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Responses of the stem borer larval endoparasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) to plant derived synomones: Laboratory and field cage experiments

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Abstract

Laboratory and field cage experiments investigated the response of females of the stem borer larval endoparasitoid *Cotesia flavipes* to two synthetic synomone components, the terpenoid (*E*)-β-farnesene and the green leaf volatile, (*Z*)-3-hexenyl acetate, both compounds identified previously in headspace volatiles of maize plants damaged by stem borer (*Chilo partellus*). In dose response tests performed in a Y-tube olfactometer, parasitoids were significantly more attracted to the arms bearing 10 or 15 μg of (*Z*)-3-hexenyl acetate and (*E*)-β-farnesene than to the control arm. (*E*)-β-Farnesene was as attractive as the essential oil from the plant *Hemizygia petiolata* (Lamiaceae) rich in the same compound (80% relative amount). The plant essential oil elicited responses from females of the parasitoid comparable to those elicited by two positive controls, stem borer larval frass and adult parasitoid diet (20% honey solution), tested in the laboratory assays. In field cage trapping experiments, captures in traps baited with the terpenoid, the plant essential oil, (*Z*)-3-hexenyl acetate and the control of 20% honey solution, were not significantly different relative to captures in unbaited traps. Addition of the green leaf volatile (*Z*)-3-hexenyl acetate to the plant essential oil to yield a 1:1 two-component blend captured significantly more female parasitoids than traps baited with either of the two components alone. The results show that blends of green leaf volatiles and sesquiterpenoids may have potential in monitoring *C. flavipes* populations in the field.

Keywords: Cotesia flavipes, Chilo partellus, *olfactometer, synomone, trap, parasitoid*

Introduction

Lepidopteran stem borers are the most injurious insect pest of cereals, including maize and sorghum, in Africa (Kfir et al. 2002). In 1993, a classical biological control program was initiated in Kenya to control the exotic Asian stem borer *Chilo partellus*

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(Swinhoe) (Lepidoptera: Crambidae), using the exotic larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). The parasitoid was imported from Pakistan, mass reared at the International Centre of Insect Physiology and Ecology, (ICIPE), Nairobi, and then released in the coastal area of Kenya. A field survey conducted a year later showed that the parasitoid had established at the coast (Overholt et al. 1997).

Although host location by *C. flavipes* has been extensively studied, there is no simple method to monitor its presence in the field. Current methods are labour-intensive and destructive, involving the dissection of maize stalks and removal and rearing of the borers to recover parasitoids that emerge (Overholt et al. 1994a; Omwega et al. 1995). The parasitoid is strongly attracted to maize plants fed on by its host, *C. partellus* larvae, and larval frass (Ngi-Song 1995; Potting 1996; Ngi-Song & Overholt 1997). The plant signalling odour attractive to the parasitoid is composed mainly of terpenoids (Ngi-Song et al. 2000), which could serve as potential lures for monitoring populations of *C. flavipes* for research purposes and the development of a management system for the stem borer.

In this study, we report laboratory and field cage experiments that tested the attractiveness of the maize stress component (*E*)-β-farnesene, the essential oil from the plant *Hemizygia petiolata* (Lamiaceae) rich in the terpenoid as a candidate substitute for the synthetic compound, and a green leaf volatile (*Z*)-3-hexenyl acetate, with the goal of developing a simple trapping system for the parasitoid.

Materials and methods

Parasitoids

A colony of *C. flavipes* was initiated from insects collected from *C. partellus* at Rawalpindi, Pakistan, by the International Institute of Biological Control (IIBC). *C. flavipes* were reared on *C. partellus* larvae according to the method described by Overholt et al. (1994b). After parasitisation, *C. partellus* were maintained on an artificial diet at 25°C, 65–70% relative humidity and 12L:12D photoperiod. Parasitoid cocoons were collected in glass vials and kept in a clean Perspex cage until emergence. On emergence, adult parasitoids were provided a 20% honey solution as diet. One-day-old naïve mated females of *C. flavipes* were used in all the experiments.

Y-tube olfactometer bioassays

A Y-tube olfactometer described by Ngi-Song et al. (1996) was used to test the attraction of 1-day-old female parasitoids to several odours. Briefly, the olfactometer consisted of a Y-shaped glass tubing, with the two arms (44 cm each) connected to a stem (16 cm). To operate the olfactometer, air was pushed from a vacuum pump (Cole-Parmer Air Cadet, IL, USA) through an activated charcoal filter and split into the arms of the olfactometer at 2.5 l/min per arm. Another vacuum pump pulled the air from the olfactometer through its stem at the same flow rate. Parasitoids were introduced singly in the stem of the olfactometer and allowed 5 min to choose one of the arms. Parasitoids passing the finish line (4 cm past the intersection) and remaining there for more than 15 s were recorded as having made a choice. After five parasitoids were tested, the Y-tube was turned 180° (interchange of positions of the arms) to eliminate the effect of any bias for one of the arms. Between tests, the Y-tube was

cleaned with acetone and then flushed with clean air to minimize contamination with volatiles. Test stimuli included (*Z*)-3-hexenyl acetate (Avocado Research Chemicals Ltd, UK), synthetic (*E*)-β-farnesene (Rothamsted Research, Harpenden, UK) and the essential oil from the plant *Hemizygia petiolata* (Lamiaceae) (Biosys Ltd, Harpenden, UK) comprising 80% (*E*)-β-farnesene and 15% linalool. The essential oil also contained trace amounts of monoterpenes (α-pinene, β-pinene, camphene, 1,8-cineole) and sesquiterpenes including, α-copaene and bourbonene. The test samples were prepared in hexane-paraffin oil (Merck, Germany), 1:1 (v/v) mixture, and were applied to cotton discs (0.5 cm thickness) cut out from dental rolls (Patterson Dental Co., Minneapolis, MN). The solvent was allowed to evaporate for 1 h prior to bioassays. Control discs contained equivalent amounts of hexane and paraffin oil only. The doses tested were 5, 10, 15, 20 and 25 μg/disc. Odours from the parasitoid diet (1.5 g of 20% honey solution) and larval frass (1.5 g of fresh frass obtained after feeding *C. partellus* larvae on fresh maize seedlings for 48 h) were also tested against clean air to serve as positive controls. Larval frass was placed directly into one arm of the Y-tube, behind a double screen mesh, and in separate tests the parasitoid diet loaded on cotton discs (0.5 cm thickness) which were cut out from dental rolls (Patterson Dental Co.), were similarly placed. Tests were conducted at 23–26°C, 65–75% relative humidity and light intensity of 350–450 lux. All tests were replicated four times with 15 parasitoids per replicate. Parasitoids were used once and then discarded.

Field cage bioassays

Field experiments were conducted in large cages (2.5 × 2.5 × 2 m) at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, in the summer of 2000 (November to December) at 24–28°C. The cages were constructed of metal frames covered with fine nylon net (400 μm) to protect experimental maize plants (4–5 weeks old) from insect attack, while preventing released parasitoids from escaping. Three experiments were conducted.

Experiment 1. This experiment tested the efficacy of a sticky trap similar to the Tangle-whitefly trap (Tanglefoot Company, MI, USA), using host plant complex and parasitoid diet (20% honey solution) as lures in separate experiments. The sticky trap consisted of a rectangular white cardboard (21 × 10 × 0.1 cm, h × w × t), covered on one side with a thin film of adhesive material (Tanglefoot Company). On the other side was a clear plastic sheet stapled to the cardboard to protect it from getting soaked with water during bioassays. The host plant complex was prepared by introducing one *C. partellus* larva into a maize stalk (4 cm long) for 48 h in an incubator maintained at 28°C. After this period, the maize stalks with frass were wrapped with a thin wire mesh and then placed 4 cm beneath the centre of the sticky trap. The parasitoid diet was prepared by mixing 20 ml of honey with 80 ml of water and then 1.5 g of this solution was loaded on a 2.0 cm long cotton dental roll plug. The loaded dental roll plug was then glued with a thin film of adhesive (Tanglefoot Company) to the surface of the sticky trap about 4 cm below the midsection of the trap. Control traps had only dental roll plugs of similar length glued to their surface. The baited and unbaited traps were suspended 1 m above ground on wooden poles 1.5 m long, placed in the middle of the maize plants.

These were repeated in six cages. One hundred *C. flavipes* females were released in each cage at 09:00 h and the number captured on the sticky trap was recorded after 8 h at 17:00 h. The test was replicated eight times per cage using fresh bait and females in each test.

Experiment 2. Based on the results in Experiment 1, tests were carried out to determine the optimal number of baited and unbaited traps/cage to be used in subsequent experiments. Since the host plant complex was as attractive as the parasitoid diet, this bioassay was carried out using traps baited with the parasitoid diet since it could be more easily standardized compared to the host plant complex. One, two, four, six and eight baited traps, with the equivalent number of unbaited traps, were tested in the same number of cages as in Experiment 1. One hundred *C. flavipes* females were released in each cage at 09:00 h and the number captured was recorded at 17:00 h the same day. Eight replicates were done.

Experiment 3. The attractiveness of *C. flavipes* females to (*E*)-β-farnesene and (*Z*)-3 hexenyl acetate and a 1:1 blend of the plant essential oil and (*Z*)-3-hexenyl acetate was assessed. Based on the results from the olfactometer assays, 150 μg of each synthetic lure was tested. The lures were first prepared in hexane and then 0.2 mg of the anti-oxidant butylated hydroxytoluene were added to each solution and then transferred into paraffin oil to minimize volatilisation. The solution was loaded on 2.0 cm long cylinders of cotton dental roll and then placed at the centre of the side of the sticky trap with a thin film of adhesive material (Tanglefoot Company). Based on the results in Experiment 2, six baited and six unbaited traps were prepared. The traps were hung 1 m above the ground as described above. Control traps contained similar amount of glue with 2.0 cm long cylinders of cotton dental roll placed 4 cm beneath the centre of the side of sticky trap with a thin film of adhesive material (Tanglefoot Company). One hundred parasitoids were released at 09:00 h in the cages containing 4–5-week-old maize plants. The number of females trapped was counted at 17:00 h. For each test compound, eight replicates were carried out.

Data analysis

For each test, the total numbers of females responding in the olfactometer were pooled across replicates and then analysed using chi-square (PROC FREQ, SAS Institute 1999–2000). Parasitoids that did not respond ('No response' group) were excluded from the analyses.

In cage bioassays involving trapping of *C. flavipes* with traps baited with the parasitoid diet and host plant complex the total number of females trapped were pooled across replicates and then analysed using chi-square (PROC FREQ, SAS Institute 1999–2000).

Numbers of females responding in the cage experiments were arcsine transformed prior to analysis. Analysis of variance (ANOVA) (PROC GLM, SAS Institute 1999–2000) was performed to compare the number of *C. flavipes* trapped using the different lures. Means were separated by Student–Newman–Keul's (SNK) multiple range test when ANOVA were significant ($P < 0.05$).

Results and discussion

Y-tube olfactometer bioassays

In Y-tube olfactometer assays, (*E*)-β-farnesene, (*Z*)-3-hexenyl acetate and the plant essential oil elicited dose-dependent responses from females of *C. flavipes* (Figures 1–3). Significantly more females responded to both (*E*)-β-farnesene and the plant essential oil at 10 μg and 15 μg/disc (70 and 67%, $\chi^2 = 12.79$; $P = 0.001$, $\chi^2 = 18$; $P = 0.001$ for (*E*)-β-farnesene; 63 and 77%, $\chi^2 = 9.98$; $P = 0.002$, $\chi^2 = 32.96$; $P = 0.001$ for the plant essential oil) than to the control discs. (*Z*)-3-Hexenyl acetate elicited strong attractive responses from females at 15 μg/disc ($\chi^2 = 16.86$; $P = 0.001$), but was weakly repellent at 5 μg/disc, while the plant essential oil elicited similar activity at five times this dose. On the other hand, the two positive controls were both attractive to the parasitoid (88%, $\chi^2 = 39.72$; $P < 0.001$ for larval frass and 65%, $\chi^2 = 11.79$; $P < 0.005$ for the 20% honey solution).

Field cage bioassays

Experiment 1. In cage bioassays, traps baited with parasitoid diet and the host plant complex caught similar numbers of parasitoids, with each bait trapping 45% of the *C. flavipes* released in the cages ($\chi^2 = 0.1905$; $P = 0.6625$). The parasitoid diet tested at a specific concentration represented a simpler method to repeat for release of odors than the host plant complex, so we chose to conduct further tests in Experiment 2 with the parasitoid diet.

Experiment 2. Increasing the number of traps per cage increased the trap catches more than 5-fold ($F = 561.96$; df = 4, 235; $P = 0.0001$) (Table I). No significant differences in catches were found between six and eight baited traps per cage (Table I). Control

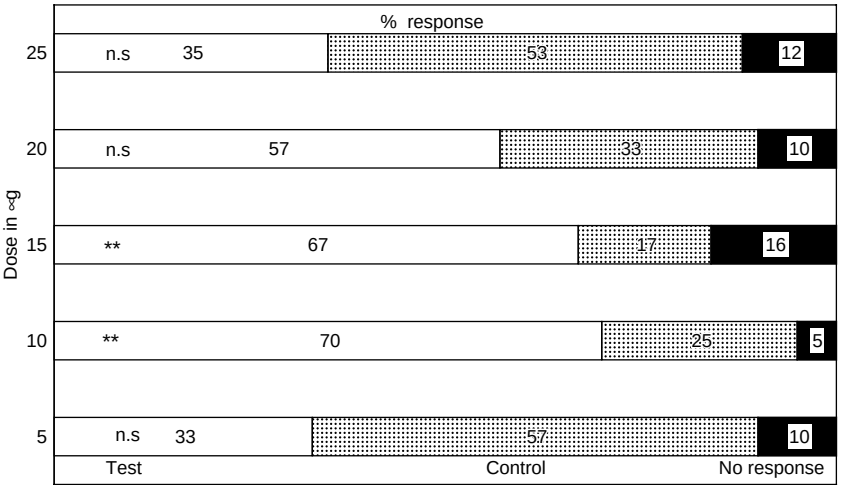

Figure 1. Response of *C. flavipes* to varying doses of (*E*)-β-farnesene in a Y-tube olfactometer. Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n = 60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: $^{**}P < 0.01$; n.s = not significant.

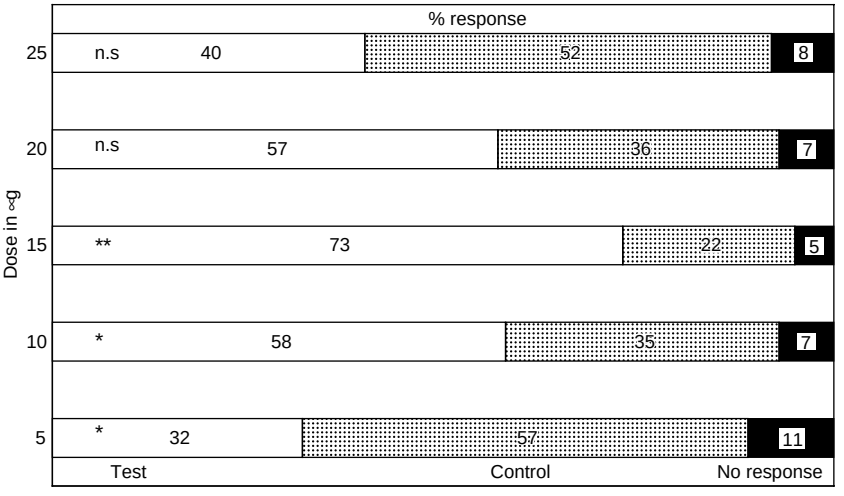

Figure 2. Response of *C. flavipes* to varying doses of (*Z*)-3-hexenyl acetate in a Y-tube olfactometer. Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n = 60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: $^{\star}P < 0.05$; $^{\star\star}P < 0.01$; n.s = not significant.

traps failed to trap any *C. flavipes* in this experiment. The host plant complex was not tested in this experiment as previously explained (see Experiment 2).

Experiment 3. In subsequent bioassays using lures containing either synthetic (*E*)-β-farnesene, (*Z*)-3-hexenyl acetate, the plant essential oil, or the combination

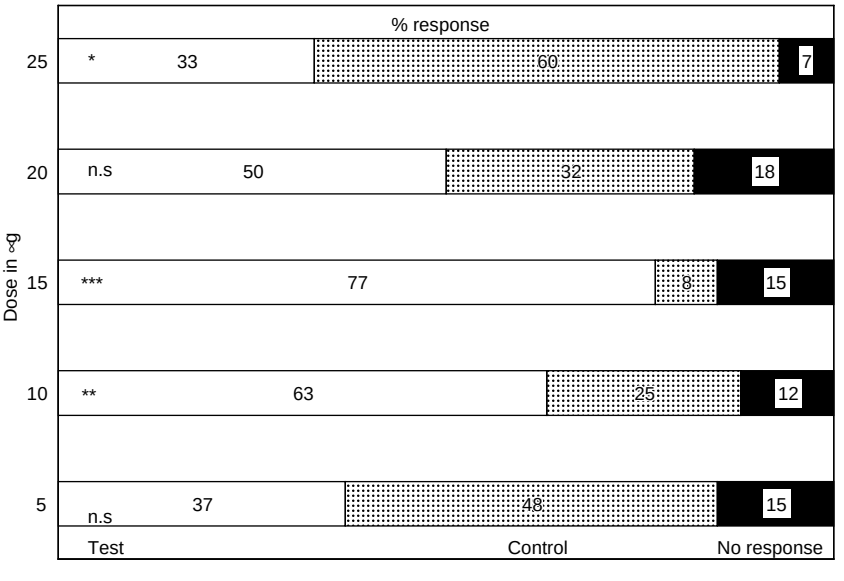

Figure 3. Response of *C. flavipes* to varying doses of the essential oil from *Hemizygia petiolata* in a Y-tube olfactometer. Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n = 60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: $^{\star}P < 0.05$; $^{\star\star}P < 0.01$; $^{\star\star\star}P < 0.001$; n.s = not significant.

(*Z*)-3-hexenyl acetate and plant essential oil, no significant differences in catches were found between traps (Table II). No females were caught in the control traps in any of the tests. Traps baited with the binary blend containing (*Z*)-3-hexenyl acetate and the plant essential oil caught moderately, but significantly more females than traps baited with the individual lures alone (Table II).

The present results using (*E*)-β-farnesene are consistent with previous findings, which reported that feeding by larvae of *Heliothis* or *Spodoptera* induces release of the terpenoid from maize plants, which serves as a kairomone for the parasitoids *Cotesia marginiventris* (Turlings et al. 1991) and *C. kariyai* (Takabayashi et al. 1995). The release of the terpenoid from larval damaged cotton plants may also serve as a kairomone for *C. marginiventris* (Loughrin et al. 1994; Pare et al. 1997). Our behavioural and cage trapping experiments clearly indicated that the plant essential oil comprised of 80% (*E*)-β-farnesene, 15% linalool and 5% of other terpenes was as attractive as (*E*)-β-farnesene alone, suggesting that the other components in the essential oil elicited no significant attractive response from the parasitoid. However, these results are of interest because synthetic (*E*)-β-farnesene has limited use in the field. It is highly volatile and is rapidly oxidized in air (Dawson et al. 1988). Thus, we cannot rule out the possibility that other components present in relatively minor amounts in it could also contribute to its overall activity. It is possible that the presence of these minor components in the essential oil could contribute to the slow release of (*E*)-β-farnesene and also serve as anti-oxidants to reduce inactivation of (*E*)-β-farnesene. They could also contribute to the repellence of the

Table I. The number of *Cotesia flavipes* females caught with different numbers of traps baited with parasitoid diet in field cage bioassays.

No. of traps	*N*	Percentage of *C. flavipes* trapped (mean ± SE)
8	48	57 ± 1.1a
6	48	54 ± 0.6a
4	48	35 ± 1.0b
2	48	19 ± 0.9c
1	48	10 ± 0.5d

Numbers followed by the same letter in a column are not significantly different (SNK); $F = 597.93$; df = 4, 235; $P = 0.0001$; N = number of parasitoids tested.

Table II. The number of *Cotesia flavipes* caught using different lures in field cage bioassays.

Compound	*N*	Percentage of *C. flavipes* trapped (mean ± SE)
(*E*)-β-Farnesene	48	53 ± 0.8b
Essential oil from *Hemizygia petiolata*	48	51 ± 0.8b
(*Z*)-3-Hexenyl acetate	48	51 ± 0.7b
(*Z*)-3-Hexenyl acetate + essential oil (1:1)	48	57 ± 0.9a

Numbers followed by the same letter in a column are not significantly different (SNK); $F = 11.28$; df = 3, 188; $P = 0.0001$; N = number of parasitoids tested.

essential oil when tested at the high dose of 25 μg/disc in the olfactometer. Our bioassays also indicate that the attractiveness of the essential oil could be enhanced moderately but significantly, by addition of the green leaf volatile (*Z*)-3-hexenyl acetate, which when tested alone at the low dose of 5 μg/disc, weakly repelled the parasitoid. Such a reversal in behavioural response to different doses of a compound by insects is not uncommon in bioassays (Torto et al. 1991). Given the possible attributes of the plant essential oil, its exploitation as a promising kairomone substitute for synthetic (*E*)-β-farnesene for field trapping of *C. flavipes* will require further research.

Previous studies have reported (*E*)-β-farnesene as a semiochemical mediating a range of insect/plant and insect/insect interactions. When released by maize plants, it serves as a kairomonal oviposition stimulant for the European corn borer, *Ostrina nubilalis* (Binder et al. 1995). It also serves as an alarm pheromone for a number of aphid species (Bowers et al. 1972; Edwards et al. 1973) and a prey finding kairomone for the seven-spot ladybird, *Coccinella semptempunctata* (Al Abassi et al. 2000) and for many carabid beetles (Kielty et al. 1996). On the other hand, (*Z*)-3-hexenyl acetate is a known constituent of mechanically damaged plants (Tumlinson et al. 1992; Agelopoulos & Pickett 1998) and stem-borer infested maize seedlings (Ngi-Song et al. 2000).

In summary, the present study has shown that there may be potential in exploiting plant essential oils rich in (*E*)-β-farnesene for use in developing cheap, inexpensive methods for monitoring *C. flavipes* populations in maize fields. One issue that will have to be resolved prior to field application is specificity. If many different species of insects are attracted to the lure, the amount of time required to separate *C. flavipes* from other species may limit the value of synomone-baited traps. From a practical standpoint of integrated pest management for the resource poor farmer, growing the plant or a related plant that emits significant levels of (*E*)-β-farnesene as an intercrop among maize plants in the field could increase attraction of the parasitoid to control stem borer larval populations.

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