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Effects of transgenic *Bt* maize pollen on longevity and fecundity of *Trichogramma ostriniae* in laboratory conditions

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Abstract

The effects of transgenic *Bt* maize pollen expressing the Cry1Ab protein of *Bacillus thuringiensis* (Berliner) as a food source on *Trichogramma ostriniae* Pang et Chen (Hymenoptera Trichogrammatidae) were examined in laboratory by studying longevity, progeny production and offspring sex ratio. Females fed on suspension of pollen of *Bt* maize or non-*Bt* maize in water lived significantly longer than those fed on water alone. However, the longevity of females fed on 10% honey alone was similar to those fed on suspension of pollen of *Bt* maize or non-*Bt* maize in 10% honey. *T. ostriniae* females fed on suspension of pollen of *Bt* maize or non-*Bt* maize in water produced significantly more progeny than those fed on water alone. The number of parasitised host eggs and number of offspring emerged by *T. ostriniae* females fed on 10% honey was not significantly different to those fed on 10% honey suspension of pollen of *Bt* maize or non-*Bt* maize. No significant differences in longevity, number of parasitised eggs, offspring emerged and the offspring sex ratio were observed between the females feeding on pollen of *Bt* maize and non-*Bt* maize, this was shown in experiments with suspension of 10% honey and with water. Maize pollen in water increased the reproduction and survival of *T. ostriniae* females compared to water alone. *Bt* maize pollen did not adversely affect *T. ostriniae*.

Key words: *Bt* maize pollen, Cry1Ab, *Trichogramma ostriniae*, longevity, fecundity, sex ratio.

Introduction

The Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera Crambidae), is the most significant economic insect pest of maize in China. Estimated average annual losses in China due to this insect ranged from 6 to 9 millions tons per year and can be much greater in an outbreak year. *O. furnacalis* also causes serious damage to sorghum, millet and cotton (Zhou *et al.,* 1995). An important biological control agent of *O. furnacalis* is the egg parasitoid *Trichogramma ostriniae* Pang et Chen (Hymenoptera Trichogrammatidae), which occurs in most maize growing regions of China (Qian *et al*., 1984; Wang *et al*., 1984; Zhang, 1988; Zhang *et al*., 1990). They are also effective biological control agents of the European corn borer, *Ostrinia nubilalis* (Hübner) (Hassan and Guo, 1991; Wang *et al*., 1999; Hoffmann *et al*., 2002; Wright *et al*., 2002). Both the Asian and European corn borers reduce maize yields and lower grain quality (Zhou *et al*., 1995; Munkvold *et al*., 1999).

Adult *Trichogramma* spp. obtain nutrients from nectar and pollen (Andow and Risch, 1987; Wellinga and Wysoki, 1989; Knutson, 1998), which enhances their longevity (Hohmann *et al*., 1988; Shearer and Atanassov, 2004), fecundity (Somchoudhury and Dutt, 1988; Shearer and Atanassov, 2004), flight propensity (Forsse *et al*., 1992) and ability to parasitise (Saavedra *et al*., 1997). The importance of nectar to parasitoids is recognised for enhancing field control of a number of lepidopteran pests (Treacy *et al*., 1987; Wu *et al*., 1988; Nagarkatti *et al*., 2003; Begum *et al*., 2006).

Genetically modified maize expressing a lepidopteran-specific toxin gene derived from *Bacillu*s *thuringiensi*s (Berliner) provides a high level of resistance against European corn borer.

The release of pollen from modified maize plants exposes non-target organisms to *Bt* toxin via direct or indirect routes of pollen-consumption. Concern regarding non-target effects of transgenic crops expressing transgenes from *Bt* arose after the publication by Losey *et al.* (1999) on the potential risk of maize pollen expressing Lepidopteran-active Cry protein to the monarch butterfly, *Danaus plexippus* (L.). Collaborative research from scientists in the United States and Canada suggests that the impact of *Bt* maize pollen from current commercial hybrids on monarch butterfly populations is negligible (Hellmich *et al*., 2001; Pleasants *et al*., 2001; Sears *et al*., 2001; Shelton and Sears, 2001). However, there still is much controversy about non-target effects of genetically modified crop pollen (Jesse and Obrycki, 2000; Zangerl *et al*., 2001; Mattila *et al*., 2005; Lang and Vojtech, 2006). Several authors studied the potential side effects of *Bt* maize pollen on predators (Pilcher *et al*., 1997; Duan *et al*., 2002), and also for beneficial insects, such as honeybee, *Apis mellifera* L. (Hanley *et al*., 2003; Babendreier *et al*., 2005) and Chinese tussah silkworm, *Antheraea pernyi* (Guérin-Méneville) (Li *et al*., 2005).

Maize pollen is an important source of food for egg parasitoids of the genus *Trichogramma* (Zhang *et al*., 2004). During maize anthesis maize typically produces a large amount of pollen, which coincides with the second generation of the Asian corn borer. The side effects of *Bt*-maize pollen expressing Cry1Ab toxin on *T. ostriniae* is not known. The goal of this study is to determine if pollen from maize hybrids that produce Cry1Ab protein (event MON810) is a potential hazard to this parasitoid. Longevity, fecundity, eclosion and progeny sex ratio of *T. ostriniae* were measured after they were fed *Bt* and non-*Bt* maize pollen.

Materials and methods

Insect source

T. ostriniae used in the study came from a colony, continuously reared on eggs of rice moth, *Corcyra cephalonica* (Stainton) for 20 generations, maintained by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS), Beijing, China. The original source of this culture was collected from parasitised Asian corn borer eggs in Hengshui, Hebei Province, China. The culture was kept at 26 ± 1 °C, 70 -80% RH, and a 16L : 8D photoperiod. The females used in the experiments were less than 24 h old, mated, and lacked egg laying experience.

Host eggs

Eggs of *C. cephalonica* were used for rearing and as host eggs in the experiment. Eggs were obtained from a culture maintained at IPP-CAAS. The culture was reared at 26 -28 °C on a diet of wheat bran and soybean flour mixture. Eggs were collected daily and stored at 4 °C no more than 2 d before exposure to UV irradiation (30 W for 40 min at 30-cm distance).

Pollen source

The maize pollen for this study originated from a transgenic *Bt* maize variety expressing the Cry1Ab protein (Event MON810) and its parental control were supplied by Monsanto Co. (St. Louis, MO). Both transgenic and nontransgenic maize pollen were collected directly from maize plants at an experimental farm of IPP-CAAS. Tassel bags were placed on shedding *Bt* and non-*Bt* maize plants for 6-7 d. After that, the tassel bags were removed from the maize stalk and air-dried for 24-48 h, and the dried pollen was sieved (200-µm mesh size). The sieved pollen was placed in plastic vials, frozen quickly in liquid nitrogen for 10 min, and stored at −20 °C until used (Jesse and Obrycki, 2000).

Experimental design

Six diet treatment combinations were designated, W for wasps that were given water alone, WP for wasps that were given non-*Bt* maize pollen (20 mg pollen in 1 ml water), WBtP for wasps that were given *Bt* maize pollen (20 mg pollen in 1 ml water), H for wasps that were given 10% honey alone, HP for wasps that were given a suspension of 10% honey and non-*Bt* maize pollen (20 mg pollen in 1 ml 10% honey solution), and HBtP for wasps that were given a suspension of 10% honey and *Bt* maize pollen (20 mg pollen in 1 ml 10% honey solution). All food sources were made daily. Newly emerged and mated females (>24 h) old were selected randomly and placed individually in test arenas containing the appropriate diet. The test arenas consisted of glass tubes (1.5 by 0.9 cm) closed with a black cotton cloth, a rectangular white paper (1.0 by 0.7 cm) containing a small piece of filter paper (0.3 by 0.8 cm) as substrate for supplying food, and 150 *C. cephalonica* eggs glued with double-sided sticky tape (0.2 by 0.4 cm). A 3-µl quantity of diet was pipetted onto the filter paper. An estimated number of pollen grains in suspension in water or 10% honey was determined by randomly selecting treated paper sheets and counting pollen grains with a stage micrometer on a dissecting microscope. The estimated number of pollen grains in 3 µl of each of the suspensions varied from 300 to 700. This procedure ensured that all females within the glass tubes were exposed to similar amounts of diet. The paper sheets with food and moisture were changed daily.

Vitality of each female parasitoid was checked daily until all died. Five days after eggs were exposed to parasitism, number of host eggs parasitised (blackened eggs) on each paper sheet were noted. After the parasitoids emerged from host eggs, their numbers were recorded and sex of each was determined. All experiments were conducted in an incubator at 25 ± 1 °C, 70 - 80% RH, and a 16L : 8D photoperiod. Thirty females were established for each of the honey only, *Bt* pollen and non-*Bt* pollen honey treatments and 35 females established for each of the water only, *Bt* pollen and non-*Bt* pollen water treatments.

Data analysis

To reduce variance heterogeneity, the data on the female longevity, the fecundity and number of offspring emerged of the different treatments were $\log_{10}$ (x+1) transformed and the data on emergence rate and sex ratio were arcsin transformed. All transformed data were analysed using analysis of variance (ANOVA) (SAS Institute Inc., 1989).

Results

Effect on of longevity of *T. ostriniae* females

The longevity of females that fed on the three honey treatments (H: honey, 10.4 ± 0.6 d; HBtP: honey, *Bt* pollen, 9.7 ± 0.5 d; and HP: honey, non-*Bt* pollen, 9.4 ± 0.5 d) were significantly greater than the longevities of females that fed on the three water treatments (W: water, 1.8 ± 0.2 d; WBtP: water *Bt* pollen, 2.5 ± 0.2 d; and WP: water non-*Bt* pollen, 2.2 ± 0.1 d) (*F*=150.86; df=5, 181; *P*<0.0001; figure 1). No significant difference in longevity was observed among the females fed on H, HBtP and HP (*F*=0.45; df=2, 80; *P*=0.6364). The longevity of the females fed on W only was significantly shorter than that fed on WBtP or WP. In comparison to water only, maize pollen and water increased the longevity of *T. ostriniae* females (*F*=5.91; df=2, 101; *P*=0.0037). No significant difference in longevity was observed between the HBtP and HP (*F*=0.24; df=1, 53; *P*=0.6236) or WBtP and WP (*F*=1.68; df=1, 68; *P*=0.1995) (figure 1). These results suggest *Bt* maize pollen did not effect longevity of the *T. ostriniae* adult females.

Daily average survival rate of *T. ostriniae* females from the water treatments was 50%, 29.41% and 23.53% on the third day, and continued to declined sharply compared with females provided one of the honey treatments (H, HBtP or HP), which had 100% survival until day five. All the *T. ostriniae* females died within 5 days when provided with W or WP, and within six days when provided with WBtP (figure 2). The females provided with H, HP, or HBtP food lived up to

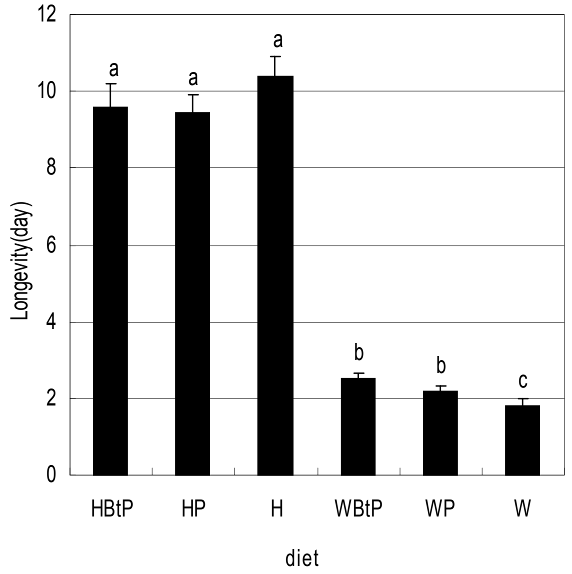

Figure 1. Longevity of *T. ostriniae* females fed on six different diets (HBtP: honey, Bt maize pollen, HP: honey, nonBt maize pollen, H: honey only, WBtP: water with Bt maize pollen, WP: water with nonBt maize pollen, W: water only). Columns with different letters indicate significant difference between different diets at P<0.0001 by ANOVA, LSD test.

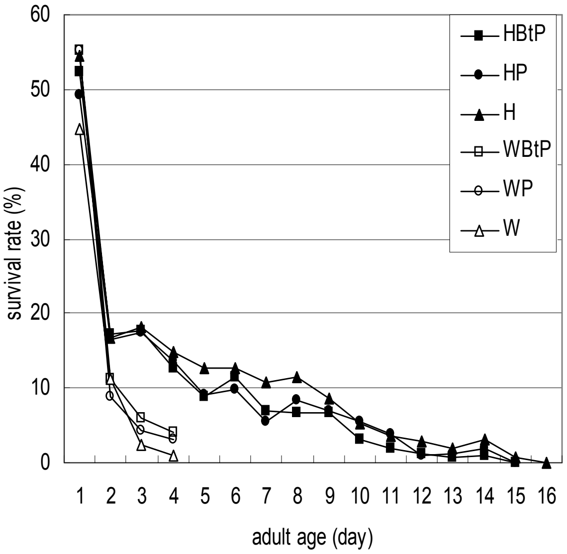

Figure 2. Daily survival rate (%) of *T. ostriniae* adult females fed on six types of foods (HBtP: honey, Bt maize pollen, HP: honey, nonBt maize pollen, H: honey only, WBtP: water with Bt maize pollen, WP: water with nonBt maize pollen, W: water only).

17, 15, and 16 days, respectively. There were no significant differences in survival between the *Bt* pollen and non-*Bt* pollen treatments for either the water or honey treatments.

Effect on fecundity, eclosion and the sex ratio

There were significant differences in female cumulative lifetime fecundity among the treatments: number of eggs parasitised (*F*=61.33; df=5, 181; *P*<0.0001) and number of emerged progeny (*F*=54.88; df=5, 181; *P*<0.0001). The number of host eggs parasitised and the number of progeny that emerged by the honey fed females (H) were numerically the highest, followed by HBtP and HP fed females, which were more than double the values of the three water treatments (table 1). Significant differences in number of host eggs parasitised and the number of progeny emerged were found among the water only, WBtP and WP treatments (*F*=7.74; df=2, 101; *P*=0.0007; *F*=5.23; df=2, 101; *P*=0.0068). Maize pollen and water fed females produced more progeny than those fed water only. Females provided with water only parasitised the fewest eggs and had the lowest number of progeny to emerge. No significant differences in number of host eggs parasitised and the number of progeny emerged were observed between HBtP and HP treatments (*F*=1.06; df=1, 53; *P*=0.3073; *F*=0.58; df=1, 53; *P*=0.4503) or WBtP and WP treatments (*F*=1.22; df=1, 68; *P*=0.2728; *F*=0.25; df=1, 68; *P*=0.6195). There were significant differences in eclosion among the six type of food treatments. The emergence rates were higher in the honey treatments compared with the water treatments (*F*=4.06; df=5, 181; *P*=0.0016) (table 1).

Table 1. Number of host eggs parasitised, number of progeny, eclosion percentage and sex ratios of progeny from *T. ostriniae* females fed six different food sources sex ratios of progeny of from *T. ostriniae* females fed six different food sources.

Food sources	No. of eggs parasitised	No. of emerged progeny	Eclosion (%)	Sex ratio (female/male)
honey	159.5 ± 38.0 a	152.3 ± 36.4 a	95.7 ± 2.4 a	2.1 ± 1.3 b
honey+ Bt-maize pollen	137.1 ± 24.4 a	131.7 ± 24.2 a	96.0 ± 2.2 a	2.5 ± 1.7 b
honey + nonBt-maize pollen	134.5 ± 36.9 a	129.1 ± 35.3 a	96.0 ± 2.4 a	2.9 ± 1.9 b
water	51.0 ± 21.2 c	48.3 ± 20.4 c	94.3 ± 2.3 b	4.4 ± 1.8 a
water + Bt-maize pollen	68.0 ± 19.1 b	64.7 ± 18.5 b	94.7 ± 2.3 b	5.7 ± 3.4 a
water + nonBt-maize pollen	64.1 ± 20.9 b	60.7 ± 20.5 b	94.6 ± 2.2 b	5.0 ± 2.6 a

Data are Mean ± SE. Means in each horizontal line followed by the same letter were statistically not significantly different at $P < 0.05$ by ANOVA, LSD test.

The *T. ostriniae* females produced the greatest number of eggs on the first day in all the treatments. Number of parasitised host eggs decreased sharply on the second day, especially for the W, WBtP and WP treatments. The number of parasitised host eggs by H, HBtP or HP treatments also declined sharply after the first day, but were significantly higher than those of W, WBtP and WP treatments (figure 3). No significant difference was observed in the offspring emerged from parasitised host eggs between the HBtP and HP and the WBtP and WP treatments.

Significant differences in sex ratio in offspring were observed when *T. ostriniae* females were provided different foods. The sex ratio was significantly higher in W (4.4 ± 1.8), WBtP (5.7 ± 3.4) or WP (5.0 ± 2.6) treatments compared to those provided H (2.1 ± 1.3), HBtP (2.5 ± 1.7) or HP (2.9 ± 1.9) (F=14.12; df=5, 181; P<0.0001). No significant differences in sex ratio were observed among the three honey treatments or water treatments (table 1). The greatest number of female offspring was obtained from the parasitised host eggs on the first day, irrespective of food; gradually the number of female offspring declined thereafter, especially in the H, HBtP or HP treatments, strongly male biased after the 6th day (figure 4).

Discussion

Studies on potential side effects of transgenic insecticidal crops on natural enemies and other non-target insects are increasingly attracting attention (O'Callaghan *et al.*, 2005; Romeis *et al.*, 2006). Such studies are necessary if these crops are to become widespread integrated pest management (IPM) tactics (Sétamou *et al.*, 2002). Pilcher *et al.* (1997) reported that pollen from *Bt* maize did not affect the survival and development of three predacious natural enemies: *Coleomegilla maculata* (De Geer), *Chrysoperla carnea* (Stephens), and *Orius insidiosus* (Say). Hanley *et al.* (2003) examined the effects of dietary transgenic *Bt* maize pollen on 4-5 day-old honey bee worker larvae, and did not observed significant differences in larval and pupal mortalities, pupal weight, and haemolymph protein concentration of newly emerged adults after they were fed non-transgenic maize pollen, Cry1Ab or Cry1F maize pollen. Bees that were fed either *Bt* maize pollen (MON 810) or a sugar solution containing either purified *Bt* toxin (Cry1Ab, 0.0014% w/v) at two concentrations (0.1% and 1% w/v) did not show any effect on bee survival or on the development of hypopharyngeal glands after feeding for 10 days (Babendreier *et al.*, 2005).

Egg parasitoids of the genus *Trichogramma* are key natural enemies of many agricultural and forestry pests (Li, 1994). The European corn borer and the Asian corn borer are the two most destructive pests of maize in the world. *T. ostriniae* is the dominant egg parasitoid of Asian corn borer in China (Zhang *et al.*, 1990), and also a strong candidate for augmentative biological control of the European corn borer (Wang *et al.*, 1999; Hoffmann *et al.*, 2002). Transgenic maize expressing Cry1Ab toxin is used commercially in North America and in some European countries. Although *Bt* maize is not grown in China, its commercialisation is currently under consideration by the Chinese government, and extensive laboratory and field trials have been con-

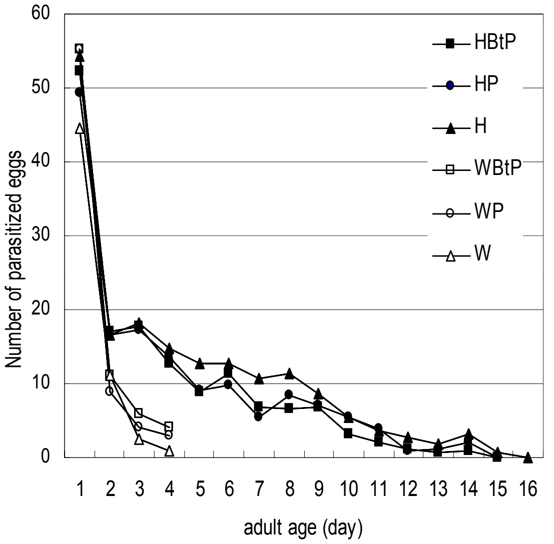

Figure 3. Daily number of host eggs parasitised by female *T. ostriniae* females provided different foods (HBtP: honey, Bt maize pollen, HP: honey, nonBt maize pollen, H: honey only, WBtP: water with Bt maize pollen, WP: water with nonBt maize pollen, W: water only).

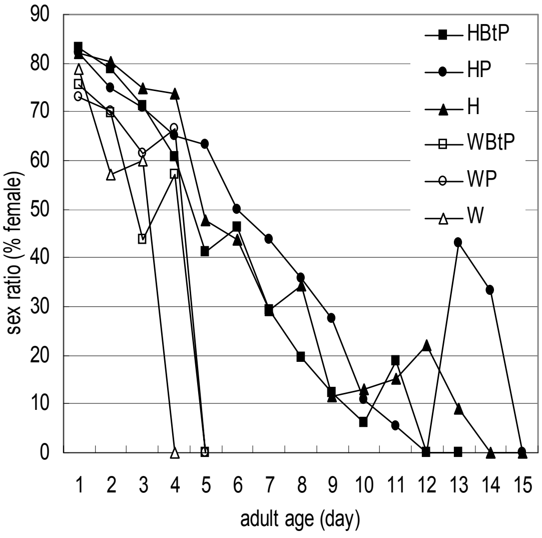

Figure 4. Daily sex ratio of progeny (% female) emerged from parasitised host eggs by *T. ostriniae* females fed on six types of food (HBtP: honey, Bt maize pollen, HP: honey, nonBt maize pollen, H: honey only, WBtP: water with Bt maize pollen, WP: water with nonBt maize pollen, W: water only).

ducted for the evaluation of the efficiency on target lepidopteran pests and potential ecological risks on non-target arthropods (He *et al*., 2003; Wang *et al*., 2004; Li *et al*., 2007). Zhang *et al.* (2004) reported that *T. brassicae* females fed on maize pollen may live longer compared to feed water alone. Geng *et al.* (2006) showed that transgenic *Bt*+CpTI cotton pollen did not adversely affect *Trichogramma chilonis* Ishii. Our experimental results suggest *Bt* maize pollen from MON810 hybrids has no adverse effects on the longevity, fecundity of *T. ostriniae* females. The sex ratio of their progeny is also not affected. We also observed that when provided water *T. ostriniae* females had enhanced longevity and fecundity when they consumed maize pollen. Zhang *et al.* (2004) reported that *T. brassicae* females fed on maize pollen plus water had significantly increased longevity and fecundity compared with those feeding on water alone. However, wasps fed on a mixture of pollen and honey had similar longevity and fecundity to those feeding on honey alone. Geng *et al.* (2006) reported that *T. chilonis* females on cotton pollen mixed with 10% honey lived significantly longer and parasitised more eggs. Females feeding on water or not feeding at all lived as long as and parasitised as many eggs as wasps feeding on cotton pollen mixed with water. Rohi *et al.* (2002) reported that pollen does not present any nutritive value to *Trichogramma bourarachae* Pintureau et Babault. The reason for the different results may be caused by the different in *Trichogramma* species, source of pollen and the concentration of pollen in water or honey.

Because some *Trichogramma* spp. tend to be proovigenic, fecundity is not likely to be increased by food availability, however, adults are more likely to survive long enough to locate sufficient hosts for all of their eggs (Fleury and Bouletréau, 1993; Hegazi and Khafagi, 1998), Honey-fed or sugar-fed females of *Trichogramma minutum* Riley or honey-fed female of *T. chilonis* lived longer and produced more offspring than those unfed females, and the offspring sex ratios of long-lived females were male-biased, while those of short-lived females were female-biased (Leatemia *et al.,* 1995; Geng *et al*., 2005). Our results also showed that the females of *T. ostriniae* fed on honey only or honey with maize pollen (*Bt* or non-*Bt*) lived longer and produced more offspring than those fed on water and water with maize pollen. The female bias sex ratios in honey treatments were significantly lower than those in water treatments, especially in the H, HBtP or HP treatments, which were strongly male biased after the 6th day. These results suggest that *T. ostriniae* probably is a proovigenic species.

Several laboratory bioassays have showed that when *Trichogramma* females feed on preparations of *B. thuringiensis* there are no effects on longevity and fecundity. Hassan and Krieg (1975) fed *Trichogramma cacoeciae* Marchal females with honey-agar contaminated with the commercial preparations of *B. thuringiensis*. No significant effect on the ability to parasitise the eggs of *Sitotroga cerealella* (Olivier) were found. Feeding adults of *T. evanescens* on a honey solution containing 500 µg of *B. thuringiensis* var. *galleriae* HD-129 for 4-5 days had no effect on their lifespan, productivity or ability to parasitise the eggs of *Spodoptera littoralis* (Boisduval) or *Ephestia kuehniella* Zeller (Salama and Zaki, 1985). No deleterious effects of *Bt* microbial preparations on longevity and total parasitism of *Trichogramma pratissolii* Querino et Zucchi and *Trichogramma pretiosum* Riley were observed when they were fed on honey with six strains of *B. thuringiensis* (Polanczyk *et al*., 2006). A field survey indicated that there were no significant differences in the percentage of parasitism, number, longevity and mortality of adults of *T. brassicae* emerging from *O. nubilalis* eggs oviposited on *Bt* or isogenic maize leaves (Manachini and Lozzia, 2004).

Our results seem to demonstrate that *Bt* maize pollen-expressing Cry1Ab toxin does not induce adverse effects on longevity, fecundity, eclosion and sex ratio of *T. ostriniae* females when fed on *Bt* maize pollen, with honey or water.

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References

ANDOW D. A., RISCH S. J., 1987.- Parasitism in diversified agroecosystems: Phenology of *Trichogramma minutum* (Hymenoptera: Trichogrammatidae).- *Entomophaga*, 32: 255-260.

BABENDREIER D., KALBERER N. M., ROMEIS J., FLURI P., MULLIGAN E., BIGLER F., 2005.- Influence of Bt-transgenic pollen, Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees.- *Apidologie*, 36: 585-594.

BEGUM M., GURR G. M., WRATTEN S. D., HEDBERG P., NICOL H. I., 2006.- Using selective food plants to maximize biological control of vineyard pests.- *Journal of Applied Ecology*, 43: 547–554.

DUAN J. J., HEAD G., MCKEE M. J., NICKSON T. E., MARTIN J. W., SAYEGH F. S., 2002.- Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*.- *Entomologia Experimentalis et Applicata*, 104: 271-280.

FLEURY F., BOULETRÉAU M., 1993.- Effects of temporary host deprivation on the reproductive potential of *Trichogramma brassicae*.- *Entomologia Experimentalis et Applicata*, 68: 203-210.

FORSSE E., SMITH S. M., BOURCHIER R. S., 1992.- Flight initiation in the egg parasitoid *Trichogramma minutum*: Effects of ambient temperature, mates, food and host eggs.- *Entomologia Experimentalis et Applicata*, 62: 47-154.

GENG J. H., SHEN Z. R., SONG K., ZHENG L., 2006.- Effect of pollen of regular cotton and transgenic *Bt*-CpTI cotton on the survival and reproduction of the parasitoid wasp *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) in the laboratory.- *Environmental Entomology*, 35: 1661-1668.

Gurr G. M., Nicol H. I., 2000.- Effect of food on longevity of adults of *Trichogramma carverae* Oatman and Pinto and *Trichogramma* nr *brassicae* Bezdenko (Hymenoptera Trichogrammatidae).- *Australian Journal of Entomology*, 39: 185-187.

Hanley A. V., Huang Z. Y., Pett W. L., 2003.- Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*.- *Journal of Apicultural Research*, 42: 77-81.

Hassan S. A., Guo M. F., 1991.- Selection of effective strains of egg parasites of the genus *Trichogramma* (Hym., Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* Hb. (Lep., Pyralidae).- *Journal of Applied Entomology*, 111: 335-341.

Hassan S. A., Krieg A., 1975.- *Bacillus thuringiensis* preparations harmless to the parasite *Trichogramma cacoeciae* (Hym.: Trichogrammatidae).- *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, 82: 515-521.

He K. L., Wang Z. Y., Zhou D. R., Wen L. P., Yao Z. Y., 2003.- Evaluation of transgenic Bt corn for resistance to the Asian corn borer (Lepidoptera: Pyralidae).- *Journal of Economic Entomology*, 96: 935-940.

Hegazi E. M., Khafagi W. E., 1998.- Studies on three species of *Trichogramma*. III. Comparison of longevity and fecundity of adult wasps fed on selected foods.- *Alexandria Journal of Agricultural Research*, 43: 79-88.

Hellmich R. L., Siegfried B. D., Sears M. K., Stanley-Horn D. E., Daniels M. J., Mattila H. R., Spencer T., Bidne K. G., Lewis L. C., 2001.- Monarch larvae sensitivity to *Bacillus thuringiensis* purified proteins and pollen.- *Proceedings of the National Academy of Sciences USA*, 98: 11925-11936.

Hohmann C. L., Luck R. F., Oatman E. R., 1988.- A comparison of longevity and fecundity of adult *Trichogramma platneri* (Hymenoptera: Trichogrammatidae) reared from eggs of the cabbage looper and the angoumois grain moth, with and without access to honey.- *Journal of Economic Entomology*, 81: 1307-1312.

Hoffmann M. P., Wright M. G., Pitcher S. A., Gardner J., 2002.- Inoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: field biology and population dynamics.- *Biological Control*, 25: 249-258.

Jesse L. C. H., Obrycki J. J., 2000.- Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly.- *Oecologia*, 125: 241-248.

Knutson A., 1998.- *The Trichogramma Manual*.- Agricultural Communications, The Texas A & M University System, College Station, USA.

Lang A., Vojtech E., 2006.- The effects of pollen consumption of transgenic Bt maize on the common swallowtail, *Papilio machaon* L. (Lepidoptera, Papilionidae).- *Basic and Applied Ecology*, 7: 296-306.

Leatemia J. A., Laing J. E., Corrigan J. E., 1995.- Effects of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae).- *Canadian Entomologist*, 127: 245-254.

Li L. Y., 1994.- Worldwide use of *Trichogramma* for biological control in different crops: A survey. pp. 37-51. In: *Biological Control with Egg Parasitoids* (Wajnberg E., Hassan S. A., Eds.).- CAB International, Oxon, UK.

Li W. D., Wu K. M., Wang X. Q., Wang G. R., Guo Y. Y., 2005.- Impact of pollen grains from Bt transgenic corn on the growth and development of Chinese tussah silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae).- *Environmental Entomology*, 34: 922-928.

Li L. L., Wang Z. Y., He K. L., Bai S. X., Hua L., 2007.- Effect of transgenic corn expressing *Bacillus thuringiensis* Cry1Ab toxin on population increase of *Rhopalosiphum maidis* Fitch.- *Chinese Journal of Applied Ecology*, 18 (5): in press.

Losey J. E., Rayor L. S., Carter M. E., 1999.- Transgenic pollen harms monarch larvae.- *Nature*, 399: 214.

Manachini B., Lozzia G. C., 2004.- Studies on the effects of Bt corn expressing Cry1Ab on two parasitoids of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae).- *Bulletin-OILB/SROP*, 27: 109-116.

Mattila H. R., Sears M. K., Duan J. J., 2005.- Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay.- *Entomologia Experimentalis et Applicata*,116: 31-41.

Munkvold G. P., Hellmich R. L., Rice L. G.., 1999.- Comparison of Fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids.- *Plant Disease*, 83: 130-138.

Nagarkatti S., Tobin P. C., Saunders M. C., Muza A. J., 2003.- Release of native *Trichogramma minutum* to control grape berry moth.- *Canadian Entomologist*, 135: 589-598.

O'Callaghan M., Glare T. R., Burgess E. P. J., Malone L. A., 2005.- Effects of plants genetically modified for insect resisitance on non-target organisms.- *Annual Review of Entomology*, 50: 271-292.

Pilcher C. D., Obrycki J. J., Rice M. E., Lewis L. C., 1997.- Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn.- *Environmental Entomology*, 26: 446-454.

Pleasants J. M., Hellmich R. L., Dively G. P., Sears M. K., Stanley-Horn D. E., Mattila H. R., Foster J. E., Clark T. L., Jones G. D., 2001.- Corn pollen deposition on milkweeds in and near cornfields.- *Proceedings of the National Academy of Sciences USA*, 98: 11919-11924.

Polanczyk R. A., Pratissoli D., Vianna U. R., Oliveira R. G. S., Andrade G. S., 2006.- Interaction between natural enemies: *Trichogramma* and *Bacillus thuringiensis* in pest control.- *Acta Scientiarum Agronomy*, 28: 233-239.

Qian Y., Cao R., Li G., 1984.- Biology of *Trichogramma ostriniae* and evaluation of its effectiveness in controlling corn borer.- *Acta Entomologica Sinica*, 27: 287-293.

Rohi L., Bourarach K., Chemseddine M., Pintureau B., 2002.- Effet de la nourriture sur la longevite de deux Hymenopteres parasitoides oophages.- *Bulletin Mensuel de la Societe Linneenne de Lyon*, 71: 118-122.

Romeis J., Meissle M., Bigler F., 2006.- Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control.- *Nature Biotechnology*, 24: 63-71.

Saavedra J. L. D., Torres J. B., Ruiz M. G., 1997.- Dispersal and parasitism of *Heliothis virescens* eggs by *Trichogramma pretiosum* (Riley) in cotton.- *International Journal of Pest Management*, 43: 169-171.

Salama H. S., Zaki F. N., 1985.- Biological effects of *Bacillus thuringiensis* on the egg parasitoid, *Trichogramma evanescens*.- *Insect Science and its Application*, 6: 145-148.

SAS Institute Inc., 1989.- *SAS/STAT User's Guider*.- Version 6, 4th Edition, Vol. 1 and 2, Cary, NC, USA.

Sears M. K., Hellmich R. L., Stanley-Horn D. E., Oberhauser K. S., Pleasants J. M., Mattila H. R., Siegfried B. D., Dively G. P. 2001.- Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment.- *Proceedings of the National Academy of Sciences USA*, 98: 11937-11942.

Sétamou M., Bernal J. S., Legaspi J. C., Mirkov T. E., 2002.- Parasitism and location of sugarcane borer (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Hymenoptera: Braconidae) on transgenic and conventional sugarcane.- *Environmental Entomology*, 31: 1219-1225.

Shearer P. W., Atanassov A., 2004.- Impact of peach extrafloral nectar on key biological characteristics of *Trichogramma minutum* (Hymenoptera : Trichogrammatidae).- *Journal of Economic Entomology*, 97: 789-792.

SHELTON A. M., SEARS M. K., 2001.- The monarch butterfly controversy: scientific interpretations of a phenomenon.- *Plant Journal*, 27: 483–488.

SOMCHOUDHURY A. K., DUTT N., 1988.- Evaluation of some flowers as a nutritional source of *Trichogramma* spp.- *Indian Journal of Entomology*, 50: 371-373.

TREACY M. F., BENEDICT J. H., WALMSLEY M. H., LOPEZ J. D., MORRISON R. K., 1987.- Parasitism of bollworm (Lepidoptera: Noctuidae) eggs on nectaried and nectariless cotton.- *Environmental Entomology*, 16: 420-423.

WANG C. L., WANG H. X., GUI C. M., LU H., 1984.- Studies on the control of the Asian corn borer, *Ostrinia furnacalis* (Guenee), with *Trichogramma ostriniae*. pp. 268–273. In: *US. National Academy of Sciences Joint Symposium on Biological Control of Insects* (ADKISSON P. L., MA S. J., Eds.).- Science Press, Beijing, China.

WANG B., FERRO D. N., HOSMER D. W., 1999.- Effectiveness of *Trichogramma ostriniae* and *T. nubilale* for controlling the European corn borer *Ostrinia nubilalis* in sweet corn.- *Entomologia Experimentalis et Applicata*, 91: 297-303.

WANG D. Y., WANG Z. Y., HE K. L., CONG B., BAI S. X., WEN L. P., 2004.- Temporal and spatial expression of Cry1Ab toxin in transgenic Bt corn and its effects on Asian corn borer, *Ostrinia furnacalis* (Guenée). - *Scientia Agricultura Sinica*, 37: 1155-1159.

WELLINGA S., WYSOKI M., 1989.- Preliminary investigation of food source preferences of the parasitoid *Trichogramma platneri* Nagarkatti (Hymenoptera, Trichogrammatidae).- *Anzeiger fur Schadlingskunde, Pflanzenschutz, Umweltschutz*, 62 (7): 133-135.

WRIGHT M. G., KUHAR T. P., HOFFMANN M. P., CHENUS S. A., 2002.- Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn.- *Biological Control*, 23: 149-155.

WU J. W., FANG H. L., YANG M. D., LIAN Y. Y., 1988.- Rearing technology of *Trichogramma dendrolimi* Matsumura for controlling pine caterpillar (*Dendrolimus punctatus* Walker) and effect of releasing *Trichogramma* to control insect pest on a large scale for sixteen successive years in Zhejiang Province.- *Colloques de l'INRA*, 43: 621-628.

ZANGERL A. R., MCKENNA D., WRAIGHT C. L., CARROLL M., FICARELLO P., WARNER R., BERENBAUM M. R., 2001.- Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions.- *Proceedings of the National Academy of Sciences USA*, 98: 11908-11912.

ZHANG Z. L., 1988.- *Trichogramma* spp. parasitizing the eggs of Asian corn borer *Ostrinia furnacalis* and its efficacy in Beijing suburbs. - *Colloques de l'INRA*, 43: 629-633.

ZHANG J., WANG J. L., CONG B., YANG C. C., 1990.- A faunal study of *Trichogramma* species on *Ostrinia furnacalis* in China.- *Chinese Journal of Biological Control*, 6: 49-53.

ZHANG G. R., ZIMMERMANN O., HASSAN S. A., 2004.- Pollen as a source of food for egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). - *Biocontrol Science and Technology*, 14: 20l-209.

ZHOU D. R., HE K. L., WANG Z. Y., YE Z. H., WEN L. P., GAO Y X., SONG Y. Y., 1995.- *Asian corn borer and its integrated management*.- Golden Shield Press, Beijing, China.

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