



BEHAVIOURAL RESPONSE OF THE PARASITOID *COTESIA FLAVIPES* TO HERBIVORE INDUCED VOLATILES IN SWEET SORGHUM

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ABSTRACT

Plants emit a variety of compounds in response to an attack by herbivores. Herbivore-induced plant volatiles (HIPVs) mediate interactions between plants and natural enemies. Volatiles were collected from sweet sorghum plants oviposited by *Chilo partellus* Swinhoe and the response of the parasitoid *Cotesia flavipes* Cameron to these volatiles were tested in four-arm olfactometer. *Cotesia flavipes* spent significantly more time (6.52 ± 0.72 min, $p = 0.0000$) in arm treated with *C. partellus* oviposited plant volatiles compared to untreated control (3.17 ± 0.19 min). These compounds were identified by GC-MS as octanal, decanal, nonanal, 6-methyl 5 heptanone and caryophyllene. Nonanal and decanal were 8.46 and 4.66%, respectively in plants with *Chilo* eggs, whereas in the control plants, it was 5.48 and 1.39%, respectively. The behavior of parasitoid towards HIPVs enhances the development of sustainable IPM strategies by manipulating the foraging behaviour of parasitoids.

Key words: Sweet sorghum, spotted stem borer, oviposition, air entrainment, volatiles, nonanal, caryophyllene, decanal, parasitoid, attraction, olfactometer, behavioural assay

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is an annual, C4 crop with sugar-rich stalks and characterized by a high photosynthetic efficiency (Lingle et al., 2012; Murray et al., 2009; Smith et al., 1987). It provides both grain and stem which will be used for sugar, alcohol, syrup, jaggery, fodder, fuel, etc.; and there are about 4,000 sweet sorghum cultivars (Rutto et al., 2013). Crop productivity is severely affected due to stem borer species observed as serious pests in Asia and Africa, of which the spotted stem borer (*Chilo partellus* Swinhoe) is the most destructive. It occurs throughout the crop growth and development, both in Asia and Africa. Within the semiarid tropics alone it causes US\$ 334 million annual loss to sorghum (Sharma, 2006). The major components of IPM are cultural practices, insecticides, biological control and host plant resistance. Chemical control is expensive and often beyond the reach of resource poor farmers. Insecticides are also ineffective for stem borer control because larvae bore into the shoot and pupate within which makes them hard to target (Khan et al., 2000). Under such circumstances, host plant resistance is the best method, and considerable progress has been made in developing techniques to screen for resistance and in identifying mechanisms of resistance. However, to date stem borer resistance has not yet been bred into high yielding cultivars. Stem borer larvae have a broad range of natural enemies which are able to locate and attack the larvae that feed inside the plant tissue. These

biological control agents are successful based on their efficiency to search and locate target hosts (Nordlund et al., 1988).

Plants have evolved sophisticated defense mechanisms which protect against insect attack. They respond to insect attack by releasing a blend of volatiles that serve as foraging cues for parasitoids. Parasitoids use volatile compounds released by insect herbivore-damaged plants to locate their hosts. These volatiles can be exploited to attract parasitoids to improve biological control in the field. Volatile chemical compounds from the host plant and the herbivores, or the interaction of the two, play an important role (Dicke, 1994), and to locate their hosts during foraging parasitoids utilize volatiles cues (Finidori-Logli et al., 1996; Potting et al., 1995; Steinberg et al., 1993; Vet and Dicke, 1992). The host plant volatile profile thus plays a key role in attracting or repelling or retaining the natural enemies (Vinson, 1975; Gohole et al., 2003). In tritrophic systems consisting of plants, herbivorous arthropods and their carnivorous natural enemies, carnivores are attracted to volatile compounds emitted by plants infested by herbivores (Takabayashi and Dicke, 1996; Arimura et al., 2009). Plant odours are used as cues by the parasitoids and predators (Vinson, 1976, 1981; Nordlund et al., 1988). These odours are inducible and only released after damage by herbivores and are termed as herbivore induced plant volatiles (HIPVs).

The quantity and the composition blend of compounds emitted by plants vary with the herbivore, the plant species and the genotype. The compositions of these volatiles are specific, depending on the species and cultivars (Takabayashi and Dicke, 1996; Arimura et al., 2009). These herbivore species-specific HIPVs facilitate the location of host-infested plants by parasitoids (Vet and Dicke, 1992); and their production is triggered on feeding damage by herbivorous larvae. Plant responses to an earlier stage of insect attack (egg deposition) (Colazza et al., 2004; Hilker and Meiners, 2006; Bruce et al., 2009; Tamiru et al., 2011). Defenses elicited due to eggs benefit plants as they enable defense to be switched on early, before leaf or stem damage is caused by larvae (Hilker and Meiners, 2006; Bruce et al., 2009). The tritrophic interactions presents an opportunity for development of cost effective and environmentally benign IPM approaches. Herein, role of chemical cues emanating from the sweet sorghum plants oviposited by *C. partellus* in the acceptance of host by searching parasitoid *C. flavipes* has been explored.

MATERIALS AND METHODS

Sweet sorghum plants (cv. 'Wray') were grown individually in pots in a nethouse. The plants were grown in plastic pots (13x 14 cm dia). Plants at the stage of five fully grown leaves were used for the volatiles collection. Plants were introduced into the cages with six *C. partellus* females and removed 24 hr later. On an average, 12 eggs per 5 plants were laid. Sweet sorghum plants with eggs were used for the collection of volatiles. Intact plants were used as controls. The *C. partellus* was maintained in the laboratory on semisynthetic diet under controlled conditions (27°C, 70% RH, 12:12 light-dark photoperiod) as described by Padmaja et al., 2012. The parasitic wasp, *C. flavipes* was reared on larvae of *C. partellus*, with the appropriate stages removed from artificial diet and fed for 24 hr on pieces of sorghum stem for acclimatization; larvae were exposed to 24 hr old mated *Cotesia* females for oviposition using the hand-stinging method; only one stinging was allowed/ larva and adult parasitoid. Parasitized larvae were placed individually in a glass vial containing a sorghum stem piece, and plugged with cotton; and these vials incubated at 27°C, at 70% RH, and 12L: 12D photoperiod for the parasite emergence. Volatiles from the sweet sorghum cultivar "Wray" grown individually in pots in a nethouse were entrained. Multiple collections were made with portable equipment that allows sampling of volatiles, for 21 days after seedling emergence, the most susceptible stage

(Padmaja et al., 2010). Aliquots of attractive headspace samples were analyzed on a Gas Chromatography (GC) - Agilent technologies 7890A system equipped with 7000 GC-MS Triple quad with column (DB - 5 MS, 30 m length, 0.25 mm i.d., 0.50 µm film thickness) directly coupled to a mass spectrometer. The oven temperature was maintained at 30°C for 5 min, rate 5°C/min to 250°C hold for 11 min. Compounds were identified by comparison of retention indices and mass spectra with those of authentic standards 6-methyl 5 heptanone, octanal, decanal, nonanal and caryophyllene obtained from Sigma Aldrich. Responses of parasitoids to plant derived volatiles were tested in a perspex four-arm olfactometer (Padmaja et al., 2010). Air was drawn through the four arms towards the center at 260 ml min⁻¹. Headspace samples (10 µl) were applied to a piece of filter paper with a micropipette and placed in an inlet port at the end of each olfactometer arm; and freshly emerged *C. flavipes* were transferred individually into the central chamber, and time spent in different regions was compared. A choice test to compare insect responses to headspace samples from oviposition induced and control (unexposed) plants was carried out by placing the test stimuli (10 µl aliquots of headspace sample) in two opposite arms. The other two arms contained filter paper with 10 µl diethyl ether, and were used as controls. Time spent in each region was recorded. Ten replicates were carried out. A paired-sample t-test was employed to analyze the differences between the time spent by *C. flavipes* in each arm of the olfactometer.

RESULTS AND DISCUSSION

Coupled gas chromatography-mass spectrometry revealed that the sweet sorghum genotype 'Wray' emitted more volatile compounds when exposed to *C. partellus* eggs compared to unexposed plants (Fig. 1). Major components that have been identified in both plant categories were 6-methyl 5 heptanone, octanal, decanal, nonanal and caryophyllene. Nonanal being the most abundant volatile compound emitted both in intact and in *C. partellus* oviposited plants. Significant differences in the abundance of nonanal, decanal, caryophyllene and octanal were observed in the headspace profile between intact plants and plants with *C. partellus* eggs. More specifically, nonanal and decanal were 8.46 and 4.66%, respectively, in plants with *Chilo* eggs, whereas in the control plants, it was 5.48 and 1.39%, respectively (Table 1). Female *C. flavipes*, spent significantly more time in the region with volatiles from 'Wray' exposed to oviposition by

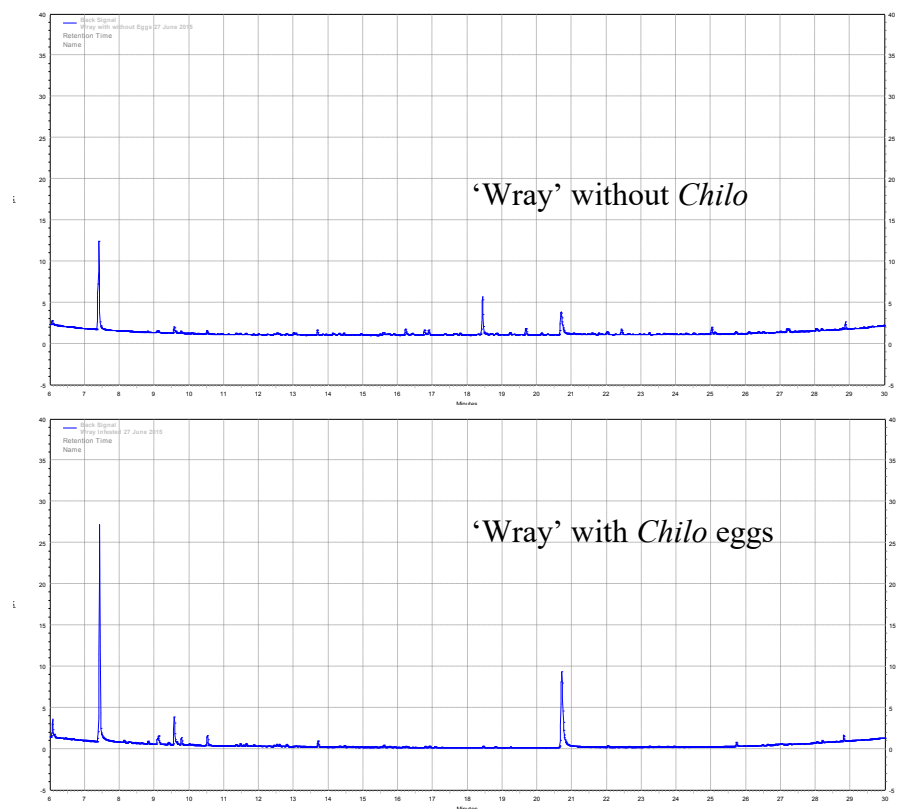


Fig. 1. Gas chromatogram (GC) traces of compounds in headspace samples of sweet sorghum

Table 1. Identification of volatile compounds (GC-MS) in sweet sorghum cultivar ‘Wray’

Peak	Without <i>Chilo</i> eggs			Peak	With <i>Chilo</i> eggs		
	RT	Area %	ID of compound		RT	Area %	ID of compound
1	16.938	2.08	6-methyl 5 heptanone	1	16.928	3.61	6-methyl 5 heptanone
2	17.614	1.16	Octanal	2	17.604	3.33	Octanal
3	21.014	5.48	Nonanal	3	21.004	8.46	Nonanal
4	24.201	1.39	Decanal	4	24.153	4.66	Decanal
5	30.102	2.28	Caryophyllene	5	30.092	2.84	Caryophyllene

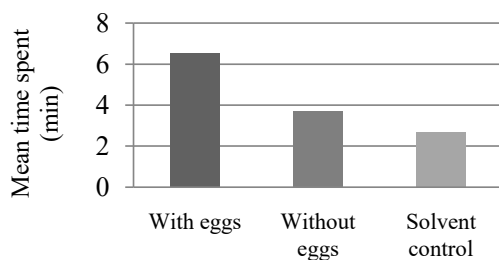


Fig. 2. Behavioural response of *C. flavipes* in a four-arm olfactometer bioassay to volatiles collected from sweet sorghum (Wray) plants (n=10)

C. partellus, compared to regions with unexposed and blank controls ($p < 0.0000$; $df = 9$) (Fig. 2). The increase in time spent is a positive response indicating that

attraction and arrestment of parasitoids increased. A number of studies have shown that OIPVs serve as cues for foraging parasitoids (Hilker and Fatouros, 2015; Colazza et al., 2004; Tamiru et al., 2012). Although it was first considered as a plant's response to wound oviposition (Hilker and Fatouros, 2015), later studies have shown that oviposition itself is responsible for the induction of qualitative and quantitative changes in the volatile profile of egg infested plants (Tamiru et al., 2011; Anastasaki et al., 2015). Plants benefit by an early activation of defense mechanisms by egg deposition, which enhances their defense before any damage can occur (Hilker and Fatouros, 2015; Bruce et al., 2009). The behavioral response of egg parasitoid *Trichogramma achaeae* females to HIPVs produced by tomato plants

infested with *Tuta absoluta* eggs or larvae in tomato when studied revealed that oviposition and larval feeding by *T. absoluta* significantly enhanced HIPV emission. The analysis of volatiles released by tomato plants, either infested or uninfested, coupled with the response of *T. achaeae* in the olfactometer tests was consistent with what was expected in terms of the foraging behavior of a generalist parasitoid (Gontijo et al., 2019).

The present study demonstrated quantitative variations in volatiles in sweet sorghum cultivar 'Wray' when *C. partellus* eggs were laid; and a preference was observed in an olfactometer bioassay of *C. flavipes* parasitoids for volatiles from plants exposed to egg deposition (Fig. 2). Attraction of larval parasitoids means that natural enemies can also attack newly hatching larvae. Volatiles emitted from apple leaves infested by two-spotted spider mite *Tetranychus urticae* attracted the *P. persimilis* and *Metaseiulus occidentalis* (Sabelis and Van de Baan, 1983). Upon infestation by *T. urticae*, Lima bean plants emitted a blend of volatiles attracting the predatory mite *P. persimilis* (Dicke et al., 1990 a,b). Corn plants damaged by *Spodoptera exigua* emitted volatiles that attracted the parasitoid *Cotesia marginiventris* Cresson (Turlings et al., 1990). Several behavioral and electrophysiological studies had revealed the attractiveness of HIPVs to predators (Drukker et al., 1995; Zhang et al., 2009; Zhang et al., 2012) and parasitoids (Turlings and Tumlinson, 1992; Yu et al., 2008; Yu et al., 2010). Maize plants under attack by larvae of *S. littoralis* attracted *C. marginiventris* and *Campoletis sonorensis* Cameron which resulted in higher parasitization and reduced feeding and weight gain of the host larvae (Hoballah and Turlings, 2001). Parasitized larvae attacked plants produced 30% more seeds than plants attacked by unparasitized larvae. Insect parasitoids are known to utilize the different volatile profile of infested plants vs non-infested plants to detect their hosts and prey. The present study is one of the first in which the egg induced volatile emission effect is studied in sweet sorghum. It is proved that the parasitoid *C. flavipes* responds to oviposition-induced volatiles released by the plants after oviposition by a herbivore. HIPVs provide parasitoids with early alert cues to enhance their foraging efficacy.

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