

Effect of plant development (age and size) on the *Mi-1*-mediated resistance of tomato to whitefly *Bemisia tabaci*

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Abstract

Whitefly, *Bemisia tabaci*, is one of the most important pests of tomato, *Solanum lycopersicum* L. The *Mi-1* gene mediates tomato resistance to the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species of *B. tabaci*, three species of root-knot nematodes, *Meloidogyne* spp., and the potato aphid, *Macrosiphum euphorbiae*. Tomato seedlings bearing the *Mi-1* gene are resistant to nematodes soon after germination but resistance to aphids is developmentally regulated; a reliable conclusion about *Mi-1* resistance to *B. tabaci* was not available to date. In the present work, 3-, 5- and 8-week-old plants of the tomato cultivars Motelle and Moneymaker (bearing and lacking the *Mi-1* gene, respectively) were simultaneously tested under free-choice (antixenosis) and no-choice (antibiosis) conditions, to assess the real influence of plant age on the *Mi-1*-mediated resistance to the MED species of *B. tabaci*. Subsequently, plants of the same age but with different level of development were compared to check whether the plant size can also affect this tomato resistance. Obtained results demonstrated that *Mi-1*-mediated resistance to *B. tabaci* is developmentally regulated, as variations in the age of bearing-*Mi-1* plants affects most infestation parameters tested. Differences between cultivars with and without the *Mi-1* gene were significant for 8- but not for 3-week-old plants. For 5-week-old plants, differences between cultivars were less pronounced than in older plants, expressing an intermediate level of resistance in Motelle. Plant size also influenced whitefly infestation and reproductive activity on the resistant cultivar. However, plant age has more impact than plant size on the *Mi-1*-mediated resistance of tomato to *B. tabaci*.

Keywords: *Bemisia tabaci*, *Mi-1* gene, plant age, plant size, resistance, tomato, whiteflies

(Accepted 4 March 2017; First published online 11 April 2017)

Introduction

Whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important pests of tomato (*Solanum lycopersicum* L.) due to the direct and indirect damage it causes to this crop worldwide. Integrated pest management combining different tactics, including the use of tolerant

or resistant varieties, was proposed for whitefly control (Ellsworth & Martínez-Carrillo, 2001). Among the genes responsible for plant resistance (R-genes) to pests or pathogens, *Mi-1* is a special case as it mediates tomato resistance to both Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species (previously B and Q biotypes) of *B. tabaci* (Nombela *et al.*, 2003), as well as three species of root-knot nematodes *Meloidogyne* spp. (Roberts & Thomason, 1986), the potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (Rossi *et al.*, 1998) and the potato psyllid *Bactericerca cockerelli* (Casteel *et al.*, 2006).

Plants reacting to pests or pathogens usually show more resistance as they advance in their natural development (Panter

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& Jones, 2002; Ebrahim *et al.*, 2011). As an example, Bas *et al.* (1992) demonstrated that resistance to the greenhouse whitefly (*Trialeurodes vaporariorum*) in wild tomato genotypes (*Lycopersicon hirsutum* f. *glabratum*) increases with plant age.

The effect of plant development (considering both plant age and size) on the resistance to pathogens or insects has also been shown on plants bearing R-genes. In some cases, plant development has been correlated to an increase on gene expression as described for the rice gene *Xa3* that confers resistance against *Xanthomonas oryzae* pv. *oryzae* (Cao *et al.*, 2007), while other R-genes show a similar expression all along plant development (Century *et al.*, 1999; Martínez de Ilarduya & Kaloshian, 2001). Among these genes, the *Mi-1* gene not only shows a similar expression during the different phases of tomato development but also after the attack of nematodes or aphids (Martínez de Ilarduya & Kaloshian, 2001).

Tomato seedlings bearing the *Mi-1* gene become resistant to nematodes soon after germination (Dropkin, 1969). On the contrary, *Mi-1*-mediated resistance of tomato to aphids is developmentally regulated as it is not expressed until plants reach 6 weeks of age (Kaloshian *et al.*, 1995). *Mi-1*-mediated resistance to *B. tabaci* was initially demonstrated in 8-week-old tomato plants (Nombela *et al.*, 2000, 2001, 2003). In another study where only younger plants were tested, no differences in relation to the presence/absence of the *Mi-1* gene were found (Pascual *et al.*, 2000). However, none of these studies was specifically designed to compare plants of different ages or sizes in order to reliably conclude whether *Mi*-mediated resistance to *B. tabaci* is (or not) developmentally regulated.

In the present work, tomato plants of different ages, bearing and lacking the *Mi-1* gene, were simultaneously tested in free-choice (antixenosis) and no-choice (antibiosis) assays, to assess the real influence of plant age on the *Mi-1*-mediated resistance to *B. tabaci*. In addition, plants of the same age but with different sizes were compared in other assays to verify whether the degree of plant development can also affect the *Mi-1*-mediated resistance to this insect pest.

Materials and methods

Insect material

Adult males and females from the MED species of *B. tabaci* (formerly Q-biotype, in accordance with De Barro *et al.*, 2011) were used in the free-choice assays, meanwhile only adult females were used in the no-choice assays. A population of this whitefly species was reared for several generations in our laboratory on susceptible tomato cv. Marmande.

Plant material and growth

A tomato cultivar carrying the *Mi-1* gene (Motelle) and another cultivar lacking this gene (Moneymaker) were used in all assays. These cultivars are near-isogenic lines (Laterrot, 1987) differing only in the presence of a 650 kb introgressed region from *Lycopersicon peruvianum* (currently *Solanum peruvianum*) containing the *Mi-1* gene, in chromosome 6 of Motelle (Ho *et al.*, 1992). Tomato seeds were germinated and grown in a climatic chamber at a temperature regime of 24:20°C (Light: Dark), L16:D8h photoperiod and 70% r.h. Plants were grown in 1-liter plastic pots filled with autoclaved vermiculite (number 3, Projar, Spain), irrigated every 15 days with a nutritive complex 20-20-20 (Nutrichem 60; Miller Chemical, Hanover,



Fig. 1. Tomato plants of cultivars Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*) compared in the free-choice and no-choice assays to study the influence of plant size. Only 8-week-old tomato plants were used. From left to right: small Motelle, big Motelle, small Moneymaker and big Moneymaker.

PA, USA) at a concentration of 3 g l⁻¹, and with water when needed in the meantime.

In the experiments to estimate the effects of plant age on the *Mi*-mediated resistance to *B. tabaci*, 3-, 5- and 8-week-old tomato plants from each genotype were simultaneously infested and compared. All these plants were grown under the previously detailed climatic conditions.

In the assays to study the influence of plant size, only 8-week-old tomato plants were used. Half of the plants of each genotype were grown as previously described, but the other half were grown from germination at a constant temperature of 19°C to get a smaller size than plants grown at 24°C. At the time of infestation, big plants had 12–13 leaves and they were 70–72 cm high, while small plants had six–seven leaves and they were 20–24 cm high (fig. 1).

Free-choice assays

The effects of plant age and plant size were evaluated in independent free-choice assays, and each assay was repeated twice. To study the influence of plant age, 20 plants from each genotype and age (120 plants total) were used in each assay. To evaluate the effect of plant size, 18 plants from each genotype and size (72 plants total) were used.

In each assay, plants were transferred from the climatic chamber to an insect-free greenhouse and randomized in a complete block design at a temperature regimen of 25:18°C, L16:D8h photoperiod and 70–88% r.h. Each plant was equidistant from the adjacent pots and the distance among plants was enough to prevent plants from touching each other. Plants were infested with more than 5000 adult whiteflies by shaking an infested plant placed in the centre of the greenhouse. After 6 days, the numbers of adult whiteflies were counted daily *in situ* on all leaves of every plant until the emergence of new adults (19 and 21 days in the studies of size and age, respectively). Counts were made early in the morning before the adults became active. Three days after the beginning of emergence of new adults, the total numbers of pupae and empty pupal cases on all leaves of every plant were recorded.

No-choice assays

Separated no-choice assays were carried out to evaluate the effects of plant age and plant size, repeating each assay twice. To check the influence of plant age, 15 plants from each genotype and age (90 plants total) were tested in each no-choice assay. To evaluate the effect of plant size, 15 plants from each genotype and size (60 plants total) were used. For each assay, plants were placed in a growth chamber at constant temperature of 24°C, L16:D8h photoperiod, and 65–75% r.h. Five adult females were confined into a plastic clip-cage (Nombela *et al.*, 2001) attached to the abaxial surface of one leaflet located in the upper-most fully expanded leaf of each plant. After 6 days, females and clip-cages were removed and eggs laid on each leaflet were recorded. Twenty-one days later, the numbers of fourth-stage (N4) nymphs on each plant were recorded. Moreover, the number of new adults was deduced from the observed number of empty pupal cases.

Statistical analysis

When data from every assay were adjusted to a normal distribution, they were analyzed by a one-way ANOVA and means compared by the Tukey's HSD test. When data were not adjusted to a normal distribution, they were \log_{10} transformed (or $\arcsin\sqrt{p}$ for percentages) before analysis. When data, after transformation, were still not adjusted to a normal distribution, means were compared by the Mann-Whitney *U* test (Statgraphics, 1997). This was the case for the mean number of adults per day in the study of age effect, and the mean number of pupae + new adults per plant in the study of size effect, both during free-choice assays.

The intrinsic growth rate (r_m) of the whitefly population on each tomato cultivar and age or size was calculated using the following expression $r_m = [\ln(Nt/No)]/t$ (Birch, 1948), where *Nt* corresponded to the total number of individuals of the new generation (N4 + new adults) found at the end of the assay, at *t* days after infestation ($t = 19$ for size-study and $t = 21$ in the case of age-study) with a known number (*No*) of females (Statsoft, 1994). Mean r_m values were compared by the Tukey's HSD test.

Results

Influence of plant age

Free-choice assays

The daily mean number of *B. tabaci* adults per plant ($n = 20$ plants) counted on the 8-week-old plants was always higher on Moneymaker than on Motelle (fig. 2a). In the same assay, the difference between cultivars on the 5-week-old plants was much less pronounced or non-existent (fig. 2a). Whitefly preference for Moneymaker was not observed on 3-week-old plants (fig. 2a) and even, more insects were occasionally recorded on Motelle than on Moneymaker plants. The mean value ($n = 21$ days) for the 8-week-old plants was statistically higher ($P = 0.000$) on Moneymaker than on Motelle (table 1). Difference between cultivars in this parameter for 5-week-old plants was also significant although less marked ($P = 0.047$). On the contrary, no significant differences ($P = 0.926$) between Moneymaker and Motelle were found on 3-week-old plants (table 1).

As all plants in the free-choice assays were infested, the daily percentages of infested plants were calculated from the

number of plants of each cultivar and age infested with a number of adults at least equal or higher than the mean number of adults averaged on that day from all 120 tested plants. Throughout the experiment, the percentages of infested plants of Moneymaker were higher than those of Motelle for 8-week-old plants only (fig. 2b). In contrast, the differences between cultivars were not so clear in the cases of 5- and 3-week-old plants (fig. 2b). When these daily percentages of infested plants were averaged (table 1), the difference between cultivars was highly significant ($P = 0.000$) for 8-week-old plants only, but not significant for younger plants ($P = 0.764$ for 3-week-old plants and $P = 0.306$ for 5-week-old plants).

At the end of the assays, when the new generation of adults started to emerge (21 post-infestation day) and the total number of pupae (N4) and new adults were counted and averaged per plant ($n = 20$ plants), significant differences ($P = 0.015$) between Moneymaker and Motelle were found on 8-week-old plants only (table 1), and no significant differences between cultivars were obtained on younger plants ($P = 0.940$ for 3-week-old plants and $P = 0.698$ for 5-week-old plants).

No-choice assays

Eggs laid during 6 days on each plant by five confined females were counted and the mean values per plant are shown in table 1. Oviposition values were similar on all Moneymaker plants independently of the plant age. On the contrary, the mean number of eggs on cultivar Motelle decreased as plant age increased. Therefore, statistically significant differences between Moneymaker and Motelle were only found on 8-week-old plants ($P = 0.001$).

Twenty-one days later, the total number of individuals (N4 + new adults) on each plant was counted and the intrinsic growth rate (r_m) of the whitefly population was calculated for each tomato cultivar and age (table 1). The value of this parameter was higher on Moneymaker than on Motelle irrespective of the plant age. However, the difference between cultivars was only significant on 8 and 5 week-old plants ($P = 0.000$ and $P = 0.011$, respectively), with the greatest difference recorded on 8-week-old plants.

Influence of plant size

Free-choice assays

The mean numbers per plant ($n = 18$ plants) of *B. tabaci* adults were daily calculated and they were always higher on Moneymaker than on Motelle for both big and small plants (fig. 3a), although the difference between both cultivars was more evident in the big plants. From these data, the mean values of daily infestation ($n = 19$ days) were calculated (table 2) and statistically significant differences between Moneymaker and Motelle were only found on the big plants ($P = 0.003$).

The percentages of infested plants were calculated every day from the number of plants of each cultivar and size infested with a number of adults at least equal or higher than the mean number of adults averaged that day from all 72 tested plants. The percentages of infested plants of Moneymaker were always higher than those of big Motelle plants (fig. 3b). On small plants, although the percentage of infested plants at the beginning of the assay was similar in both, Moneymaker and Motelle, this value was higher on Moneymaker than on Motelle from the seventh day of

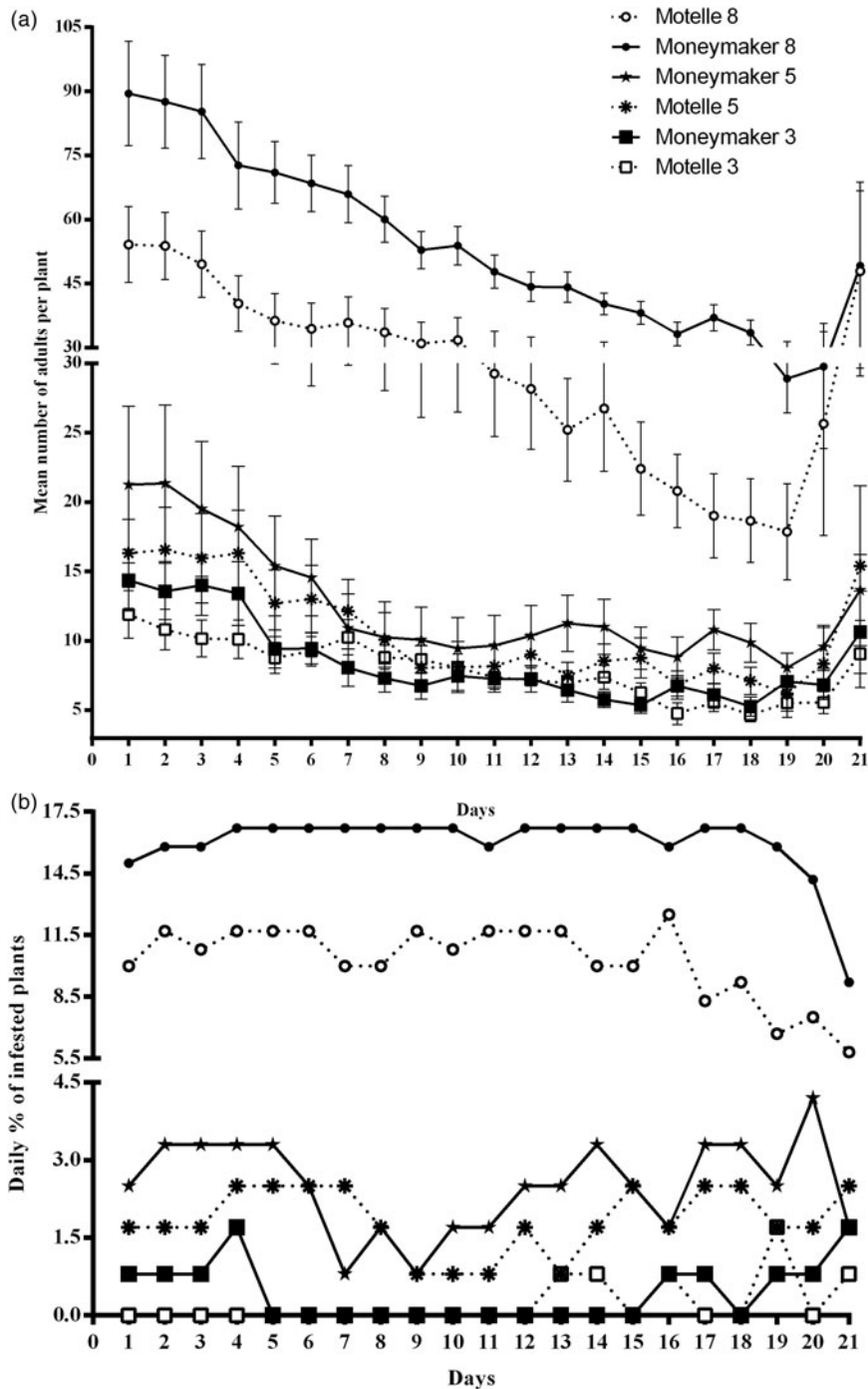


Fig. 2. Daily infestation of tomato cultivars Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*), during the free-choice assays to test the influence of plant age under greenhouse conditions. The daily counting started at 6 days post-infestation. (a) Infestation rates of adult whiteflies daily recorded on 8-, 5- and 3 week-old plants of Motelle and Moneymaker. Values represent the mean of 20 plants of each age and genotype. (b) Percentage of infested plants calculated every day from the number of plants of each cultivar and age infested with a number of adult whiteflies at least equal or higher than the mean number of adults averaged that day from all 120 tested plants of the free-choice assay.

counting (fig. 3b). When these daily percentages of infested plants were averaged (table 2), the difference between cultivars was significant for both big ($P=0.000$) and small ($P=0.012$) plants.

At the end of the assays (19 days post-infestation), the numbers of pupae and new adults per plant ($n=18$ plants) were higher on Moneymaker than on Motelle, although with not statistically significant differences, for any plant size

Table 1. Means \pm standard errors of the parameters evaluated in the free-choice and no-choice assays of the study to analyze the effect of the plant age on the *Mi-1*-mediated resistance of tomato to *B. tabaci*. Plants of Motelle (*Mi-1/Mi-1*) and MoneyMaker (*mi-1/mi-1*) cultivars were compared, separately for each plant age (8-, 5- and 3-week old).

	8 week-old		5 week-old		3 week-old	
	MoneyMaker	Motelle	MoneyMaker	Motelle	MoneyMaker	Motelle
Free-choice assays						
Mean number of adults per day ¹	1079.00 \pm 84.20 a	649.80 \pm 49.13 b	250.80 \pm 18.29 a	212.30 \pm 15.79 b	169.80 \pm 12.91 a	159.80 \pm 9.11 a
Averaged percentage of infested plants ²	15.93 \pm 0.37 a	10.25 \pm 0.40 b	2.50 \pm 0.20 a	1.83 \pm 0.14 a	0.43 \pm 0.12 a	0.23 \pm 0.10 a
Mean number of pupae + new adults per plant ³	1958.00 \pm 347.30 a	1159.00 \pm 245.90 b	432.50 \pm 81.07 a	333.00 \pm 71.30 a	226.00 \pm 19.86 a	209.80 \pm 32.47 a
No-choice assays						
Mean number of eggs per plant ⁴	63.91 \pm 3.72 a	29.07 \pm 4.71 b	64.10 \pm 9.11 a	41.20 \pm 3.95 a	63.27 \pm 5.68 a	44.90 \pm 5.29 a
<i>r_m</i> value ⁵	0.103 \pm 0.003 a	0.035 \pm 0.009 b	0.104 \pm 0.006 a	0.067 \pm 0.006 b	0.104 \pm 0.006 a	0.074 \pm 0.009 a

¹Mean number of adult whiteflies recorded daily on 20 tomato plants. Each value represents the mean of 21 days of counting. Different letters for the same plant age indicate significant differences ($P < 0.05$) between cultivars by the Mann–Whitney test.

²Averaged percentage of plants infested every day with a number of adults at least equal or higher than the mean number of adults averaged on that day from all 120 tested plants. Each value represents the mean of 21 days of counting. Different letters for the same plant age indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

³Mean number of new-generation whiteflies [pupae (N4) + new adults (empty pupal cases)] recorded at the end of the assay. Each value represents the mean of 20 plants. Different letters for the same plant age indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

⁴Mean number of eggs laid on each plant after five adult female whiteflies were confined to a single leaflet per plant for 6 days. Each value represents the mean of 16 plants. Different letters for the same plant age indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

⁵Intrinsic growth rate (r_m) of the whitefly population, calculated using the expression $r_m = [\ln(Nt/No)]/t$, where Nt corresponded to the total number of individuals of the new generation (N4 + new adults) found at the end of the assay, at $t = 21$ days after infestation with a known number (No = 5) of females. Each value represents the mean of 16 plants. Different letters for the same plant age indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

($P = 0.304$ for big plants and $P = 0.557$ for small plants) (table 2). Nevertheless, this difference between cultivars was much more evident on the big plants, as pupae counted on Motelle were approximately 60% of those on MoneyMaker, while the difference in pupae production on the small plants was only 20%.

No-choice assays

After 6 days, the mean number of eggs per plant was very similar on big and small MoneyMaker plants (table 2), but this value on Motelle was significantly reduced on the big plants ($P = 0.003$), compared with the small ones. However, no significant differences were found between both cultivars on small plants ($P = 0.741$).

The whitefly intrinsic growth rate (r_m) was calculated, as described for the plant-age experiments, for each plant cultivar and size considering the total number of individuals (Nt = N4 + new adults) on each plant. The r_m was significantly higher on MoneyMaker than on Motelle only for big plants ($P = 0.030$), but not for the small plants ($P = 0.321$) (table 2).

Discussion

Effect of plant age on *Mi-1*-mediated resistance against *B. tabaci*

These results demonstrated that plant age is a very important factor on *Mi-1*-mediated resistance against *B. tabaci*, as variations in the age of tomato plants affect most parameters of whitefly infestation on bearing-*Mi-1* tomato plants. So, daily

infestation in the free-choice assays of the present work was always greater on MoneyMaker than on Motelle for 8-week-old plants, with significant differences in the mean number of daily adults, confirming results from previous studies (Nombela *et al.*, 2000, 2001, 2003). On the contrary, 3-week-old plants did not show differences between both cultivars and the difference among plants with and without the *Mi-1* gene was minimal on 5-week-old plants. Moreover, the percentage of plants infested with number of adults equal to or greater than the average obtained daily for each cultivar significantly differed between MoneyMaker and Motelle only for 8-week-old plants. Consequently, when adults of the new generation emerged, differences between cultivars were observed in the final infestation levels of 8-week-old plants but not of younger plants.

This plant age-related trend was reinforced by results from the no-choice assays where differences between both cultivars on female oviposition were found only for 8-week-old plants. This is consistent with results from a previous study on young plants, where no differences were found between plants with or without *Mi-1* (Pascual *et al.*, 2000). In studies with aphids it was observed that 5- and 7-week-old plants showed differences between MoneyMaker and Motelle, although the differences were more evident and constant with 7-week old plants (Kaloshian *et al.*, 1995). Differences due to plant age have been also detected on susceptible and resistant tomato varieties during infestation with *Tuta absoluta* (Leite *et al.*, 2001). In that case, an increase in the trichomes density with plant age was correlated to a slower larval development on the resistant variety compared with the susceptible one. However, it has

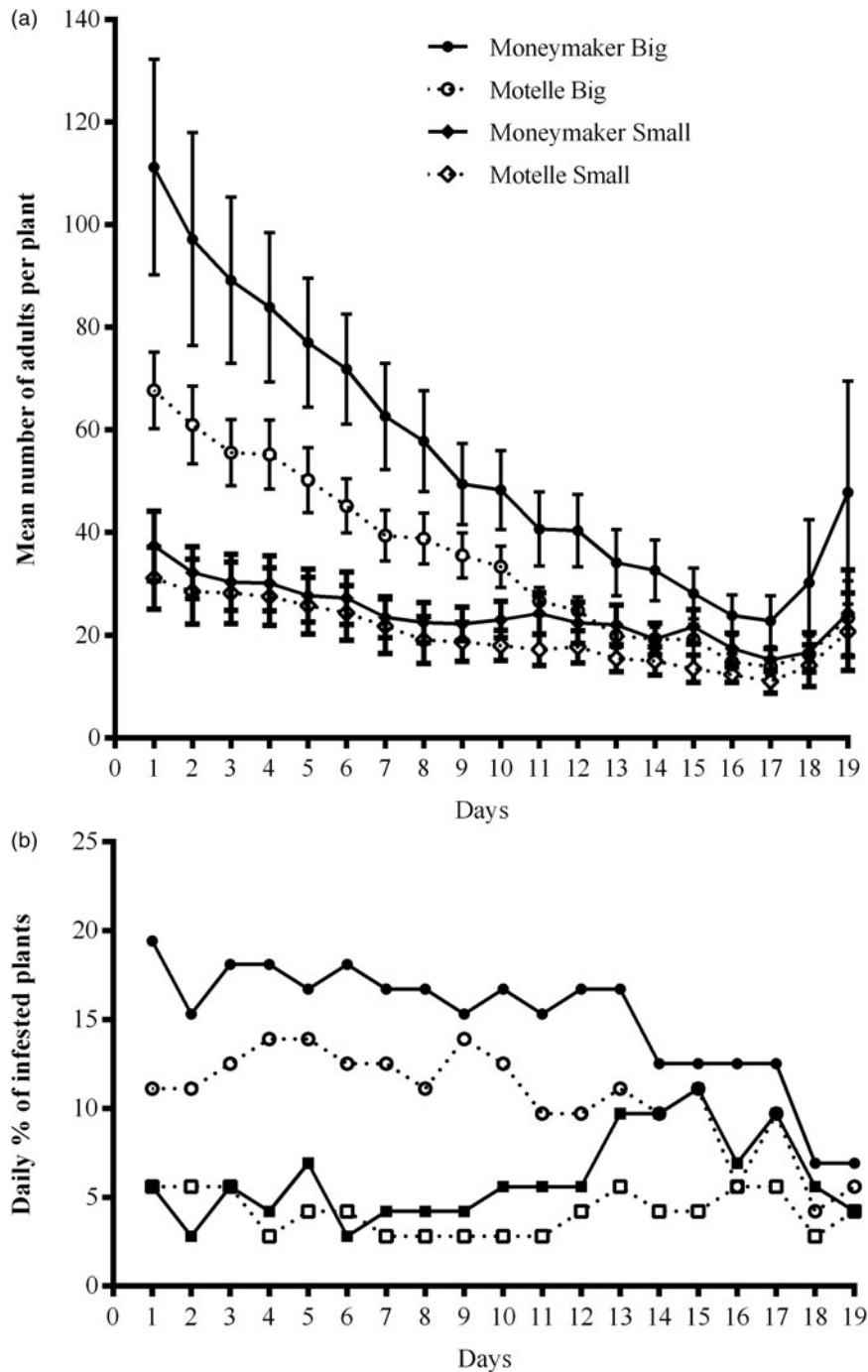


Fig. 3. Daily infestation of tomato cultivars Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*), during the free-choice assays to test the influence of plant size under greenhouse conditions. The daily counting started at 6 days post-infestation. (a) Infestation rates of adult whiteflies daily recorded on big and small plants of Motelle and Moneymaker. Values represent the mean of 18 plants of each size and genotype. (b) Percentage of infested plants calculated every day from the number of plants of each cultivar and size infested with a number of adult whiteflies at least equal or higher than the mean number of adults averaged that day from all 72 tested plants of the free-choice assay.

been proven that *Mi-1*-mediated resistance to *B. tabaci* is independent of the presence of glandular trichomes or their exudates (Nombela *et al.*, 2000). This age-related enhanced

resistance of plants seems to be a common phenomenon as it has been described for many additional plant–pathogen interactions (reviewed by Ebrahim *et al.*, 2011).

Table 2. Means \pm standard errors of the parameters evaluated in the free-choice and no-choice assays of the study to analyze the effect of the plant size on the *Mi-1*-mediated resistance of tomato to *B. tabaci*. Plants of Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*) cultivars were compared, separately for each plant size (big and small plants).

	Big plants		Small plants	
	Moneymaker	Motelle	Moneymaker	Motelle
Free-choice assays:				
Mean number of adults per day ¹	1028.00 \pm 118.90 a	657.20 \pm 74.56 b	443.20 \pm 24.51 a	365.70 \pm 27.25 a
Averaged percentage of infested plants ²	15.87 \pm 0.54 a	11.27 \pm 0.50 b	6.14 \pm 0.61 a	4.20 \pm 0.29 b
Mean number of pupae + new adults per plant ³	1810.00 \pm 461.40 a	1097.00 \pm 253.40 a	772.20 \pm 233.60 a	612.60 \pm 182.20 a
No-choice assays				
Mean number of eggs per plant ⁴	75.00 \pm 6.66 a	44.20 \pm 6.71 b	73.23 \pm 5.23 a	68.80 \pm 11.44 a
<i>rm</i> value ⁵	0.090 \pm 0.007 a	0.041 \pm 0.020 b	0.086 \pm 0.005 a	0.092 \pm 0.009 a

¹Mean number of adult whiteflies recorded daily on 18 tomato plants. Each value represents the mean of 19 days of counting. Different letters for the same plant size indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

²Averaged percentage of plants infested every day with a number of adults at least equal or higher than the mean number of adults averaged on that day from all 72 tested plants. Each value represents the mean of 19 days of counting. Different letters for the same plant size indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

³Mean number of new-generation whiteflies ['pupae (N4) + new adults (empty pupal cases)'] recorded at the end of the assay. Each value represents the mean of 18 plants. Different letters for the same plant size indicate significant differences ($P < 0.05$) between cultivars by the Mann-Whitney test.

⁴Mean number of eggs laid on each plant after five adult female whiteflies were confined to a single leaflet per plant for 6 days. Each value represents the mean of 14 plants. Different letters for the same plant size indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

⁵Intrinsic growth rate (r_m) of the whitefly population, calculated using the expression $r_m = [\ln(Nt/No)]/t$, where Nt corresponded to the total number of individuals of the new generation (N4 + new adults) found at the end of the assay, at $t = 21$ days after infestation with a known number (No = 5) of females. Each value represents the mean of 14 plants. Different letters for the same plant size indicate significant differences ($P < 0.05$) between cultivars by the Tukey's HSD test.

One of the parameters used in studies about insect biology is the intrinsic growth rate of a population (r_m), which allows to analyze the adaptability of the insect to the host plant, giving an idea about how the insect population changes (González-Zamora & Gallardo, 1999; Qiu *et al.*, 2003; Musa & Ren, 2005; Yang & Chi, 2006; Bonato *et al.*, 2007; Islam & Slunziang, 2007; Mansaray & Sundufu, 2009). Significant differences between Moneymaker and Motelle were found in 8 and 5 week-old plants for the intrinsic growth rate of *B. tabaci*, although the most dramatic difference was observed on 8-week-old plants. The reason for this was a decrease in the intrinsic growth rate associated with an increase of the plant age on resistant Motelle, but not on susceptible Moneymaker. This indicates that *B. tabaci* population increased to a lower extent in the next generation on 8-week-old Motelle plants than on 3- and 5-week old plants of the same cultivar.

Effect of plant size on *Mi-1*-mediated resistance against *B. tabaci*

We found that, in addition to plant age, plant size is also important for *Mi-1*-mediated resistance against whiteflies. During the free-choice assays, differences between both cultivars were evident on big plants but not on the small ones. Regarding the final level of infestation, the mean number of pupae and new adults on Moneymaker was almost double than on Motelle, on big plants, while the difference was lower on small plants, although with no significance in either cases. Assuming that pupae survival at this stage is almost 100%, we might suggest a tendency that the difference between both cultivars will be higher for big plants than for small ones. A previous study comparing the survival of two whitefly species (*Bemisia tabaci* and *Trialeurodes vaporariorum*) on tomato leaves with different developmental stage indicated that development plays an important role in plant-pests

interactions (Zhang & Wan, 2012). Besides, tomato resistance to aphids depends on leaf development irrespective of its position (Kaloshian *et al.*, 1997). However, the leaf position could be an important factor in the resistance against pathogens (Visker *et al.*, 2003).

Another study revealed that plant size or growth rate contributed more than other plant traits, such as secondary metabolites, to herbivore resistance (Carmona *et al.*, 2011). Our results are in agreement with that study as plant size also influenced whitefly reproductive activity during no-choice assays, with significant differences in oviposition between both cultivars only for big plants. Besides, the intrinsic growth rate of *B. tabaci* on Moneymaker was constant, irrespective of plant size, while this parameter was lower on the big Motelle plants than on the small ones. Only for big plants this parameter was statistically higher in Moneymaker than in Motelle, suggesting that a decrease in *B. tabaci* population in the next generation would happen only on big plants while it would have a similar increase on both cultivars on smaller plants. A review of Boege & Marquis (2005) suggested that the differences occurring during plant growth can influence the allocation of resources to defense against herbivores. This can suggest that other component(s) influenced by plant development can be important for the *Mi-1*-mediated resistance against whiteflies.

Age-related influence on known *R*-gene mediated resistances

Taking together all results from both studies, effect of age and size, it can be concluded that *Mi-1*-mediated resistance against *B. tabaci* is developmentally regulated, although plant age has more impact than plant size on this resistance. The *Mi-1* gene is not the only R gene that confers development-dependant plant resistance. In tomato, the *Cf-9B* gene,

belonging to the gene family *Cf-9*, confers resistance to *Cladosporium fulvum* in mature (flowering) plants, while this resistance was not found on seedlings (Panter *et al.*, 2002). Our results are also in agreement with those from previous studies (Qi & Mew, 1985; Mew, 1987; Goel & Gupta, 1990; Ogawa, 1993), which demonstrated that rice R genes conferred resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) only on adult plants. Those studies suggested a correlation between plant resistance and expression level of the corresponding R gene, as described for *Xa3* (also known as *Xa26*), which increases its expression in old rice plants, being correlated with a greater resistance to the pathogen *Xoo* (Cao *et al.*, 2007). However, this correlation has not always been found: *Xa21* resistance gene has the same expression on susceptible and resistant rice plants indicating that this developmentally regulated resistance is not correlated with its expression levels (Century *et al.*, 1999). *Mi-1* gene shows a similar expression during the different phases of tomato development, and also after the attack of nematodes or aphids (Martínez de Ilarduya & Kaloshian, 2001). During our study of tomato-*B. tabaci* incompatible interactions (manuscript in preparation), the expression of *Mi-1* gene was constant as no differential regulation was detected by microarray analysis. Overexpression of *Mi-1.2* gene on transgenic tomato plants did not increase its resistance against aphids nor triggered resistance on smaller plants (Goggin *et al.*, 2004).

Regulation of resistance levels by plant development can limit, in some cases, its implementation as a crop protection system. Plants are susceptible to the attack of pathogens and herbivores during a period of plant development (Century *et al.*, 1999), but the mechanisms regulating this phenomenon are still unknown. Two hypotheses that might explain this developmentally regulated resistance on plants bearing the *Mi-1* gene has been suggested (Martínez de Ilarduya & Kaloshian, 2001): (1) *Mi-1.2* is post-transcriptional regulated differently in roots and leaves, or (2) other component, regulated by plant development, is necessary for the resistance against insects but not for nematodes. Since the attempts to generate an antibody specific to *Mi-1.2* protein were unsuccessful (Goggin *et al.*, 2004), no advances in the first hypothesis have been made. Although a *Mi* polyclonal antibody has been developed (Van Ooijen *et al.*, 2008), it is not *Mi-1.2*-specific and does not distinguish among most tomato *Mi* homologous proteins (all these *Mi* like proteins are also endogenous). In relation to the second hypothesis, the existence of another component has been demonstrated as the *Rme1* gene is necessary for the *Mi-1*-mediated resistance against nematodes, aphids and whiteflies (Martínez de Ilarduya *et al.*, 2001, 2004). *Rme1* has not been cloned to date, so it cannot be assured that its function is regulated by plant development similarly to *Rcr3* gene in the resistance to *C. fulvum* mediated by *Cf-2* (Dixon *et al.*, 2000). More studies are necessary to better understand the mechanisms that regulate this *Mi-1*-mediated resistance to be used as a part of the crop protection system against pests and diseases.

Acknowledgements

This research was funded by a Project (AGL2007-65854/AGR) from the Plan Nacional I + D + I, Spanish Ministry of Education and Science. Clara I. Rodríguez Álvarez was financially supported by a fellowship/contract (AP2006-1035) from the Spanish FPU Program.

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