

Interactions of Bt-cotton and the omnivorous big-eyed bug *Geocoris punctipes* (Say), a key predator in cotton fields

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Abstract

The continuous expression of the Bt (= *Bacillus thuringiensis*) toxin in plants during the growing season may bring Bt toxin into contact with the omnivorous big-eyed bug *Geocoris punctipes* (Say) (Hemiptera: Geocoridae), an important predator in cotton fields, through prey fed Bt-cotton or through plant feeding. Therefore, an experiment was set up during two seasons (2003 and 2004) in the field to assess host plant- and prey-mediated effects on development and reproduction of the predator. We used a combination of Bt and non-Bt cotton plants, and of prey, with one treatment conveying Bt Cry1Ac toxin to the upper trophic level [*Spodoptera exigua* (Hübner) larvae (BAW)] and the other prey item free of toxin [*Helicoverpa zea* (Boddie) eggs (CEW)]. This design allowed us to differentiate direct and indirect effects of the toxin in the host plant from effects attributable to prey quality under field conditions. The experiment began with newly hatched big-eyed bug nymphs and ending when the last female died. The combination of prey and Bt-cotton plants did not exert interactive effects on development and reproduction of the omnivorous predator. The prey effect was independent of either host plant type. Nymphs fed BAW larvae developed slower and produced smaller adults with no difference between cotton genotypes. Reproductive output and longevity were similar between cottons for both prey types, and were consistently lower for predators fed BAW larvae. Cry1Ac was detected in Bt-cotton and in BAW larvae fed Bt-cotton and offered to the predators, but not in the predators' bodies. The results do not indicate any lethal or sublethal effect of transgenic Bt-cotton or of Cry1Ac conveyed through prey on development and reproduction of *G. punctipes* in our field experiments.

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1. Introduction

Insecticide-transgenic plants have become important components of maize and cotton production in various areas of the world (James, 2004). These plants have been modified with genes from other species (in most cases the bacterium *Bacillus thuringiensis* Berliner; hereafter referred to as Bt) that constitutively express toxins. In crop systems where such plants are used, omnivorous generalist predators

are exposed to natural and transgenic plant defenses through direct feeding on plants and via herbivorous prey.

Numerous tritrophic studies have elucidated host plant traits that may affect the life histories of natural enemies when implemented in pest management programs (Bottrell et al., 1998; Hagen, 1986). The widespread adoption of plants expressing transgenic toxins adds another element of interaction to the multitrophic interactions in agroecosystems that may exert a variety of effects (Obrycki et al., 2004). There are indications of some insecticide-transgenic plants exerting at least indirect adverse effects on omnivorous predators (Bell et al., 2003). Given the high dose strategy being used in the Bt-transgenic plants, the season-long constitutive expression of the toxins, and the dual routes of

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exposure to omnivores, it is important to ask the question: Do Bt-transgenic plants exert negative effects on the life histories of important omnivorous predators?

Laboratory studies have indicated that there are no short-term indirect effects of prey fed Bt-plants on some predatory heteropterans (Al-Deeb et al., 2001; Bernal et al., 2002; Pilcher et al., 1997; Zwahlen et al., 2000). However, there are no long-term studies of the effects of Bt toxins on life histories of omnivorous predators that account for prey quality, direct and indirect routes of exposure, and the effects of field conditions, all of which are highly relevant to understanding environmental impacts of insecticide-transgenic plants. Our study was designed to address the question of direct and indirect effects of Bt-transgenic cotton on an important omnivorous predator, *Geocoris punctipes* (Say) under field conditions, accounting for effects of prey quality and multiple routes of exposure.

Omnivorous predators are able to use food resources from different trophic levels, which buffer them against the unpredictability of spatial and temporal occurrence of food resources in patchy environments (Coll and Guershon, 2002; Pimm and Lawton, 1978). The ability to switch among prey and to survive on plant material allows omnivorous predator populations to persist under conditions inimical to specialized entomophages, and to be present when alternative prey begins colonizing a habitat and occur at low densities. These attributes may be particularly valuable for predators in ephemeral agricultural systems, where prey resources can be quite variable (Coll and Guershon, 2002). Generalists (including omnivores) tend to be most effective at maintaining incipient pest populations at low levels, but do not necessarily exhibit numerical responses to particular prey, as is the case with specialists (Symondson et al., 2002; Whitcomb and Godfrey, 1991). This can be exemplified in cotton fields with the omnivorous generalist predator *G. punctipes* that feeds on a diverse prey suite varying in quality (Crocker and Whitcomb, 1980) and uses host plants as a food supplement (Naranjo and Stimac, 1985; Stoner, 1970). Three years of field data showed that *G. punctipes* dynamics do not correlate with populations of high-quality prey (heliathine eggs) in cotton fields (Torres and Ruberson, 2006). *G. punctipes* populations increase steadily as the cotton season progresses, regardless of presence of high-quality prey. Big-eyed bugs also clearly preferred plant terminals and young leaves—soft plant tissues that could serve as ready food supplements for nymphs—for oviposition, indicating the importance of cotton plants in the ecology of these predators (Torres and Ruberson, 2006).

Despite its potential advantages, omnivory also brings with it the risk of exposure to a wide range of suitability in dietary constituents (Bozer et al., 1996; Weiser and Stamp, 1998). Thus, the fitness of omnivores can vary widely depending on habitat and available prey and plant resources (Coll, 1998). Plants can exert effects on omnivorous predators directly and indirectly through prey (Coll and Guershon, 2002). There is a large body of literature

addressing the direct effects of plant feeding on life histories of omnivorous heteropteran predators (e.g., chapters in Alomar and Wiedenmann, 1996). Prey-mediated effects of plant allelochemicals also have been reported (Bozer et al., 1996; Weiser and Stamp, 1998). Thus, omnivorous predators are exposed to plant defensive compounds via two routes, which may intensify selective pressure on these species.

To examine the effects of Bt-cotton plants and prey quality interactions on life history parameters of the omnivorous predator big-eyed bug, *G. punctipes*, we conducted experiments over two growing seasons using bugs caged on plants in the field. We used a combination of Bt and non-Bt cotton plants, and of prey, with one treatment conveying Bt Cry1Ac toxin to the upper trophic level [*Spodoptera exigua* (Hübner) larvae] and the other prey item free of toxin [*Helicoverpa zea* (Boddie) eggs]. This design allowed us to differentiate direct and indirect effects of the toxin in the host plant from effects attributable to prey quality under field conditions.

1.1. Study system

Cotton is a key crop in a number of countries, and the adoption of Bt-transgenic cotton varieties has been extensive (James, 2004). In the United States approximately 50% of the total cotton production is planted with Bt-transgenic varieties. All of the Bt-transgenic varieties currently in use express the Cry1Ac toxin, which is specific for lepidopteran larvae, although its efficacy varies with species (Stewart et al., 2001). Caterpillars that are partially susceptible to Bt toxins exhibit delayed development and smaller size (Jenkins et al., 1993; Mascarenhas and Luttrell, 1997), and are more readily predated (Roush, 1996). An example of a lepidopteran that is only partially susceptible to Cry1Ac is the beet armyworm, *S. exigua*, a noctuid pest of cotton and other crops in North America. The beet armyworm also is typically controlled in cotton in the US by the activity of natural enemies (Ruberson et al., 1994). The partial susceptibility of this species, coupled with its importance in cotton and the important role of predators in suppressing it, make it an ideal and relevant organism for testing the transfer of Bt toxin across trophic levels in a crop system.

Geocoris punctipes is an important omnivorous predator in the eastern half of the US Cotton Belt. The efficacy of this predator against various pests has been documented (Ali and Watson, 1982; Lingren et al., 1968; Ruberson et al., 1994). However, prey vary considerably in their suitability for predator development, survival, and reproduction—lepidopteran eggs are typically much more suitable than are lepidopteran larvae and aphids (Cohen and Debolt, 1983; Dunbar and Bacon, 1972a; Eubanks and Denno, 2000; Lawrence and Watson, 1979; Torres et al., 2004). *Geocoris punctipes* also feeds frequently directly on plants (Stoner, 1970; Tillman and Mullinix, 2003), and can acquire direct benefits from phytophagy (Naranjo and Stimac, 1985). The broad prey range of *G. punctipes* and its frequent phytoph-

agous behavior can place this predator in indirect contact with Bt toxins through contaminated prey, and its active plant-feeding behavior may provide direct contact to toxins. Nymphal stages of *G. punctipes* were adversely affected when fed prey contaminated with a commercial Bt formulation (Herbert and Harper, 1986), whereas adults were not so strongly affected. Thus, there is potential for the Bt cotton to adversely affect this important predator.

2. Materials and methods

2.1. Field description

Two parallel experimental plots of cotton, 20 rows wide and 85 m long, were planted at the Coastal Plain Experiment Station (CPES) of the University of Georgia, Tifton, GA. Two varieties of cotton were used in the study: non-Bt cotton variety DPL 5690RR and transgenic Bt-cotton variety DPL 458RR containing genes for expression of the toxin Cry1Ac from *B. thuringiensis* (Bt). Seeds were planted at a rate of 9 kg/ha on 8 May 2003 and on 2 June 2004. Bt and non-Bt cotton fields were treated with aldicarb to suppress thrips (Temik 560 g a.i./ha) at planting. Temperature was monitored using a WatchDog logger (Spectrum™ Technologies, Inc.) held inside the cages and set to record at 30-min intervals, and rainfall data were obtained from a local weather station of the Coastal Plain Experiment Station (www.georgiaweather.net).

2.2. Prey types

Two different prey types were used in the study to permit differentiation of the effects of prey quality and of Bt toxin. The first prey type, larvae of the beet armyworm, *S. exigua* (hereafter BAW), is able to survive ingestion Cry1Ac toxin (Stewart et al., 2001) and can expose predators indirectly to the toxin. This prey also is of relatively low quality for *Geocoris* spp. (Torres et al., 2004). The second prey type was eggs of the corn earworm, *H. zea* (hereafter CEW), which do not expose the predator indirectly to Cry1Ac toxin, and which are high-quality food for *Geocoris* spp. The beet armyworm neonate larvae (<24 h after hatching) were obtained from the Biological Control Laboratory of the Tifton Campus of the University of Georgia, and corn earworm eggs provided by USDA-ARS-CPMRL, Tifton, GA. The prey were offered ad libitum to the predators and were changed at two-days intervals.

2.3. 2003 Season

Newly hatched nymphs (<24 h old) of the big-eyed bug, *G. punctipes*, were caged on Bt and non-Bt cotton plants in the field plots on 17 June 2003 (32 days-old plants). The nymphs were caged in organdy bags (~20 cm length and ~15 width) with five nymphs per bag (15 replicates), for a total of 75 nymphs per treatment. The bags containing prey and predator nymphs were tied to the leaf petiole enclosing

the uppermost fully expanded cotton leaf. On each evaluation date (ca. two-day intervals) the bags with nymphs and cages with adults (see below) were moved up to new leaf to keep the same location on the plant and also because cotton leaves infested with BAW larvae inside the bags/cages were partially destroyed. At the beginning of the experiment, bags containing prey and predators were attached to the plants and supported with bamboo rods because the plants were not strong enough to hold the bags, especially under windy and rainy conditions. The experiment consisted of two host plant types (Bt and non-Bt) and two prey types (BAW larvae and CEW eggs) arranged in a 2 × 2 design. Nymphal developmental times, mortality, and adult weight at emergence were monitored at two-day intervals during development and daily when the nymphs started molting to adult. Adults were paired on the day of emergence and maintained in the same treatments to which they had been exposed as immatures. Pairs were held individually in 500-ml styrofoam cups with the bottoms removed and wrapped in knee-high stretch hose. The cages enclosed one leaf each and were tied to the cotton leaf petioles. To facilitate location and counting of eggs in the cages, a small square of cotton batting (~1 cm²) was inserted in each cage as an oviposition substrate; in practice, eggs were also laid on the cup wall, knee-high stretch hose and on the cotton leaves. Egg collections and prey replacement were carried out at two-day intervals. Eggs were counted using a 10× magnifying lamp, and the eggs were subsequently incubated in plastic cups with a piece of green bean pod to determine egg viability.

2.4. 2004 Season

The same treatments and procedures used in 2003 were repeated in 2004, except that the timing of nymphal placement in the cages coincided with the appearance of nymphs in feral populations in the field (i.e., 22 June, according to a survey of bugs in the plots). Newly hatched (<24 h) big-eyed bug nymphs obtained from the laboratory colony were caged on Bt and non-Bt cotton plants in the field on 30 June 2004 (28 days-old plants). As in 2003, nymphs were caged in organdy bags with five nymphs per cage (15 replicates), and a total of 75 nymphs per treatment. The prey used, cage types, and data collection followed the same procedures used in 2003. Time of caging bugs in the field was conducted based on predator dynamics using data from previous years, thus cotton planting in 2004 was delayed compared to 2003 to produce plants roughly at the same age used in 2003.

Weight of feral adults developing at the same experimental location in Bt and non-Bt cotton fields and preying on any available prey were measured. The collection took place during the week that nymphs caged on both cotton plants and received plentiful prey molted to adult (i.e., 1st week of August). The collection was carried out using drop cloths (1-m-long white canvas cloth laid on the ground between two cotton rows, and plants on the adjacent rows

are shaken vigorously over the cloth). Adults that fell on the cloth were collected. Twenty females and 20 males were collected in each cotton type and taken to the laboratory where they were weighed, and subsequently released in their plots of origin. The weights of feral adults that presumably developed on available prey in the respective cotton types were compared to the weights of adults that developed in the cages of the various treatments.

2.5. Toxin (*Cry1Ac*) in trophic levels of cotton ecosystem during 2004 season

To verify exposure of *Cry1Ac* to predators, materials representing the three trophic levels (plant, prey, and predators) were assayed for *Cry1Ac* using enzyme-linked immunosorbent assay (ELISA). Cotton leaves and unconsumed BAW (2 days old) inside cages were collected and frozen from 2 July to 22 September 2004 covering the period of the predators' nymphal development and the peak of their adult reproduction. Predator adults assayed consisted of 15 males that were superfluous and unpaired and males from pairs in which the female died prior to 30 September. The materials were assayed to determine the levels of Bt toxin (*Cry1Ac*). The cotton leaf sample was collected from leaves caged with predators and BAW or CEW by pressing the lid of a microcentrifuge tube through the cotton leaf folded on the main vein.

All frozen materials were thawed and weighed in a 1.5-ml centrifuge tube and mixed with phosphate-buffered saline solution in Tween 20 (1 × PBST) (Agdia[®] Inc., Elkhart, IN). Non-fat dried milk (0.4% w/v) and Tween 20 (0.5% v/v) were added to PBST to compose the final extraction buffer, which was mixed with sample material at a rate of 1:10 (w/v). The toxin levels in the samples were assayed using antibody-coated wells in PathoScreen[®] plates for Bt-*Cry1Ac*/*Cry1Ab* ELISA kit using peroxidase enzyme conjugate (Agdia[®] Inc., Elkhart, IN). Standards of *Cry1Ac* at concentrations 0.625, 1.25, 2.5, 5, 10, 20, and 40 ng/ml (ppb) were used to build a standard optical density curve for estimating protein content of sampled material.

2.6. Statistical analysis

Nymphal survival was rated per cage ($n = 5$ nymphs each cage) from a total of 15 cages, except in cases where cages were lost to damage or other factors. Big-eyed bug nymphal developmental time, survival, weights of newly emerged adults, and adult reproductive parameters were submitted to a normality test (Kolmogorov-D:Normal test, Proc Univariate of SAS; SAS Institute, 1999–2001) and square-root ($x + 0.5$) transformed when needed to meet assumptions of analysis of variance (ANOVA). Because the variables required transformations due to the skewed distributions, the means are accompanied by 95% confidence intervals rather than standard errors or deviations (Sokal and Rohlf, 1995). The effects of prey (BAW and CEW) and plants (Bt and non-Bt) were analyzed with a 3-way ANOVA design

with prey and cotton as main fixed and year as random factor, and the model was further reduced to 2- or 1-way ANOVA when appropriate. All analyses were performed using the Proc GLM of SAS (SAS Institute, 1999–2001) and significant treatment means were compared using the Tukey HSD test. Further, a retrospective power analysis for major predator life history parameters (nymphal survival and developmental time, female longevity, and number of eggs produced) was conducted to detect an effect corresponding to a 20% difference between treatments (Marvier, 2002). This analysis was conducted to avoid accepting a false null hypothesis of no difference between treatments having Bt and non-Bt cotton as main and fixed effect (Sahai and Ageel, 2000). For all analyses, the effect size is given by d and defines the absolute difference (untransformed value) between treatments in the parameter of interest, determined using the within-population standard deviation. The intrinsic rate of natural increase was estimated adapting the procedure written by Maia et al. (2000) using SAS software, which relies on a Jackknife method to estimate confidence intervals, and to allow comparison between treatments.

3. Results

3.1. *Cry1Ac* toxin in cotton plants and prey 2004 season

Average levels of *Cry1Ac* toxin in cotton leaves from cages holding nymphs from 2 July to first week of August and adult during reproductive peak from first week of August to 30 September were (mean \pm SD) 0.23 ± 0.04 and $0.25 \pm 0.03 \mu\text{g Cry1Ac g}^{-1}$ of fresh tissue, respectively. From this original concentration of toxin expressed in Bt-cotton leaves nearly 81 and 76% was exposed to the predator nymphs and adults through the BAW larvae (0.18 ± 0.03 and $0.19 \pm 0.02 \mu\text{g Cry1Ac g}^{-1}$ of fresh weight). Despite the amount of *Cry1Ac* toxin detected in the plants and prey, and, therefore directly and indirectly exposed to the big-eyed bug nymphs and adults, no toxin was detected in the body of adult predators.

3.2. Predator nymphal survival and development

The survival of big-eyed bug throughout nymphal stages was variable for each treatment, ranging from 0 to 100% per cage (Table 1). Survival variability was certainly influenced by the variable environmental conditions of the field during the experimental periods (Fig. 1). In addition, 12 cages were lost (from 0 to 3 cages out of 15 cages per treatment) due to ant attack (mainly *Solenopsis invicta* Buren) among all treatments during the two seasons, and these replications were dropped from analyses. A 3-way ANOVA (cotton type, prey type as main fixed factors and year as random factor) indicated no differences in nymphal survival (mean \pm 95% CI) between Bt- (44.8 ± 7.36) and non-Bt cotton (46.7 ± 7.7), with cotton as main effect ($F_{1,100} = 0.14$; $P = 0.7063$, $d = 5.19$; Power = 0.8712), or between prey

Table 1

Survival (%), development from newly hatched nymphs to adult emergence (days), fresh body weight (mg), and sex ratio of *Geocoris punctipes* reared on *Helicoverpa zea* eggs (CEW) or *Spodoptera exigua* first instar larvae (BAW) caged on Bt or non-Bt cotton plants in the field during cotton seasons 2003 and 2004

Cotton	Prey	Survival (95% CI) ^A	Developmental time (95% CI)	Body Weight (95% CI)		Sex ratio
				♀	♂	
<i>Season 2003—nymphs caged on 17 June</i>						
Bt	CEW	45.9 (30.1–63.0)	27.2 b ^B (26.7–27.6)	5.29 a (4.9–5.6)	3.16 a (2.9–3.4)	0.53
	BAW	51.6 (39.1–64.1)	29.9 a (29.2–30.5)	3.83 b (3.6–4.0)	2.90 b (2.6–3.2)	0.62
Non-Bt	CEW	61.3 (43.5–70.2)	26.4 b (25.9–26.8)	4.92 a (4.7–5.2)	3.32 a (3.1–3.4)	0.55
	BAW	45.2 (28.2–59.3)	30.5 a (29.8–31.1)	3.83 b (3.6–4.1)	2.98 b (2.7–3.2)	0.58
<i>Season 2004—nymphs caged on 30 June</i>						
Bt	CEW	46.6 (37.1–58.9)	24.3 b (23.6–25.0)	4.86 a (4.5–5.2)	3.00 a (2.8–3.1)	0.56
	BAW	37.3 (14.7–55.9)	29.1 a (27.4–30.8)	3.44 b (3.1–3.7)	2.67 b (2.4–2.9)	0.54
Non-Bt	CEW	36.9 (19.2–54.6)	23.8 b (22.8–24.7)	4.34 a (3.9–4.8)	3.18 a (2.9–3.4)	0.50
	BAW	42.8 (30.2–55.3)	30.8 a (29.6–32.2)	3.45 b (3.0–3.8)	2.88 b (2.6–3.1)	0.45

^A 95% confidence intervals of mean.

^B Means followed by the same letters within column and season do not differ significantly by Tukey HSD test ($P > 0.05$).

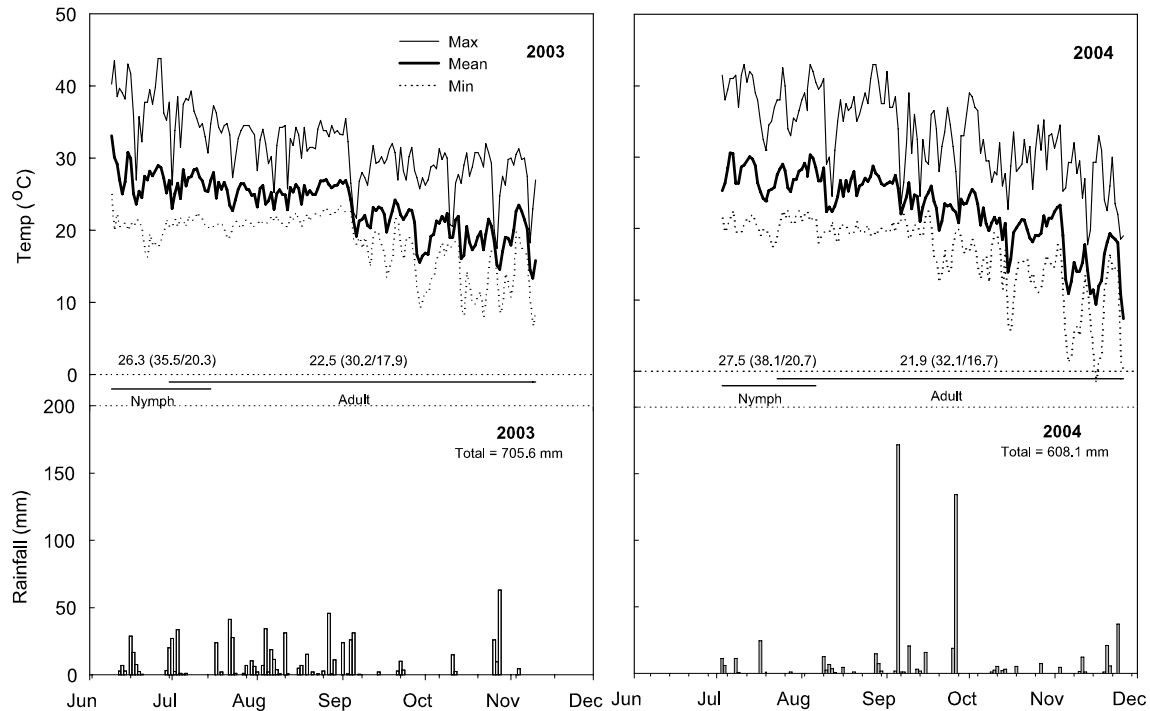


Fig. 1. Temperature and rainfall data measured during the experimental period in 2003 and 2004. Horizontal lines represent the time period of nymphal and adult stages present in the field, and the numbers stand for average temperature for that period and numbers inside parenthesis for maximum and minimum average temperatures.

($F_{1,100} = 1.66$; $P = 0.2913$). However, nymph survival varied significantly between years ($F_{1,100} = 5.16$; $P = 0.0252$). On average, nymphal survival was lower in 2004 (mean \pm SE = $40.7 \pm 3.71\%$) compared to 2003 (mean \pm SE = $51.2 \pm 3.27\%$) across all treatments, but nymphs survived equally among treatments (Table 1) regardless of prey or cotton types used in 2003 (2-way ANOVA, $F_{1,47} = 0.44$; $d = 12.75$; Power = 0.9112) and 2004 (2-way ANOVA, $F_{1,53} = 0.04$; $d = 2.86$; Power = 0.9612).

Newly hatched nymphs of big-eyed bug caged either on Bt or non-Bt cotton exhibited similar developmental time, survival, sex ratio and adult weight. On the other hand, prey type significantly affected nymphal developmental

time, with nymphs fed BAW larvae requiring 3–7 additional days to reach the adult stage and weighing significantly less than nymphs fed CEW eggs (Table 1). From a complete model including cotton, year, gender, and prey as main factors, gender was dropped out after the first ANOVA run because it was not significant (gender, $F_{1,280} = 0.41$; $P = 0.5230$). Among the remaining main factors, cotton type was not significant ($F_{1,280} = 0.08$; $P = 0.7762$) but this factor was maintained in further analysis because it significantly interacted with year. Then, using a 3-way ANOVA, nymphal developmental time (mean days \pm 95% CI) did not differ between Bt (27.7 ± 0.54) and non-Bt cotton (27.8 ± 0.57) ($F_{1,288} = 0.52$; $P = 0.4748$;

$d=7.34$; Power=1), but it varied as function of year ($F_{1,288}=25.74$; $P<0.0001$) and prey type ($F_{1,288}=254.24$; $P<0.0001$). Nymphal development was 2 days faster in 2004 compared to 2003, and about 5 days faster when bugs were fed CEW eggs than BAW larvae. However, these differences were unaffected by being caged on either Bt or non-Bt cotton. The first level of interaction between year and prey was significant ($F_{1,288}=10.78$; $P=0.0016$) with nymphs fed CEW eggs developing faster in 2004 than in 2003 but similar developmental times were observed for nymphs fed BAW larvae in both years.

The weight of newly emerged adult predators varied as expected for gender (Male vs. Female, $F_{1,280}=405.63$; $P>0.0001$), with males smaller than females (Table 1). Prey type also significantly affected adult weight (BAW vs. CEW, $F_{1,280}=183.5$; $P<0.0001$), as did year (2003 vs. 2004, $F_{1,280}=8.15$; $P<0.0001$), and only the interactions between prey type and gender ($F_{1,288}=56.36$; $P<0.0001$), and between year and gender ($F_{1,288}=4.81$; $P=0.0291$) were significant. Independent of gender, nymphs fed CEW eggs produced larger adults than those fed BAW larvae, and females fed CEW eggs were larger in 2003 than in 2004 (Table 1). However, no effect of being caged on Bt-cotton was observed for either prey type [means for Bt = 3.5 mg and for non-Bt cotton = 3.6 mg ($F_{1,107}=1.09$; $P=0.2264$)] (Table 1).

Adult weight for females and males caged on plants and fed exclusively with BAW larvae or CEW eggs and weights of feral adults ($n=20$ adults for each gender per cotton type), collected in the same plots in the field feeding on any available prey, showed no significant effect of cotton genotype ($F_{1,181}=1.87$; $P=0.2731$) (Fig. 2). No significant interaction was found between cotton type and gender ($F_{1,181}=1.61$; $P=0.2931$), or cotton type and prey ($F_{1,181}=1.72$; $P=0.1812$). However, adult weight was significantly affected by gender as a main effect ($F_{1,181}=332.00$;

$P<0.0001$), and by prey type ($F_{2,181}=36.37$; $P<0.0001$), and there was a significant interaction between prey and gender ($F_{2,181}=15.95$; $P<0.0001$). The results indicated that adults of big-eyed bug were smaller when fed exclusively BAW larvae in comparison to feral adults preying on any available prey in the fields or exclusively fed CEW eggs for both cotton types (Fig. 2).

3.3. Adult life history parameters

All females (sample size specified in Table 2) produced viable eggs both years. However, of the 15 paired females per treatment in 2003, some were lost due to ant's attack inside the cages (mainly *S. invicta*). In 2004, only 12 females were paired in the BAW larvae treatments (Bt and non-Bt) and 14 females from CEW eggs in non-Bt cotton due to more variable nymphal survival. However, further reduction in sample size occurred due to ant attack or female escape. Final number of females per treatment is presented in Table 2. Egg hatching ranged from 62.4 to 92.8% and it was not significant across all treatments. Reductions in hatching were likely due to various factors, and one was the egg parasitoid, *Telenomus reynoldsi* Gordh & Coker. For both years and across plant/prey combinations, egg parasitism by *T. reynoldsi* ranged from 1.2 to 2.1% across all treatments.

Geocoris punctipes that were reared as nymphs and subsequently maintained as adults fed BAW larvae or CEW eggs showed no measurable difference in number of eggs produced per female (3-way ANOVA, $F_{1,77}=0.76$; $P=0.3863$; $d=35.52$; Power=1) between Bt-cotton (mean \pm 95% CI = 172.8 ± 38.9) and non-Bt cotton (159.3 ± 36.0). Nor was female longevity affected (3-way ANOVA, $F_{1,77}=0.06$; $P=0.8093$; $d=14.89$; Power=1) when compared between Bt-cotton (61.2 ± 8.3) and non-Bt cotton (59.1 ± 6.6). Other possible effects of cotton type

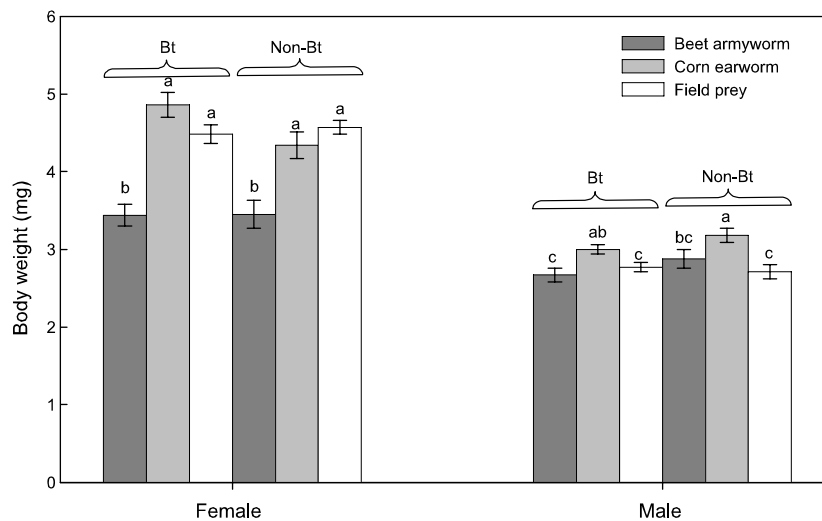


Fig. 2. *Geocoris punctipes* adult fresh body weight (\pm SE) reared caged in Bt and non-Bt cotton plants fed beetle armyworm neonate (BAW) larvae or corn earworm (CEW) eggs and from field collection from both cottons and fed on available field prey in 2004. Bars under different letters are different across all prey but within same gender by Tukey HSD test at 0.05 levels of significance.

Table 2

Means (95% Confidence intervals) of reproductive characteristics of *Geocoris punctipes* fed *Helicoverpa zea* eggs (CEW) or beet armyworm (BAW) first instar larvae caged on Bt or non-Bt cotton plants in the field [temp. °C (2003, mean = 22.8, min = 6.8, and max = 39.3); 2004, mean = 22.4 (min = -1.5 and max = 43.4) and natural photoperiod]

Season	Characteristics ^A	Bt-cotton		Non-Bt cotton		One-way ANOVA Statistics [F_{df}] ^P
		CEW eggs (n = 10)	BAW larvae (n = 10)	CEW eggs (n = 10)	BAW larvae (n = 13)	
2003	Age at 1st oviposition (days) ^B	6.4 b (5.2–7.0)	9 a (6.3–11.7)	6.4 b (4.7–5.5)	9.4 a (7.0–11.7)	$F_{3,39} = 3.26^{0.0315}$
	Mean no. of eggs per female	217.5 a (154.8–280.7)	94.9 b (80.0–112.5)	255.8 a (202.3–356.3)	89.1 b (72.1–106.5)	$F_{3,39} = 24.10^{<0.0001}$
	Female longevity (days)	48.7 a (34.4–62.9)	55 a (45.7–64.3)	57.6 a (46.8–68.4)	54.5 a (49.3–61.2)	$F_{3,39} = 1.22^{0.3147}$
	Post-reproductive period (days)	1.8 a (0.4–3.2)	3.2 a (0.9–5.4)	0.8 a (0.4–2.0)	1.8 a (0.9–2.7)	$F_{3,39} = 2.09^{0.1169}$
2004	Age at 1st oviposition (days) ^B	5 b (4.3–5.6)	9 a (7.8–11.2)	5.4 b (4.6–6.1)	8.1 a (4.8–11.6)	$F_{3,38} = 10.59^{<0.0001}$
	Mean no. of eggs per female	300 a (240.4–359.5)	38.5 b (25.5–51.6)	209.2 a (145.2–273.2)	41.8 b (27.6–56.1)	$F_{3,38} = 41.49^{<0.0001}$
	Female longevity (days)	85.4 a (69.2–102.7)	49.7 b (32.4–67.1)	72 a (53.4–90.6)	45.8 b (32.4–59.4)	$F_{3,38} = 5.91^{0.0021}$
	Post-reproductive period (days)	12 a (3.8–20.1)	7.5 a (0.1–15.3)	7 a (2.0–12.1)	7 a (0.6–14.6)	$F_{3,38} = 0.69^{0.6184}$

^A Means followed by the same letter within rows do not differ significantly (One-way ANOVA; Tukey HSD test; $P > 0.05$).

^B Time from adult emergence to initial oviposition.

with prey and year interactions were not significant (3-way ANOVA, $P > 0.05$). Year factor had no significant effect on pre-oviposition periods and number of eggs per female (2-way ANOVA, $P > 0.05$) but it had a marginal effect on female longevity ($F_{1,77} = 3.42$; $P = 0.0684$) and a highly significant effect on the duration of the post-reproductive period ($F_{1,77} = 22.64$; $P > 0.0001$). Females fed CEW eggs lived longer—78.4 days in 2004 compared to 53.1 days in 2003—but for females fed BAW larvae there was no differences between 2003 and 2004 (55.2 and 47.9 days). Prey quality (BAW larvae vs. CEW eggs) was the most important factor interfering with all evaluated adult reproductive parameters (1-way ANOVA, Table 2) with no effects attributable to cotton type. Two-way ANOVA indicated that females fed CEW eggs initiated oviposition earlier ($F_{1,94} = 74.19$; $P > 0.0001$), produced more eggs per female ($F_{1,77} = 184.15$; $P > 0.0001$), and lived longer ($F_{1,77} = 7.40$; $P = 0.0081$) than females fed BAW larvae, regardless of which cotton they were provided (Table 2). An unexpected interaction was observed for prey type and year for number of eggs per female ($F_{1,77} = 9.17$; $P = 0.0033$) and female longevity ($F_{1,77} = 14.71$; $P = 0.0003$), with females fed BAW larvae producing more eggs (92.3 vs. 40.1 eggs per female) and living longer (55.3 vs. 47.9 days) during 2003 than 2004, while the opposite was observed for females fed CEW eggs (Table 2).

Sex ratio, nymph survival, and number of eggs per female were not significantly affected by cotton type (Tables 1 and 2). However, accumulated small differences were reflected in the estimation of the intrinsic rate of increase (r_m) in 2004. Therefore, those females feeding on CEW eggs and caged on Bt-cotton produced greater r_m than females caged on non-Bt cotton (Table 3). Also, females caged on non-Bt cotton fed CEW eggs exhibited a relatively lower initial peak of oviposition than females caged on the same prey and on Bt-cotton (Fig. 3). No further effect is observed on r_m comparing cotton types. As expected, females fed better quality prey (CEW eggs) yielded greater r_m than females fed BAW larvae independent of the cotton type on which they were reared.

Among adult reproductive parameters, period of post-reproductive survival was longer in 2004 than in 2003. In 2004, a linear relationship was observed between period of post-reproductive survival and female longevity for all females reared on Bt and non-Bt cotton and feeding either on CEW eggs ($y = -0.50 + 0.04 (\pm 0.007)x$, $r^2 = 0.58$; $F = 31.71$; $P > 0.0001$) or BAW larvae ($y = -0.57 + 0.06 (\pm 0.01)x$, $r^2 = 0.69$; $F = 34.91$; $P > 0.0001$). This relationship was not significant for females in 2003 feeding on either prey. Females in 2004 living longer than 70 days experienced low temperatures because they were still alive later in the season than most females in 2003, which were caged

Table 3

Mean intrinsic rates of natural increase [r_m (95% Confidence intervals)] of *Geocoris punctipes* fed *Helicoverpa zea* eggs (CEW) or *Spodoptera exigua* first instar larvae (BAW) caged on Bt or non-Bt cotton plants in the field [temp. °C (2003, mean = 22.8, min = 6.8 and, max = 39.3); 2004, mean = 22.4 (min = -1.5 and max = 43.4) and natural photoperiod]

Season	Bt-cotton		Non-Bt cotton	
	CEW eggs	BAW larvae	CEW eggs	BAW larvae
Season 2003—nymphs caged on 17 June	0.076 (0.071–0.080)	0.057 (0.054–0.060)	0.069 (0.066–0.073)	0.053 (0.050–0.058)
Season 2004—nymphs caged on 30 June	0.078 (0.075–0.082) ^a	0.042 (0.032–0.051)	0.068 (0.063–0.073)	0.044 (0.033–0.054)

Values between parenthesis comprehend the 95% confidence interval for treatment means estimated through Jackknife method (Maia et al., 2000).

^a Indicates that the pairwise Bt and non-Bt for CEW means are different at 95% confidence interval of mean within season 2004.

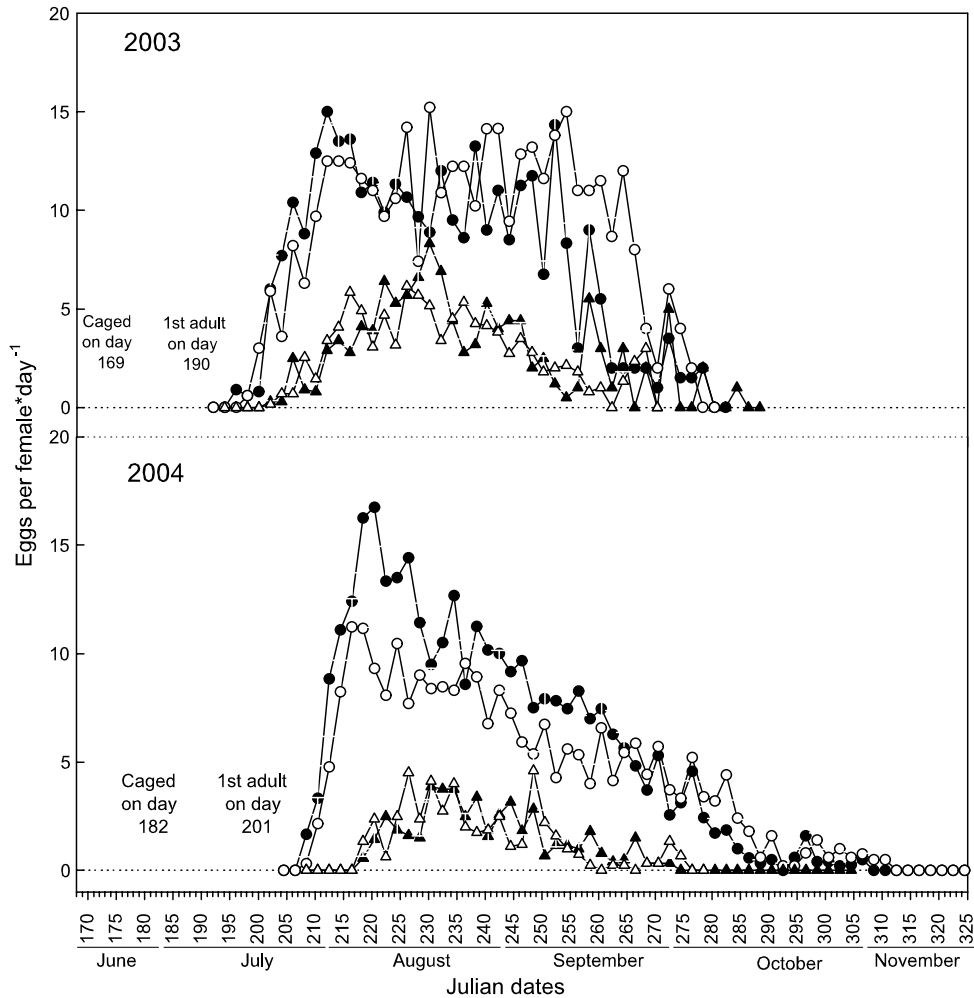


Fig. 3. Age-specific oviposition of *Geocoris punctipes* females fed *Spodoptera exigua* (BAW) larvae (triangle) or *H. zea* (CEW) (circle) eggs caged on Bt (closed symbol) and non-Bt cotton (open symbol) during two seasons in the field.

earlier in that year (Fig. 1). For females in 2004 living less than 70 days (i.e., dying before November), post-reproductive survival was short and similar to females in 2003. In contrast, those females surviving until November 2004 died up to 30 days after stopping oviposition.

4. Discussion

Although it was apparent that Cry1Ac toxin passed from the plant to the herbivore-prey, there was no significant effect on the life history of *G. punctipes* attributable to this toxin. The most evident changes in nymphal development and adult reproduction of *G. punctipes* occurred when predators were reared on different prey types, with lower performance for those bugs feeding on BAW larvae compared to CEW eggs, regardless of cotton genotype. Variations in big-eyed bug life history due to the use of lepidopteran larvae or eggs as prey have been previously reported (Cohen and Debolt, 1983; Dunbar and Bacon, 1972a; Lawrence and Watson, 1979; Torres et al., 2004). Therefore, there is no evidence of negative effect from Bt-BAW reared larvae or from direct feeding on Bt-cotton for

this omnivorous predator. BAW larvae are only partially susceptible to the Cry1Ac toxin (Stewart et al., 2001) enabling larvae to acquire the toxin from the plant and expose it to predators. Therefore, nymphs and adults of *G. punctipes* in the BAW treatment with Bt-cotton were exposed to Cry1Ac toxin through sickened or relatively healthy larvae containing the toxin. Nevertheless, despite consuming relatively large numbers of intoxicated prey throughout their lifetimes, development and reproduction of *G. punctipes* were unaffected by the presence of toxin in the prey.

No Cry1Ac toxin was detected in *G. punctipes* adults provided CEW eggs on Bt-cotton plants or in predators fed Bt-reared BAW larvae on Bt-cotton plants. Similarly, in other studies no Cry1Ac toxin was detected in the bodies of *G. punctipes* fed Bt-reared BAW larvae in confined experiments in a greenhouse or in bodies of these predators collected in Bt-cotton fields over three cotton seasons, although Bt was detected in a larger predatory heteropteran, *Podisus maculiventris* (Say) (Torres et al., 2006). These greenhouse and field outcomes confirm the findings presented here. In this study, Torres et al. (2006) demonstrated

that *G. punctipes* is able to ingest purified Cry1Ac toxin that is detectable above 4 ppm. The results clearly suggest that although *G. punctipes* can ingest Cry1Ac toxin, but that the predator does not acquire sufficient toxin from prey or from direct plant feeding to be detectable by ELISA. This result agrees with results reported for *Nabis* sp., *Geocoris* sp., *O. tristicolor*, and *L. hesperus* confined for short periods on Bt-potato foliage and deprived of prey (Armer et al., 2000). The lack of effects of Cry1Ac on *G. punctipes* when provided Bt-fed BAW larvae may be due to removal of toxin from the predator's body. Although Cry1Ac can be ingested by *G. punctipes* from purified Cry1Ac-water concentrations higher than 4 ppm, we found that the toxin was largely eliminated through feces (Torres et al., 2006) and not detectable after 72 h either in the predator body or in their feces.

The year of the study (2003 or 2004) significantly affected nymphal survival across all treatments, and influenced longevity and post-reproductive survival for females fed CEW eggs. However, no interactions were observed between years and cotton types. Reduced nymphal survival in 2004 may have resulted from many causes, but temperature and moisture play significant roles in development and survival of insects in the field. Nymphal survival was low in the first and second instars, when dehydration risk would be the greatest (data not shown). Rainfall during the period from placement of neonate predators in cages until the last nymph molted to the adult stage was 211.1 mm in 2003, compared to only 68.6 mm in 2004. There also were more hours of high temperatures in 2004 than in 2003 (average of 38.1 °C for maximum temperature during the period compared to 35 °C in 2003; Fig. 1). Although capable of tolerating relatively high temperatures, *G. punctipes* requires a reliable source of free water because this predator has low resistance to water loss (Cohen, 1982).

Caging nymphs 13 days later in 2004 accelerated nymphal development ($F_{1,294} = 13.0$; $P = 0.0004$) (28.3 days in 2003 vs. 26.9 days in 2004) in all four treatments. More rapid preimaginal development in 2004 can be explained by accumulated thermal units during the nymphal period. Considering the period during which preimaginal development occurred in both seasons, and using an estimated lower developmental threshold for nymphs of 13.3 °C (estimated from Dunbar and Bacon, 1972b), nymphs were exposed to almost similar degree-days (336 °C in 2003 vs. 343.3 °C in 2004). The DD result indicates that nymphs in 2004 were exposed to sufficient thermal units in a shorter period to complete development compared to 2003. This accelerated nymphal development, however, had a slight cost in adult weight for bugs developing more rapidly, with larger adults in 2003 than in 2004 (1-way ANOVA, $F_{1,294} = 8.61$, $P = 0.0036$) across all treatments independent of gender, prey, and cotton type (Table 1).

Although body weight sometimes correlates with female fecundity in predatory heteropterans (Honek, 1993), the variation in body weight was not enough to produce significant correlations between number of eggs per female or

weight and female longevity in our study. Independent of weight and within the same prey item, females fed high-quality prey (CEW eggs) in 2004 tended to be more fecund than in 2003, which is due to greater oviposition immediately after adult emergence compared to females in 2003 (Fig. 3). Indeed, increased longevity of females fed CEW eggs in 2004 may explain the greater fecundity, because number of eggs per female correlated significantly with female longevity on both prey and independent of cotton genotype (CEW, $r = 0.46$, $P < 0.0001$ and BAW, $r = 0.19$, $P = 0.0006$).

Since the predator diet (prey and plant) was similar between seasons, it appears that the temperature decline during November (Fig. 1) was the major factor affecting the duration of the post-reproductive period. The accumulated DD over the reproductive temperature threshold (18 °C; Davis, 1981) during the adult period was quite similar (2003 = 651.1 and 2004 = 645.0 °C), although the last surviving females in 2003 and 2004 died on 10 and 28 November, respectively. The average extended period of living females in 2004, however, exposed them to temperatures below that favorable for reproduction, but not low enough to cause mortality, resulting in longer post-reproductive survival of females in 2004 compared 2003.

The results reported here with close control of prey and plant types available to predators, in combination with natural environmental variability in the fields, support the data from several field surveys of predators in Bt and non-Bt cotton that found no effect of Bt-cotton on populations of predatory heteropterans, including *G. punctipes* (Hagerty et al., 2005; Torres and Ruberson, 2005). The present study further demonstrates that there is no measurable life-history impact of Cry1Ac at the individual level. The ability of predators to compensate for variable prey was strongly indicated by the size of feral predators collected in our experimental Bt and non-Bt cotton fields (Fig. 2). The quality of food resources available to the predators can be indirectly assessed by comparing the size of field-collected females with experimental ones. In this instance, feral females were significantly larger than experimental females reared on BAW larvae and similar in size to those fed CEW eggs (Fig. 2), a high-quality prey for big-eyed bugs. A treatment without prey was not adopted in our study because in the field *G. punctipes* can feed on a wide variety of prey, and feral adult weights observed justify this procedure, although a treatment with absence of prey is often used in evaluations of host plant effects on zoophytophagous predators.

The Bt-cotton plants and Bt-fed prey did no interact to produce measurable effects on the life history of the omnivorous, predatory big-eyed bug *G. punctipes*, regardless of prey quality. Nor have any effects on populations of *G. punctipes* been observed in Bt-cotton. The diversity of prey typically available in cotton ecosystems probably compensates readily for reductions in the numbers of caterpillars that are targeted by Cry1Ac toxin. Also, the field densities of lepidopteran eggs, the bollworm stage preferred by big-eyed bugs, are not

directly affected by Bt-cotton (Torres and Ruberson, 2006). Indeed, field-collected big-eyed bug females were similar in weight to bugs caged under the same field conditions and fed abundant, high-quality prey (CEW eggs) (Fig. 2). Considering the detrimental impact on predatory heteropterans of broad-spectrum insecticides used to manage bollworm infestations in non-Bt cotton (Eveleens et al., 1973; Hagerty et al., 2005; Naranjo et al., 2003), the use of Bt-cotton seems to be a suitable strategy for conserving big-eyed bugs in the cotton ecosystem to help manage pest populations not targeted by Bt-cotton, as has been demonstrated with commercial formulations of Bt (Ali and Watson, 1982). This is important because, although Bt transgenic cotton has provided excellent control of tobacco budworm, the cotton bollworm and some other lepidopterans can exceed economic thresholds in Bt cotton fields when predatory heteropterans are disrupted with use of broad-spectrum insecticide (Hagerty et al., 2005).

Based on our results, we reject the hypothesis that modified Bt-cotton expressing Cry1Ac toxin and other physiological changes in traits relative to insect resistance affect the life history of the omnivorous predatory heteropteran *G. punctipes*.

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