

## RESPONSE OF *GONIOZUS INDICUS* (HYMENOPTERA: BETHYLIDAE) TO SUGARCANE AND *CYPERUS PAPYRUS* VOLATILES

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### Abstract

Gas chromatography revealed different volatile emissions from uninfested and infested sugarcane, and uninfested *Cyperus papyrus* L, when compared with infested *C. papyrus*. In addition, *Goniozus indicus* Ashmead showed an attraction to samples containing frass from *Eldana saccharina* Walker that had fed on *C. papyrus*, and no attraction to samples containing frass from *E. saccharina* that had fed on sugarcane. These results indicate that chemical cues are important for *G. indicus* to locate a host habitat, and that the cues may be missing from *E. saccharina* infested sugarcane.

**Keywords:** gas chromatography, SPME, chemical cues, parasitoids, *Cyperus papyrus*, *Eldana saccharina*, *Goniozus indicus*

### Introduction

*Eldana saccharina* Walker (Lepidoptera: Pyralidae) is indigenous to South Africa, living in *Cyperus papyrus* L (Atkinson, 1979). Here it is successfully parasitized by nine indigenous parasitoids including *Goniozus indicus* Ashmead (Hymenoptera: Bethyridae) (Conlong, 1990). It successfully moved into sugarcane in the 1940s (Carnegie, 1974), and has become increasingly abundant throughout the industry (Atkinson and Nuss, 1989). In contrast to the situation in its indigenous plant hosts, negligible parasitism has been recorded on any of its life stages in sugarcane (Conlong and Hastings, 1984). It is hypothesised that there may be chemical cues missing in the sugarcane habitat, which indigenous parasitoids, such as *G. indicus*, use/need to find their hosts (Conlong and Kasl, 2001).

### Materials and Methods

Volatiles were collected from infested and uninfested *C. papyrus* and sugarcane variety N11 using two methods: static and dynamic headspace collections. For both methods, aerial portions of plants were enclosed in glass jars (length 20 cm, diameter 7 cm) and sealed with aluminium foil (Paré and Tumlinson, 1997). For static headspace collection, a solid phase micro extractor (SPME) was injected through the aluminium foil into the glass jar, for the collection of the volatiles (Rohloff, 1999). For dynamic headspace collection, air was drawn through the glass jar, using a vacuum pump, and over Supelpack 2 adsorbent resin that was packed into a glass vial (length 6 cm, diameter 7 mm) (Gouinguéné *et al*, 2001; Turlings *et al*, 1998). Volatiles were eluted from the resin using 10 µl of dichloromethane (Paré *et al*, 1998; Tooker *et al*, 2002).

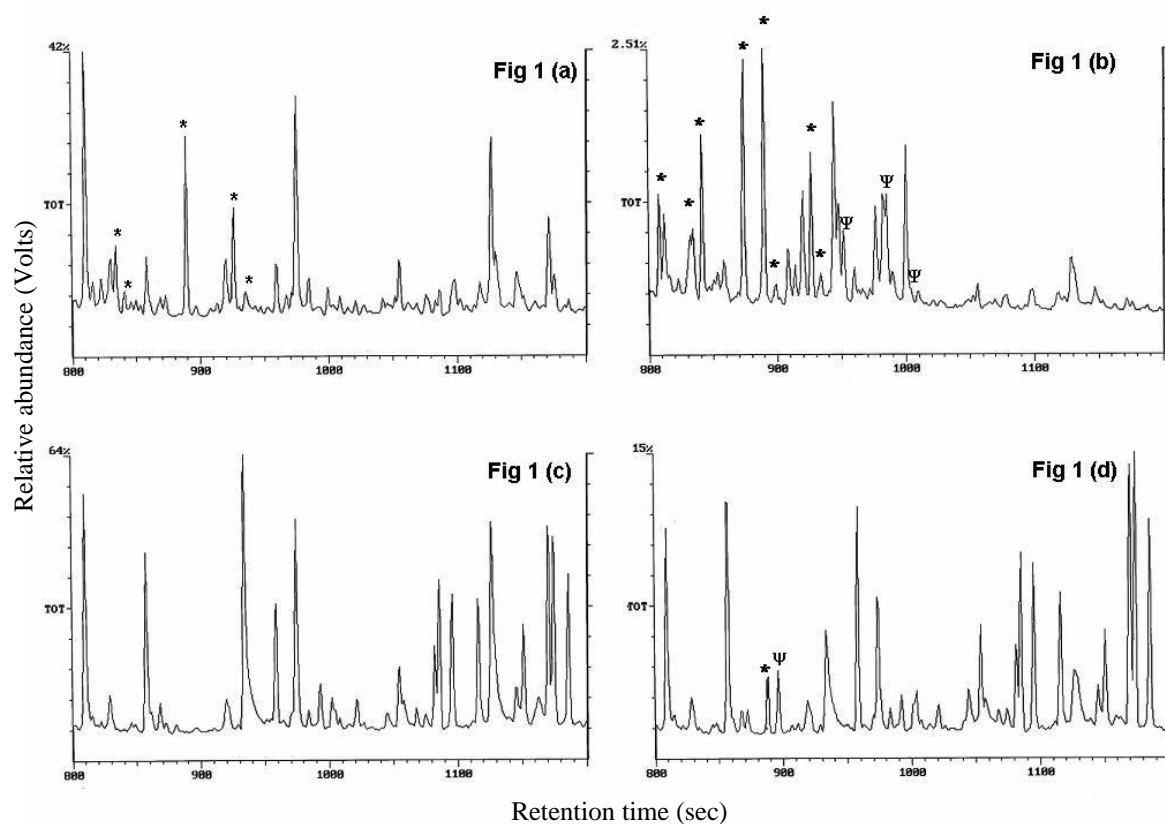
Volatiles were also eluted from frass found on infested *C. papyrus* and sugarcane variety N11 by soaking the frass in dichloromethane (Hibbard *et al*, 1997). The volatiles dissolved in the dichloromethane were then used for GC analysis and behavioural bioassays. Volatile analysis was completed using a Varian ion gas chromatograph (GC). SPME were injected directly into the GC, and extracts were injected using a calibrated syringe.

A four-way olfactometer (Vet *et al*, 1983) was used to determine behavioural responses of *G. indicus* to different plant/host odours. Odours tested included frass from *E. saccharina* infested *C. papyrus*; *C. papyrus* wash; *C. papyrus* frass extract, extract from an *E. saccharina* infested *C. papyrus* umbel, extract from an uninfested *C. papyrus* umbel on an *E. saccharina* infested plant, frass from *E. saccharina* infested sugarcane and cane wash. Washes were made by soaking portions of plants in dichloromethane. Odours were presented to *G. indicus* by placing 30  $\mu$ l of extract on glass filter paper, and into a glass jar connected to an arm of the olfactometer via a silicon pipe. One odour was presented at a time, the other three arms being blank. The response of four insects per odour was recorded. Insects used were 3-10 day old, naive mated females ready for oviposition.

## Results

### 'Long-range' attractants

Quantitative and qualitative differences in emissions from uninfested *C. papyrus* umbels compared with *E. saccharina* infested *C. papyrus* umbels (Figures 1a and 1b respectively), and between *E. saccharina* infested *C. papyrus* umbels compared with infested sugarcane (Figures 1b and 1d). Not much difference was notable between volatiles of *E. saccharina* infested and uninfested sugarcane (Figures 1c and 1d).

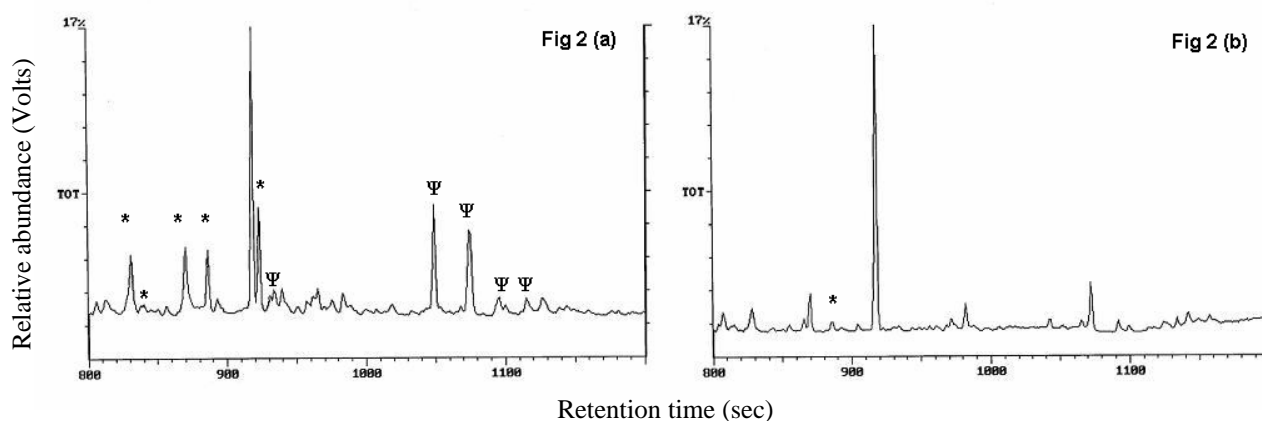


**Figure 1. Gas chromatograms of volatiles released by (a) uninfested *C. papyrus*, (b) infested *C. papyrus*, (c) uninfested sugarcane variety N11 and (d) infested sugarcane variety N11 (\*=common compounds; Ψ=unique compounds).**

The most notable differences were in the compounds in the middle of the volatile profile (Figure 1). These compounds have not yet been identified; however, now that important differences between volatiles have been noted and compounds of interest identified, they can be named using a GC -mass spectrometer.

#### 'Short-range' attractants

GC analysis of frass extracts collected from *E. saccharina* infested *C. papyrus* and sugarcane variety N11 plants (Figures 2a and 2b), show different compounds present, and that compounds common to the two samples are found in varying quantities.



**Figure 2. Chromatograms of volatiles released by frass of *E. saccharina* feeding on (a) *C. papyrus* and (b) sugarcane variety N11 (\*=common compounds; Ψ=unique compounds).**

#### *Goniozus indicus* behaviour to host plant volatiles

In the four-way olfactometer, 75% of the *G. indicus* females tested showed an attraction to (i) infested *C. papyrus* umbel extract, (ii) *C. papyrus* frass extract and (iii) *C. papyrus* frass. The other 25% made no choice or chose a blank arm of the olfactometer. There was no response to the other four odours.

### Discussion

#### 'Long-range' attractants

Howse *et al* (1998) showed that plant volatiles are used by parasitoids as long-range signals in host finding. This allows them to find their hosts' habitat first. It was hypothesised that such 'cues' were missing from sugarcane. Preliminary results show that this assumption may be correct, as *E. saccharina* infested *C. papyrus* umbels emitted a very different volatile profile to uninfested *C. papyrus*, and, more importantly, was also vastly different to *E. saccharina* infested N11 sugarcane. As parasitoids have been reported to be able to recognise their hosts' environment, regardless of whether the host was present or not (Ananthkrishnan, 1992), it is unlikely that *G. indicus* would be attracted to sugarcane when the cues it releases are so different from those to which the parasitoid is accustomed. Further, infested sugarcane does not release a vastly different volatile profile from uninfested sugarcane, therefore parasitoids may not be able from a distance to identify host locations. The paucity of parasitoids reported from sugarcane (Conlong and Hastings, 1984) bears testimony that this may be the case.

### *'Short-range' attractants*

Should parasitoids be able to find their host's habitat, they still have to be able to locate their host within this habitat. To do this, they use short-range attractants (Geervliet *et al.*, 1994). It is known that *G. indicus* uses frass for short-range location of hosts (Smith *et al.*, 1994). Results presented show very few common compounds within the volatiles emitted from frass of *E. saccharina* feeding on *C. papyrus* and N11 sugarcane, and that compounds in N11 frass occur in very small amounts. Signals used by parasitoids to identify their hosts need to be clear enough to be distinguished from 'background noise'. Most behaviourally attractive volatile compounds are produced at higher levels than usual (Turlings *et al.*, 1995). It is possible that the amounts of compounds in the volatiles released by N11 frass are too small to be attractive to the parasitoids. Further, Mattiacci *et al.* (1999) concluded that, although a host specific kairomone is present in the frass, independent of the host food source, plant cues must play a role in the short-range host location. Thus, the absence of the usual habitat of the parasitoid host (*C. papyrus*) may render the N11 frass unattractive to *G. indicus*.

### *Goniozus indicus behavioural responses*

Often compounds within different volatiles are common, however, the specificity of the information conveyed to the parasitoids through these 'cues' is achieved through variation in the quantity and/or quality of the volatile signal (Ngi-Song *et al.*, 2000). Olfactometer bioassays show that *G. indicus* responds to the quantitative and qualitative differences between the volatile emissions seen on the GC traces. Only the extract from infested *C. papyrus* umbels, the frass, and the frass extract of *E. saccharina* feeding on *C. papyrus* were found to be behaviourally attractive. In the olfactometer, *G. indicus* was not attracted to frass collected from *E. saccharina* that had fed on sugarcane variety N11. This is in contrast to the findings of Smith *et al.* (1994), who showed *G. indicus* to respond to the sugarcane frass. A possible explanation for this is that he used insects that had previously been exposed to sugarcane frass (Grasswitz, 1998). Increased responsiveness of parasitoids to innately recognised cues is possible if they have previously been exposed to them (Lewis *et al.*, 1975, Vet and Groenewold, 1990, Steinberg *et al.*, 1992).

Results at this stage of the study imply that, even if parasitoids such as *G. indicus* were to be released in an *E. saccharina* infested sugarcane field, they may be unable to locate their hosts, unless they had been previously exposed to frass of sugarcane feeding *E. saccharina* and had learnt to identify the volatiles from this frass as a short range, host finding cue (Grasswitz, 1998).

### **Conclusion**

Preliminary GC results show very different chemical volatile profiles from an *E. saccharina* infested indigenous host plant (*C. papyrus*) and sugarcane. These differences can have important implications for parasitoid host habitat finding. In addition, differences in volatiles from their host frass feeding on different host plants, could further limit parasitoid effectiveness in its short-range host finding ability. Preliminary olfactometer studies indicate that *G. indicus* only responds to volatiles produced by its indigenous host plant, *C. papyrus*, when infested by *E. saccharina*, and shows no response to volatiles produced from *E. saccharina* infested sugarcane. Thus, by combining chemical (GC) studies with behavioural (olfactometer) assays, this study has enabled a better understanding of the relationship between plants, pests and parasitoids.

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