

## Resistance of squash cultivars to *Aphis gossypii*

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### ABSTRACT

The Cucurbitaceae plants are damaged by attack of a wide spectrum of insects and microorganisms. Among the sucker insects causing damages on squash *Cucurbita pepo* (L.), the aphid *Aphis gossypii* (Glover) (Hemiptera: Aphididae) is pointed as one of the most important, once their nymphs and adults suck the sap of the leaves continuously, besides being potential vector of virus. The present research evaluated different cultivars, aiming to identify the resistance against this aphid. The cultivars Novita, Sandy, Caserta Cac Melhorada, Novita Plus, Samira, AF-2858 and Caserta TS were used in laboratory assays (T= 25±2°C; RH= 70±10% and fotophase= 12 h). In the immature phase the duration of nymphal instars was evaluated, the total duration and their viability, confining individuals on leaf disks from cultivars. In the adult phase the duration of reproductive period, the fecundity and the biological cycle were observed. The cultivar 'Sandy' expressed high level of antibiosis and feeding non-preference against *A. gossypii*, increasing the nymphal stage and causing mortality near to 70%. Besides, this cultivar reduced the production of nymphs and the longevity of the insects. The 'Novita Plus' cultivar also induced significant nymphal mortality, however in lower levels than those verified in 'Sandy', indicating a moderate resistance.

**Keywords:** *Cucurbita pepo*, host plant resistance, Aphididae, antibiosis, agricultural entomology.

### RESUMO

#### Resistência de cultivares de abobrinha italiana a *Aphis gossypii*

As plantas da família Cucurbitaceae são prejudicadas pelo ataque de um amplo espectro de insetos e microrganismos. Dentre os insetos sugadores que atacam a abobrinha *Cucurbita pepo* (L.), o pulgão *Aphis gossypii* (Glover) (Hemiptera: Aphididae) merece destaque, uma vez que suas ninfas e adultos sugam a seiva das folhas constantemente, além de ser potencial vetor de vírus. A presente pesquisa foi realizada com o objetivo de avaliar diferentes cultivares de abobrinha italiana quanto à resistência a esse pulgão. Utilizaram-se as cultivares Novita, Sandy, Caserta Cac Melhorada, Novita Plus, Samira, AF-2858 e Caserta TS em ensaios de laboratório (T= 25±2°C; UR= 70±10% e fotoperíodo= 12 h). Na fase jovem avaliou-se a duração dos estádios ninfais do pulgão, a duração total da fase jovem e suas viabilidades, confinando indivíduos sobre discos foliares das cultivares. Na fase adulta, observou-se a duração do período reprodutivo, a fecundidade e o ciclo biológico. Com base nos resultados obtidos, constatou-se a ocorrência de elevado nível de antibiose e não-preferência para alimentação em 'Sandy', que prolongou a duração da fase jovem, causou mortalidade ninfal próxima a 70%, além de reduzir a produção de ninfas e a longevidade dos insetos. A cultivar 'Novita Plus' também induziu significativa mortalidade ninfal, porém inferior à constatada em 'Sandy', indicando uma resistência moderada dessa cultivar.

**Palavras-chave:** *Cucurbita pepo*, resistência de plantas a insetos, Aphididae, antibiose, entomologia agrícola.

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The Cucurbitaceae family has over 800 species of plants, which are grouped into about 80 genotypes, some of which hold great importance to the economical horticulture worldwide (Oliveira *et al.*, 2000). Among these cucurbitaceas, the *Cucurbita pepo* L. specie, popularly called Italian squash, is extremely important in São Paulo State, where one can find the largest areas with this crop in the whole country (Koch, 1995).

São Paulo State is the most important Brazilian producer of vegetables holding the largest market share in the country (Melo & Vilela, 2007). In 1990, 1.365 ha were used for squash cultivation, producing approximately 17,000 t. In 1999, the area increased to 3,781 ha, and its productivity to 37,140 t. According to

Agriannual (2009), the amount of squash commercialized from January to July in 2008 was 19,996 t.

França & Castelo Branco (1987) suggest that the yield of this vegetable could be increased if this vegetable were not so susceptible to attacks of insects and microorganisms. The most common pests in cucurbitaceous are aphids, thrips and whiteflies, causing substantial losses, especially when intense infestations occur on the beginning of the vegetative cycle.

*Aphis gossypii* (Glover) (Hemiptera: Aphididae) is one of the most troublesome pests for the cucurbitaceous. Its nymphs and adults suck the sap of the plants producing various harmful effects to the crops. Among the symptoms to be highlighted is the yellowing of the

plant, deformation of tissues, foliar necrosis, reduction in vegetative growth, wilt, and, eventually, death. Damages are more severe in young plants, in fleshy and juicy tissues, and they can become even sharper in hot and dry seasons (Latorre *et al.*, 1990). Moreover, its sucking mouthparts promote the transmission of different types of viruses since they feed themselves from sick and healthy plants successively (Gallo *et al.*, 2002).

The most commonly used control method in the management of *A. gossypii* in squash is still chemical; however, this technique is being substituted by other less aggressive to the environment and to human kind (Costa *et al.*, 2001). In addition, this method has not been effective to reduce the population

of aphids in cucurbitaceous, and in the dissemination of viruses in these crops (Kurozawa & Pavan, 2005). Ultimately, it should be taken into account that successive applications of the same chemical product select insect populations with resistance to certain active principles (Prabhaker *et al.*, 1985; Stansly & Schuster, 1992; Oliveira & Silva, 1997).

Because of the necessity of new control methods against *A. gossypii* in squash, the use of resistant genotypes seem to be a feasible option for the management of this insect in crops. According to Lara (1991), this approach is close to the ideal, since it is of easy use for the producers; it is not harmful to the environment, inexpensive, and also more stable in the long run. This research aimed to evaluate different Italian squash cultivars in relation to its resistance to aphid *A. gossypii*, under laboratory conditions.

## MATERIAL AND METHODS

The research was carried out in laboratory and in greenhouse, in Botucatu, São Paulo State (22° 52' 20" S, 48° 26' 37" W Gr), in 2008.

**Stock rearing of *A. gossypii* in greenhouse** – In order to perform the tests, aphids of *A. gossypii* were kept inside two wooden cages (1.0 x 1.0 x 0.8 m), the ceiling and sides were covered with white anti-aphid screen, providing adequate light and ventilation. Pots with cotton plants were placed inside the cages ('Delta Opal'), which were used for the maintenance of the insects. Fortnightly, the pots were monitored and the deteriorated plants were replaced by healthy ones, aiming at keeping the vigor of the rearing. Cotton cultivation was chosen, opposed to squash, in order to avoid the pre-imaginal conditioning mentioned by Lara (1991).

**Collection of nymphs** – Initially, adult females were collected from the stock rearing, and later taken to the laboratory (T= 25±2°C, UR= 70±10% and photoperiod= 12h) to obtain the collection of nymphs necessary for this research. Three Petri dishes (6 cm diameter) were used for each cultivar.

These dishes held 15 mL of solid solution of water agar (1%) and a 3-cm-diameter foliar disc (removed with a leaker) from the cultivars, where the abaxial face was placed facing upwards. With the aid of a brush, 15 adult females apterous of *A. gossypii* were placed in the center of the dish, where the nymphs originated from. The dishes were covered with laminated PVC and perforated with stylus in order to prevent water condensation and promote ventilation. Later, the dishes were placed in the opposite position on a platter simulating the natural conditions of a leaf in the plant. After 16 hours, the females were removed, and the nymphs were randomly selected for the next phase (Michelotto & Busoli, 2003; Pessoa *et al.*, 2004; Leite, 2006).

### Resistance to *A. gossypii* evaluation

– The development of the nymphs of the aphid confined to the following cultivars were evaluated: Caserta Cac Melhorada, Novita, Novita Plus, Samira, Sandy, AF-2858, Menina Brasileira and Caserta TS. For each cultivar, 20 Petri dishes were used (prepared according to the description mentioned before), each of them corresponding to a replicate. The leaves used to obtain the discs were collected from the upper part of the plants, cultivated in greenhouse and were free from phytosanitary products. Prior to use, the leaves were washed with running water and later placed in a solution with 5% sodium hypochlorite for 5 minutes, and washed right after with distilled water twice. Leaf discs were made (3 cm diameter) with the aid of a leaker. A nymph was transferred to each Petri dish with leaf discs, which were then closed and labeled according to the treatment. Evaluation was carried out daily and, when a leaf disc in a dish was found to be deteriorated, the nymph was transferred to a new dish. Discs were considered deteriorated when they became yellowish and dry, which generally took place between one to three days of use, making its replacement necessary. During the evaluation period, the dishes with the nymphs of the aphid were kept inside a B.O.D. incubator, under the same environmental conditions mentioned before.

Evaluations aimed at determining

the duration of the nymphs stages, total duration of its youth and viability. The presence of exuviae was adopted as the criterion to detect changes of instar, which was removed among evaluations using a fine brush. In the adult phase, the reproductive period, fecundity and biological cycle were evaluated.

**Statistical analyses** – Once Hartley (Banzatto & Kronka, 2006) test showed homogeneity between the variances, the data obtained in the tests were submitted to F Test and the means were compared by Tukey ( $p \leq 0,05$ ), with the statistic software ESTAT 2.0. When necessary, data were transformed in  $(x + 0.5)^{1/2}$  or arcsine of  $[(x + 0.5)/100]^{1/2}$ .

## RESULTS AND DISCUSSION

The analysis of the mean duration of the stages of *A. gossypii* kept in squash, revealed no difference between the cultivars for the first stage (Table 1). In the second nymphal stage, cultivars 'Samira' and 'Sandy' reduced the period of duration when compared to the other treatments, with means from 1.14 to 1.20 days, respectively. These means are also lower than the ones found by Aldyhim & Khalil (1993), who reported 1.50 days of duration in these stages. 'Menina Brasileira', 'Novita', 'Caserta TS', 'Novita Plus' and 'AF-2858' induced to longer periods of time of the nymphs in the second stage.

In the third stage (Table 1), 'Sandy' presented a shorter period of duration (1.00 day), differing from 'Novita Plus' (1.54 days) and 'Samira' (1.43 days). In the other treatments, intermediate means were obtained in this phase. In the last nymphal stage, the mean of 'Sandy' (2.33 days) was higher than of the other cultivars, increasing significantly the duration period and suggesting the occurrence of resistance in this cultivar, probably due to antibiosis and feeding non-preference, which may, according to Lara (1991), promote the prolonging of the nymphal stages of the insects.

As related to the total duration of the nymphal phase (Figure 1), 'Sandy' was the cultivar that prolonged most this period, differing from 'Samira', which required less time to conclude

**Table 1.** Average duration of nymphal stadiums of *A. gossypii*, in eight cultivars of *Cucurbita pepo* (duração média dos estádios ninfais de *A. gossypii*, em oito cultivares de *Cucurbita pepo*). T= 25±2°C, R.H.= 70±10% and photoperiod= 12h. Botucatu, UNESP, 2008.

Cultivars	Duration/stadium (days) <sup>1</sup>			
	First	Second	Third	Fourth
Caserta Cac	1.74 ± 0.10 a	1.26 ± 0.10 bc	1.26 ± 0.10 ab	1.42 ± 0.11 b
Sandy	1.73 ± 0.10 a	1.20 ± 0.05 c	1.00 ± 0.00 b	2.33 ± 0.04 a
Novita Plus	1.69 ± 0.12 a	1.43 ± 0.10 abc	1.54 ± 0.09 a	1.38 ± 0.09 b
AF-2858	1.64 ± 0.12 a	1.31 ± 0.09 abc	1.31 ± 0.09 ab	1.31 ± 0.09 b
Novita	1.60 ± 0.12 a	1.69 ± 0.11 ab	1.31 ± 0.09 ab	1.33 ± 0.08 b
Caserta TS	1.53 ± 0.11 a	1.47 ± 0.10 abc	1.20 ± 0.08 ab	1.40 ± 0.12 b
Samira	1.43 ± 0.10 a	1.14 ± 0.07 c	1.43 ± 0.10 a	1.36 ± 0.09 b
M. Brasileira	1.32 ± 0.10 a	1.74 ± 0.16 a	1.28 ± 0.10 ab	1.50 ± 0.11 b
F	1.85 <sup>ns</sup>	4.79*	3.47*	12.88*
CV (%)	30.84	31.93	29.40	28.04

<sup>1</sup>Original data; means followed by the same letter in the column did not differ from each other; Tukey, 5% (<sup>1</sup>dados originais; médias seguidas de mesma letra na coluna não diferem entre si pelo teste de Tukey a 5% de probabilidade).

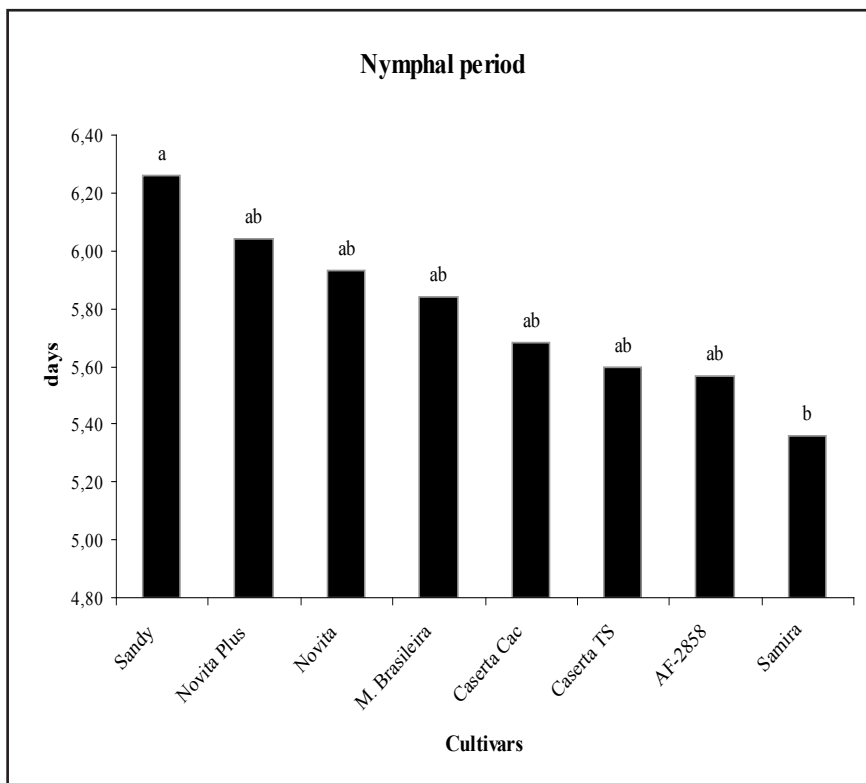
this phase, being considered the most adequate cultivar; therefore, susceptible to the development of nymphs of *A. gossypii*. In the other cultivars were found intermediate means, similar to the results observed by Aldyhim & Khalil

(1993) in cultivar Clarita, at 25°C, where the mean nymphal period of the aphid was 5.6 days, also similar to the results of this research. However, data obtained in this research differ from the ones observed by Satar *et al.* (2005),

where the mean nymphal period was of 4.6 days at 25°C in *Cucumis sativus*, in cultivar 'Beit Alpha' and by Leite *et al.* (2008), who checked the total duration of the nymphal phase of 4.73 days at 27°C, using cultivar 'Caserta TS'.

'Sandy' affected negatively the development of the nymphs of aphids (Table 2), leading to a mortality rate of 68.7% of the individuals. The fact that the highest mortality rate during the first stage (45.0%) was found in this cultivar should be highlighted, suggesting a high level of antibiosis in this cultivar, whose characteristic is to promote high levels of mortality rate in the early stages of the insects. In 'Novita Plus' and 'Caserta TS', in the second instar, a significant mortality rate occurred (26.3 and 25.0%, respectively), which suggests the occurrence of moderate resistance. In cultivar 'Sandy' occurred 75.0% of mortality rate, corroborating the data found in the first stage. The other cultivars showed to be more viable to the development of nymphs of aphid, similarly to what has been reported by Leite *et al.* (2008), who found 100.0% nymphal viability in 'Caserta'.

Concerning the other biological aspects of the insect, 'Sandy' stood out as the most resistant cultivar in all parameters (Table 3), showing the lowest mean (2.25 days) in the reproductive period, differing from all other treatments, especially 'Novita' (14.73 days). The mean values for the duration of the reproductive period were



**Figure 1.** Total nymphal duration of *A. gossypii*, in eight cultivars of *Cucurbita pepo*. Means followed by the same letter did not differ from each other; Tukey, 5%. CV= 16.08%; F= 2.60\* (duração total da fase ninfal de *A. gossypii*, em oito cultivares de *Cucurbita pepo*. Médias seguidas de mesma letra não diferem entre si pelo teste de Tukey a 5% de probabilidade. CV= 16,08%; F= 2,60\*). T= 25±2°C, R.H.= 70±10% and photoperiod= 12h). Botucatu, UNESP, 2008.

**Table 2.** Means of viability per stadium and total of nymphs of *A. gossypii*, in eight cultivars of *Cucurbita pepo* (médias de viabilidade por estágio e total de ninfas de *A. gossypii*, em oito cultivares de *Cucurbita pepo*; T= 25±2°C, R.H.= 70±10% and photoperiod= 12h). Botucatu, UNESP, 2008.

Cultivars	Viability/stadium (%)				Total viability <sup>1</sup>
	First	Second	Third	Fourth	
Caserta Cac	100.00	100.00	100.00	94.74	98.68 a
Samira	100.00	100.00	100.00	92.86	98.21 a
AF-2858	100.00	92.85	92.85	92.85	94.64 a
M. Brasileira	100.00	100.00	94.74	94.74	93.37 ab
Novita	100.00	93.75	81.25	75.00	87.50 abc
Caserta TS	85.00	75.00	75.00	75.00	77.50 bc
Novita Plus	85.21	73.68	68.42	68.42	73.68 c
Sandy	55.00	25.00	25.00	20.00	31.25 d
<b>F</b>	---	---	---	---	20.42*
<b>CV (%)</b>	---	---	---	---	76.53

<sup>1</sup>Original data; transformed in arc sen [(x+0.5)/100]<sup>1/2</sup> for statistical analysis. Means followed by the same letter in the column did not differ from each other; Tukey, 5% (1 dados originais; para análise estatística foram transformados em arco seno [(x+0,5)/100]<sup>1/2</sup>. Médias seguidas de mesma letra na coluna, não diferem entre si pelo teste de Tukey a 5% de probabilidade).

lower than the ones found by Aldyhim & Khalil (1993), who reported 20.80 days in genotype Clarita and similar to the ones found by Leite *et al.* (2008), who reported 12.81 days at 24°C and 10.38 days at 27°C, using 'Caserta'.

The insect kept in 'Sandy' showed low fecundity (Table 3), and low production of nymphs/female/day (1.61 individuals), differing from all the other cultivars. The mean observed in 'Sandy' is lower than the one found by Leite *et al.* (2008) in 'Caserta', who mentioned

4.09 nymphs/female/day. Considering the means of nymphs/female, the results obtained for 'Sandy' differed from the other cultivars, which were all similar among themselves. This cultivar stood out once again as the least adequate concerning the biology of the insect, producing 0.70 nymphs/female, that is, this rate is about 57 times lower than the one found in 'AF-2858' (39.71 nymphs/female).

The mean of the biological cycle (11.67 days) obtained with confined

insects in 'Sandy' was practically half of that observed in 'Novita' (22.25 days), highlighting the adverse effects of the first over the biology of *A. gossypii* (Table 3), classifying it as resistant. It is important to note that, even though 'Sandy' prolonged the young phase of the insect (Figure 1), the same did not occur in the adult phase (Table 3), showing that the reservation acquired during the immature phase was insufficient or inadequate to the biological development of the aphids. In general, the means of the cycle obtained in this research were lower than the ones reported by Aldyhim & Khalil (1993) and Leite *et al.* (2008), who found 29.50 and 24.17 days, respectively, in 'Caserta'. However, it is important to highlight that these researchers used different genotypes.

The fact that 'Sandy' presented higher total duration in the nymphal phase, less viability in the stages and in the nymphal phase, in addition to the shortest reproductive and fecundity period, indicate the occurrence of resistance of antibiosis type in this cultivar. However, it is possible that this material also has shown feeding non-preference. Yet, the occurrence of this mechanism has not been investigated in isolation. Besides, the high mortality rate in initial phases of the insects upholds the hypothesis of the expression of antibiosis as the most active mechanism.

**Table 3.** Biological aspects (M±SE) of *A. gossypii*, in eight cultivars of *Cucurbita pepo* (aspectos biológicos (M±EP) de *A. gossypii*, em oito cultivares de *Cucurbita pepo*; T= 25±2°C, R.H.= 70±10% and photoperiod= 12h). Botucatu, UNESP, 2008.

Cultivars	Reproductive period (days) <sup>1</sup>	N° nymphs/female/day <sup>1,2</sup>	N° nymphs/female <sup>1,2</sup>	Life cycle (days) <sup>1</sup>
Novita	14.73 ± 0.77 a	3.23 ± 0.08 abc	32.81 ± 5.82 a	22.25 ± 0.98 a
AF-2858	12.00 ± 0.78 ab	3.73 ± 0.13 ab	39.71 ± 5.49 a	18.58 ± 0.79 ab
Novita Plus	11.08 ± 0.79 b	2.68 ± 0.19 c	20.58 ± 4.53 a	20.15 ± 1.09 ab
Caserta Cac	11.00 ± 0.97 b	3.71 ± 0.24 ab	38.32 ± 5.36 a	17.61 ± 1.03 ab
Samira	10.93 ± 1.17 ab	3.66 ± 0.25 ab	36.86 ± 4.67 a	20.85 ± 1.29 a
Caserta TS	9.57 ± 0.83 b	4.12 ± 0.19 a	29.15 ± 5.82 a	18.64 ± 1.24 a
M. Brasileira	8.83 ± 0.91 b	3.20 ± 0.22 bc	29.21 ± 4.69 a	16.61 ± 1.01 ab
Sandy	2.25 ± 0.23 c	1.61 ± 0.06 d	0.70 ± 0.46 b	11.67 ± 0.22 b
<b>F</b>	19.35*	19.40*	4.96*	12.88*
<b>CV (%)</b>	36.05	11.66	76.53	28.04

<sup>1</sup>Original data; means followed by the same letter in the column did not differ from each other; Tukey, 5%. <sup>2</sup>Data transformed in (x+0.5)<sup>1/2</sup> for statistical analysis (1 dados originais; médias seguidas de mesma letra, dentro das colunas, não diferem entre si pelo teste de Tukey (P<0,05). <sup>3</sup>Para análise estatística os dados foram transformados em (x+0,5)<sup>1/2</sup>).

Therefore, this high mortality rate found in 'Sandy' suggests the existence of a chemical compound with antinutritional and/or antibiotic characteristics, which cause a delay or is detrimental to the development of the nymph of *A. gossypii* in this cultivar. The influence of the allelochemical compounds and its nutritional stage on the biology of this aphid has been reported by various researchers (Dussourd, 1997; Cook & Neal, 1999; Bonjour et al., 1993). Leite et al. (2002) identified two chemical compounds in the cultivar 'Menina Brasileira': 3-tetradecen-5-ino/alpha-humulen/mircenol and acid 11,14,17, eicosatrienoic metil ester/1-dodecen-3-ino, which may have a negative effect in the biology of the insect.

'Sandy' also showed to be less oviposited by whitefly *Trialeurodes vaporariorum* (West.) in tests with or without choice performed with other pumpkin cultivars in the field and in greenhouse (Alves et al., 2006), suggesting the expression of resistance in comparison to other sucker insects.

A general analysis of the findings lead us to the conclusion that 'Sandy' expresses high levels of antibiosis against *A. gossypii*, and 'Novita Plus' expresses moderate resistance level.

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