# Development of leaf silvering in squash cultivars infested by silverleaf whitefly

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### **ABSTRACT**

The silverleaf whitefly Bemisia tabaci biotype B is one of the most harmful insect pests for agricultural and vegetable crops. Beside the direct damage, it transmits pathogenic virus and induces plant physiological disorders, such as the squash silverleaf disorder. In this research we evaluated the development of leaf silvering in squash cultivars submitted to artificial infestation of B. tabaci biotype B. An experiment was conducted under field conditions, in Campinas, São Paulo State, Brazil, during the season 2003-2004. The germplasm (Cucurbita spp.) comprised of seven cultivars of summer squash and nine of bush squash. The experiment used a complete randomized blocks design, with 16 treatments (cultivars) and five replications. Each plot consisted of two plants. The artificial infestation was done when the plants emitted the third pair of leaves, by transplanting soybean plants infested with silverleaf whitefly between the squash plots. The leaf silvering was evaluated every two or three days, using a rating scale varying from 1 (no symptom) to 5 (leaves completely silvered). The highest level (5.0) was observed in 'Baianinha', 'Golden Delight', 'Caravela' and 'Arlika', while 'Novita' (2.5), 'Atlas' (2.0) and 'Atlanta AG-303' (1.5) showed light symptoms, indicating that these have low sensibility to this physiological disorder.

**Keywords:** *Cucurbita* spp., *Bemisia tabaci* (Genn.) biotype B, Aleyrodidae, silverleaf whitefly, squash silverleaf disorder.

### **RESUMO**

## Prateamento das folhas em cultivares de aboboreira infestadas por mosca-branca

A mosca-branca (Bemisia tabaci biótipo B) é uma das mais nocivas pragas da agricultura. Além de danos diretos, transmite vírus e também induz desordens fisiológicas, como o prateamento-dasfolhas-da-aboboreira. No presente trabalho, avaliou-se o prateamento em folhas de cultivares de aboboreiras, submetidas à infestação artificial de B. tabaci biótipo B. Um experimento foi conduzido em condições de campo, em Campinas-SP, no ano agrícola 2003-2004, com 16 cultivares de aboboreira (Cucurbita spp.), sendo sete de hábito de crescimento rasteiro e nove de moita. Utilizou-se delineamento de blocos ao acaso, com 16 tratamentos (cultivares) e cinco repeticões, sendo cada parcela formada por duas plantas. Procedeu-se à infestação da área experimental por meio de transplante de plantas de soja infestadas pela mosca-branca entre as aboboreiras, quando estas emitiram o terceiro par de folhas. A evolução do prateamento nas folhas foi avaliada a cada dois ou três dias, mediante uso de escala de notas variando de 1 (sem sintomas) a 5 (folha completamente prateada). 'Baianinha', 'Golden Delight', 'Caravela' e 'Arlika' apresentaram grau máximo de prateamento das folhas (média 5,0) enquanto 'Novita' (2,5), 'Atlas' (2,0) e 'Atlanta AG-303' (1,5) exibiram sintomas amenos, caracterizando-se como menos sensíveis a essa desordem fisiológica.

**Palavras-chave:** *Cucurbita* spp., *Bemisia tabaci* (Genn.) biótipo B, Aleyrodidae, mosca-branca, desordem fisiológica, prateamento-das-folhas-da-aboboreira.

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The whitefly *Bemisia tabaci* (Genn.) biotype B, also known as B. argentifolii Bellows & Perring, is one of the most destructive agricultural pests, attacking vegetables, ornamental species and field crops in the field or in greenhouse, in tropical and subtropical regions worldwide (Chen et al., 2004). High infestations of whitefly may alter plant physiological processes, reducing transpiration and photosynthesis, causing loss of vigor and subsequent reduction in plant development, due to the feeding of nymphs and adults (Inbar & Gerling, 2008; Mc Auslane et al., 2004). Secretions deposited by the insect on the leaves are a suitable medium for

the development of sooty mold, which impairs photosynthesis (Davidson *et al.*, 1994) and can also contaminate products from plants, which occurs, for example, in the staining of the fibers in cotton (Henneberry *et al.*, 2000). *B. tabaci* biotype B is also an efficient vector of dozens of viruses, some of them important for crops in different regions of the world (Brown & Czosnek, 2002; Hilje & Morales, 2008).

Another type of damage that this biotype can cause is the induction of physiological disorders such as irregular ripening of tomato fruits and the squash leaf silvering. The squash leaf silvering was first recorded in Israel in the 1960's

and was attributed to drought stress (Burger et al., 1988). In the 1980's, this abnormality was observed in Florida and it was associated to the presence of B. tabaci (Maynard & Cantliffe, 1989). Yokomi et al. (1990) found that leaf silvering symptoms appeared three days after the initiation of nymph feeding and that adult feeding was not associated to the disorder. Later studies also demonstrated the association between the feeding of *B. tabaci* biotype B nymphs and the squash leaf silvering (Schuster et al., 1991; Hoelmer et al., 1991; Costa et al., 1993). Later, De Barro & Khan (2007) showed that the adult stage of this biotype of B. *tabaci* is also capable of inducing leaf silvering, provided there is adequate adult infestation for more than 11 days.

After the discovery of the ability of the biotype B to induce this disorder, other biotypes were also found to induce silvering in squash leaves: biotype Ms (Delatte *et al.*, 2005) and biotype Ug6 (Sseruwagi *et al.*, 2005). The current distribution of these two biotypes is restricted to islands in the Indian Ocean and Africa.

When the infestation of whitefly on the squash is controlled with insecticides or removal of infested leaves, the symptomatic leaves remain symptomatic, but the new leaves are normal (Yokomi *et al.*, 1990). However, if the infestation continues until the reproductive phase, reduction in yield and fruit quality may occur; according to Chen *et al.* (2004), fruits of plants infested by *B. tabaci* biotype B present reduction in the levels of chlorophyll and carotenoids, consequently, the fruits become bleached with lower commercial value.

Not only plants of the three main cultivated species of Cucurbita [C. pepo L., C. maxima Duch. ex Poir and C. moschata (Duch. ex Lam.)] present symptoms of this disorder, showing sensitivity to that whitefly (Paris et al., 1993) but also wild species such as C. ecuadorensis and C. martinezii (McAuslane et al., 1996). Nevertheless, differences in susceptibility to the leaf silvering have been observed in species of Cucurbita. Evaluating accessions of the U.S. National Germplasm System, USA, planted in an area naturally infested by B. tabaci biotype B in Puerto Rico, Wessel-Beaver (1997) found genotypes of the species C. moschata, C. pepo and C. maxima with high resistance to this disorder.

This study aimed to identify among summer and bush squash cultivars, the ones which do not show symptoms of leaf silvering or which express it in a less intense way, when infected by B. *tabaci* biotype B.

### MATERIAL AND METHODS

Stock-rearing of B. tabaci biotype

**B** - To initiate the rearing, adults of whitefly were collected from a tomato field in Paulínia, São Paulo State, and transferred to plants of soybean and cabbage under greenhouse in the Instituto Agronômico de Campinas (IAC), São Paulo State. After advancing a few generations, adults were sent to Dr. Judith K. Brown, University of Arizona, USA, who identified them as belonging to the biotype B of B. tabaci. This identification is confirmed periodically through the cultivation of squash plants within the rearing greenhouse inducing these plants to express the leaf silvering, the physiological disorder caused by the feeding of immature insects of that biotype (Brown et al., 1995).

Installation of experiments and evaluations - Under field conditions, in an area of the Centro Experimental of Campinas (IAC), São Paulo State, (22°53'S, 47°04'W, 674 m height), the assay to evaluate the evolution of the leaf-silvering was settled with sixteen squash cultivars of the species C. maxima, C. moschata, C. pepo and C. maxima x C. moschata. (Table 1). These cultivars comprise the main hybrids and varieties of squash developed and released from private and official companies. Four of them are referred as tolerant to viruses species: 'Atlanta AG-303' to Papaya ring spoty virus (PRSV); 'Golden Delight' to Zucchini yellow mosaic virus (ZYMV) and Watermelon mosaic virus (WMV); 'Revenue' to ZYMV, WMV and Cucumber mosaic virus (CMV) and 'Sandy' to PRSV and to powdery mildew strain 1.

For seeding, polystyrene trays with 72 cells were used, which were filled with the substrate Plantmax® and kept in a greenhouse for two weeks. For transplanting, which was carried out on November 27th, 2003, 80 pits of about 25 cm depth, fertilized with 200 g of the formula 4-14-8 were prepared. Spacing was 4 x 3 m, which provided 12 m<sup>2</sup> for each cultivar, regardless the growth habit. Each pit received two plantlets. Top-dressing was carried out with 30 g of the formula 20-5-20, and an irrigation with nitrocalcium according to the soil analysis and the references for this crop (Raij et al., 1997).

Irrigation was carried out daily

through watering can in order to meet the water necessity of the plants. Despite the proximity between the experimental area and the place of rearing of the whitefly, with possible infestation by the insect without human interference, we chose to perform an artificial infestation in the experiment. Thus, pots containing two highly-infested soybean plants were transferred from the insect rearing greenhouse to the experimental area two weeks after transplanting, after the emission of the third pair of leaves of the squash plants. Each pot, presenting on average eight leaflets with about 300 adults per leaflet was transplanted equally spaced from the plots, one for four pits. The leaf-silvering evolution was evaluated in each cultivar through a graded scale varying from 1 to 5 (Alves, 2005), adapted from the scale proposed by Paris et al. (1987). The grade 1 means absence of symptoms on leaves and the others indicate the gradual development of the symptoms, it means, the silvering intensity, up to the grade 5, which refers to the totally silver leaf. The field evaluations were carried out each two or three days, resulting 18 reads, assigning one grade representative of each plant of the plot, considering the leaves with the most expressive symptom.

Statistic procedures - The experimental design was of randomized blocks, composed of sixteen treatments (Table 1) repeated five times, totalling 80 plots. Each plot was a pit containing two squash plants. In each plot, the value was obtained by averaging the scores assigned to each plant. Due to the absence of data normality, data were analyzed through the Friedman nonparametric test and the means were compared using the multiple comparison test, at 5% of probability using the statistic program BioEstat.

### RESULTS AND DISCUSSION

The first evaluation was performed the day after the artificial infestation, two weeks after transplanting, when there was no difference between the grades of the cultivars (Table 2). However, 'Golden Delight' and 'Baianinha' had average scores of 3.5 and 2.0 respectively, and, also, 'Golden Dawn

**Table 1.** Squash cultivars (*Cucurbita* spp.) evaluated for leaf silvering induced by *Bemisia tabaci* biotype B (cultivares de aboboreiras (*Cucurbita* spp.) avaliadas em relação ao prateamento das folhas induzido por *Bemisia tabaci* biótipo B). Campinas, IAC, 2003/2004.

Group	Cultivar	Species				
	Arlika	С. реро				
	Atlanta AG-303	C. pepo				
Bush	Caserta	С. реро				
	Golden Dawn III	С. реро				
	Golden Delight	С. реро				
	Novita	С. реро				
	Raven	С. реро				
	Revenue	С. реро				
	Wafra	С. реро				
Summer	Atlas	C. moschata				
	Baianinha	C. moschata				
	Bárbara	C. moschata				
	Caravela	C. moschata				
	Menina Brasileira	C. moschata				
	Exposição	C. maxima				
	Tetsukabuto	C. maxima x C. moschata				

III' and 'Atlanta AG-303', scored 1.5, which indicates that, even before the artificial infestation, the squash plantlets had already been infested by B. tabaci biotype B, since the silverleaf symptoms appear three to five days after the exposure to nymphs (Yokomi et al., 1990) or from nine to eleven days after the exposure to adults (Hoelmer et al., 1991). By the second evaluation, carried out two days after the first one, the leaves of 'Golden Delight' showed intense silvering, with average grade 4.0; by this evaluation, except for 'Golden Dawn III' and 'Baianinha', with average grades of 2.5, the other cultivars showed a less intense degree of leaf-silvering. In the third evaluation, there was little change in relation to the grades of the previous evaluation. From the fourth evaluation, the most sensitive cultivars to this physiological disorder began to express more intensively the leaf-silvering, such as 'Baianinha' which, in the fifth assessment, averaged 5.0, the highest possible score. The second cultivar to achieve the maximum grade of silvering was 'Golden Delight', eleven days after infestation, in the 7th evaluation. 'Caravela' and 'Arlika', in the  $10^{th}$  and  $17^{th}$  evaluations, respectively, also achieved grade 5.0,

which characterized them as highly sensitive. Even eighteen days after the infestation of *B. tabaci* biotype B, in the 8th evaluation, 'Exposição' differed from the most sensitive genotypes. However, in the following evaluations, the symptoms of this genotype changed and reached an average of 4.0 in the last evaluation. Thus, this cultivar behaved as susceptible.

Among the cultivars with lower rates of silvering, 'Novita', 'Atlas' and 'Atlanta AG-303', had the lowest grades of silvering throughout the crop season. It should be mentioned that 'Novita' and 'Atlas' are among the cultivars less attractive and less oviposited by B. tabaci biotype B while 'Atlanta GA-303' is one of the cultivars with the greatest oviposition by this insect (Alves et al., 2005). Thus, the factors which determine the sensitivity to the leafsilvering may be different from the ones which determine the resistance against the insect. In this way, Cardoza et al. (1999) submitted the strains ZUC76-SLR and ZUC33-SLR/PMR, tolerant to leaf-silvering, and the cultivar Zucchini Elite, to different levels of infestation of adults of B. tabaci biotype B. They found that, even in the presence of 160 couples (maximum infestation), 'ZUC76-SLR'

showed no symptoms, whereas half of that infestation (80 couples) was enough to induce 'ZUC33-SLR/PMR' to present a moderate leaf-silvering. 'Zucchini Elite' developed the maximum degree of silvering with lower infestation, 40 couples of whitefly. Differences in the intensity of the leaf-silvering in strains of zucchini squash (C. pepo), infested by B. tabaci biotype B were also found by McAuslane et al. (2004) in the U.S.A. In an experiment carried out in the summer, these authors found intense silvering in plants of 'ZUC61', whereas 'ZUC76-SLR' plants did not exhibit symptoms. They also found the effect of planting date on the expression of this disorder, since, in an experiment carried out in the fall, plants of the strain 'ZUC76-SLR' showed light symptoms of silverleaf. Given this fact, it is of interest to install experiments with the cultivars of this research in order to evaluate the development of the leaf-silvering symptoms in this germplasm, especially for the least sensitive genotypes, such as 'Novita', 'Atlas' and 'Atlanta AG-303'in different seasons in Brazilian conditions.

Besides being highly oviposited, 'Golden Delight' is one of the most attractive varieties to adults (Alves et al., 2005). This genotype also provides the shortest development time from egg to adult and the highest adult emergence of B. tabaci biotype B (Alves et al., 2005). In agreement with these data, in the present research we found that 'Golden Delight' is also, among the most sensitive genotypes to the leafsilvering. Given these characteristics, growing this cultivar should be avoided under high pressure of *B. tabaci* biotype B or proper management of this insect should be carried out in these occasions. 'Golden Delight' can also be used as a susceptible control for studies of sensitivity to leaf-silvering and of resistance to whitefly in squash.

In this work, cultivars such as 'Novita', 'Atlas' and 'Atlanta AG-303' were found to be less sensitive to the leaf-silvering. Similarly to the American genotypes that show no symptoms or very light symptoms (McAuslane *et al.*, 1996), these genotypes exhibited light symptoms of leaf-silvering even under

**Table 2.** Development of leaf silvering in sixteen squash cultivars submitted to artificial infestation *of B. tabaci* biotype B, evaluated through a rating scale<sup>1</sup> (evolução do prateamento em folhas, avaliado mediante escala de notas<sup>1</sup>, em dezesseis cultivares de aboboreira, submetidas em campo à infestação de *B. tabaci* biótipo B). Campinas, IAC, 2003/2004.

Cultivar -	Average scores attributed to each cultivar in each evaluation <sup>2</sup>									
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	
Baianinha	2.0a	2.5ab	2.5abc	4.0a	5.0a	5.0a	5.0a	5.0a	5.0a	
Golden Delight	3.5a	4.0a	4.5a	4.5a	4.5ab	4.5a	5.0a	5.0a	5.0a	
Caravela	1.0a	1.0c	1.0d	2.0abc	3.5abc	4.5a	4.5ab	4.5ab	4.5ab	
Arlika	1.0a	1.0c	1.5bcd	2.5abc	3.0abc	4.0ab	4.0abc	4.0abc	4.0ab	
Golden Dawn III	1.5a	2.5ab	3.5ab	3.5ab	3.5abc	4.0ab	4.0abc	4.0abc	4.0ab	
Raven	1.0a	1.5bc	2.0bcd	3.0abc	3.5abc	3.5ab	4.0abc	4.0abc	4.0ab	
Menina Brasileira	1.0a	1.5bc	1.5bcd	2.0abc	2.5bcd	2.5ab	3.0abc	3.5abcd	4.0ab	
Wafra	1.0a	1.0c	1.5abcd	2.0abc	3.0abc	3.5ab	3.5abc	4.0abc	4.0ab	
Bárbara	1.0a	1.5c	2.0abcd	2.5abc	3.0abc	3.0ab	3.5abc	3.5abcd	3.5bc	
Exposição	1.0a	1.0c	1.0d	1.5c	1.5d	2.0b	2.0bc	2.5bcd	3.0bc	
Tetsukabuto	1.0a	2.0bc	2.0abcd	2.5abc	3.0abc	3.0ab	3.0abc	3.0abcd	3.0bc	
Revenue	1.0a	1.5bc	1.5bcd	1.5c	2.0cd	2.5ab	2.5abc	3.0abcd	3.0bc	
Caserta	1.0a	2.0bc	2.0abcd	2.5abc	3.0abc	3.0ab	3.0abc	3.0abcd	3.5bc	
Novita	1.0a	1.0c	1.0d	1.5c	2.0cd	2.0b	2.0bc	2.0cd	2.0cd	
Atlas	1.0a	1.0c	1.0d	1.5c	1.5d	1.5b	1.5c	1.5d	1.5d	
Atlanta AG-303	1.5a	1.5bc	1.5bcd	1.5c	1.5d	1.5b	1.5c	1.5d	1.5d	
Average value	1.3	1.7	1.9	2.4	2.9	3.1	3.3	3.4	3.5	
	10 <sup>th</sup>	11 <sup>th</sup>	12 <sup>th</sup>	13 <sup>th</sup>	14 <sup>th</sup>	15 <sup>th</sup>	16 <sup>th</sup>	17 <sup>th</sup>	18 <sup>th</sup>	
Baianinha	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	
Golden Delight	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	
Caravela	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	
Arlika	4.0ab	4.0ab	4.0ab	4.0ab	4.5ab	4.5ab	4.5a	5.0a	5.0a	
Golden Dawn III	4.0ab	4.0ab	4.0ab	4.5a	4.5ab	4.5ab	4.5a	4.5a	4.5ab	
Raven	4.0ab	4.0ab	4.0ab	4.0ab	4.5ab	4.5ab	4.5a	4.5a	4.5ab	
Menina Brasileira	4.0ab	4.0ab	4.0ab	4.0ab	4.0abc	4.5ab	4.5a	4.5a	4.5ab	
Wafra	4.0ab	4.0ab	4.0ab	4.0ab	4.0abc	4.0abc	4.0ab	4.0ab	4.0ab	
Bárbara	3.5abc	3.5abc	3.5ab	4.0ab	4.0abc	4.0abc	4.0ab	4.0ab	4.0ab	
Exposição	3.0bcd	3.0bcd	3.5ab	3.5ab	3.5abc	3.5abc	3.5ab	3.5ab	4.0ab	
Tetsukabuto	3.5abc	3.5abc	3.5ab	3.5ab	3.5abc	3.5abc	3.5ab	4.0ab	4.0ab	
Revenue	3.0bcd	3.0bcd	3.0ab	3.5ab	3.5abc	3.5abc	3.5ab	3.5ab	3.5bc	
Caserta	3.5abc	3.5abc	3.5ab	3.5ab	3.5abc	3.5abc	3.5ab	3.5ab	3.5bc	
Novita	2.0cd	2.0cd	2.0b	2.0b	2.0bc	2.0bc	2.0b	2.0b	2.5bc	
	1.5d	1.5d	1.5b	1.5b	1.5c	1.5c	2.0b	2.0b	2.0cd	
Atlas							4 61			
Atlas Atlanta AG-303	1.5d	1.5d	1.5b	1.5b	1.5c	1.5c	1.5b	1.5b	1.5d	

<sup>1</sup>Rating scale for leaf silvering, ranging from 1 (no symptom) to 5 (highest intensity of leaf-silvering) (escala de notas para o prateamento das folhas, variando de 1 a 5, onde 1= ausência do sintoma; ... 5= grau máximo de prateamento na folha); <sup>2</sup>The evaluations, from the 1<sup>st</sup> to the 9<sup>th</sup>, were performed respectively, on 12<sup>th</sup>, 15<sup>th</sup>, 17<sup>th</sup>, 19<sup>th</sup>, 22<sup>th</sup>, 24<sup>th</sup>, 26<sup>th</sup>, 29<sup>th</sup>, 31<sup>th</sup> December, 2003 and, the evaluations from the 10<sup>th</sup> to the 18<sup>th</sup>, were performed respectively, on 2<sup>nd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup>, 16<sup>th</sup>, 19<sup>th</sup> and 21<sup>th</sup> January, 2004 (as avaliações, da primeira à nona, foram realizadas respectivamente nas datas de 12, 15, 17, 19, 22, 24, 26, 29 e 31/12/2003 e, da décima à décima oitava, foram realizadas respectivamente nas datas de 2, 5, 7, 9, 12, 15, 16, 19 e 21/01/2004); \*Averages followed by different letters in column are different by the Friedman test and by the nonparametric test of multiple comparisons (médias seguidas de letras distintas nas colunas diferem entre si pelo teste de Friedman e teste não paramétrico de comparações múltiplas (p<0,05)).

high infestation of *B. tabaci* biotype B throughout the cycle.

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