

Within-plant distribution of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), in Bt and non-Bt cotton fields

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Abstract

Knowledge of the vertical and horizontal distribution of *Aphis gossypii* Glover (Hemiptera: Aphididae) on genetically modified cotton plants over time could help optimize decision-making in integrated cotton aphid management programs. Therefore, the aim of the present study was to determine the vertical and horizontal distribution of *A. gossypii* in non-transgenic Bt cotton and transgenic Bt-cotton over time during two cotton seasons by examining plants throughout the seasons. There was no significant interaction between years and cotton cultivar treatments for apterous or alate aphids. Considering year-to-year data, analyses on season-long averages of apterous or alate aphids showed that aphid densities per plant did not differ among years. The number of apterous aphids found per plant for the Bt transgenic cultivar (2427 apterous aphids per plant) was lower than for its isoline (3335 apterous aphids per plant). The number of alate aphids found per plant on the Bt transgenic cultivar (12.28 alate aphids per plant) was lower than for the isoline (140.56 alate aphids per plant). With regard to the vertical distribution of apterous aphids or alate aphids, there were interactions between cotton cultivar, plant age and plant region. We conclude that in comparison to non-Bt cotton (DP 4049), Bt cotton (DP 404 BG (Bollgard)) has significant effects on the vertical, horizontal, spatial and temporal distribution patterns of *A. gossypii*, showing changes in its distribution behaviour inside the plant as the cotton crop develops. The results of our study are relevant for understanding the vertical and horizontal distribution of *A. gossypii* on Bt cotton cultivar (DP 404 BG (Bollgard)) and on its isoline (DP 4049), and could be useful in decision-making, implementing controls and determining the timing of population peaks of this insect.

Keywords: cotton aphid, transgenic plants, non-target effect, dynamic, behaviour, vertical distribution, horizontal distribution, Cry1Ac protein

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Introduction

Genetically modified cotton (*Gossypium hirsutum* L.) has proved to be one of the main socio-ecological alternatives for textile agribusiness in the central-western region of Brazil, since it generates employment and creates income for rural and industrial producers, as well as cutting the quantity of chemicals used to control the pests that attack the conventional cotton crop. After more than a decade of commercial cultivation, transgenic Bt cotton (cotton genetically modified with Cry toxic protein genes produced by gram-positive bacteria, *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) is now considered a major advance in lepidopteran pest management in all the main cotton-producing countries (James, 2004; Guoping *et al.*, 2010).

The growth and development of this crop in Brazil could be affected by constant attacks by non-target pests. *Aphis gossypii* Glover (Hemiptera: Aphididae) is one of the main non-target pests that attacks transgenic cotton, causing direct damage by sucking of phloem and harming the plant indirectly through the transmission of virus infections (Sujii *et al.*, 2008). Studies carried out to date have not produced any evidence that Bt crops, expressing the Cry1A protein used for controlling lepidopteran pests, have any direct effect on aphids (Schuler *et al.*, 2001, 2005). This is because the Bt protein is not ingested by aphids, since they feed directly on the sap in the phloem. However, some studies have documented significant quantities of Cry proteins in aphid samples (Zhang *et al.*, 2006; Burgio *et al.*, 2007). According to Liu *et al.* (2005), the aphid population may develop more rapidly on Bt cotton plants than non-Bt plants. Rodrigues *et al.* (2010) reported that Bt cotton changes the normal dispersion pattern of *A. gossypii* in the crop. Therefore, there is no exact knowledge of how genetically modified cotton plants affect populations of *A. gossypii* that interact within the agro-ecosystem. Knowledge of a possible alteration in the distribution of *A. gossypii* when infesting Bt cotton crops could change the way that this species is sampled on this host.

Knowledge of the vertical and temporal distributions of insect pests on the host plant is important for developing effective Integrated Pest Management (IPM) programmes (Trichilo *et al.*, 1993), saving time and cutting costs of pest monitoring, without adversely affecting the reliability of the results (Wilson *et al.*, 1982). Aphids are generally observed with greater frequency on the upper and middle parts of plants, possibly because leaf tissue is softer and easier for the aphids to penetrate and extract carbohydrates and amino acids (Weathersbee & Hardee, 1994; Fernandes *et al.*, 2001). According to Gonzaga *et al.* (1991), the vertical distribution of *A. gossypii* on cotton varies with plant age. However, the development of aphids on the host plant depends on two main factors, plant characteristics and climatic conditions (Ghovlanov, 1976). There are three properties that can be measured to assess how the host plant influences the success of a plant-eating insect: (i) stimuli that lead the insect to locate and choose the plant; (ii) plant conditions that lead the pest to begin and continue feeding; and (iii) the nutritional characteristics of the plant that guarantees the development of the insect and its progeny (Ghovlanov, 1976). Sucrose is the primary form of carbon translocated in the leaves of the majority of higher plants, including cotton, and is the only sugar found in the sap inside the cotton phloem (Hendrix & Huber, 1986). *A. gossypii* converts most of the sucrose that it ingests into oligosaccharides (Hendrix, 1999). Furthermore,

the carbohydrate concentration and content in cotton vary as the plant ages (Wells, 2002).

Many studies have underlined the importance of arthropod distribution on cotton (Kuehl & Fye, 1972; Wilson *et al.*, 1983, 1984; Idris & Roff, 2002; Fernandes *et al.*, 2003; Rodrigues *et al.*, 2010). However, there is no information on the vertical and horizontal distribution of *A. gossypii* as a function of the age of cotton genetically modified with Bt proteins. Given the need for effective control of this pest on Bt cotton, it is important to determine whether the Bt protein affects the behaviour of *A. gossypii* in relation to its distribution within the plant over time. Information on the vertical and horizontal distribution of *A. gossypii* within genetically modified cotton plants of different ages could help optimize decision making in integrated cotton aphid management programs. Therefore, the aim of this study was to determine vertical and horizontal distribution of *A. gossypii* in transgenic Bt-cotton (DP 404 BG (Bollgard)) and non-transgenic Bt cotton (DP 4049) during two cotton seasons by examining plants throughout the seasons. DP 404 BG is a Cry1Ac transgenic Bt cotton cultivar (Bollgard, Monsanto, St Louis, MO, USA) whilst DP 4049 is its isoline.

Materials and methods

Study location

The study was performed at the Estação Experimental da Embrapa Algodão (Embrapa Cotton Experimental Station), Campina Grande, Paraíba State, Brazil. The experiment design consisted of randomized blocks with two treatments for the Cry1Ac transgenic Bt cotton cultivar (*Gossypium hirsutum* L.) DP 404 BG (Bollgard, Monsanto, St Louis, MO, USA) and its isoline DP 4049, with five replications, each represented by a plot measuring 0.25 ha. The field plots were planted in the first week of March 2008 and between the second and the third week of April 2009. The cotton plot consisted of rows spaced 1.00 m apart with 0.20 m between the plants in the row.

The plots were not sprayed with any insecticide to allow natural aphid infestation. There were not recorded incidences of lepidopteran pests in the cotton plots.

Aphid sampling

The distribution and dynamics of aphid populations on the cotton plants were determined at intervals of seven days, from plant emergence until the first bolls appeared. The numbers of wingless (apterous) and winged (alate) aphids and their locations were recorded for 25 randomly-selected plants in each plot, using the nodes on the plants' main stems as reference points (from node zero to the final node), as well as the leaves and fruit structures. Each plant was divided into three regions: bottom (lower third of plant), middle (middle third of plant) and top (upper third of plant). The counts were done by visual inspection of each plant in the field. We use different random sample of each sampling date.

Statistical analysis

The numbers of apterous and/or alate aphids counted per plant were tested for normality (Kolmogorov D: normal test) and homogeneity of variance (Bartlett's test) and, where necessary, converted into square roots of $(x+0.5)$. Note that the tables contain the original mean figures.

The mean number of alate and apterous aphids counted on the first leaves appearing on main stem nodes were subjected

Table 1. Summarized models of three-way analysis of variance (ANOVA $P < 0.05$) of the effects of cotton cultivars, plant age and plant region³) on the number of apterous or alate aphids *A. gossypii* found per cotton plant.

Source	Models	DF	F ratio	Prob > F
Apterous aphids (<i>n</i>)	Model	87	46.91	0.0001
	Cultivar (C)	1	4.40	0.0036
	Age (A)	13	118.55	0.0001
	Plant region (R)	2	92.59	0.0001
	C × A	13	142.22	0.0001
	C × R	2	1.51	0.2228
	A × R	26	10.42	0.0001
	C × A × R	26	7.89	0.0001
Alate aphids (<i>n</i>)	Model	87	41.79	0.0001
	Cultivar (C)	1	236.76	0.0001
	Age (A)	13	94.37	0.0001
	Plant region (R)	2	50.54	0.0001
	C × A	13	116.79	0.0001
	C × R	2	22.63	0.0001
	A × R	26	8.39	0.0001
	C × A × R	26	10.15	0.0001

¹ Cultivars: non-transgenic Bt cotton and transgenic Bt-cotton.

² Ages: 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 days.

³ Plant regions: bottom, middle and top.

to three-way ANOVA (cotton cultivar, cotton age and plant region) and the Student-Newman-Keul test ($P < 0.05$).

The percentages of alate and apterous aphids were referenced according to vertical node positions on the plant (lowest node = 1 and highest node = 20) or against the position of the leaf and/or fruit structure on the limbs using PROC REG (SAS Institute, 2006). To test the hypothesis that the vertical distribution of aphids on the plant varies according to cultivar, we compared the linear regression coefficients of the models adjusted to the data. Next, the regression coefficients of the adjusted models of the percentages of alate or apterous aphids per vertically or horizontally distributed structure (leaves and reproductive structures) were compared for the Bt cultivar (DP 404 BG (Bollgard)) and its isoline (DP 4049) using PROC MIXED (SAS Institute, 2006). PROC MIXED also tests the uniformity of the linear coefficients.

Results

There were no significant interaction between years and cotton cultivar treatments for apterous ($F_{1,12} = 1.47, P = 0.6188$) or alate aphids ($F_{1,12} = 1.89, P = 0.5288$).

Considering year-to-year data, analyses on season-long averages of apterous ($F_{1,12} = 1.13, P = 0.7234$) or alate ($F_{1,12} = 0.77, P = 0.8316$) aphids showed that aphid densities per plant did not differ among years for the two years studied. Thus, the analyses were performed with the pooled data.

The number of apterous aphids found per plant for the Bt transgenic cultivar (2427 apterous aphids per plant) was lower than for its isoline (3335 apterous aphids per plant) ($F_{(1,12)} = 21.24, P < 0.0320$). Similar results were obtained for alate aphids: the number of alate aphids found per plant on the Bt transgenic cultivar (12.28 alate aphids per plant) was significantly lower than for the isoline (140.56 alate aphids per plant) ($F_{(1,12)} = 55.19, P < 0.0018$).

With regard to the vertical distribution of apterous aphids ($F_{\text{Cultivar} \times \text{age} \times \text{region}} (26,332) = 7.89, P > 0.0001$) and alate aphids

Table 2. Number of apterous aphids (mean ± SD) found on structures¹ of non-transgenic Bt cotton and transgenic Bt-cotton as a function of age and plant region ($F_{\text{Cultivar by region by age}} (4,332) = 7.89; P < 0.0001$).

Age	Region	Cultivar	
		Non Bt-cotton	Bt-cotton
1	Bottom	0.00 ± 0.00 aA	0.00 ± 0.00 aA
	Middle	0.00 ± 0.00 aA	0.00 ± 0.00 aA
	Top	0.00 ± 0.00 aA	0.00 ± 0.00 aA
7	Bottom	0.00 ± 0.00 aA	0.00 ± 0.00 aA
	Middle	0.00 ± 0.00 aA	0.00 ± 0.00 aA
	Top	0.00 ± 0.00 aA	0.00 ± 0.00 aA
14	Bottom	0.00 ± 0.00 aA	0.96 ± 0.13 aA
	Middle	0.00 ± 0.00 aA	0.76 ± 0.10 aA
	Top	0.00 ± 0.00 aA	0.68 ± 0.09 aA
21	Bottom	0.00 ± 0.00 aA	1.44 ± 0.45 aA
	Middle	0.00 ± 0.00 aA	0.80 ± 0.11 aA
	Top	0.04 ± 0.01 aA	1.72 ± 0.65 aA
28	Bottom	0.00 ± 0.00 aA	1.16 ± 0.34 aA
	Middle	0.00 ± 0.00 aA	1.28 ± 0.33 aA
	Top	0.04 ± 0.01 aA	0.16 ± 0.09 aA
35	Bottom	0.00 ± 0.00 aA	4.60 ± 1.32 aA
	Middle	0.04 ± 0.01 aA	11.40 ± 3.02 aA
	Top	0.16 ± 0.08 aA	14.04 ± 5.12 aA
42	Bottom	0.00 ± 0.00 bA	16.28 ± 6.19 aA
	Middle	0.04 ± 0.01 bA	52.64 ± 15.83 aA
	Top	0.00 ± 0.00 bA	21.92 ± 7.05 aA
49	Bottom	2.64 ± 0.32 bA	64.52 ± 14.10 aA
	Middle	5.68 ± 1.23 bA	98.88 ± 13.77 aA
	Top	0.84 ± 0.22 bA	57.48 ± 14.14 aA
56	Bottom	1.76 ± 0.29 bA	195.64 ± 39.99 aA
	Middle	10.80 ± 3.12 bA	192.92 ± 35.23 aA
	Top	0.40 ± 0.10 bA	139.96 ± 30.34 aB
63	Bottom	5.64 ± 1.98 bA	246.84 ± 40.65 aA
	Middle	14.64 ± 4.33 bA	319.64 ± 50.34 aA
	Top	0.72 ± 0.12 bA	58.88 ± 12.12 aB
70	Bottom	41.64 ± 12.13 bA	320.00 ± 49.12 aA
	Middle	73.16 ± 15.85 bA	244.00 ± 38.12 aA
	Top	1.28 ± 0.17 aB	13.12 ± 5.22 aB
77	Bottom	99.28 ± 20.12 aA	125.36 ± 14.14 aA
	Middle	137.24 ± 31.99 aA	136.12 ± 13.34 aA
	Top	22.52 ± 6.88 aB	19.40 ± 7.12 aB
84	Bottom	537.36 ± 54.54 aA	28.68 ± 8.01 bA
	Middle	644.40 ± 80.32 aA	25.20 ± 6.08 bA
	Top	102.24 ± 17.12 aB	7.24 ± 3.01 bA
91	Bottom	684.24 ± 79.99 aB	0.24 ± 0.08 bA
	Middle	1071.88 ± 97.87 aA	0.16 ± 0.05 bA
	Top	200.04 ± 35.87 aC	0.00 ± 0.00 bA

¹ Leaves and fruits (squares, flowers and bolls).

² Means with the same lower case letter within rows and within the same age, and means with a common upper case letter within columns and within the same age do not differ significantly by the Student-Newman-Keuls test ($P = 0.05$).

($F_{\text{Cultivar} \times \text{age} \times \text{region}} (26,332) (26,332) = 10.15, P > 0.0001$), there were interactions between cotton cultivar, plant age and plant region (table 1).

This means that the vertical distribution of *A. gossypii* on the cotton plant varies according to the cultivar, age and region of the plant. From emergence up to 35 days, distributions of apterous (table 2) and alate aphids (table 3) were not affected by the cultivar, age and region of the plant.

Table 3. Number of alate aphids (mean \pm SD) found on structures¹ of non-transgenic Bt cotton and transgenic Bt-cotton in function of age and plant region ($F_{\text{Cultivar by region by age (4,332)}}=10.15$; $P<0.0001$).

Age	Region	Cultivar	
		Non Bt-cotton	Bt-cotton
1	Bottom	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
	Middle	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
	Top	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
7	Bottom	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
	Middle	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
	Top	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
14	Bottom	0.00 \pm 0.00 aA	0.16 \pm 0.03 aA
	Middle	0.00 \pm 0.00 aA	0.32 \pm 0.09 aA
	Top	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
21	Bottom	0.00 \pm 0.00 aA	0.16 \pm 0.05 aA
	Middle	0.00 \pm 0.00 aA	0.04 \pm 0.01 aA
	Top	0.04 \pm 0.01 aA	0.00 \pm 0.00 aA
28	Bottom	0.00 \pm 0.00 aA	0.04 \pm 0.01 aA
	Middle	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
	Top	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
35	Bottom	0.00 \pm 0.00 aA	0.04 \pm 0.01 aA
	Middle	0.00 \pm 0.00 aA	0.08 \pm 0.02 aA
	Top	0.00 \pm 0.00 aA	0.00 \pm 0.00 aA
42	Bottom	0.00 \pm 0.00 aA	0.28 \pm 0.09 aA
	Middle	0.00 \pm 0.00 bA	1.00 \pm 0.12 aA
	Top	0.00 \pm 0.00 aA	0.20 \pm 0.05 aA
49	Bottom	0.04 \pm 0.01 aA	0.72 \pm 0.10 aB
	Middle	0.04 \pm 0.01 bA	2.16 \pm 0.98 aA
	Top	0.00 \pm 0.10 aA	0.68 \pm 0.10 aB
56	Bottom	0.12 \pm 0.09 aA	1.68 \pm 0.77 aA
	Middle	0.28 \pm 0.12 bA	1.96 \pm 0.83 aA
	Top	0.04 \pm 0.01 aA	0.56 \pm 0.12 aB
63	Bottom	0.04 \pm 0.02 aA	0.88 \pm 0.12 aA
	Middle	0.20 \pm 0.03 aA	0.80 \pm 0.09 aA
	Top	0.00 \pm 0.00 aA	0.24 \pm 0.05 aA
70	Bottom	0.64 \pm 0.13 aAB	0.16 \pm 0.03 aA
	Middle	1.76 \pm 0.74 aA	0.12 \pm 0.02 bA
	Top	0.16 \pm 0.07 aB	0.04 \pm 0.01 aA
77	Bottom	3.40 \pm 1.09 aB	0.04 \pm 0.01 bA
	Middle	15.44 \pm 3.99 aA	0.12 \pm 0.03 bA
	Top	5.12 \pm 1.22 aB	0.00 \pm 0.00 bA
84	Bottom	13.96 \pm 4.12 aB	0.04 \pm 0.01 bA
	Middle	46.48 \pm 8.98 aA	0.00 \pm 0.00 bA
	Top	8.48 \pm 2.23 aC	0.00 \pm 0.00 bA
91	Bottom	10.24 \pm 3.43 aB	0.00 \pm 0.00 bA
	Middle	26.52 \pm 7.76 aA	0.00 \pm 0.00 bA
	Top	8.54 \pm 2.01 aB	0.00 \pm 0.00 bA

¹ Leaves and fruits (squares, flowers and bolls).

² Means with the same lower case letter within rows e within the same age, and means with a common upper case letter within columns within the same age do not differ significantly by the Student-Newman-Keuls test ($P=0.05$).

Irrespective of the plant region, from 42 to 70 days, the number of apterous aphids per plant was higher on the Bt cultivar than on its isoline. However, irrespective of the plant region, from 77 to 91 days the number of alate aphids per plant was higher on the isoline than on the Bt cultivar (table 3).

On non-transgenic plants, from emergence to 63 days, apterous (table 2) and alate (table 3) aphids were distributed

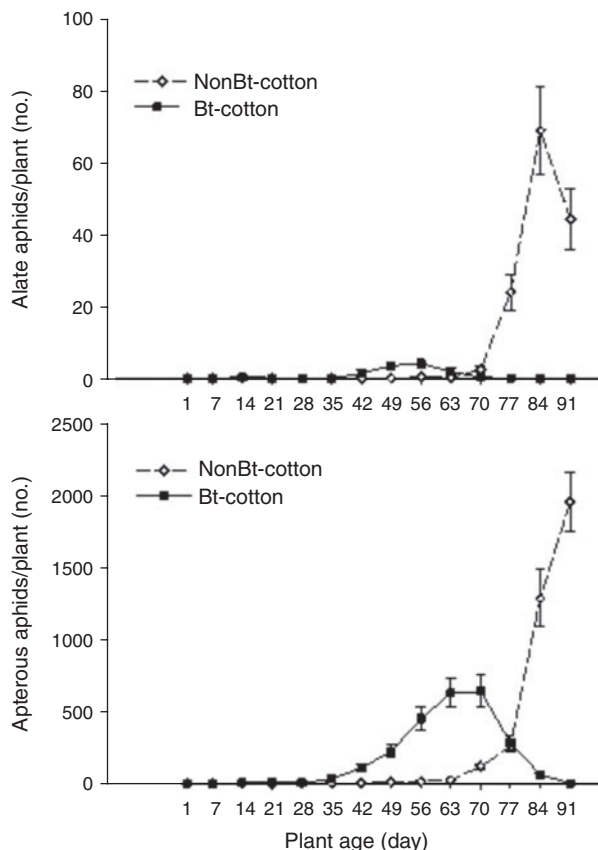


Fig. 1. Dynamics of apterous and alate aphids within a season (2008–2009; data pooled) in non-transgenic Bt cotton and transgenic Bt-cotton. Each data point represents the mean of 25 cotton plants. Note different scale of y-axis.

uniformly over the regions of each plant. From 70 days onwards, there were more apterous aphids in the bottom and middle regions of the non-transgenic plants than in the top region (table 2). For the Bt cultivar, the distribution of apterous aphids was uniform over the plant, from emergence to 49 days and from 84 days to 91 days. From 63 days to 77 days on Bt cultivars, the bottom and middle regions of the plants exhibited more apterous aphids than the top region (table 2). Alate aphids were distributed uniformly over the Bt cultivar for the entire lifetime of the plant, except during the period from 49 to 56 days when the number of alate aphids per plant was higher in the middle region than in the top region (table 3). However, on the non-transgenic plant, the distribution of alate aphids over the plant was uniform from emergence to 63 days. From 91 days onwards, the middle region exhibited more alate aphids than the top region (table 3).

On the Bt cultivar, apterous aphid populations peaked at 63 and 70 days (fig. 1), whereas on the isoline, peaks were reached at 91 days. The alate aphids on the Bt cultivar did not exhibit any population peaks, maintaining a relatively low and constant population density throughout the crop cycle (fig. 1). Alate aphids on the isoline exhibited a low, constant population density from plant emergence to 70 days, with a peak at 84 days (fig. 1). On the Bt cultivar, we observed that, during apterous aphid population peaks (fig. 1), there was a proportionally greater number of these aphids on the nodes in

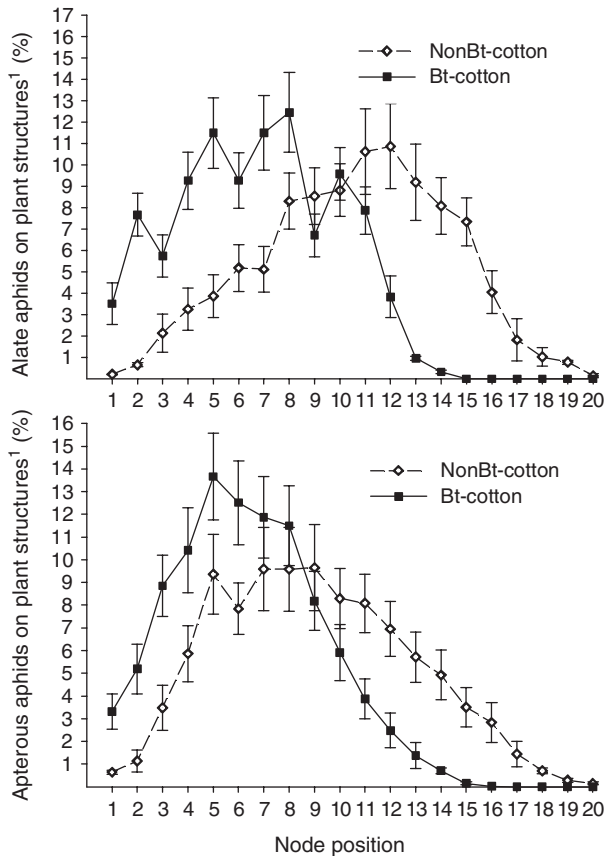


Fig. 2. Vertical distribution (%) of cotton aphids on nodes within non-transgenic Bt cotton (Apterous aphids: $y=3.44+2.96x-0.16x^2$, $F_{\text{model}(2,17)}=99.06$, $P<0.0001$, $F_{(1,17)}=165.20$, $R^2=0.92$, $P<0.0001$ and alate aphids: $y=2.11+3.80x-0.18x^2$, $F_{\text{model}(2,17)}=163.99$, $P<0.0001$, $F_{(1,17)}=327.95$, $R^2=0.95$, $P<0.0001$) and transgenic Bt-cotton (Apterous aphids: $y=22.84-1.41x+0.01x^2$, $F_{\text{model}(2,17)}=18.44$, $P<0.0001$, $F_{(1,17)}=36.45$, $R^2=0.68$, $P<0.0001$ and alate aphids: $y=14.65+0.91x-0.09x^2$, $F_{\text{model}(2,17)}=33.02$, $P<0.0001$, $F_{(1,17)}=10.81$, $R^2=0.79$, $P<0.0043$) based on plant node position from bottom (node 1) to plant apex (node 20).

¹All plant structures (leaves, squares, flowers and bolls). Each data point was the mean of 25 cotton plants.

the bottom and middle regions of the plants (table 2 and fig. 2). However, on the non-transgenic cultivar, the greatest numbers of alate and apterous aphids were found on the nodes in the middle region of the plants (fig. 2).

We verified that on nodes 2 to 11 and 3 to 15 of the Bt cultivar and isoline (fig. 2), the percentages of apterous aphids found were 91.9% and 92.1%, respectively. However, 91.3% of alate aphids were found on nodes 2 to 11 of the Bt cultivar and 90.0% on nodes 5 to 16 of the non-transgenic cultivar (fig. 2).

Based on the fact that alate and apterous aphids were found to be concentrated in the bottom and middle regions of Bt cotton and isoline plant, we applied quadratic models to test the distribution of aphids on the plant (fig. 2), since such models are best suited to representing the vertical distribution behaviour of the apterous aphids (Bt cultivar: $y=22.43-1.41x+0.01x^2$, $F_{\text{model}(2,17)}=18.44$, $P<0.0001$, $F_{(1,17)}=36.45$, $R^2=0.68$, $P<0.0001$ and isoline: $y=3.44+2.96x-0.16x^2$, $F_{\text{model}(2,17)}=99.06$, $P<0.0001$, $F_{(1,17)}=165.20$, $R^2=0.92$,

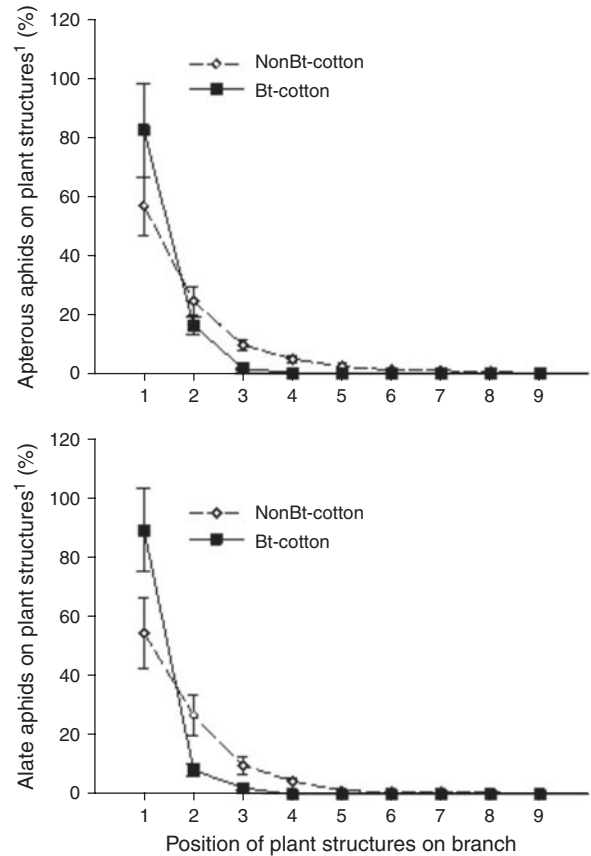


Fig. 3. Horizontal distribution (%) of cotton aphids within non-transgenic Bt cotton (Apterous aphids: $y=58.70-15.29x+1.03x^2$, $F_{\text{model}(2,6)}=72.30$, $P<0.0001$, $F_{(1,6)}=26.24$, $P<0.0022$, $R^2=0.96$) and alate aphids: $y=58.40-15.44x-1.04x^2$, $F_{\text{model}(2,6)}=77.51$, $P<0.0001$, $F_{(1,6)}=27.85$, $P<0.0019$, $R^2=0.96$) and transgenic Bt-cotton (Apterous aphids: $y=77.32-26.21x+2.04x^2$, $F_{\text{model}(2,6)}=21.29$, $P<0.0019$, $F_{(1,6)}=16.58$, $P<0.0066$, $R^2=0.82$ and alate aphids: $y=80.40-27.88x-2.20x^2$, $F_{\text{model}(2,6)}=13.19$, $P<0.0064$, $F_{(1,6)}=11.08$, $P<0.0158$, $R^2=0.82$) cotton plants based on position of plant structures¹ on branch from inside leaf (leaf 1 including bud) toward outside cotton plants.

¹All plant structures (leaves, squares, flowers and bolls).

$P<0.0001$) and alate aphids (Bt cultivar: $y=14.65+0.91x-0.09x^2$, $F_{\text{model}(2,17)}=33.02$, $P<0.0001$, $F_{(1,17)}=10.81$, $R^2=0.79$, $P<0.0043$ and isoline: $y=-2.11+3.80x-0.18x^2$, $F_{\text{model}(2,17)}=163.99$, $P<0.0001$, $F_{(1,17)}=327.95$, $R^2=0.95$, $P<0.0001$) on the Bt cultivar and isoline plants. Comparing the linear coefficients of the models for the Bt cultivar and the isoline (Sas Proc Mixed applied to the linear coefficient uniformity), we observed that the vertical distribution pattern for apterous aphids ($t_{(1,572)}=51.17$, $P<0.0001$) and alate aphids ($t_{(1,219)}=32.77$, $P<0.0001$) on the plants were different for the Bt and non-transgenic cultivars.

We observed proportionally more apterous aphids (Bt cultivar: 92.84% and non-transgenic cultivar: 80.85%) (fig. 3) and alate aphids (Bt cultivar: 97.65% and non-transgenic cultivar: 81.57%) (fig. 3) on the structures produced by positions 1 and 2 of vegetative and fruiting limbs than in the other positions on the cotton cultivars. Since the incidence of apterous aphids (Bt cultivar: $y=77.32-26.21x+2.04x^2$,

$F_{\text{model}(2,6)}=21.29$, $P<0.0019$, $F_{(1,6)}=16.58$, $P<0.0066$, $R^2=0.82$ and isoline: $y=58.70-15.29x+1.03x^2$, $F_{\text{model}(2,6)}=72.30$, $P<0.0001$, $F_{(1,6)}=26.24$, $P<0.0022$, $R^2=0.96$ (fig. 3) and alate aphids (Bt cultivar: $y=80.40-27.88x-2.20x^2$, $F_{\text{model}(2,6)}=13.19$, $P<0.0064$, $F_{(1,6)}=11.08$, $P<0.0158$, $R^2=0.82$ and isoline: $y=58.40-15.44x-1.04x^2$, $F_{\text{model}(2,6)}=77.51$, $P<0.0001$, $F_{(1,6)}=27.85$, $P<0.0019$, $R^2=0.96$) (fig. 3) was concentrated in positions 1 and 2 of plant limbs, the quadratic model (fig. 3) was best suited in terms of representing the decrease in the percentage of aphids found on the structures produced by the positioning of limbs located in the internal region of the plants outwards to the external region.

Comparing the linear coefficients of the models for the Bt cultivar and the isoline (Sas Proc Mixed applied to the uniformity of the linear coefficients), we observed that the horizontal distribution pattern for apterous aphids ($t_{(1,412)}=49.99$, $P<0.0001$) and alate aphids ($t_{(1,199)}=29.31$, $P<0.0001$) on limbs differed for the Bt cultivar and isoline.

Discussion

The results of this study clearly show that *A. gossypii* responds differently to Bt and non-Bt cotton, since the number of apterous and alate aphids found per plant on the Bt transgenic cultivar (DP 404 BG (Bollgard)) was lower than that found on the isoline (DP 4049). According to Liu *et al.* (2005), the aphid population may develop more rapidly on Bt cotton plants than non-Bt plants. Rodrigues *et al.* (2010) observed a greater number of aphid colonies on Bt cotton than on non-Bt cotton, due to interspecies competition between the various populations forming the community of arthropods on the crop. Nevertheless, it is known that there is no direct relationship between a greater number of colonies and a greater number of aphids. The differences in numbers of alate and apterous aphids found on Bt cotton cultivar and its isoline are probably due to genetic modification, since it is likely that transgenic cotton plants secrete less amino acids and soluble sugars than non-transgenic plants (Yan *et al.*, 2007); also, the Bt toxin reduces the absorption of amino acids and glucose (Fast & Angus, 1965; Gringorten, 2001). According to Faria *et al.* (2007), lines of transgenic corn (*Zea mays* Linné) generally exhibit lower concentrations of leucine and isoleucine in the sap than non-transgenic lines. Therefore, food quality is probably the main factor in determining the distribution, abundance and food usage of many plant-eating insects (Hódar *et al.*, 2002); and, in addition, the components of the host plant directly affect the development, fecundity and consumption of these insects (Awmack & Leather, 2002). As host plant quality is widely known to affect insect distribution, abundance, development and fecundity, it seems reasonable to us to suspect that factors affecting host plant quality are responsible for the observed decrease of *A. gossypii* on Bt cotton.

Although the Bt toxin is passed on from the oily plant sap to insects of the family Aphididae (Zhang *et al.*, 2006; Burgio *et al.*, 2007), it is otherwise only known that there are subtle changes in arthropod communities between Bt and non-Bt plants, possibly driven by the drop in target lepidoptera numbers (Whitehouse *et al.*, 2005). Thus, although *A. gossypii* feeds directly on the phloem, we still need to conduct further investigations in order to discern differences if any in the nutritional quality of the phloem in transgenic and non-transgenic cotton plants, and its relationship to aphid population growth parameters. The uptake of Bt proteins by

non-target plant-eating insects with a lower susceptibility to Bt transgenic crops indicates that Bt proteins could be transferred from one trophic level to another, interfering directly in already established food chains (Torres & Ruberson, 2006). In Brazil, *A. gossypii* is a key pest for IPM strategies, due to the fact that it plays a very important role in the food chains of the cotton agro-ecosystem. This insect pest acts as host or prey for a variety of parasitoids and predators, and certainly they contribute to reducing pest population growth in the cotton agro-ecosystem (Ramalho, 1994).

Species of the *Aphis* spp. complex, including *A. gossypii*, *Aphis fabae* Scopoli and *Aphis spiraecola* Pagenstecher, are often present in agricultural crops (Blackman & Eastop, 2000). However, the distribution pattern within the plants can vary in the different botanical species (Carvalho *et al.*, 2002). Variations in the pattern of vertical distribution of aphids within cotton plants are due to the chemical or physical attraction exerted, such as the nutritional contribution or incident wavelength of each cultivar, the plant's age and anatomical plant region (Celini & Vaillant, 2004; Santos *et al.*, 2004; Afshari *et al.*, 2009; Kianpour *et al.*, 2010; Rodrigues *et al.*, 2010). In fact, the results of our study showed that the vertical distribution of apterous *A. gossypii* ($F_{\text{cultivar} \times \text{age} \times \text{region}(26,332)}=7.89$, $P>0.0001$) and alate *A. gossypii* ($F_{\text{cultivar} \times \text{age} \times \text{region}(26,332)}=10.15$, $P<0.0001$) on the cotton plant varies according to cultivar, plant age and plant region (table 1) and that the pattern of vertical distribution for apterous ($t_{(1,572)}=51.17$, $P<0.0001$) and alate aphids ($t_{(1,219)}=32.77$, $P<0.0001$) on the plants is different for Bt and non-transgenic cultivars. These results thus demonstrate that vertical distribution within the cotton plant changes according to the cultivar, plant region and age of the plant.

On non-transgenic plants, the distribution of alate aphids was uniform from emergence to 63 days. After this period and up to 91 days, the middle region of the plant exhibited more alate aphids than the top region. This probably relates to the phenological phase of the crop, since the incidence of aphids increased during this phase (1–60 days), when photosynthetic assimilates are used for leaf production and development (Furtado *et al.*, 2007). According to Shibuya *et al.* (1988), the behaviour of glycoproteins with high mannose-type glycan chains depends on the structure of each glycan chain found in each cultivar, which can interfere with plant aphid distribution. Another related factor is the maximum and minimum translocation of photo-assimilates along the phloem from synthesis or remobilization to locations for storage or use (Hussain *et al.*, 2008). According to Yan *et al.* (2004), the insertion of Bt genes in plants is known to induce changes in secondary compounds that are extremely important for herbivore/cotton plant interactions.

Kindlmann & Dixon (1996) suggested that the dynamics of aphids on their hosts are not evidenced by a clearly-defined pattern and are considered fairly variable throughout the year, mainly in comparison to the mechanism that these insects have for creating similar or different dynamics. Population peaks for apterous aphids found on the Bt cotton cultivar (63 and 70 days) indicate that this cultivar could produce two clearly-defined peaks, whereas the non-transgenic isoline tends to show only one such peak (fig. 1). Zhang *et al.* (2008), studying the dynamic of *A. gossypii* in transgenic (GK-12 and NuCOTN 33B) and non-transgenic (Simian 3) cotton cultivars, also observed two clearly-defined population peaks, the Simian 3 population exhibiting a higher population peak than the GK-12 one, but lower than that of the NuCOTN 33B

population. On the other hand, the results obtained for the non-transgenic cultivar were similar to those obtained by Pinto *et al.* (2000) when studying the dynamics of *Myzus persicae* (Sulzer) in a non-transgenic potato crop (*Solanum tuberosum* L.). Patterns in the production of population peaks can occur when the plants are all approximately the same physical age (Celini & Vaillant, 2004). However, the population peak production pattern for alate aphids (fig. 1) in our study indicates that there is no population peak on the Bt cultivar, with population density kept relatively low and constant during the crop cycle, whereas on the isoline, the alate aphid population peaked at 84 days (fig. 1). Although there was no *A. gossypii* population peak on the Bt cultivar, as such, this cultivar did host adults of *A. gossypii* and allow colonies to form.

According to Gonzaga *et al.* (1991), a higher proportion of *A. gossypii* is distributed in the top region than in the middle and bottom regions of non-transgenic plants when the plant is 93 days old. Yet Cividanes & Santos (2003) observed that there were more *Brevicoryne brassicae* (L.) on the middle leaves than on the top and bottom ones. The results obtained here show that the Bt cultivar hosts greater numbers of *A. gossypii* in bottom and middle region nodes. For the isoline, the highest number of alate and apterous aphids was found on the middle region nodes, with figures of 91.9% of apterous aphids on nodes 2 to 11 of the Bt cultivar, and 92.1% of apterous aphids on nodes 3 to 15 of the isoline. For alate aphids, 91.3% were found on nodes 2 to 11 of the Bt cultivar and 90.0% on nodes 5 to 16 of the isoline (fig. 2). These results may be due to variation between cultivars in respect of the relative proportion of the various amino acids to the quantity of aphids. According to Auclair (1976), the performance of *Aphis pisum* (Harris) is affected not only by the total concentration of amino acids but also by the relative concentrations of the different amino acids which can lead to a possible nutritional imbalance and/or drop in food stimulus. The concentration of amino acids differs between transgenic and non-transgenic corn cultivars, so that transgenic plants contain greater proportions of alanine, arginine and proline (Faria *et al.*, 2007).

We observed higher numbers of apterous aphids (Bt cultivar: 92.84% and isoline: 80.85%) (fig. 3) and alate aphids (Bt cultivar: 97.65% and isoline: 81.57%) (fig. 3) on the structures produced by positions 1 and 2 of vegetative and fruiting branches than on the other branch positions. Proportions of carbohydrates vary in the different parts of the plants (Wells, 2002). Lawo *et al.* (2009) verified the presence of 11 sugars in the honeydew of *A. gossypii* aphids studied on transgenic and non-transgenic cotton cultivars. Sucrose and fructose are dominant in the cotton plant phloem, as well as erlose (glucosylsucrose, a trisaccharide) synthesized by the aphid, which together make up 73% (Bt Mech 184) to 94% (Mech 162) of total sugars (Lawo *et al.*, 2009). Fructose is found in higher quantity in the honeydew from non-Bt cotton plants, and glucose is amplified in the honeydew of Bt cotton plants (Lawo *et al.*, 2009). Erlose differs visibly from one cotton cultivar to another. Thus, we believe that the composition of sugars present in positions 1 and 2 of these cultivars of cotton varies and influences the distribution of *A. gossypii* accordingly. This present uncertainty concerning this situation is why other studies are important so as to verify or disprove the hypothesis.

Some species belonging to the order Hemiptera are present in greater numbers on the limbs of plants, whilst other species of this order prefer the branches and leaves, and some are

distributed over the entire plant (Kozar, 1976). In our study, the horizontal distribution pattern for apterous ($t_{(1,412)} = 49.99$, $P < 0.0001$) and alate aphids ($t_{(1,199)} = 29.31$, $P < 0.0001$) on the branches of plants differed for the Bt cultivar and its isoline when the linear coefficients of the models are compared for these cultivars. There are some factors on the surface and in mesophyll cells or phloem sap of Bt cotton affecting non-Bt cotton aphids (cotton aphids reared on non-Bt cotton line); but, as shown in earlier studies, it took several generations for non-Bt cotton aphids to completely adapt to Bt cotton as Bt-aphids (cotton aphids reared on Bt cotton) fed on two cotton lines with no apparent differences between them (Xue *et al.*, 2009).

Since Bt cottons were approved for commercial planting, observations on piercing-sucking insect populations in the transgenic plant fields have been reported and the results are controversial. The outbreaks of piercing-sucking insects, such as whiteflies, bugs and aphids, have been reported in Bt cotton fields (Cui & Xia, 2000; Reed *et al.*, 2001; Deng *et al.*, 2003). However, in other investigations, the populations of these insects in the Bt cotton and non-Bt cotton fields were found to be quite similar (Wan *et al.*, 2003; Wu & Guo, 2003). According to our results, we conclude that in comparison to non-Bt cotton (DP 4049), Bt cotton (DP 404 BG (Bollgard)) had significant effects on the vertical, horizontal, spatial and temporal distribution patterns of *A. gossypii*, showing changes in its distribution behaviour inside the plant as the cotton crop develops. This may be due to the fact that introducing Bt genes into cotton plants can induce changes in the secondary compounds responsible for herbivore/cotton plant interactions. Therefore, the results of our preliminary study are highly relevant for understanding the vertical and horizontal distribution of *A. gossypii* on Bt cotton cultivar (DP 404 BG (Bollgard)) and on its isoline (DP 4049) and may well be useful in decision-making, implementing controls and determining the timing of population peaks in this important pest insect.

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