

Spatial and temporal dynamics of oviposition behavior of bollworm and three of its predators in Bt and non-Bt cotton fields

Jorge B. Torres* & John R. Ruberson

Department of Entomology, University of Georgia, Tifton, GA 31794, USA

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Abstract

Host plants exhibiting insect resistance traits have long been known to influence within-plant distributions of pests and their natural enemies. Sites and timing of egg deposition are particularly important for synchrony of predators and their prey in the field. Temporal and spatial distribution of eggs of the cotton bollworms [*Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)] and that of the predators *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), and *Micromus* spec. (Neuroptera: Hemerobiidae) were determined during three cotton seasons, from 2002 to 2004, by collecting and examining plants throughout each season. Comparisons also were made between Bt and non-Bt cotton to investigate possible changes in oviposition behavior on Bt cotton. The study was conducted in commercial fields with insecticide use to manage pests when economic thresholds were exceeded in both cotton types. Egg densities for predators and bollworms varied among years, but were similar on Bt and non-Bt cottons. Oviposition of bollworms and *G. punctipes* correlated spatially within plants, with most eggs laid on structures in the top five nodes of cotton plants and on the three outermost leaves on lateral branches regardless of cotton type. Bollworm oviposition dynamics exhibited two peaks within the season (early July and early August). Eggs of all predators and bollworms collected from the field and incubated in the laboratory had high hatching rates throughout each season (74–100%). Temporal association of predator with bollworm oviposition showed a significant correlation with green lacewings, a delay of 10 days for big-eyed bugs, and no correlation with brown lacewings. Furthermore, Bt cotton plants exerted no significant effect on temporal or spatial patterns of oviposition of bollworms or the predators, indicating no change in oviposition behavior of bollworm females within plant structures after almost one decade of widespread planting of Bt cotton.

Introduction

The budworm–bollworm complex, *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) (hereafter ‘bollworm’), is one of the most serious pest lepidopteran groups for several crops, including cotton (Fitt, 1989). Despite many control practices targeting this pest complex in cotton, the use of insecticides is often required to obtain

a profitable yield, although efficacy of insecticides is hampered in some areas by insecticide resistance (Wolfenbarger et al., 1981; McCaffrey, 1998). Almost one decade after Bt-transgenic cotton [cotton plants genetically modified with a Cry toxin gene from the bacterium *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (hereafter referred to as ‘Bt cotton’)] was first cultivated commercially, Bt cotton has become a major tool for managing bollworms in major cotton-producing countries (James, 2004). Although resistance to the Cry1Ac toxin of Bt has not been detected in field populations of *Heliothis* or *Helicoverpa*, populations with high levels of resistance to Bt toxins have been selected in the laboratory (Gould et al., 1995; Fengxia et al., 2003).

*Correspondence and present address: Jorge B. Torres, Departamento de Agronomia/Entomologia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros S/N, Dois Irmãos, 52171-900 Recife, PE, Brasil. E-mail: jtorres@ufrpe.br

Therefore, cropping practices to avoid, or at least delay, the spread of resistance in field populations have been recommended (Roush, 1997).

Because the expression of Cry1Ac toxin in Bt cotton varies among plant structures (Greenplate, 1999), changes in behavior of the larvae within the plant are a concern and have been investigated as a potential mechanism contributing to control failures and selection for resistance. Neonate bollworm larvae are able to recognize Bt plants and move from previous feeding sites, and older larvae are less susceptible to Cry1Ac toxin (Parker & Luttrell, 1999; Gore et al., 2001, 2002; Zhang et al., 2004). Studies on oviposition behavior of bollworm females have demonstrated that ovipositing moths clearly prefer the upper third of cotton plants for egg placement (Wilson et al., 1980; Farrar & Bradley, 1985). This behavior results in contact of neonate larvae with terminal plant tissues that have higher Cry1Ac toxin expression. However, bollworm females laying eggs on flower structures and other structures with low toxin expression within the plant canopy increase the likelihood of survival for their offspring (Gore et al., 2001, 2002). Oviposition in flower structures, for example, allows initial larval development to an older stage more tolerant of Cry1Ac and can lead to control failures and increase resistance selection. Therefore, a behavioral change in bollworm egg placement may facilitate escape from exposure to the highest dosages of Cry1Ac toxin expression.

The tops of cotton plants, the preferred oviposition site of bollworm females, are also the preferred foraging location of immatures and adults of many species of their predators in the plant canopy, including the big-eyed bug, *Geocoris punctipes* (Say) (Heteroptera: Geocoridae) (Van den Bosch & Hagen, 1966; Wilson & Gutierrez, 1980; Nuessly & Sterling, 1994). This pattern, however, may change for both pest and predator if the environment of the plant canopy is changed with the expression of a new plant trait for pest resistance. Morphological traits are the ones most often responsible for altering pest and predator behavior on plants exhibiting insect resistance (e.g., Butter & Singh, 1996), but changes in plant physiology also can affect prey and predator behavior (Cortesero et al., 2000).

Spatial patterns of predator oviposition are critical for ensuring interaction between immature predators and their prey (Sadeghi & Gilbert, 2000) and can be very important for escaping competition among predator species (Schellhorn & Andow, 1999). Predators in cotton fields tend to have weak temporal associations with pest populations (Ellington et al., 1997), suggesting that other important factors besides pestiferous prey underlie predator dynamics in cotton fields. Among these factors, alternative prey, host-plant quality, and plant phenology may be quite significant.

In addition, for predators that supplement their diets with plant products, plant species, and oviposition site selection within the plant canopy can be important by providing close contact of their offspring with plant tissues or structures that facilitate acquisition of the food supplement (sap, pollen, nectar, etc.). Evaluation of site-specific predator oviposition on plants in the field may provide a more accurate understanding of the temporal and spatial association of predators with the host plant and prey.

Cotton supports a large suite of predator species. *Geocoris punctipes* is one of the most abundant Geocoridae ('big-eyed bugs') of the US Cotton Belt, and the species is also common in Central and South America (Sweet, 2000). Lacewings also are important predators in cotton fields (López et al., 1996). Predators from natural populations or those from inundative releases (Lingren et al., 1968; Nuessly & Sterling, 1994; López et al., 1996) can significantly reduce bollworm populations and populations of other cotton pests not targeted by Bt toxin, reducing the frequency of insecticide sprays (Wu & Guo, 2003; Hagerty et al., 2005). Given the continuing need for effective biological control in Bt cotton, it is important to determine whether Bt cotton affects the behavior of natural enemies relative to pest prey. In this study, we investigated the site- and time-specific oviposition dynamics of bollworms and of predators [big-eyed bugs, and green *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) and brown lacewings *Micromus spec.* (Neuroptera: Hemerobiidae)] common in cotton fields in relation to Bt cotton plants.

Materials and methods

Study area and pest management

Three paired Bt and non-Bt cotton fields, located in different farms across years (farm names: Marchant, Old House, Frazier, Ty Ty, and Chula), were surveyed from 2002 to 2004. All farms were located in Tift County, GA, USA, between coordinates 31°45'N, 83°63'W to 31°51'N, 83°55'W, and were 3–17 km from one another. The fields ranged in size from 5.5 to 15 ha and were planted from the last week of April to the second week of May in 2002 (25 April–14 May) and between the first and the second week of May in 2003 (9–13 May) and 2004 (6–12 May).

Crop management followed standard agronomic practices, including insecticide applications based on scouting data (Table 1). Insecticide applications were based on established economic thresholds for pest infestations in both Bt and non-Bt fields for Georgia (GA Pest Management Handbook; Guillebeau, 2004). All fields received preventative in-furrow treatments at planting to control thrips in all 3 years. Based on scouting data, non-Bt fields received insecticide applications to control bollworm larvae, and

Table 1 Timing, materials, and rates of insecticide applications for management of pest infestations in experimental cotton fields, 2002–2004

Dates (fields) ¹	Non-Bt cotton	Bt cotton	Targeted pest ²
2002			
26 April–14 May (C,M,T)	Aldicarb 15G (560 g ha ⁻¹) ^{3,4}	Aldicarb 15G (560 g ha ⁻¹)	Thrips
8–9 July (C,M,T)	Spinosad (100 g ha ⁻¹)	–	Heliothines
10–12 August (C,M)	Lambda-cyhalothrin (34 g ha ⁻¹) + thiodicarb (680 g ha ⁻¹)	–	Heliothines
14 August (T)	–	Dicrotophos (390 g ha ⁻¹)	Stinkbugs
2003			
7–14 May (C,M,O)	Aldicarb 15G (560 g ha ⁻¹)	Aldicarb 15G (560 g ha ⁻¹)	Thrips
8 July (O)	Spinosad (100 g ha ⁻¹)	–	Heliothines
13 July (M)	Spinosad (100 g ha ⁻¹)	–	Heliothines
14 July (C)	Lambda-cyhalothrin (30 g ha ⁻¹)	–	Heliothines
21 July (C)	Lambda-cyhalothrin (45 g ha ⁻¹)	–	Heliothines
3–5 August (M,O)	Lambda-cyhalothrin (45 g ha ⁻¹)	–	Heliothines + stinkbugs
2004			
6–12 May (C,M,F)	Aldicarb 15G (560 g ha ⁻¹)	Aldicarb 15G (560 g ha ⁻¹)	Thrips
2–7 July (C,M,F)	Lambda-cyhalothrin (45 g ha ⁻¹)	–	Heliothines
15 July (C,M)	Spinosad (100 g ha ⁻¹)	–	Heliothines
29 July (C)	–	Dicrotophos (420 g ha ⁻¹)	Stinkbugs
5 August (C)	Zeta-cypermethrin (160 g ha ⁻¹)	–	Heliothines + stinkbugs
17 August (M,F)	Zeta-cypermethrin (210 g ha ⁻¹)	Zeta-cypermethrin (210 g ha ⁻¹)	Heliothines + stinkbugs

–, no insecticide applied.

¹C = Chula, M = Marchant, T = Ty Ty, O = Old House, and F = Frazier fields.

²Thrips (*Frankliniella occidentalis*, *Frankliniella fusca*, and *Thrips tabaci*), Heliothines (*Helicoverpa zea* and *Heliothis virescens*), stinkbugs (*Nezara viridula* and *Euschistus servus*).

³Rates in grams of active ingredient per hectare.

⁴Aldicarb insecticide was applied in furrow during planting.

both field types (Bt and non-Bt) were sprayed to control stink bugs and whitefly infestations.

Bollworm and predator (big-eyed bug, green lacewing, and brown lacewing) egg survey

To determine egg distribution and oviposition dynamics on plants during the cotton season, plants were harvested in plastic bags from each of the cotton fields at approximately 10-day intervals (hereafter ‘sampling dates’) in each growing season. Transparent plastic bags, 110 cm wide × 125 cm long (Stone Container Corporation, Mansfield, OH, USA), were used to bag the plants. The bottoms of the bags were cut off, and bags were tied around the base of the cotton plant and pressed on the ground around the plant 5–10 days prior to collection. On the day of collection, the plastic bags set previously around the plants were pulled quickly over the plant and tied at the top, and the cotton plant was cut off at ground level and transferred to the laboratory for examination. In the laboratory, the plants were maintained in a cold chamber (~5 °C) until inspection, which occurred within 24 h after collection. The specific locations of eggs of bollworms and big-eyed bugs were recorded in reference to nodes on the principal stem (node 0 to terminal) and

leaves on the branches, plant structure, and location in the structure. Eggs of green lacewing and brown lacewing were scattered within plants and were not mapped. Following mapping of the eggs, each egg was removed carefully by cutting a small piece of the substratum or severing the stalk (green lacewings) and incubated in 1.5-ml centrifuge tubes in the laboratory to evaluate egg viability. A total of 414 and 424, 581 and 607, and 516 and 517 Bt and non-Bt cotton plants were collected and evaluated in 2002, 2003, and 2004, respectively.

Statistical analysis

Prior to analyses, the number of eggs collected was transformed into a standardized unit of eggs per plant. The total number of eggs collected per sampling date was averaged as eggs per plant, then per field ($n = 3$ pairs) to correct the unbalanced number of plants evaluated per field (minimum of 20 plants per field per sampling date). Furthermore, the number of eggs per plant was tested for normality (Kolmogorov D: normal test) and homogeneity of variance (Bartlett’s test), and square root ($x + 0.5$) or $\log(x + 1)$ transformations were used when necessary; however, untransformed means are presented in tables and figures.

The results were submitted to two-way analysis of variance (ANOVA) with a repeated measure on one factor (sampling dates) within each season (i.e., years) with field as a blocking factor and cotton type as fixed effect. Separate two-way ANOVA (years and cotton types) were performed to evaluate changes on egg densities among years and between cotton types considering season-long means over all sampling dates. When year or cotton effects were significant by ANOVA, a Tukey studentized range test was performed to separate the means among years, and ANOVA results from an F-test were used to conclude on differences between cotton types (d.f. = 1) within each year. Correlations between bollworm and predator egg dynamics were assessed using concurrent dynamics or predator egg numbers 10-, 20-, and 30-day intervals (i.e., sampling dates) following bollworm oviposition (big-eyed bug, and green and brown lacewings) to explore a possible time delay in predator oviposition relative to timing of bollworm oviposition.

Overall seasonal means of bollworm egg distribution within plant structures were subjected to two-way ANOVA (cotton type and plant structure) and Tukey studentized range test, with $P < 0.05$. Bollworm and big-eyed bug eggs found on the plants were mapped from the uppermost node downward to the cotyledon node and from the outermost leaf of the vegetative/fruitlet branches toward the interior of the plants. The egg counts were averaged per field ($n = 3$ each year) and transformed to percentage of eggs per structure from the total number of eggs collected. The percentage of eggs per node was regressed against node position vertically in the plant (uppermost node = 1 and downward) or against leaf position in the branch using

PROC REG of SAS (SAS Institute, 1999–2001). Furthermore, to test the hypothesis that bollworm females might be changing oviposition behavior, spreading more of their eggs in Bt cotton to avoid plant terminals with more concentration of Bt toxins in comparison to non-Bt cotton, the linear portion of fitted models was compared. Thus, the linear slopes of fitted models (i.e., first- or second-order models) of percentage of eggs per vertical structure (node on mainstem or leaf on vegetative branch) were compared between cottons using PROC MIXED to test the equality of linear slopes (SAS Institute, 1999–2001).

Results

Bollworm oviposition

We found 642 bollworm eggs on field-collected plants. Repeated measures ANOVA showed that the numbers of bollworm eggs per plant were similar on both cotton types in 2002 ($F_{1,4} = 0.00$, $P = 0.9983$), 2003 ($F_{1,4} = 0.98$, $P = 0.3774$), and 2004 ($F_{1,4} = 0.54$, $P = 0.5024$). However, egg densities varied significantly across sampling dates in all 3 years: 2002 ($F_{7,28} = 10.63$, $P < 0.0001$), 2003 ($F_{7,28} = 5.39$, $P = 0.0006$), and 2004 ($F_{7,28} = 2.88$, $P = 0.0214$). Despite this variation in egg counts, there was no interaction between cotton and sampling dates ($P > 0.05$).

Considering year-to-year data, analyses on season-long averages of bollworm eggs showed that egg densities per plant differed among years ($F_{2,12} = 4.86$, $P = 0.0284$). More bollworm eggs were recovered from non-Bt cotton plants in 2002 ($F_{2,6} = 5.49$, $P = 0.0441$) than in the two following years (Table 2). In Bt cotton, however, there were similar

Cotton seasons	Bt cotton	Non-Bt cotton	Statistics between cottons ¹
Bollworms			
2002	0.337 ± 0.045	0.335 ± 0.092a	F = 0.00, P = 0.998
2003	0.155 ± 0.051	0.078 ± 0.028b	F = 3.41, P = 0.051
2004	0.226 ± 0.081	0.130 ± 0.038ab	F = 8.31, P = 0.032
Big-eyed bug			
2002	0.181 ± 0.030	0.221 ± 0.050	F = 0.34, P = 0.593
2003	0.170 ± 0.039	0.142 ± 0.042	F = 0.37, P = 0.575
2004	0.231 ± 0.032	0.267 ± 0.050	F = 1.05, P = 0.364
Green lacewing			
2002	0.14 ± 0.05	0.11 ± 0.04b	F = 0.21, P = 0.669
2003	0.21 ± 0.04	0.20 ± 0.04ab	F = 0.04, P = 0.857
2004	0.19 ± 0.03	0.25 ± 0.12a	F = 0.18, P = 0.695
Brown lacewing			
2002	0.06 ± 0.03b	0.03 ± 0.01b	F = 1.57, P = 0.578
2003	0.20 ± 0.06a	0.24 ± 0.04a	F = 0.31, P = 0.609
2004	0.15 ± 0.07ab	0.27 ± 0.10a	F = 1.24, P = 0.327

Table 2 Seasonal means (± SE) of eggs of bollworm (*Heliothis* and *Helicoverpa*), big-eyed bug (*Geocoris punctipes*), green lacewing (*Chrysoperla rufilabris*), and brown lacewing (*Micromus spec.*) per plant of Bt and non-Bt cotton

¹Results from one-way ANOVA (F-test) between Bt and non-Bt cotton for each year.

²Different letters within a column indicate that means are significantly different by Tukey studentized range test at 0.05 significance level.

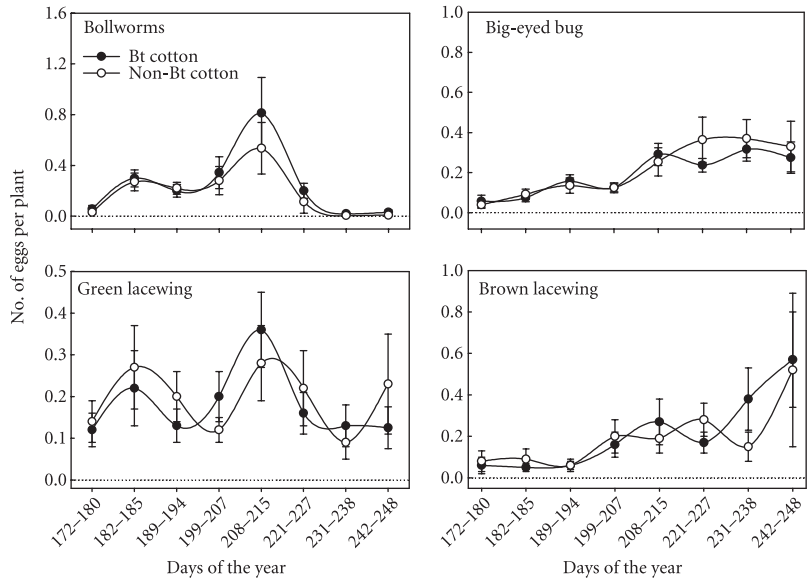


Figure 1 Oviposition dynamics within a season (2002–2004; data pooled) of bollworm (*Heliothis* and *Helicoverpa* spp.) and big-eyed bug (*Geocoris punctipes*), green lacewing (*Chrysoperla rufilabris*), and brown lacewing (*Micromus* spec.), in Bt and non-Bt cotton fields, Tift County, GA, USA. Note different scale of y-axis.

egg densities across the years ($P > 0.05$). Despite variation across years for non-Bt cotton, there was no interaction of cotton type and year for egg densities per plant ($P > 0.05$). Analyses of each year comparing Bt vs. non-Bt cotton verified that more bollworm eggs were recovered from Bt cotton in 2003 and 2004 (Table 2), a tendency observed specifically during the second peak of oviposition (Figure 1).

Bollworm oviposition produced two oviposition peaks each season, in early July and early August (Figure 1). Because the second oviposition peak contributed nearly twice as many eggs as the first peak (Figure 1), the second oviposition peaks in 2003 and 2004 contributed to a signi-

ficantly higher seasonal average of bollworm eggs in Bt cotton compared to non-Bt cotton (Table 2).

For both cotton types, and during both oviposition peaks, there were proportionally more eggs laid in the top nodes of the plants. The top 5–10 nodes averaged approximately 80–95% of bollworm eggs laid on both types of cotton (Figure 2). As oviposition was concentrated in the top nodes, a quadratic model was the best fit to represent the decrease in percentage of eggs laid per node from top to bottom of plants, and the pattern was similar comparing the linear coefficients of the models between Bt and non-Bt cotton (PROC MIXED of SAS for equality of linear coefficient: $t_{1,236} = 0.08, P = 0.9332$). Likewise, a similar pattern

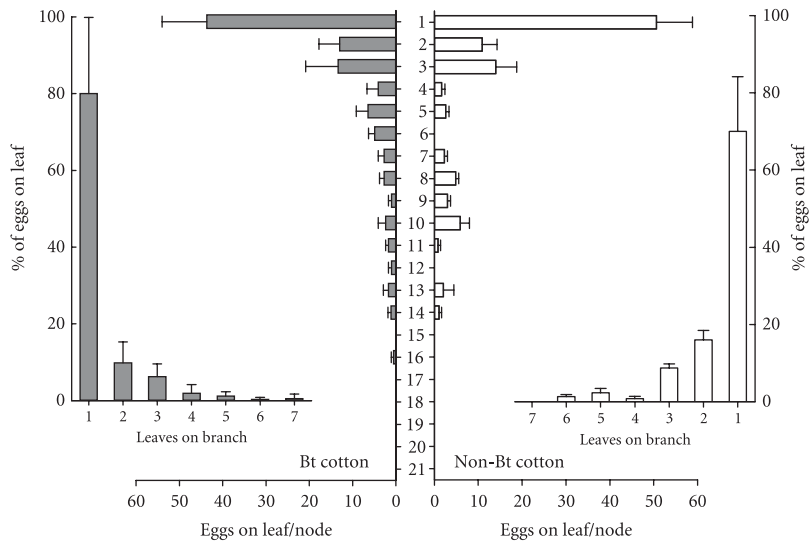


Figure 2 Vertical distribution (%) of bollworm eggs within Bt ($y = 29.12 - 4.58x + 0.167x^2, R^2 = 0.55, F = 71.65, P < 0.0001$) and non-Bt ($y = 29.81 - 4.75x + 0.17x^2, R^2 = 0.44, F = 44.72, P < 0.0001$) cotton plants based on plant node position from plant apex (node 1) to bottom (node 21), and on leaves/nodes of vegetative/fruitleaves from outside leaf (leaf 1 including bud) toward inside Bt ($y = 105.21 - 44.95x + 4.44x^2, R^2 = 0.78, F = 69.80, P < 0.0001$) and non-Bt ($y = 95.17 - 39.12x + 3.78x^2, R^2 = 0.87, F = 87.29, P < 0.0001$) cotton plants collected in the field during cotton seasons 2002–2004, Tift County, GA, USA.

Table 3 Seasonal means (\pm SE) of bollworm eggs found on different cotton plant structures throughout the 2002–2004 cotton seasons, Tift County, GA, USA

Plant structures	Cotton types ¹	
	Bt	Non-Bt
Bud (pinhead square)	15.1 \pm 3.43a	11.6 \pm 3.35a
Fruit structures ²	8.2 \pm 1.99ab	7.3 \pm 2.04ab
Uppermost expanded leaf	4.7 \pm 1.29ab	3.7 \pm 0.94abc
Mainstem leaf	5.8 \pm 1.27bc	3.2 \pm 0.83abc
Leaf petiole	1.8 \pm 0.78cd	1.9 \pm 0.75bc
Dried petal (boll tag)	1.1 \pm 0.42d	1.2 \pm 0.36c
Mainstem ³	0.9 \pm 0.26d	0.8 \pm 0.22c

¹Means within a column followed by different letters differ significantly by Tukey studentized range test at 0.05 significance level.

²Fruit structures include squares, flowers, bracts, and bolls, but exclude dried petals.

³Mainstem includes main and branching stems.

was observed between cotton types (PROC MIXED of SAS for equality of linear coefficient: $t_{1,80} = -0.25$, $P = 0.8028$) for eggs laid on vegetative/fruitletting branches where terminals (bud and outermost leaf on the branch) were the location of 77 and 80% of the eggs collected on Bt and non-Bt cotton, respectively (Figure 2).

Bollworm females showed similar oviposition preference for plant structures in both cotton types (Table 3). Plant terminals (i.e., bud pinhead and upper expanded leaves) were the preferred sites for oviposition, followed by fruit structures (flower structures) (Table 3). Over 3 years, only three eggs were found on bracts of developed bolls, which were included with fruit structures, while bracts of squares (flower buds) were the preferred location in the fruit structures. Although the dry flower petals are also a fruit structure, they were treated separately and hosted more eggs than other boll components, but with significantly fewer eggs relative to the other upper plant structures (Table 3).

Bollworm eggs collected throughout the season and incubated in the laboratory showed relatively high viability, with 82–100% of eggs hatching. Eggs not hatching were either parasitized by *Trichogramma spec.* or failed to hatch for unknown reasons. Parasitism by *Trichogramma spec.* accounted for 13.3 and 12.5% of egg mortality, and non-viability reduced egg hatch 3.7 and 2.3% in Bt and non-Bt cotton, respectively.

Big-eyed bug oviposition

Examination of whole Bt and non-Bt cotton plants throughout each cotton season allowed us to map the within-plant distribution of 589 big-eyed bug eggs and to determine

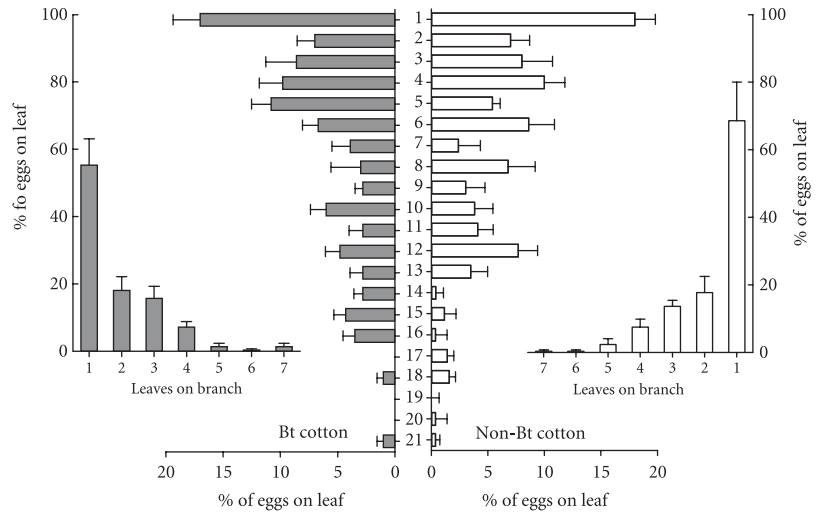
big-eyed bug oviposition dynamics. From these eggs, only three yielded nymphs of the big-eyed bug, *Geocoris uliginosus* (Say); therefore, all recovered eggs are treated as belonging to *G. punctipes*. *Geocoris uliginosus* is a big-eyed bug typically found on the ground, but it also can be found on cotton plants, especially when abundant in the field, as was the case in whole-plant and drop-cloth samples during 2004 compared to 2002 and 2003 (Torres & Ruberson, 2005).

The average number of big-eyed bug eggs per plant was quite variable within and among years (Figure 1 and Table 2), but repeated-measures ANOVA did not detect any difference on average of eggs per plant between cotton types in any year ($P > 0.05$) nor interactive effects of cotton types and sampling dates were observed ($P > 0.05$). The only significant variation was yielded for sampling dates with egg densities per plant increasing progressively on both cotton types during the season in 2002 ($F_{7,28} = 5.09$, $P = 0.0017$) and in 2004 ($F_{7,28} = 3.59$, $P = 0.0070$). The number of eggs per plant on each sampling date in 2003, although tending to increase as the season progressed, was quite variable, with no significant difference between sampling dates for either cotton type ($F_{7,28} = 1.74$, $P = 0.1392$). Likewise, despite a trend of more eggs found per plant in 2004 in both cotton types compared to 2002 and 2003 (Table 2), there were no differences for season-long means across years.

At the beginning of the season, big-eyed bug oviposition occurred at low densities, but increased as the season progressed (Figure 1). This oviposition pattern of the predator resulted in a lack of temporal correlation with the prey (i.e., bollworm oviposition) in both cotton types (Bt: $r = -0.02$, $P = 0.861$; non-Bt: $r = 0.06$, $P = 0.577$), with bollworm females exhibiting two distinct peaks of oviposition over the season (Figure 1). However, there was a significant correlation between big-eyed bug egg numbers on a given date and bollworm egg numbers 10 days previously (Bt: $r = 0.39$, $P = 0.001$; non-Bt: $r = 0.66$, $P < 0.0001$), indicating a time delay in the predator oviposition relative to its prey.

Big-eyed bug egg distribution within plants showed a linear decrease from the plant top toward the lowest node on the plant stem (Figure 3) and exhibited a similar vertical distribution pattern between Bt and non-Bt cotton (PROC MIXED, comparing linear coefficients on node height: $t_{1,236} = 0.77$, $P = 0.4439$). From the plant top, 46% of big-eyed bug eggs were found from node 1–5 and 73% from nodes 1–10. Egg distribution on fruiting/vegetative branches, however, was concentrated on the plant periphery, with the three outermost nodes/leaves on fruiting/vegetative branches accounting for nearly 90% of the eggs, and showing a quadratic decrease toward the plant's interior (Figure 3). Similar to the vertical distribution on nodes of the main stem, big-eyed bug egg distribution on fruiting/

Figure 3 Vertical distribution (%) of *Geocoris punctipes* eggs on nodes within Bt ($y = 11.07 - 0.57x, r^2 = 0.29, F = 48.51, P < 0.0001$) and non-Bt ($y = 12.88 - 0.67x, r^2 = 0.39, F = 51.25, P < 0.0001$) cotton plants based on plant node position from plant apex (node 1) to bottom (node 21); and per leaf of vegetative/fruiting branches from outside (leaf 1 including branch bud) toward inside plant of Bt ($y = 70.26 - 24.89x + 2.17x^2, R^2 = 0.76, F = 96.82, P < 0.001$) and of non-Bt ($y = 76.89 - 28.53x + 2.57x^2, R^2 = 0.78, F = 108.56, P < 0.001$) cotton plants collected in the field during cotton seasons 2002–2004, near Tifton, GA, USA.



vegetative branches was similar between Bt and non-Bt cotton (PROC MIXED, comparing linear coefficients on leaf/node of branches: $t_{1,373} = -1.36, P = 0.1748$).

Within plant structures, big-eyed bugs preferred leaves as oviposition sites on both cotton types (Table 4), and more than 93% of the eggs were found on the lower surface of leaves. Furthermore, big-eyed bugs preferred laying their

eggs along veins of the leaves. Plant terminals (‘pinhead structures’), fruit structures (i.e., squares, flowers, bracts, bolls, and open lint), and stems were also sites for egg laying, but in very low frequencies (Table 4).

Eggs of big-eyed bugs collected and incubated in the laboratory exhibited high egg hatching (>74%) from both cotton types. Among the remaining eggs, 13.3 and 12.8%

Table 4 Seasonal means (\pm SE) of *Geocoris punctipes* eggs per field of Bt and non-Bt cotton collected throughout the 2002–2004 cotton seasons, according to plant structures, Tift County, GA, USA

Cotton	Main plant components	Eggs	Leaf components	Eggs
Bt	Leaf	24.8 \pm 4.82	Lower surface	23.8 \pm 4.63
			Along vein	10.2 \pm 1.76
			Edge	1.2 \pm 0.74
			Petiole	1.3 \pm 0.75
			Petiole junction	3.0 \pm 0.78
			Middle leaf	8.1 \pm 2.87
			Upper surface	1.1 \pm 0.39
			Bud (pinhead square)	2.2 \pm 0.83
Non-Bt	Leaf	21.2 \pm 3.10	Lower surface	20.0 \pm 3.14
			Along vein	11.1 \pm 1.48
			Edge	0.6 \pm 0.18
			Petiole	0.8 \pm 0.28
Non-Bt	Upper expanded leaf	2.2 \pm 0.66	Petiole junction	2.6 \pm 0.47
			Middle	5.0 \pm 1.90
			Upper surface	1.5 \pm 1.04
			Bud (pinhead square)	1.8 \pm 0.83
Non-Bt	Flower and bolls ¹	2.2 \pm 1.01		
			Stem	0.3 \pm 0.25

¹Fruit structures include squares, flowers, bracts, bolls, and open lint; stem includes main and branch stems.

were parasitized by *Telenomus reynoldsi* Gordh in Bt and non-Bt cotton, respectively. Eggs not hatching or parasitized were considered non-viable (12.1 and 12.8% for Bt and non-Bt). Nonviable eggs were usually white or pale, with no sign of development (e.g., red eyespots), and eventually collapsed.

Green lacewing oviposition

Plant inspection resulted in 595 green lacewing eggs counted. Repeated-measures ANOVA did not indicate differences in densities of green lacewing eggs between cotton types for any of the 3 years of investigation ($P > 0.05$) or in cotton and sampling dates interactive effects ($P > 0.05$). Egg densities, however, had significant variation across sampling dates in 2002 ($F_{7,28} = 9.35, P < 0.0001$) and 2003 ($F_{7,28} = 4.69, P = 0.001$) but not in 2004 ($F_{7,28} = 1.60, P = 0.176$). These results explain the overall variation in green lacewing egg densities throughout sampling dates that exhibited two oviposition peaks in the first week of July and of August, respectively (Figure 1).

A two-way factor ANOVA (year and cotton) considering season-long means over all sampling dates did not show variation in green lacewing egg densities ($P > 0.05$). Thus, although relatively fewer eggs were collected in 2002 on both cotton types, green lacewing oviposition was similar across years and between cotton in each year (Table 2).

Green lacewing oviposition peaks occurred simultaneously with bollworm egg peaks (Figure 1), resulting in a positive and significant temporal correlation in both cotton types (Bt: $r = 0.25, P = 0.040$; and non-Bt: $r = 0.39, P = 0.013$). Consideration of a time delay on predator oviposition (10, 20, and 30 days following bollworm oviposition) did not yield any enhancement of the relationship between predator and bollworm oviposition dynamics. In the laboratory, more than 93% of the collected chrysoptid eggs hatched successfully, while 1 and 2.3% were found non-viable, and 5.8 and 1.8% were parasitized by *Telenomus spec.* in Bt and non-Bt cotton, respectively.

Brown lacewing oviposition

Plant inspection resulted in collection of 606 brown lacewing eggs. Repeated-measures ANOVA for brown lacewing eggs was significant only for sampling dates across season in 2002 ($F_{7,28} = 2.70, P = 0.038$), in 2004 ($F_{7,28} = 3.32, P = 0.017$), and partially significant in 2003 ($F_{7,28} = 2.17, P = 0.069$). There was no significant difference between cotton types in hemerobiid egg dynamics in each of the 3 years ($P > 0.05$). Brown lacewing eggs per plant increased slowly throughout the season, with significantly higher densities at the end of the season, but with similar egg densities in both cottons (Figure 1). Although the number of eggs per plant within the season varied, no interactive effects were detected between

cotton type and sampling date ($P > 0.05$) in any of the years.

Considering the season-long means over all sampling dates for each year and cotton type, a significant difference was found among years ($F_{2,12} = 9.95, P = 0.002$) but not between cotton ($F_{1,2} = 1.50, P = 0.244$) nor an interaction between the two. Fewer eggs of brown lacewing per plant were found on both cotton types in 2002 (Bt: $F_{2,6} = 4.99, P = 0.047$; non-Bt: $F_{2,6} = 5.94, P = 0.037$) than in 2003 and 2004 (Table 2).

The concurrent oviposition dynamics of brown lacewing and bollworms exhibited a lack of temporal correlation in both cotton types (Bt: $r = 0.17, P = 0.161$; non-Bt: $-0.16, P = 0.197$). The asynchronous oviposition pattern between brown lacewings and bollworm females resulted from bollworms concentrating oviposition in two peaks, while brown lacewings increased oviposition toward the end of the season (Figure 1). Further analysis of the predator and prey association, anticipating sampling date of predator oviposition did not generate any significant temporal association between brown lacewing and bollworm female oviposition.

Brown lacewing eggs collected from plants and incubated in the laboratory showed more than 91% of viability. The remaining eggs were parasitized at rates of 7.9 and 3.5% by *Trichogramma spec.* or were considered non-viable (0.9 and 1.1%) from Bt and non-Bt cotton fields, respectively.

Discussion

Bollworms and their predators (big-eyed bugs and lacewings) apparently did not discriminate between Bt and non-Bt cotton in the field, laying similar numbers of eggs on both cotton types. The large size of the fields minimized intrafield movement; thus, our results strongly suggest that these predators are developing populations equally in both cotton types, which agrees with surveys of immature or adult predators conducted simultaneously in these same fields (Torres & Ruberson, 2005). Bollworm oviposition patterns observed in our study did not support behavioral change toward egg placement on lower plant structures to escape high Bt toxin expression in the peripheral, younger plant parts. These findings agree with results reported by Parker & Luttrell (1998) in a study of oviposition behavior of *H. virescens*. They used field cages with Bt cotton mixed from 0 to 100% with non-Bt cotton in 1994, prior to commercial release of Bt cotton. Evaluating the top five nodes, the authors reported no difference in tobacco budworm egg distribution between cotton types or among the plant structures selected for oviposition by artificial moth infestations in caged plants. Parker & Luttrell (1998) also reported no difference in egg densities on plant terminals (only the upper three nodes) between Bt and non-Bt

cotton fields during 1995, as we found from 2002 to 2004. However, those authors did not evaluate entire plants, and eggs laid on structures lower on the plant, where Cry1Ac toxin expression is reduced, were not considered.

Oviposition by bollworms on plant structures with reduced Bt toxin expression is a concern. Significant differences in Cry toxin levels among cotton plant structures have been reported (Greenplate, 1999; Greenplate et al., 2003). Although many environmental factors affect levels of Cry toxin detected in cotton plant tissues, levels of Bt toxin in plant terminals are significantly higher than the amount of toxin expressed in old leaves and squares (Greenplate et al., 2003). Although Parker & Luttrell's (1998) studies were conducted prior to large-scale use of Bt-transgenic cotton with no previous exposure of bollworms to Bt cotton plants in the field, their results provide an excellent standard for further monitoring of bollworm oviposition behavior, as females with no prior experience with Bt cotton did not discriminate between Bt-transgenic and regular cotton. Therefore, any further change in bollworm moth oviposition on Bt cotton relative to the findings of Parker & Luttrell (1998) would indicate that populations are beginning to behave differently in relation to Bt cotton. Our results match those of Parker & Luttrell (1998), indicating that no such changes have occurred yet in the past decade of Bt cotton use.

The periodic monitoring of pest behavior is not only important for bollworms in cotton but also for other important pests targeted by Bt-transgenic crops. Bt-transgenic cotton exhibits physiological changes other than direct toxicity that can affect life history traits of larval bollworm. Although an oligophagous species (Fitt, 1989), *H. virescens* females responded positively to extracts of their suitable host plants and did not fly upwind in response to odors of a resistant tobacco cultivar or to extracts of non-host plants (Tingle et al., 1990), suggesting that specific volatiles play a role in host location and probably for oviposition. Also, after landing on a host plant, *H. virescens* was able to discriminate among host plants through chemicals on the leaf surface using chemoreceptors in the tarsi (Ramaswamy et al., 1987). The insertion of Bt genes into cotton plants is known to have induced changes in important secondary compounds related to herbivore–cotton plant interactions (Jallow et al., 1999; Zhang et al., 1999; Yan et al., 2004). For instance, alpha-pinene and beta-pinene are among the major volatile compounds triggering antennal response in Old World bollworm, *Helicoverpa armigera* Hübner (Jallow et al., 1999; Yan et al., 2004). These compounds are 5.5 and 2.85 times higher in Bt cotton than in non-Bt cotton, respectively (Yan et al., 2004) and could induce more oviposition on Bt cotton than in regular cotton in the field. In addition, condensed tannins that play a role in cotton

resistance to arthropod pests were significantly lower in Bt cotton (Zhang et al., 1999).

Adult moth behavioral adaptation to differential Cry1Ac expression among plant structures, expressed as site selection for oviposition, might be a phenomenon that never occurs because there are many additional factors underlying oviposition behavior in the field (Renwick & Chew, 1994). The phenology of bollworm females during the cotton season indicates that they complete generations on other crops or alternative native hosts before and during the cotton season (Fitt, 1989), thereby escaping continuous selection pressure from Bt toxins in cotton. Moreover, bollworm moths are active in short-range movement among crops, but can also migrate long distances, such as into the USA from Mexico and among US states, to cope with food and environmental changes (Westbrook et al., 1998). Nevertheless, widespread planting of Bt-transgenic cotton may contribute to oviposition modifications, and any behavioral change that could interfere with the efficacy of Bt crops to control bollworms would be a great threat. Therefore, further studies of bollworm oviposition in the years ahead and in different cotton regions, and comparisons with the results reported here and those of Parker & Luttrell (1998) will be important to detect possible behavioral changes in this key cotton pest.

Regardless of cotton genotype, two major differences were observed in bollworm egg dynamics. First, higher egg densities were observed in both cottons in 2002 compared to the following two seasons (Table 2). Second, higher egg densities were noted in Bt cotton fields than in non-Bt fields during the second peak of oviposition in 2003 and 2004. Higher egg counts in 2002 are probably a result of high bollworm female abundance that year. The average moth captures in pheromone traps [*Helicoverpa zea* (Boddie)] from four counties around our fields during the same survey period was 32.5% greater in 2002 (mean \pm SE, 173.0 ± 24.2 moths per pheromone trap) than in 2003 (116.8 ± 19.8) (Ruberson et al., 2003; Diffie et al., 2004). Second, the difference in bollworm egg densities between Bt and non-Bt cotton observed during the second oviposition peak in 2003 and 2004 (Figure 1) does not necessarily indicate that moths preferred Bt cotton to lay their eggs. Differences in egg numbers between cotton types might be related to insecticide use to control bollworms and stink bugs. All non-Bt cotton fields in 2003 and 2004 required second insecticide applications, including the broad-spectrum insecticides lambda-cyhalothrin in 2003, and lambda-cyhalothrin and zeta-cypermethrin in 2004. No pyrethroids were used in Bt cotton fields in 2003 or 2004. Therefore, considerable insecticide pressure was imposed on all life stages of bollworms in non-Bt cotton fields in 2003 and 2004, besides the overall repellency of lambda-cyhalothrin

to insects and mites. In addition, one Bt cotton field (Chula field) was treated with organophosphate (dicrotophos) on 29 July 2004 to control stink bugs immediately before the second bollworm oviposition peak. Broad-spectrum organophosphate insecticides such as dicrotophos have been known to induce lepidopteran outbreaks in cotton because of the elimination of natural enemies in treated fields (Eveleens et al., 1973). Predation in cotton fields can account for considerable reduction of bollworm eggs in the tops of cotton plants and is similar between Bt and non-Bt cotton fields (Obrycki et al., 2004). The abundance of key bollworm egg predators [*G. punctipes*, *Orius insidiosus* (Say), and *C. rufilabris*] on the two sampling dates following dicrotophos application on Bt cotton (e.g., Chula field) averaged 0.13 predators per plant, compared to 0.41 and 0.43 predators per plant in untreated Bt cotton and non-Bt cotton fields, respectively. At the same time, bollworm egg densities averaged 1.11 eggs per plant in the dicrotophos-treated Bt cotton field (Chula field) compared to 0.11 and 0.19 eggs per plant in untreated Bt cotton and non-Bt cotton fields, respectively. A similar trend was reported by Mellett et al. (2004), who found twice as many eggs of the Old World bollworm, *H. armigera*, in cotton fields following two foliar sprays with endosulfan compared to Bt and non-Bt untreated fields. Therefore, the absence of pyrethroid pressure on bollworm populations after the first oviposition peak in Bt cotton fields and the use of an organophosphate insecticide to control stink bugs, with the strongly detrimental impact of the insecticide on predators, might have increased egg counts by relieving predation pressure on eggs in Bt cotton during the second peak of oviposition.

Among the predators evaluated, only oviposition by green lacewings was concurrent with bollworm oviposition (Figure 1), while oviposition by big-eyed bugs exhibited a 10-day delay relative to bollworm oviposition. Brown lacewing oviposition did not correlate at all with bollworm oviposition. Bollworm females showed two well-defined peaks of oviposition, whereas the big-eyed bugs and brown lacewings tended to progressively increase their oviposition throughout the season. Lack of correlation between oviposition of *G. punctipes* and brown lacewings with bollworm eggs would be expected. Big-eyed bugs are generalist feeders, use a variety of arthropods as prey in cotton fields, and increase oviposition steadily throughout the season (Figure 1). Moreover, strong association between big-eyed bug eggs and bollworm eggs is of risk for the predator. The incubation period of big-eyed bugs is approximately three times longer than that of bollworms, thus big-eyed bug nymphs would always be behind relative to the availability of bollworm prey (eggs and young larvae). Nevertheless, in general, both big-eyed bug and bollworm eggs overlapped

considerably in their spatial distribution within cotton plants, bringing the predator in close contact with potential prey (Figures 2 and 3 and Tables 3 and 4). This pattern may be an explanation for greater mortality of bollworm eggs toward the tops of the plants (Nuessly & Sterling, 1994), which correlates with the preferred foraging location of *G. punctipes* and other small predatory heteropterans (e.g., *Orius*) within cotton plants (Wilson & Gutierrez, 1980).

Green and brown lacewings are more specialized on aphids, and their population dynamics tend to be more closely related to aphid abundance (Agnew et al., 1981; Szentkirályi, 2001). Although green lacewings prey on eggs and larvae of bollworms, the positive correlation of green lacewing and bollworm eggs seems more accidental than biologically meaningful. In our fields, infestations of cotton aphids (*Aphis gossypii* Glover), a preferred prey of green and brown lacewings, peaked in late June and early July, providing abundant food for population growth and coinciding with the first bollworm and green lacewing egg peaks. The generation time of the green lacewings would place their next ovipositional peak about the time of the second bollworm egg peak in late July and early August.

All predators studied had already deposited eggs in the fields by the time of the first sampling date in all three seasons but at very low densities that were similar in both cotton types (Figure 1). In this context, reduced insecticide use in Bt cotton fields opens opportunities for conservation of these predator populations. For this reason, cropping systems favoring early cotton field colonization and further reproduction by these predators will be important to foster presence of predators when oviposition by major pests such as bollworms occurs and young bollworm larvae first appear in cotton fields. The data indicate that factors other than bollworm eggs determine dynamics of the three studied predators in cotton fields. Previous studies found that *G. punctipes* lays eggs on various surfaces, but tends to prefer certain plants when given a choice, and preferentially oviposits on leaves rather than other plant structures (Naranjo, 1987), and this result was not related to prey availability (Naranjo & Stimac, 1987). Among factors influencing oviposition of predatory heteropterans, plant type and plant structure seem to be very important (Naranjo & Stimac, 1987; Coll, 1996; Pfannenstiel & Yeagan, 1998; Evangelista et al., 2003), especially for those species with strong plant-feeding behavior. Plants preferred for oviposition by some predatory heteropterans have been found to correlate with nymphal (development and survival) and adult (longevity and fecundity) performance (Coll, 1996; Evangelista et al., 2003). Therefore, *G. punctipes* laying eggs on soft and young cotton plant tissues may be advantageous for the predator for two reasons: first, soft and young cotton plant tissues may facilitate acquisition

of moisture and nutrients from plant feeding by young nymphs; and second, the spatial match with a highly suitable prey (bollworm eggs) in cotton plant terminals (Figures 2 and 3).

It is apparent that Bt cotton plants exerted no significant effect on temporal or spatial patterns of oviposition of bollworms or three of their selected predators, indicating no change in oviposition behavior of bollworm females within plant structures after almost one decade of widespread planting of Bt cotton. Furthermore, the lack of differences in oviposition by predators throughout the season and over 3 years between Bt and non-Bt cotton suggests that population dynamics of important predators species are not impaired by Bt cotton.

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