天敌昆虫生态学与生物学专栏

Development and reproduction of *Chrysopa sinica* (Neuroptera: Chrysopidae) reared on *Aphis gossypii* (Homoptera: Aphidinae) fed transgenic cotton*

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Abstract The effects of transgenic cotton containing *Bacillus thuringiensis* (Berliner) Cry1Ac toxin on the survival rate, developmental duration, and fecundity of a predatory Chinese green lacewing, *Chrysopa sinica* (Tjeder), fed cotton aphids, *Aphis gossypii* (Glover), were evaluated. There were no significant difference in total survival rates, or in larval and pupal durations of *C. sinica* supplied with aphids fed on either transgenic or non-transgenic cotton. There were also no significant difference in longevity, fecundity, or weight of adult lacewings. The results indicated that transgenic Bt cotton has little effect on the biological characteristics of *C. sinica*.

Key words *Chrysopa sinica*, *Aphis gossypi*, Bt cotton, development, fecundity

Genetic transformation of crops with enhanced resistance to insect pests and diseases are increasing (Romeis *et al.*, 2008; Wang, 2011). Growers throughout the world are rapidly adopting genetically transformed crops, with 170 million hectares produced globally in 2012 (James, 2013). About a third of this production involves cotton and maize plants that have been engineered to produce one or more insecticidal proteins (Cry toxins) naturally produced by the common soil microbe *Bacillus thuringiensis* Berliner (Bt) for control of lepidopteran and coleopteran pests (Wolfenbarger *et al.*, 2008; Zhang *et al.*, 2011), which is considered a safer option for pest control than the application of pesticides, and is widely used in many biological and integrated pest management programs (Ge *et al.*, 2011).

Transgenic Bt crops with a δ-endotoxin from Bt Berliner had great effect on the control of lepidopteran pests (Wolfenbarger *et al.*, 2008; Zhang *et al.*, 2011). However, one of the widely discussed environmental impacts of Bt crops was their potential effect on non-target organisms, including biological control agents (Conner *et al.*, 2003). Hellmich *et al.* (2008) reported no effects, while Andow *et al.* (2006) demonstrated some effects of Bt crops on non-target insects and natural enemies . In fact, Wolfenbarger *et al.* (2008) found no uniform effects of Bt crops on the functional guilds of non-target arthropods in their a meta-analysis. The effects of Bt toxin on predators at the 3rd trophic level could occur via a tri-trophic interaction including a plant, a herbivore, and a predator specie (Giles *et al.*, 2000). Thus, there were needed to study the interactions of natural enemies, herbivores, and transgenic plants at tri-trophic level (Ozder and Saglam, 2003).

The cotton aphid, *Aphis gossypii* Glover, is an important pest of cotton in China. The Chinese green lacewing, *Chrysopa sinica* (Tjeder), is the major predator of this pest (Ge and Ding, 1996).

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Transgenic Bt cotton, *Gossypium hirsutum* (L.) has been extensively used in China since 1998 to control the cotton bollworm (Lu *et al.*, 2010) and changes biocontrol services (Lu *et al.*, 2010; Ouyang *et al.*, 2013). Wu and Guo (2003) reported that transgenic Bt cotton could efficiently suppress the normal population dynamics of cotton aphids in the field. However, little is known of the effects of transgenic Bt on *C. sinica*. The aim of this study was to determine the effects of Bt cotton on the survival rates, development, fecundity, weight, longevity, and egg hatch of *C. sinica* consuming cotton aphids fed transgenic cotton.

1 Materials and methods

1.1 Materials

Cotton cultivars: There were two cultivars were used in the experiment. GK-12 expressed the Cry 1Ac endotoxin of Bt, and SM-3 was used as the conventional variety for comparison. These two cultivars were grown in plastic pots (10 cm × 20 cm) in 2 separate climatic chambers [(26 ± 1)℃, 60% – 80% RH, L: D = 14: 10]. Cotton plants were fertilized with 40 mL of an N: K: P (10: 4: 1) solution every 2 d, and used for experimental purposes when they were at the 6-8-leaf stage (≥ 60 cm in height).

Insect species: *Chrysopa sinica* (Tjeder) and cotton aphids were all collected from the cotton fields and reared in the laboratory for at least three generations. Aphids were reared on SM-3 to obtain a homogenous colony, and then transferred to GK-12 and SM-3 plants in two separate climatic chambers and maintained for at least three generations for experimental purposes.

1.2 Biological parameters of *C. sinica*

There were two experimental treatments of lacewings supplied with aphids reared on GK-12 cotton, and on SM-3 cotton (control). For each treatment, a piece of fresh cotton leaf (6 cm × 3 cm) was added to 15 glass tubes (4 cm × 8 cm), and 15 newly hatched lacewing larvae were individually placed into each tube. For 1st, 2nd, or 3rd instar lacewing larvae, 20, 80, or 150 even-sized aphids older than the 4th instar were placed daily onto the fresh cotton leaf as prey, respectively. Cotton leaf pieces were replaced daily, and numbers of molting and dead lacewing larvae in each tube were checked at 8 h intervals each day. This setup was replicated three times, resulting in a sample size of 45 larvae for each treatment.

After pupal eclosion, adults were weighed immediately using an automatic electrobalance. For each treatment, one male and one female lacewing were placed together in a larger glass container (6 cm × 8 cm) to allow them to mate and lay eggs. Number of eggs laid by female in each container was recorded every 12 h until adults died and hatching was complete.

1.3 Statistical analyses

Data including survival rate, development, pupal duration, weight and longevity of adult lacewings, and the number and hatching ratio of eggs were analyzed using analysis of variance (ANOVA) in SPSS (19.7) for Windows statistical software (Chicago, IL, USA). The proportion survival rate and hatching ratio of eggs were arcsine-transformed before analysis to stabilize variance. Difference between means was determined using a least significant differences (LSD) test at $P < 0.05$ level.

2 Results

The Bt cotton (GK-12) had little effect on the survival rate of *C. sinica*. Among *C. sinica* larvae that were fed aphids reared on GK-12 and SM-3, 69.8% and 77.8% developed into adults, respectively. There were no significant difference between treatments with respect to survival rates of 1st ($F = 3.493$, $df = 1,5$, $P = 0.135$), 2nd ($F = 0.377$, $df = 1,5$, $P = 0.572$), or 3rd instar larvae ($F = 0.920$, $df = 1,5$, $P = 0.391$), pupae ($F = 1.249$, $df = 1,5$, $P = 0.311$), or larvae + pupae ($F = 2.915$, $df = 1,5$, $P = 0.163$, Table 1).

Table 1　Survival rates of *Chrysopa sinica* that were fed *Aphis gossypii* reared on either GK-12 or SM-3 (%)

Cotton cultivar	1st instar	2nd instar	3rd instar	Pupal stage	Preimaginal stage
SM-3	93.3 ± 3.8a	92.8 ± 0.3a	94.7 ± 2.7a	94.6 ± 2.8a	69.8 ± 2.2a
GK-12 (Bt)	88.9 ± 2.2a	92.5 ± 0.2a	83.5 ± 5.0a	84.6 ± 4.8a	77.8 ± 5.9a

Note: Data in the table are mean ± SE. Data followed by the same lowercase letters are not significantly different at $P > 0.05$ level by LSD test. The same below.

There were no significant difference in the developmental rates of 2nd instar larvae (F = 1.851, df = 1, 81, P = 0.171), 3rd instar larvae (F = 0.043, df = 1,77, P = 0.837), and pupae (F = 3.600, df = 1,67, P = 0.070) between two cotton cultivar (Fig. 1). The developmental time of 1st instar larvae, however, was significantly extended when they were fed on GK-12 cotton-fed prey (F = 9.083, df = 1,89, P = 0.006).

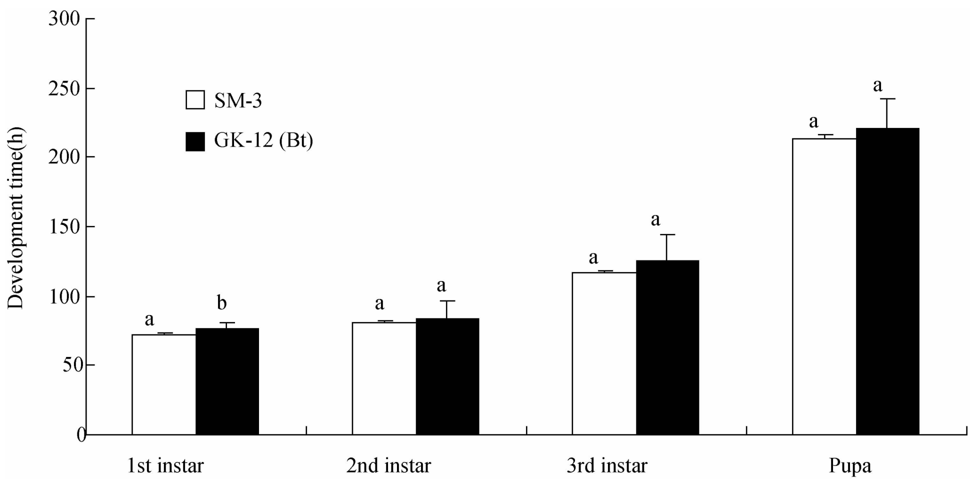

Fig. 1　Developmental rate and pupal duration of *Chrysopa sinica* reared on *Aphis gossypii*, fed GK-12 and SM-3 cotton

Bars in the figure with different lowercase letters indicate significantly different at $P < 0.05$ level by LSD test.

No significant difference in adult weight (female: F = 3.683, df = 1, 35, P = 0.084; male: F = 1.431, df = 1, 35, P = 0.259), lifespan (female: F = 0.156, df = 1, 35, P = 0.702; male: F = 0.428, df = 1, 35, P = 0.528), or fecundity (F = 0.212, df = 1, 35, P = 0.655) of *C. sinica* was observed between treatments (Table 2). Meanwhile, the cotton cultivars showed no significant difference on the hatching ratio of *C. sinica* (F = 0.440, df = 1, 5, P = 0.522) (Table 2).

Table 2　Biological characteristics of adults and the hatch percentage of eggs of *Chrysopa sinica*, supplied with *Aphis gossypii* reared on either GK-12 or SM-3 cotton

Cotton cultivar	Weight (mg)		Life span (d)		Eggs laid per female	Hatch rate (%)
	Females	Males	Females	Males		
SM-3	16.7 ± 0.8a	10.2 ± 0.4a	47.3 ± 1.9a	34.6 ± 0.7a	675.4 ± 24.2a	69.9 ± 1.9a
GK-12 (Bt)	15.4 ± 1.2a	9.4 ± 0.6a	45.9 ± 1.6a	32.6 ± 1.2a	651.4 ± 20.3a	67.7 ± 1.4a

3 Discussion

Extensive disagreement exists about the effect of transgenic crops on predators higher in the food chain. For example, Rice and Wilde (1989) and Power (1992) believed that chemical constituents of transgenic plants might result in the occurrence of toxic or nutritionally unsuitable herbivorous prey, which might cause increased mortality and developmental times in predators. Conversely, Helllmich *et al.*, (2008) found that Bt maize had no effects on predators. Dogan *et al.* (1996) found that Bt potatoes had no effects on the developmental rates of *Hippodamia convergens* when fed *Myzus persicae*, that were reared on transgenic potatoes expressing the δ-endotoxin of Bt. Lundgren and Wiedenmann (2002) observed that there were no effects of mixtures of Bt-corn pollen on survival or developmental times of *C. maculata* fed on aphids. However, Levidow (2003) reported that lacewing larvae that had fed on corn borers reared on Bt leaves had a lower survival rate. Hilbeck *et al.* (1998) found that except for the 1st instar, *Chrysoperla carnea* Stephens larvae reared on Bt-fed *Spodoptera littoralis* (Boisduval) (a lepidopteran non-target pest for Bt) developed equally as fast as those in the Bt-free treatment. Romeis *et al.* (2004) believed that *C. carnea* larvae are not sensitive to Cry 1Ab. Our results in the present study indicated that *C. sinica* larvae that were fed aphids reared on Bt cotton experienced no adverse effects in survival rate or developmental time. This could have been because the Bt toxin protein was not toxic to the aphids or the predator, or the amount of Bt protein ingested by the aphids might have been too low to influence older *C. sinica* larvae.

C. sinica reared on Bt-fed prey had slightly shorter adult lifespans and lower reproductive capacity, but the difference was not statistically significant. Similarly, Dogan *et al.* (1996) observed no significant difference in the effect of Bt-fed aphids *Myzus persicae* (Sulzer) on ladybeetles *Hippodamia convergens* (Guérin-Ménéville). Our results were also consistent with those of Duan *et al.* (2002), who showed that ladybeetles, *Coleomegilla maculate*, fed aphids from Bt corn had no adverse effects on adult reproductive capacity or lifespan.

Sundby *et al.* (1968) believed that the size of adult coccinellids might significantly influence subsequent populations because smaller females were less fecund. In this study, the female adult weights of green lacewings were 15.4 mg and 16.7 mg in the transgenic and non-transgenic treatments, respectively. These weights did not differ significantly across treatments.

Our results demonstrated that Bt cotton had no significant difference in the developmental times of instars, pupal duration, total developmental times from hatching to adult emergence, the survival rate from hatching to adult, adult weight, or the hatching ratio of eggs, indicating that fed on aphids reared on Bt cotton had no adverse effects on *C. sinica* larvae and adults. These results are in accord with those of previously published studies on the non-target effects of Bt crops (Romeis *et al.*, 2006a, 2006b). Romeis *et al.* (2006a, 2006b) argued that the green lacewing, *Chrysoperla carnea*, was unaffected either when fed exclusively with Bt-insensitive spider mites containing high amounts of biologically active Cry 1Ab toxin, or when fed on Bt maize-reared aphids, and concluded that *C. carnea* was at negligible risk from Bt toxin protein.

The lack of effect could be due to two factors: (1) the aphids did not ingest the Cry protein when fed on Bt cotton, so *C. sinica* was not exposed to the toxin, or (2) *C. sinica* ingested the Cry protein when fed on Bt-fed aphids but was not susceptible to the toxin. One study showed that aphid prey reared on Bt maize did not contain Cry protein (Dutton *et al.*, 2002). Another lacewing species, *C. carnea*, was shown to be insensitive to Cry1 toxins in several direct feeding studies (Romeis *et al.*, 2004; Lawo and Romeis, 2008). Obrist *et al.* (2006) provided further evidence that *C. carnea* is not susceptible to Cry 1Ab toxin when fed with spider mites containing the toxin.

Hilbeck *et al.* (1998) reported reduced survival of immature *C. carnea* when purified and trypsinized Cry 1Ab toxin produced by *Escherichia coli* was mixed into an artificial diet at a concentration of 100 μg /mL. However, another study by Romeis *et al.* (2004) revealed that larvae of *C. carnea* were not affected when toxin from the same source was incorporated into a sucrose solution. In tri-trophic studies, *C. carnea* was negatively affected in terms of both development and survival. Dutton *et al.* (2003) and Romeis *et al.* (2004) suggested that the negative effects on *C. carnea* were most likely not caused by susceptibility of the predator to the toxin but were due to the lepidopteran larvae being affected by the toxin.

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转 Bt 基因抗虫棉对中华草蛉生物学特性的影响*

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摘 要 为进一步揭示转 Bt 基因抗虫棉对中华草蛉 *Chrysopa sinica* (Tjeder)生物学特性的影响,以泗棉 3 号和 GK-12 为材料,以棉蚜为猎物,研究了转基因抗虫棉对中华草蛉存活率、发育历期和繁殖力的影响。结果表明,转基因抗虫棉对中华草蛉幼虫期存活率、幼虫发育历期、蛹发育历期与对照差异均不显著;对成虫寿命、体重以及雌虫繁殖力也无显著差异。表明转 Bt 抗虫棉对中华草蛉的生物学特性影响较小。

关键词 中华草蛉, 棉蚜, 转 Bt 基因抗虫棉, 生长发育, 繁殖力