Attraction of the parasitoid *Cotesia marginiventris* to host (*Spodoptera frugiperda*) frass is affected by transgenic maize

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Abstract We assessed in the laboratory the attraction of the parasitoid *Cotesia marginiventris* (Cresson) toward odors emitted by conventional maize (*Zea mays* L. ssp. *mays*) and *Bt* (*Bacillus thuringiensis*) maize seedlings following actual or simulated injury by *Spodoptera frugiperda* (Smith), the parasitoid's host, and emitted by the host's frass, produced following consumption of conventional or *Bt* maize seedlings. Females of *C. marginiventris* exhibited similarly strong responses to conventional and *Bt* maize seedlings injured by the host or with simulated injury, and these were stronger than responses to clean air. In contrast, the responses of *C. marginiventris* females were consistently weaker toward host frass derived from *Bt* maize tissue compared to frass derived from conventional maize tissue. We hypothesized that the weakened response was due to a detrimental effect of *Bt* endotoxins, present in the *Bt* maize tissue, on the bacterial community present in the host's gut and frass, including bacteria that produce odors attractive to *C. marginiventris*. As an initial test of our hypothesis, we compared between the responses of *C. marginiventris* females to host frass produced following consumption of *Bt* maize and frass produced from conventional maize which had been treated with an antibiotic (tetracycline) to eliminate host gut bacteria. Our results showed that *C. marginiventris* females responded similarly weakly to host frass derived from conventional maize tissue treated with antibiotic and to frass derived from *Bt* maize tissue, treated or untreated with antibiotic, while they responded strongly to frass derived from conventional maize untreated with antibiotic, so provided initial, partial support for our hypothesis. We discussed the weakened response of *C. marginiventris* females to host frass derived from *Bt* maize in the context of plausible impacts of transgenic crop cultivars on parasitoid foraging and populations, and the implications for biological control of non-target, polyphagous pests, such as *S. frugiperda*.

Keywords *Bacillus thuringiensis* · *Bt* · *Spodoptera frugiperda* · Biological control · Kairomones · Sublethal effects · Non-target effects

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Introduction

Successful host location by parasitoid females is essential for parasitoid reproduction, and typically is dependent on chemical communication between parasitoids and hosts, and between parasitoids and host plants. In order to locate hosts, parasitoid females systematically narrow their search by locating their host's habitat (e.g., a host plant) and then locating the host within the habitat (Vinson 1984, 1991). Females typically locate their hosts by exploiting volatile chemicals released by host plants at high concentrations and induced by herbivores (Turlings et al. 1990; Vet and

Dicke 1992). Subsequently, upon locating a plant with hosts, parasitoid females typically exploit host-derived cues active at close range to locate hosts. Such cues may include sounds produced by hosts during feeding (Meyhöfer et al. 1994; Tomov et al. 2003), odors emanating from wounded plant tissues (Vet and Dicke 1992), or odors from host products, such as frass (Mattiacci and Dicke 1995; Rose et al. 1997; Weiss 2006). In particular, parasitoid females can use odors to discriminate between frass produced by host and non-host species (Agelopoulos et al. 1995; Ngi-Song and Overholt 1997; Rose et al. 1997), by hosts of different ages/instars (Mattiacci and Dicke 1995), and by hosts feeding on different host plants (Inayatullah 1983; van Leerdam et al. 1985).

Genetically modified (GM) or transgenic crop cultivars are increasingly ubiquitous features of agricultural landscapes, and *Bt* maize is among the most widely grown GM crops worldwide (James 2004). *Bt* maize includes maize (*Zea mays* L. ssp. *mays*) cultivars that were genetically modified to produce *Bacillus thuringiensis* (Berliner) endotoxins, which cause lesions in the midgut epithelium of susceptible insects (Hakim et al. 2010), including lepidopteran or coleopteran maize pests. In general, studies addressing the effects of GM crops on beneficial insects have reported minimal side effects, though there is cause for concern, according to some authors (O'Callaghan et al. 2005; Andow et al. 2006; Lovei et al. 2009). In contrast to the case of pesticides (e.g., Desneux et al. 2007), few studies have investigated sublethal effects of *Bt* crops on beneficial insect physiology or behavior (Ramírez-Romero et al. 2005, 2008; Romeis et al. 2006), and even fewer studies have focused on parasitoids in particular (Turlings et al. 2005; Vojtech et al. 2005; Ramírez-Romero et al. 2007; Sanders et al. 2007; Walker et al. 2007). Most such studies addressed potential host-mediated effects of GM crops, including *Bt* crops, on parasitoid (or predator) development, adult lifespan, and reproduction (reviews in Bernal et al. 2004; Hilbeck and Schmidt 2006; Lovei et al. 2009, Bernal 2010), while only a few studies addressed potential effects on parasitoid foraging behavior (e.g., Schuler et al. 1999, 2003).

Unplanned effects of plant genetic modification on GM crop phenotypes are well known, and can be due to pleiotropy, epistasis, or insertional mutagenesis. Such changes include alteration of biochemical, physiological, and morphological traits unrelated to the intended genetic modification (Olesinski et al. 1995; Überlacker et al. 1996; Wang et al. 1998; Lumbierres et al. 2004). Particularly in reference to plant-produced volatiles used in plant–herbivore communication, one study uncovered consistent differences between GM and conventional crop cultivars in their attractiveness to herbivore females foraging for oviposition sites (Bernal and Sétamou 2003). However, genetic transformation has not been found to affect communication between GM crops and parasitoids (Schuler et al. 1999, 2003; Turlings et al. 2005; Dean and De Moraes 2006), though the effects (if any) of GM crops on chemical communication (directly) between hosts or their products and parasitoids have received little attention (Schuler et al. 2003). Effective chemical communication between parasitoids, hosts, and host plants is essential for agricultural pest management generally, and for biological pest control in particular because of the frequent use of parasitoids for control of agricultural pests.

In the present study, we initially assessed whether *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a parasitoid of fall armyworm [*Spodoptera frugiperda* (Smith), Lepidoptera: Noctuidae] is equally attracted to host-related volatiles derived from *Bt* and conventional maize plants. Fall armyworm (FAW) was selected for our study because while it is variably targeted by commercially available *Bt* maize cultivars, and not by the cultivar used in our experiments, it is a common and important pest of maize in the Americas and is variably susceptible to *Bt* endotoxins, including the particular endotoxin evaluated in this and other studies (Bokonon-Ganta et al. 2003; Monnerat et al. 2006; Virla et al. 2008; de Polanía et al. 2009). Thus, variable numbers of FAW are expected to survive exposure to *Bt* maize in the field and, consequently, to be subject to parasitism by *C. marginiventris*. Specifically, in this study we compared the attraction of *C. marginiventris* to odors emitted by: (i) *Bt* or conventional maize plants that were injured by FAW; (ii) *Bt* or conventional maize plants following simulated FAW injury (i.e. artificial injury and topical application of host regurgitate), and; (iii) frass produced by FAW fed *Bt* or conventional maize tissue. Subsequently, we tested whether the observed effect of *Bt* maize on the parasitoid's response to FAW frass (see Results) could be explained as a detrimental effect of *Bt* endotoxins on bacteria present in the FAW midgut and frass. This was done by comparing between the responses of *C. marginiventris* females towards odors from frass derived from *Bt* maize tissue and responses towards odors from frass derived from conventional maize tissue treated with the broad-spectrum antibiotic tetracycline. Previous studies showed that bacteria present in the gut and frass of insects are responsible for producing volatiles that are attractive to parasitoid females (Auger et al. 1990; Thibout et al. 1993, 1995), and that lepidopteran gut bacteria are generally susceptible to tetracycline (Visotto et al. 2009). Our study is the first to question whether GM crops, including *Bt* maize, negatively affect chemical communication between foraging parasitoids and their hosts via alteration of host frass, an important source of odors that are useful for locating hosts at short ranges.

Materials and methods

Insects and plants

Cotesia marginiventris is a solitary larval endoparasitoid that parasitizes many lepidopterous pest species (Ashley 1986), and utilizes kairomones (e.g., frass odors) and host-induced plant odors (synomones) to locate hosts (Loke and Ashley 1984; Turlings et al. 1990). Parasitoid females used in experiments were obtained from a laboratory culture maintained at 25 ± 2°C, 50–70% RH, 14:10 L:D. Parasitoids obtained from collaborators at USDA-ARS, Tifton, Georgia, were frequently added to the laboratory culture to maintain culture vigor. The parasitoid culture was maintained by exposing 4 day-old FAW larvae for 1 h to female parasitoids at a 3:1 ratio in covered plastic vials (148 ml, 49 × 85 mm). Exposed larvae were then placed individually in small plastic cups containing ~5 g of artificial diet (see Ramírez-Romero et al. 2007) until they yielded parasitoid cocoons, which were collected and placed individually in glass vials plugged with cotton wool until adults emerged. Parasitoid females were offered honey solution (20% v:v) immediately upon emergence, allowed to mate, and used in experiments when they were 24–48 h old.

FAW is a widespread and migratory pest of many crops including maize (Sparks 1979; Ashley 1986). It is variably susceptible (~20–98% larval mortality) to the Cry1Ab Bt maize used in this study (see below), particularly in 5 day-old or older larvae, because activated Cry1Ab toxin binds weakly on its epithelial brush border membrane (Aranda et al. 1996; Bokonon-Ganta et al. 2003; Ramírez-Romero 2004; Monnerat et al. 2006; Ramírez-Romero et al. 2007; Virla et al. 2008). The larvae used in the experiments, and for maintaining the *C. marginiventris* culture, were obtained from a laboratory culture maintained at 25 ± 2°C, 50–70% RH, and a 14:10 photoperiod. Pupae were placed inside cylindrical waxed paper cartons (17 cm diam × 18 cm deep) where emerging adults were allowed to mate and lay eggs on paper lining the interior of the cartons. Newly-laid eggs were incubated to larval emergence in 1 l wide-mouth glass jars under the conditions described above. Neonate larvae were transferred to small plastic cups covered with a waxed-paper lid and containing ~5 g of artificial diet until they were used in the experiments or yielded pupae. Pupae were then collected and transferred to waxed paper containers as indicated above.

Bt and conventional maize seedlings were grown individually in small pots (100 mm diam × 80 mm height) in a greenhouse. *Bt* maize plants were of a hybrid (Pioneer 35N05) expressing a Cry1Ab endotoxin gene (Event MON810), while conventional plants were of a second, near-isogenic hybrid (Pioneer 34A55), which does not express a *Bt* endotoxin gene. Maize seeds were sown one per pot in a 1:1 mixture of moist vermiculite and potting soil (SunGro Horticulture Inc., Vancouver, Canada). Conventional and *Bt* maize seedlings were used in experiments when they were 10–15 days old (three or four leaves).

Experiments 1–3: Parasitoid responses to plant- and host derived odors from *Bt* and conventional maize

Responses toward test odors were observed in a four-arm olfactometer (described by Vet et al. (1983), 90° arc, 27 cm diameter). All trials were conducted at 25 ± 2°C and 80–90% R.H. The airflow in each of the four arms was equalized at 30 cm/s using flowmeters at each arm and a terminal flowmeter between the arena and a pump. Closed glass bells (d: 15 cm, h: 43 cm) (for maize plants, see below) or 125 mm glass flasks (for frass, see below) were used to contain odor sources, and were fitted to tubing leading from the four olfactometer arms. Three odor sources were tested in separate experiments: (1) maize plants injured by FAW; (2) maize plants with simulated FAW injury (artificially injured), and; (3) FAW frass.

Experiment 1: Host-injured plants

Two days prior to a trial, potted *Bt* and conventional maize seedlings were infested with two pairs of FAW larvae (one pair 4–6 days old, and one pair 8–10 days old). The 8–10 day-old larvae were removed from seedlings immediately before trials, leaving only the 4–6 day-old larvae to ensure continued volatile production by seedlings during trials. Thus, host-injured plants represented complete plant–host systems, i.e. a maize seedling, FAW larvae, and FAW frass. The plants were then placed individually in the closed glass bells to serve as odor sources.

Experiment 2: Artificially injured plants

In this experiment, we used artificially injured seedlings to which FAW regurgitate was applied to exclude potential effects of FAW feeding differences on *Bt* or conventional maize seedlings. Previous studies showed that maize seedlings thus injured were as attractive to parasitoids as seedlings injured by FAW (Turlings et al. 1993). Maize seedlings were artificially injured by excising the whorl with scissors, making four vertical 5 mm-long incisions at the point of excision, and applying FAW regurgitate to the incisions. Regurgitate was applied with a micropipette at a rate of 20 µl regurgitate per plant, and was collected from 8 to 10 day-old larvae fed artificial diet, following the method described by Turlings et al. (1993). FAW regurgitate was stored at 3°C and used within 24 h in every case. Artificially injured seedlings were placed individually in

closed glass bells, and were used between 120 and 250 min after application of regurgitate to injured seedlings.

Experiment 3: Host frass

Conventional and *Bt* maize seedlings (8–10 days old) grown in a greenhouse were transplanted individually in small pots (148 ml), and the soil surface was covered with Parafilm M® (Pechiney Plastic Packaging Co., Chicago, USA). Single FAW larvae (8 days old) were placed on individual seedlings, which were enclosed in a cage made of a plastic vial (148 ml) with its bottom replaced with fine-mesh metal screen. Pots and cages were joined with their corresponding lids (with a 4 cm-diam hole to allow passage of seedlings between pot and cage). FAW larvae were starved for 4 h, then placed on maize seedlings and allowed to feed for 48 h. Larvae were then removed, and frass was collected from the cage interior with a fine camel hair brush. Frass was used immediately or stored for fewer than 48 h at 3°C. Frass was used in experiments by placing 100 mg in individual 125 ml glass flasks.

Observations in *Experiments 1–3* were conducted in the mornings between 9.00 and 12.00 h. Parasitoid females were exposed to two treatment odor fields, i.e. corresponding to conventional and *Bt*-maize, and two clean air fields) simultaneously. A parasitoid female was introduced into the center of the olfactometer and observed during 10 min. The number of visits (i.e. the number of times the female entered in a field) and time spent in each of the four odor fields were recorded. The olfactometer was cleaned with 70% alcohol and odor sources replaced and rotated after testing eight females. Forty-nine, 37, and 43 replicate trials, each with a different parasitoid female, were conducted for *Experiments 1, 2*, and *3*, respectively.

The numbers of visits and the time spent in each olfactometer field were analyzed via Friedman's two-way ANOVA by ranks with experiment (host injured plants, artificially injured plants, frass) and odor treatments (*Bt* seedlings, conventional seedlings, clean air fields) as factors. Subsequently, the effect of treatment within experiments was evaluated using Friedman's one-way ANOVA by ranks, and means were separated using Student–Newman–Keuls tests.

Experiment 4: Comparative effects of *Bt* maize and tetracycline on parasitoid responses to host frass

Using a four-arm olfactometer (described above) we compared how the responses of *C. marginiventris* females to odors from FAW frass were affected by consumption of *Bt* maize and the antibiotic tetracycline. Thus, we conducted four sets of assays, each set with three clean air fields and one test odor field corresponding to frass produced by FAW that were fed: (i) conventional maize seedlings (=*Bt*−An−); (ii) *Bt* maize seedlings (=*Bt*+An−); (iii) conventional maize seedlings treated with tetracycline (=*Bt*−An+), or; (iv) *Bt* maize seedlings treated with tetracycline (=*Bt*+An+). Tetracycline is an antibiotic with broad-spectrum activity against lepidopteran gut bacteria, and the most effective midgut bactericide among 22 antibiotics evaluated in one study (Broderick et al. 2009; Visotto et al. 2009). It was applied topically to maize seedlings at a rate of 0.5 ml of 20 µg/ml suspension per seedling, and seedlings were allowed to dry for 15 min before offering excised leaf disks (5 cm diam) to FAW larvae in Petri dishes (5.2 cm diam). The olfactometer was cleaned with 70% alcohol and odor sources replaced and rotated after testing eight females. Between 21 and 24 replicates trials were conducted per each set of assays.

Assays were conducted as described previously, with the following exceptions. Single odor sources were compared against three clean air fields in olfactometer trials, as noted above. Frass was obtained from larvae fed maize leaf disks, as noted above. Observation time in olfactometer trials was 60 s, which minimized odor habituation occurring in the absence of a reward (i.e. hosts) in parasitoid females (Monteith 1963; Dudai 1989; Desneux et al. 2004). Pilot experiments showed that odor habituation occurred faster when using odors of frass from larvae fed maize leaf disks than when using odors of frass from larvae fed on whole maize plants (as in *Experiment 3*). We recorded only the time spent in each olfactometer field, but not visits because these were infrequent in our pilot experiments, given the 60 s observation period.

Three independent statistical analyses were conducted. First, parasitoid attraction to frass odor versus clean air was assessed within each of the four sets of assays by comparing the frequency of parasitoids selecting (=spending most of their time in a particular odor field) (Sandoz et al. 2000) the frass odor field or clean air fields to a 1:3 distribution using a log-likelihood ratio test (*G* test). Second, the influences of *Bt* maize and tetracycline on parasitoid attraction to frass were assessed by comparing the frequencies of parasitoid females selecting the frass odor in the assay with conventional maize without tetracycline (i.e. *Bt*−An−) against the frequencies of females selecting the frass odor in each of the three remaining sets of assays (i.e. assays with *Bt*−An+, *Bt*+An−, or *Bt*+An+) using permuted Fisher's exact tests (proc multtest, SAS/STAT Version 9.1, SAS Institute Inc., Cary, NC, USA). Third, a Kruskal–Wallis test was used to compare the times spent in the frass odor fields among the four sets of assays (i.e. among *Bt*−An−, *Bt*+An−, *Bt*−An+, and *Bt*+An+).

Results

Experiments 1–3: Parasitoid responses to plant- and host derived odors from *Bt* and conventional maize

The type of experiment (i.e. *Experiments 1*, *2*, and *3*) did not influence the effects of the odor treatments, as measured by visit frequencies ($P = 0.65$, $F = 0.54$, df $= 2$) and time spent in odor fields ($P = 0.57$, $F = 0.76$, df $= 2$), though the effects of the odor treatments were consistently significant within the experiments ($P < 0.001$, df $= 3$; visit frequencies, $F = 0.38.03$; time in odor field, $F = 52.87$). However, the interaction between experiment type (*Experiments 1*, *2*, and *3*) and odor treatments (clean air 1, clean air 2, and conventional or *Bt* maize) was significant ($P \leq 0.003$, df $= 6$; visit frequencies, $F = 3.31$; time in odor field, $F = 5.77$). Hence, subsequent analyses focused on treatment effects within each of *Experiments 1–3*.

Experiment 1: Host-injured plants

Parasitoid females more frequently visited ($P < 0.001$) (Fig. 1a) and spent more time ($P < 0.001$) (Fig. 1b) in odor fields corresponding to host-injured seedlings, *Bt* or conventional, compared to clean air fields. Specifically, parasitoids more frequently visited the field containing the odor of host-injured conventional seedlings than of injured *Bt* seedlings (Fig. 1a), but the time spent per field did not differ between these odor fields (Fig. 1b).

Experiment 2: Artificially injured plants

Parasitoid females more frequently visited ($P = 0.002$) (Fig. 1c) and spent more time ($P < 0.002$) (Fig. 1d) in odor fields corresponding to artificially injured seedlings, conventional or *Bt*, compared to clean air fields. However, differences between artificially injured conventional and *Bt* seedlings were not significant.

Experiment 3: Frass

Parasitoid females more frequently visited ($P < 0.001$) (Fig. 1e) and spent more time ($P < 0.001$) (Fig. 1f) in fields corresponding to frass derived from conventional maize tissue, followed by fields corresponding to frass from *Bt* maize tissue, and finally to fields corresponding to clean air.

Experiment 4: Comparative effects of *Bt* maize and tetracycline on parasitoid responses to host frass

Parasitoid females selected the frass odor field with a greater than random frequency in the assays involving hosts fed conventional maize without tetracycline (=*Bt*−An−) ($G = 6.33$, $P = 0.011$, 1 df), but not in the remaining assays (*Bt*+An−: $G = 0.58$, $P = 0.444$, 1 df; *Bt*−An+; $G = 2.31$, $P = 0.128$, 1 df; *Bt*+An+: $G = 0.02$, $P = 0.899$, 1 df) (Fig. 2a). Moreover, parasitoid females more frequently selected (i.e. spent most of their time) the frass odor field in the assays involving conventional maize seedlings without antibiotic (=*Bt*−An−) compared to the remaining assays (permuted Fisher's exact tests, all $P \leq 0.035$) (Fig. 2a). Finally, parasitoid females appeared to spend more time in the frass odor field in the assays involving conventional maize seedlings without antibiotic (=*Bt*−An−) compared to the remaining assays ($K = 10.23$, $P = 0.017$) (Fig. 2b).

Discussion

The results of our experiments showed that *C. margini-ventris* females responded positively to host-associated and host-induced odors derived from both conventional and *Bt* maize seedlings when searching for FAW hosts. Compared to clean air fields, parasitoid females were attracted to odors emitted by maize plants injured by FAW (with larvae and frass present), by maize plants with artificial FAW injury (without larvae and frass), and by FAW frass (Fig. 1a–f). Notably, however, the responses of parasitoid females were consistently weaker toward frass derived from *Bt* maize tissue compared to conventional maize tissue (Fig. 1e, f). It is plausible that the weaker responses were due to detrimental effects of *Bt* endotoxins, present in *Bt* maize, on the bacterial community present in frass, including bacteria responsible for producing volatiles attractive to parasitoid females (Auger et al. 1990; Thibout et al. 1993, 1995). *Bt* endotoxins are active against various species of bacteria (Pendleton 1969; Krieg 1970; Favret and Yousten 1989; Yudina et al. 1996; Yudina and Burtseva 1997), and bacteria responsible for producing odors attractive to parasitoids are susceptible to *Bt* endotoxins (Pendleton 1969; Favret and Yousten 1989). If indeed the weaker responses of parasitoid females towards frass derived from *Bt* maize tissue were the result of detrimental effects of the *Bt* endotoxins on FAW gut and frass bacteria, then the responses of parasitoid females to frass from conventional maize tissue treated with the broad-spectrum antibiotic tetracycline (i.e. *Bt*−An+) would be similarly weak, and weaker than the response to frass derived from conventional maize tissue free of tetracycline (i.e. *Bt*−An−). Tetracycline has a broad range of activity across lepidopteran taxa, and eliminated all culturable gut bacteria from *Anticarsia gemmatalis*, a species in the same family as FAW (Broderick et al. 2009; Visotto et al. 2009). Our results, in effect, showed that the responses of parasitoid females to

Number of visits to olfactometer fields (±SE)

Time spent in olfactometer fields (sec) (±SE)

Fig. 1 Responses of *Cotesia marginiventris* females to odors from host-injured (**a**, **b**) or artificially injured (**c**, **d**) conventional (*Conv*) and transgenic (*Bt*) maize seedlings, and odors from frass (**e**, **f**) produced by fall armyworm (*Spodoptera frugiperda*) larvae fed either conventional (*Conv*) or transgenic (*Bt*) maize seedlings in a four-arm olfactometer. Two clean air fields (*C1*, *C2*) were present in the olfactometer. Response variables measured were the number of visits and the time spent (s) in each olfactometer field. *Different letters* indicate significant differences among columns (*P* < 0.05, Student–Newman–Keuls test)

(A) Host-injured
(B) Host-injured
(C) Artificially-injured
(D) Artificially-injured
(E) Frass
(F) Frass

Conv Bt C1 C2
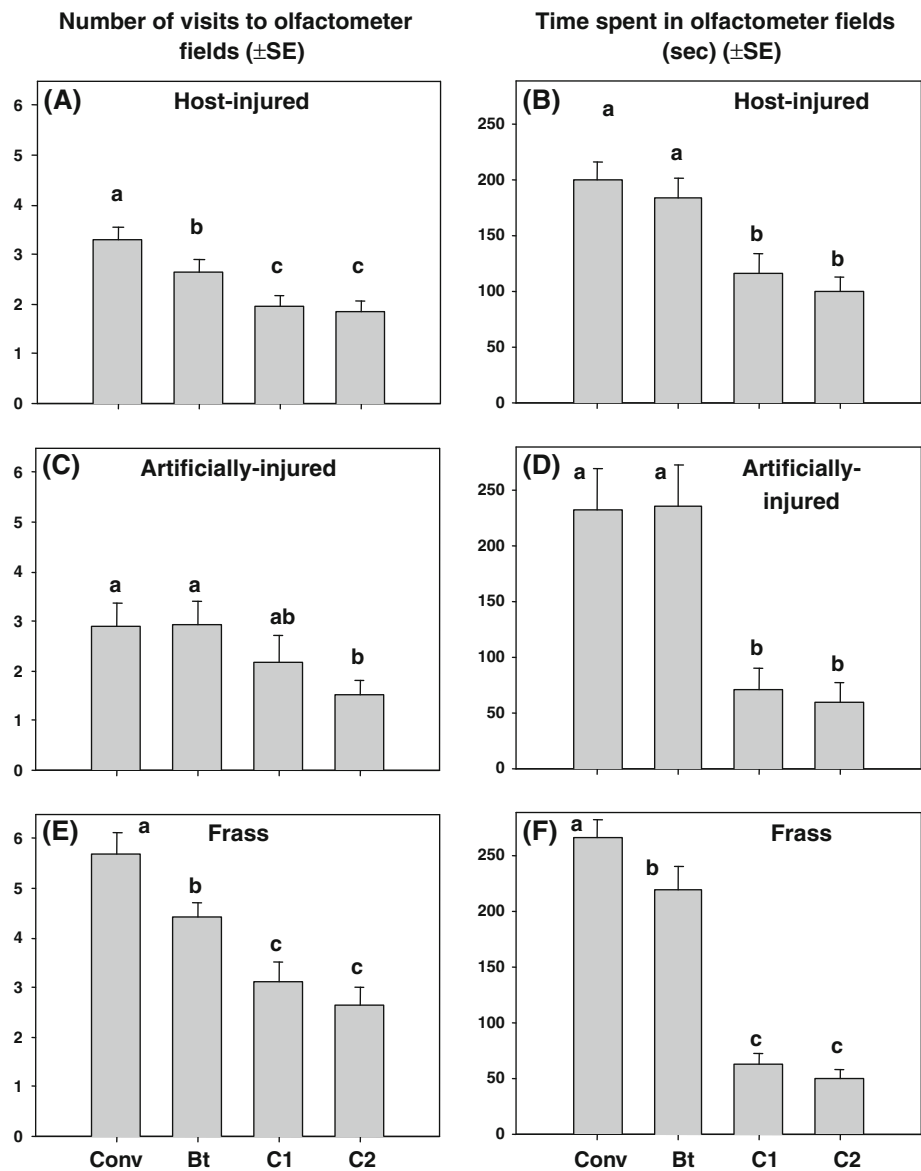
frass derived from conventional maize tissue treated with tetracycline were weak and comparable to the responses of females toward frass derived from *Bt*-maize seedlings (independently of treatment with tetracycline), which contrasted with the stronger response toward frass derived from conventional maize tissue free of tetracycline.

Altogether, our results suggested a novel mechanism by which *Bt* maize may affect parasitoids present in such crops. Namely, our results suggested that while the responses of foraging parasitoids to odors that are useful at medium to long ranges, such as those emitted by host-injured plants, may not differ between conventional and *Bt* maize plants, their responses to odors useful at close-range, such as those emitted by frass, may be weakened by a host's ingestion of *Bt* maize tissue. Prior research has shown that different cues are differently important for

foraging parasitoids, and that cues produced by host plants are important at medium to long ranges, while cues produced by hosts or their products are important at close range (Vinson 1984, 1991; Vet and Dicke 1992). Specifically, attraction of parasitoids to host products, such as frass, is an important component of the host location process (Vinson 1984, 1991), and has been reported previously for a variety of parasitoids (Vet and Dicke 1992; Chuche et al. 2006; Weiss 2006; Chiu-Alvarado et al. 2009), including species of *Cotesia* (Agelopoulos et al. 1995; Potting et al. 1995; Ngi-Song and Overholt 1997). If host location by *C. marginiventris* females is impaired because an important source of close-range cues, such as frass, is rendered less attractive by *Bt* endotoxins, then FAW encounter and parasitism rates may be reduced within *Bt* crop fields. In the case of FAW, which is an important pest
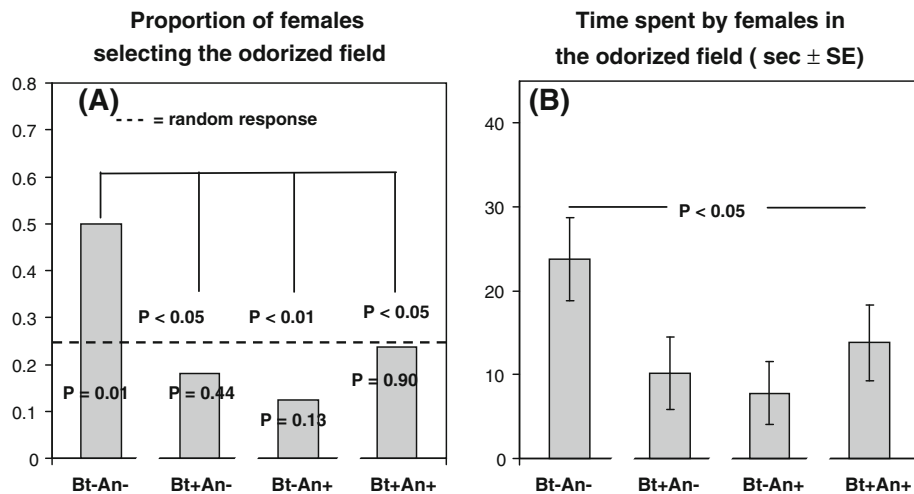
Proportion of females selecting the odorized field

Time spent by females in the odorized field (sec ± SE)

Fig. 2 a Proportions of parasitoid females selecting, and **b** mean times spent by *Cotesia marginiventris* females in an olfactometer field with odor of frass from fall armyworm (*Spodoptera frugiperda*) fed maize leaves (conventional or transgenic) treated or untreated with antibiotic. Each column in both (**a**) and (**b**) corresponds to the results of one set of trials in which *C. marginiventris* females were exposed to one frass odor and three clean air fields. *Bt*−An−, conventional maize without antibiotic; *Bt*+An−, *Bt* maize without antibiotic; *Bt*−An+, conventional maize with antibiotic; and *Bt*+An+, *Bt* maize with antibiotic. *Dashed line* in (**a**) indicates expected proportion under random response to the four odor sources; *P* values above the dashed line indicate significance of differences relative to column *Bt*−An− (permuted Fisher exact test), and below the dashed lined they indicate significance of differences relative to a random response (*G* tests). Sole *P* value in (**b**) indicates significance of difference among columns (Kruskal–Wallis test)

of numerous crops, lower parasitism rates of those FAW surviving exposure to *Bt* maize could lead to higher populations of the pest in neighboring and subsequent crops.

Our *Experiments 1* and *2* revealed significant differences between parasitoid responses to injured plants and clean air, though not between injured plants of the conventional and *Bt* maize cultivars. To date very few studies have compared the olfactory responses of parasitoids to injured *Bt* versus conventional plants, and the results of our study are in line with previous findings on *Cotesia* spp. (Schuler et al. 1999, 2003; Sétamou et al. 2002; Turlings et al. 2005; Lovei et al. 2009). Females of *Cotesia* spp. rely in part on odors emanating from frass when searching for hosts on a host plant (Agelopoulos et al. 1995; Mattiacci and Dicke 1995; Potting et al. 1995; Ngi-Song and Overholt 1997), and our results showed that the response of *C. marginiventris* females to frass odors derived from *Bt* maize tissue was weaker than to frass odors derived from conventional maize. This suggested that the *Bt* endotoxins present in *Bt* maize tissue diminished the attractiveness of FAW frass to *C. marginiventris*. Moreover, the response to the *Bt*+An+ treatment was similar to those toward the *Bt*+An− or *Bt*−An+ treatments, but weaker than toward the *Bt*−An− treatment, which suggested that the effects of *Bt* maize and tetracycline were not additive (e.g., Fig. 2a), and of similar magnitudes (Fig. 2a, b).

A plausible hypothesis explaining our results, and derived directly from our findings, is that the weakened response of *C. marginiventris* females to frass derived from

Bt maize (Fig. 1e, f) was due to a detrimental effect of the *Bt* endotoxins on the bacterial community present in frass and responsible for producing volatiles attractive to foraging females, though it remains to be rigorously tested. Gut bacterial communities contribute significantly to the health of lepidopteran insects (Dillon and Dillon 2004) and are responsible for producing odors attractive to parasitoids foraging for hosts (Auger et al. 1990; Thibout et al. 1993, 1995), and *Bt* endotoxins are known to alter the composition of these communities (Pendleton 1969; Favret and Yousten 1989; Broderick et al. 2006, 2009). Broadly, our results suggested that *Bt* endotoxins had a detrimental effect on the bacterial community of the FAW gut, so qualitatively altered the blend of odors emitted from FAW frass, and partially impaired host foraging by *C. marginiventris*.

The weakened response of *C. marginiventris* females to FAW frass derived from *Bt* maize shown here, together with the known effects of *Bt* maize on *C. marginiventris* (Ramírez-Romero 2004; Ramírez-Romero et al. 2007), suggested that *Bt* maize could impair the host finding process and reduce the biological control impact of this parasitoid of FAW, an important pest that is variably susceptible to commercially available *Bt* maize cultivars. Moreover, previously it was noted that an important contribution of biological control with parasitoids to pest management strategies based on GM crops was that parasitism of pest individuals surviving exposure to such crops would contribute to delaying the evolution of pest

resistance to GM crops (Bernal et al. 2004; Lundgren et al. 2009). Because FAW is variably susceptible ($\sim$20–98% larval mortality) to *Bt* maize cultivars (e.g., Bokonon-Ganta et al. 2003; Monnerat et al. 2006; Virla et al. 2008; de Polanía et al. 2009) it is plausible that it could quickly evolve resistance to *Bt* in areas where selection pressure is high and maize cultivation and *Bt* cultivars are predominant, and such evolution would be minimally delayed through parasitism by *C. marginiventris*, according to our results. Moreover, evolution of resistance to *Bt* maize would be especially problematic in the case of FAW, which is a widespread, polyphagous, and migratory pest (Sparks 1979; Ashley 1986), because its management in various crops from the southern USA to Argentina could be compromised, particularly if management relies on *Bt* toxins or *Bt* cultivars. Such unintended, though plausible, effects of *Bt* maize raise concerns over the compatibility of *Bt* crops with ecology-based pest management tactics, such as biological control, and suggest that closer examination of this technology is warranted, especially in countries where the *Bt* maize event evaluated in our study (MON810) was recently granted regulatory approval (AgBios 2009). In particular, the compatibility of *Bt* crop cultivars with biological control of both target and non-target pests within and outside the crop should be examined. *Cotesia marginiventris* is a common and frequent parasitoid of FAW and other pests on various crops (Marsh and Carlson 1979; Ashley 1986; Ruberson et al. 1994), and additional studies on potential effects of *Bt* crop cultivars, including recent *Bt* maize events, on this parasitoid would provide insight into plausible spill-over effects of widespread planting of *Bt* crops on biological pest control in transgenic and surrounding crops.

Overall, our results are important because they suggest a hitherto unexamined mechanism by which *Bt* crops may affect parasitoid populations in the field, and at the same time they point to a testable hypothesis explaining the mechanism at the level of individual parasitoids. Future studies should include the most recent *Bt* maize events, and compare the gut bacterial communities of fall armyworm exposed to *Bt* maize or conventional maize to identify which, if any, bacteria are eliminated by ingestion of *Bt* endotoxins, and should compare the volatile profiles of fall armyworm frass derived from both types of maize. Our research is part of a growing body of literature addressing potential, unintended effects of GM crops, including the commercially available *Bt* crops, on natural enemies and other non-target organisms (reviews in Bernal et al. 2004; Hilbeck and Schmidt 2006; Lovei et al. 2009; Lundgren et al. 2009; Bernal 2010). Additional related research is necessary because these cultivars are rapidly becoming widespread and stand-alone pest management tactics, and some researchers have hypothesized that GM crop cultivars

may negatively impact parasitoids and biological control in unanticipated ways (e.g., Bernal et al. 2004; Romeis et al. 2006; Lundgren et al. 2009; Bernal 2010).

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