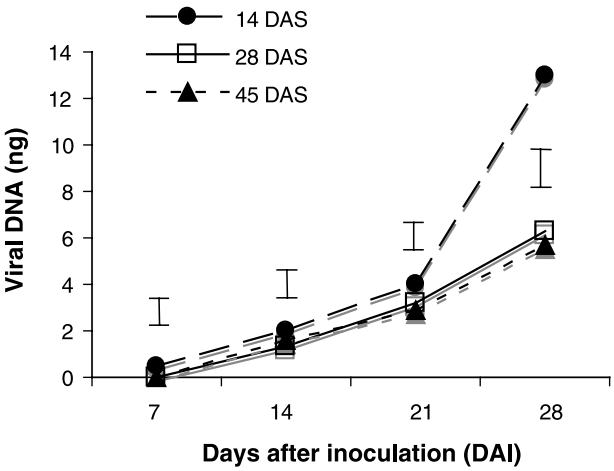

Fig. 1. Accumulation of TYLCV DNA in upper leaves of a susceptible tomato variety (cv. 144) following inoculation at 14, 28 and 45 DAS. Tomato plants (cv. 144) were grown in a glasshouse and inoculated at 14, 28 and 45 days after sowing (DAS). The plants were inoculated using clip cages, one clip cage per plant, 50 whiteflies per clip cage. TYLCV DNA was quantitated by dot blot hybridization as described in the text. Samples were collected at 7, 14, 21 and 28 days after inoculation (DAI). The results are the means of eight replicates. The least significant difference (Fisher's PLSD test) is marked on the graph for each time point

Effect of age on TYLCV-induced yield reduction – glasshouse experiment

The plants that were used for assaying virus levels were kept in the glasshouse and tested for yield as well (Table 2). It should be noted that due to different growth conditions between the field- and glasshouse-grown plants – the glasshouse plants were trained on a single stem, tips removed once the upper level reached the pruning string (see Materials and methods section) – the yield per plant was much lower in the glasshouse-grown plants compared to

Table 2. The effect of plant age at the time of inoculation with tomato yellow leaf curl virus following inoculation with clip cages and growth in a glasshouse

Cultivar	Plant age at inoculation (DAS)[a]	Av. yield (g/plant)	Av. fruit no. per	Av. fruit size per plant (g)
144	non-inoculated	2104[a]	22.8[a]	92.1[a]
	14	62[b]	1.2[b]	50.9[b]
	28	110[b]	1.9[b]	56[b]
	45	1628[c]	18.4[c]	88.5[a]
TY-199	non-inoculated	1564[a]	21.3[a]	73.6[a]
	14	751[b]	13.8[b]	54.3[b]
	28	1018[c]	14.5[b]	70.4[a]
	45	1163[c]	14.7[b]	79.1[a]
3193	non-inoculated	1623[a]	18.3[a]	88.7[a]
	14	781[b]	13.4[b]	58.3[b]
	28	1291[c]	16.2[a,b]	79.7[a]
	45	1488[a,c]	18.7[a]	79.6[a]
Anastasia	non-inoculated	1859[a]	22.0[a]	84.5[a]
	14	115[b]	2.4[b]	47.9[b]
	28	1122[c]	15.9[c]	70.6[c]
	45	1537[d]	19.4[a]	79.2[a,c]

[a] *DAS* Days after sowing.
Means with different letters differ significantly at $P < 0.05$ when analyzed by one-way ANOVA.

the field experiment. Moreover, glasshouse plants are usually kept yielding for 9 or so months, and we had to terminate the experiment after 4 months. However, although glasshouse yield was lower than that in the field, plant age at time of inoculation had the same effect on TYLCV-induced yield reduction as in the field experiment (Tables 1 and 2). Plants inoculated at 14 DAS suffered the highest TYLCV-induced yield loss, followed by plants inoculated at 28 DAS, and the highest yield (lowest TYLCV-induced yield reduction) was reached by plants inoculated at 45 DAS (Table 2).

Discussion

In the present work, we examined whether plant age at the time of inoculation has any effect on the expression of genetic resistance to TYLCV. Tomato plants were inoculated at three different ages-14, 28 and 45 DAS. We chose to inoculate at 14 DAS since this is the first true-leaf stage-in practice, the earliest stage for efficient inoculation of TYLCV. Inoculation at 28 DAS was selected to represent inoculation just prior to transplant to the field-transplanting tomato plants 30 DAS is a common agricultural practice. Inoculation at 45 DAS was selected to represent inoculation of plants following transplant to the field, but not too long after transplant, since in many whitefly-stricken areas, the plants are infected shortly after exposure to open-field conditions. At the two earlier dates (14 and 28 DAS), inoculation was performed in the greenhouse, while at the later date of 45 DAS, the plants were inoculated in the field.

Six different TYLCV-resistant tomato varieties, as well as two susceptible varieties, were tested. Plant age at the time of inoculation had no effect on the disease-severity score of the susceptible varieties, and a very small effect (if any) on the disease-severity score of the resistant varieties. In contrast, plant age at the time of inoculation had a significant effect on the total yield of all of the varieties tested, susceptible and resistant alike. However, it should be noted that although inoculation of older susceptible plants did result in increased yield, the yield of the TYLCV-infected susceptible plants was very low for all of the ages tested.

The different resistant and susceptible tomato varieties were tested for TYLCV-induced yield reduction, which is the ultimate test for viral (or any

other pathogen) resistance. The yield of each infected entry was compared with that of its control, uninfected counterpart. All tested varieties suffered a significant yield reduction due to inoculation with TYLCV at all three tested ages. The lowest yield was produced by plants inoculated at 14 DAS. The susceptible varieties produced practically no yield following inoculation at 14 DAS, whereas the yield produced by the resistant varieties varied according to their resistance level, ranging between 18 and 45% of the yield of their non-inoculated counterparts. A smaller TYLCV-yield reduction – a substantial yield increase of between 50 and 100%, depending on the resistance level displayed by the tomato variety, was achieved following inoculation at 28 DAS. A further decrease in TYLCV-induced yield reduction (yield increase of 30 to 40%) was achieved following inoculation at 45 DAS. Moreover, the yield produced by TYLCV-resistant tomato plants inoculated at 45 DAS was from 100 to 300% higher than that produced by plants inoculated at 14 DAS (Table 1).

Like total yield, the number of fruits produced by the inoculated plants increased markedly following inoculation at a more advanced age. Plant height was also affected by plant age at the time of inoculation-all tested varieties that were inoculated at 28 DAS were taller than their counterparts inoculated at 14 DAS. This was not necessarily the case for plants inoculated at 45 DAS-four varieties reached the same height, while two varieties showed increases in this parameter. Interestingly, the two varieties showing a statistically significant effect of inoculation at 45 DAS on plant height were those showing the lowest level of TYLCV resistance (Table 1).

In a separate glasshouse experiment, we tested TYLCV levels in the inoculated plants. To make sure that viral inoculum is indeed the same between all the different samples, plants were inoculated with clip cages, one clip cage per plant, with 50 whiteflies per cage. Looking at the susceptible variety 144, it was found that until 21 DAI, TYLCV accumulated to the same level (not-significant statistical differences) regardless of plant age at the time of inoculation (Fig. 1). Moreover, the increase in virus level between 7 and 21 DAI was moderate, with a slope decrease from 0.25 for inoculation at 14 DAS to 0.23 for 28 DAS and to 0.21 for plants inoculated at 45 DAS. However, from 21 DAI to 28 DAI there was a sharp increase in TYLCV accumulation (slope of 1.3) in plants inoculated at 14 DAS. This was in contrast to plants inoculated at 28 and 45 DAS, which showed a much milder increase in TYLCV inoculation (slope of 0.4) (Fig. 1). We also assayed TYLCV accumulation levels in three resistant varieties, but the TYLCV level was too low for accurate detection by dot-blot hybridization in the plants inoculated at 28 and 45 DAS (not shown). This is in agreement with previous results showing an inverse correlation between resistance level and TYLCV accumulation-the higher the resistance level the lower the TYLCV accumulation level in these plants [4, 9, 18, 19].

When yield of the glasshouse grown plants was assayed, despite differences in yield per plant due to different growth methods, in essence, results were the same as in the field experiment – plants that were inoculated at older age suffered a reduced TYLCV-induced yield reduction (Table 2).

In conclusion, the results from this study clearly demonstrate the occurrence of mature-plant resistance in tomato plants that are susceptible and resistant to TYLCV. However, while plant age at time of inoculation had a strong effect on yield, it barely affected the disease-severity score. This may indicate that older plants are not necessarily more resistant *per se* to TYLCV than younger plants, but are merely able to dampen down the devastating effect of the virus since they are in an advanced developmental stage, or are simply stronger than the younger plants. This is supported by our results showing a major decrease in the TYLCV accumulation level 28 DAI in susceptible plants that were inoculated at an older age. Considering our results, maybe the term mature-plant tolerance is a more accurate description of the phenomenon.

These results raise another question-when is the ‘‘correct’’ or best time to inoculate tomato plants when screening for TYLCV resistance? This may depend on the genetic material being screened: if segregating populations are being screened for individual resistant plants, then it is best to inoculate at the earliest possible stage, when the effect of the viral infection is most severe. This way the selected

plants will indeed be those showing the highest level of TYLCV resistance. If, on the other hand, commercial varieties or hybrids are being tested for level of resistance, then inoculation at 28 DAS may be most suitable as it represents inoculation just prior to transplanting to the field. Since most commercial tomato plants are sown in specialized and protected nurseries, from an agricultural point of view, 28 DAS may be the most relevant stage for testing commercial hybrids.

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