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Development and Reproduction of *Propylaea japonica* (Coleoptera: Coccinellidae) Raised on *Aphis gossypii* (Homoptera: Aphididae) Fed Transgenic Cotton

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Sanrong Zhu, Jianwei Su, Xianghui Liu, Li Du, Erdal N. Yardim, and Feng Ge (2006) Development and reproduction of *Propylaea japonica* (Coleoptera: Coccinellidae) raised on *Aphis gossypii* (Homoptera: Aphididae) fed transgenic cotton. *Zoological Studies* **45**(1): 98-103. A laboratory experiment was designed to evaluate the effects of transgenic cotton containing *Bacillus thuringiensis* (Berliner) Cry1Ac toxin on the survival, development, and fecundity of a predatory ladybeetle, *Propylaea japonica* (Thunberg), through a food chain using cotton aphid, *Aphis gossypii* Glover as an herbivorous prey. No significant differences were observed in total survival from hatching to adult, or in larval and pupal durations of *P. japonica* supplied with aphids fed on either transgenic or non-transgenic cotton. Similarly, no significant differences in longevity, reproduction, weight, or fatty acid contents of adult beetles were detected. Our results suggest that this type of transgenic cotton might have little effect on the survival, development, and fecundity of *P. japonica* through this food chain. http://zoolstud.sinica.edu.tw/Journals/45.1/98.pdf

Key words: Development, Fecundity, *Bt*, *Propylaea japonica* (Thunberg), *Aphis gossypi* Glover.

Many crops have been genetically transformed to provide enhanced resistance to insect pests and diseases. Among them, those transformed with *Bacillus thuringiensis* Berliner (*Bt*) are some of the most widespread. Farmland planted with *Bt* cultivars increased from 1.7 x 10^6 ha in 1996 to over 52.6 x 10^6 ha worldwide in 2001, representing over a 30-fold increase in that specific period (Dale et al. 2002). Because *Bt* toxin is considered a safer option for pest control than the application of pesticides to environment effect , it is used in many biological and integrated pest management programs (Ge and Li 1997).

Transgenic *Bt* crops expressing a δ-endotoxin from *Bt* Berliner hold great promise for the control of lepidopteran pests population (Wilson et al. 1992, Jenkins et al. 1993, Flint et al. 1996, Luttrell et al. 1999). For instance, *Bt*-cotton produces high mortality in cotton bollworms, *Helicoverpa armigera* (Hübner), through exposure to the *Bt* toxin (Forrester et al. 1993). However, the availability of such a toxin in plant tissues in high doses throughout the season may pose potential dangers to other non-target herbivorous insects and their natural enemies (Wilson et al. 1992, Snow and Palma 1997, Baur and Boethel 2003, Wu and Guo 2003).

Some studies have investigated the effects of transgenic crops on non-target organisms. The results of those studies are inconsistent. Some of them reported no effects (Dogan et al. 1996, Pilcher et al. 1997), while others demonstrated some effects of *Bt*-crops on non-target insects and natural enemies (Hibeck et al. 1998, Dutton et al. 2002). Possible effects of *Bt*-toxin on predators in the 3rd trophic level can occur through a tri-trophic

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interaction including a plant, an herbivore, and a predator species (Botter et al. 1998, Giles et al. 2000). Therefore, it is important to generate data in tri-trophic-level studies including natural enemies, herbivores, and transgenic plants (Lozzia et al. 1998, Ozder et al. 2003); interactions between trophic levels through a food chain need to be fully documented for specific agroecosystems.

The cotton aphid, *Aphis gossypii* Glover, is an important pest of cotton in China. The ladybeetle, *Propylaea japonica* (Thunberg), is the major predator of this pest (Ge and Ding 1996). Transgenic *Bt*-cotton, *Gossypium hirsutum* (L.), expressing the Cry 1Ac insecticidal δ-endotoxin protein of *Bt* Berliner is commercially available and has been extensively used in China since 1998 against the cotton bollworm, *Helicoverpa armigera* (Hübner), a major pest in the cotton zone (Jia et al. 2001). Wu and Guo (2003) reported that transgenic *Bt*-cotton can efficiently suppress population dynamics of cotton aphids in the field. However, little evidence exists on the effects of transgenic *Bt* on *P. japonica*. The aim of our study was to determine the effects of *Bt*-cotton on the population parameters of *P. japonica* consuming cotton aphids that have fed on transgenic cotton. Parameters studied include stage-specific mortality, development, and weight gain; fecundity, longevity, and fatty acid content of adult beetles as well as the hatching ratio of eggs.

MATERIALS AND METHODS

Cotton

Two cultivars, a conventional (SM-3) and a transgenic *Bt*-cotton (GK-12), were supplied by the Institute of Cotton, Chinese Academy of Sciences, China, were used in the experiment. GK-12 contains a truncated gene for expression of the Cry 1Ac endotoxin of *Bt* SM-3 and was used as the conventional variety for comparison because it is the parental line of GK-12, into which the *Bt* gene was transferred. The SM-3 and GK-12 cultivars were grown in plastic pots (10 cm in diameter x 20 cm high) in 2 separate climatic chambers, kept at 26 ± 1°C and 60%-80% RH under a 14:10 h L:D photoperiod throughout the experiment. Cotton plants were individually planted in the pots, fertilized with 40 ml of an N:K:P (10:4:1) solution every 2 d, and used for experimental purposes only when they reached a height of ≥ 60 cm (the 6-8-leaf stage).

Insect species

Propylaea japonica (Thunberg), collected from cotton fields, was reared in the laboratory for at least 2 generations, and was supplied with aphids reared on SM-3 cotton. Cotton aphids were collected from the same fields, and reared in the laboratory for at least 2 generations on the SM-3 cotton cultivar to obtain the same colonies. Aphids from the same colony were then transferred to transgenic *Bt*-cotton (GK-12) and non-transgenic cotton (SM-3) plants in 2 separate climatic chambers and maintained for at least 4 generations.

Experimental observations

Treatments of the experiment consisted of (1) ladybeetles supplied with aphids reared on GK-12 cotton and (2) ladybeetles supplied with aphids reared on SM-3 cotton (control). For each treatment, 10 newly hatched ladybeetle larvae were individually placed into 10 glass tubes (4 cm in diameter x 8 cm high), replicated 3 times, so a treatment consisted of 30 larvae. A piece of fresh cotton leaf (6 x 3 cm) was added to each tube. Ten even-sized aphids, older than the 4th instar (0.223 ± 0.002 mg/individual), were daily placed onto the fresh leaves to prey on 1st instar larvae. Similarly, 20 aphids were supplied daily for 2nd instars, 30 aphids for 3rd instars, 50 aphids for 4th instars, and 100 aphids for adult beetles. Dead and uneaten aphids in each tube were removed every 12 h. Numbers of molting and dead beetle larvae in each tube were checked at 8 h intervals. Cotton leaf pieces were replaced every day. Adults were weighed immediately after pupal eclosion using a Cahn 20 automatic electrobalance (St. Louis, MO, USA). Male and female adults were determined. One male and 1 female ladybeetle which had fed on aphids from the same cultivar (treatment) were placed together in a larger glass container (6 cm in diameter x 8 cm high), to allow them to mate and lay eggs. The number of eggs laid in each container was recorded every 12 h until adults died and hatching was completed. All materials were kept in the climatic chambers throughout the observations.

Chemical analysis

Twenty newly hatched larvae were reared for each treatment until the emergence of adults. Adults were then placed in a 5 ± 1°C chamber for chemical analyses. Free fatty acid contents of the aphids and ladybeetles were measured using a 1-step extraction calorimetric assay (Wei 1979).

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using SPSS for Windows, vers. 10.0 (Chicago, IL, USA). The response parameters included the stage-specific survival rate; developmental time; pupal duration; the weight, longevity, and fatty acid content of adult beetles; and the number and hatching ratio of eggs. The proportion surviving was arcsine-transformed before analysis. Untransformed means ± SEM are presented in the tables. Means were further separated by comparing each pair for the least significant difference between treatments using Student's *t*-test.

RESULTS

Among *P. japonica* larvae fed aphids reared on *Bt*-cotton (GK-12) and non *Bt*-cotton (SM3), 79.4% and 73.8% were able to develop to adults, respectively. No significant differences occurred between treatments with respect to survival rates of 1st instar larvae (t = 0.000, df = 3, p = 1.000), 2nd instar larvae (t = 1.367, df = 3, p = 0.265), 3rd instar larvae (t = 2.032, df = 3, p = 0.135), 4th instar larvae (t = 1.000, df = 3, p = 0.391), pupae (t = 1.000, df = 3, p = 0.391), or larvae + pupae (t = 0.831, df = 3, p = 0.467) (Table 1).

The developmental times of 2nd instar larvae (t = 1.930, df = 19, p = 0.069), 3rd instar larvae (t = 1.089, df = 19, p = 0.290), 4th instar larvae (t = 0.000, df = 19, p = 1.000), and pupae (t = 0.742, df = 19, p = 0.467) were not significantly influenced by the cultivar used (Fig. 1). However, development of 1st instar larvae was significantly extended when they were fed on transgenic cotton-fed prey (t = 2.663, df = 19, p = 0.015).

No significant differences in adult weight (female: t = 2.022, df = 8, p = 0.078; male: t = 1.410, df = 10, p = 0.189), lifespan (t = 0.448, df = 7, p = 0.668), or fecundity (t = 1.002, df = 7, p = 0.350) of *P. japonica* were observed between treatments (Table 2), although individuals in the control lived slightly longer and laid slightly more eggs than those in the *Bt*-cotton treatment (Table 2). Moreover, the cultivars did not influence the hatching ratio (t = 0.325, df = 12, p = 0.683) or the fatty acid content of *P. japonica* through the designed food chain (Table 2).

DISCUSSION

Transgenic cotton, expressing the δ-endotoxin gene from the bacterium, *B. thuringiensis* (*Bt*), appears to be a promising new technology for managing cotton bollworm (Forrester et al. 1993). It also offers the potential to dramatically reduce the overall use of broad-spectrum chemical insecticides to control lepidopteran pests (Gary and Fitt

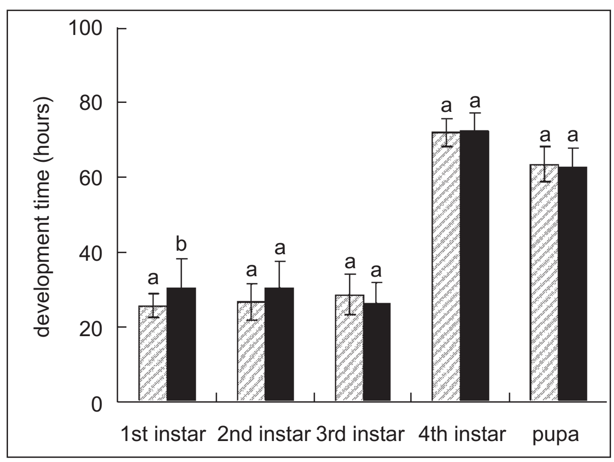

Fig. 1. Stage-specific development time and pupal duration of immature *Propylaea japonica* larvae raised on *Aphis gossypii*, fed transgenic *Bt* (solid) cotton and non-transgenic cotton (hatched). Bars with different letters indicate means that significantly differ (*t*-test).

Table 1. Stage-specific survival rate (%) (mean ± SE) of immature *Propylaea japonica* larvae raised on *Aphis gossypii*, fed transgenic *Bt*-cotton and non-transgenic cotton

Cotton cultivar	1st instar	2nd instar	3rd instar	4th instar	Pupal stage	Preimaginal stage
SM3	96.4 ± 3.6[a]	95.8 ± 4.1[a]	88.6 ± 3.8[a]	95.0 ± 5.0[a]	95.8 ± 4.2[a]	73.8 ± 4.1[a]
GK-12(*Bt*)	96.4 ± 3.6[a]	86.6 ± 7.8[a]	96.4 ± 3.6[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]	79.4 ± 3.7[a]

[a]Means in a column followed by different letters significantly differ (*t*-test).

1994), and may have fewer side effects on non-target organisms (Meeusen and Warren 1989, Huang et al. 1999).

Chemical constituents of transgenic plants may result in the occurrence of toxic or nutritionally unsuitable herbivorous prey, which in turn may cause increased mortality and development times and reduced fecundity in predators (Rice and Wilde 1989, Power 1992). Nicholas et al. (1998) reported that the antibiosis by *Bt*-crops produced adverse effects on predators. Conversely, Dongan and Berry (1996), investigating the development time of a predator ladybeetle, *Hippodamia convergens*, fed the green peach aphid, *Myzus persicae*, which had been reared on transgenic potatoes expressing δ-endotoxin of *Bt*, found that the *Bt*-potato had no effects on development times of the beetles. Duan et al. (2002) showed that feeding on aphids and pollen of *Bt*-corn had no adverse effect on adult reproductive capacity and lifespan of the ladybeetle, *Coleomegilla maculata*. Lundgren et al. (2002) studying the effect of mixtures of *Bt*-corn pollen and contaminated aphids on *C. maculata* reported that there were no effects on survival or development times of the beetles.

Clearly, the results of this study indicated that feeding on aphids reared on *Bt*-cotton had no adverse effects on *P. japonica* larvae and adults. Except for the 1st instar, *Bt*-cotton did not influence the development times of instars, pupal duration, total development times from hatching to adult emergence, the survival rate from hatching to adult, adult weight, or the hatching ratio of eggs. Hilbeck et al. (1998) studied the effects of the *Bt*-fed herbivorous *Spodoptera littoralis* (Boisduval) (a lepidopteran non-target pest for *Bt*) on the predator, *Chrysoperla carnea* Stephens, and they also found that except for the 1st instar, chrysopid larvae reared on *Bt*-fed *S. littoralis* developed equally as fast as those with *Bt*-free treatment. This could have been because the amount of *Bt*-protein ingested by the aphids was too low to have any affect on older larvae. Or, simply, *Bt* toxin protein is not toxic to the aphids or the predator, *P. japonica*, at all.

A slightly shorter adult lifespan and lower reproductive capacity of *P. japonica* occurred when they were reared on *Bt*-fed prey. However, a statistically significant degree of difference was not achieved. When Dogan et al. (1996) studied the effect of *Bt*-fed aphids (*Myzus persicae* (Sulzer)) on a ladybeetle (*Hippodamia convergens* (Guérin-Ménéville), they did observe some differences, but the differences did not achieve statistical significance, and to consider such minor effects as being biologically relevant would be questionable.

Fatty acids are used as energy sources by aphidophagous predators (Kaplan et al. 1986). The size of adult coccinellids may significantly influence subsequent populations because smaller females are less fecund (Sundby et al. 1968). The adult weights and fatty acid contents of ladybeetles were 3.59 and 3.87 mg, and 14.68 and 14.99 μgmol/g protein in the transgenic and non-transgenic treatments, respectively. Although the values were higher in favor of the non-transgenic cotton treatment, there was no statistically significant difference between the 2 treatments.

Risk assessments and a long history of safe use indicate that *Bt*-crops produce less risk to human health and the environment than do the chemical alternatives. Most available data indicate that *Bt*-transgenic crops have no effects on populations of beneficial predator insects; by contrast, even drifts of chemical sprays clearly affect the abundance of beneficial insects (Dutton 2002). Our experiments suggest that transgenic cotton had no indirect effect on this non-target predatory organism. However, widespread use of *Bt*-crops may foster the development of resistance by insect pests. *Bt*-crops will then require higher doses of or higher *Bt* expression in plants to manage insect resistance (Macdonald and Yarrow 2003). Therefore, further environmental risk assessment

Table 2. Weight, life span, fecundity, and fatty acid contents (mean ± SE) of adults and the hatching rate of eggs (mean ± SE) of the ladybeetle, *Propylaea japonica*, supplied with *Aphis gossypii* reared on transgenic *Bt*-cotton and non-transgenic cotton

Cotton	Weight (mg)		Life span	Eggs laid	Hatch rate	Free fatty acid
cultivar	Females	Males	(d)	per female	(%)	(μmol/g protein)
SM3	4.30 ± 0.19[a]	3.52 ± 0.48[a]	46.3 ± 3.4[a]	293.9 ± 17.9[a]	51.9 ± 10.1%[a]	14.99
GK-12 (*Bt*)	4.19 ± 0.21[a]	3.10 ± 0.83[a]	42.9 ± 4.4[a]	275.5 ± 6.4[a]	61.8 ± 15.1%[a]	14.86

[a]Means in a column followed by the same letter do not significantly differ (*t*-test)

studies and field tests must be done in addition to laboratory experiments. The data generated in the present study are important in evaluating and comparing the potential environmental effects of *Bt*-cotton in risk assessment analyses.

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