

SHORT COMMUNICATION

**IDENTIFICATION OF HERBIVORE INDUCED PLANT VOLATILES FROM PUSH-PULL PLANTS AND *FUSARIUM* SPECIES: AIDS FOR THE MANAGEMENT OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) IN SUGARCANE?**

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**Abstract**

The stalk borer *Eldana saccharina* continues to be the most serious pest in the sugarcane industry and research into integrative pest management strategies is ongoing. This study investigates the impact of plant and fungal derived volatiles on the behaviour of *E. saccharina* in sugarcane. The push plants chosen for this study were the grasses *Melinis minutiflora* and *Brachiaria humidicola*. Plant entrainment studies and analyses of compounds released have shown that the volatiles 4,8-dimethyl-1,3,7-nonatriene (DMNT), hexenyl acetate,  $\beta$ -caryophyllene, methyl salicylate and 4,8,12-trimethyl-1,3,7,11 tridecatetraene (TMTT) are common to both grasses. These volatiles are well recognised plant stress signals, which are usually released by a plant as a consequence of insect herbivory. However, *M. minutiflora* and *B. humidicola* are emitting volatiles without incurring any insect damage. In addition to plant volatiles, it appears that endophytic fungal volatiles are also effective repellents and attractants of *E. saccharina*. In previous behavioural experiments, first instar larvae have found *Fusarium sacchari* to be repulsive and *Fusarium pseudonygamai* attractive. Both *Fusarium* species emit the volatiles 4-heptanone; 1,2,3-trimethylbenzene; 2-pentyl furan; t-butyl isobutyl ketone; 4-ethyl-3-methyl phenol; 6-methyl-3,5-heptadiene-2-one; 4-ethyl-1,2-dimethoxy benzene; spiro[4.5]dec-7-ene; 1H-3a,7-methanoazulene. In addition to these volatiles, *F. pseudonygamai* produces 3-nonanone and *F. sacchari* emits styrene.

*Keywords:* push-pull plants, endophytic fungi, volatiles, insect chemical ecology, *Eldana saccharina*

**Introduction**

The sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is indigenous to Africa, and well established populations have been reported throughout much of sub-Saharan Africa (Conlong, 2000). Although *E. saccharina* has been found to feed on plants that originate from four different families, namely Cyperaceae, Poaceae, Typhaceae and Juncaceae, wetland sedges and grasses make up a large proportion of its natural host plants (Conlong et al., 2007). *E. saccharina* has been the major pest in the sugarcane industry since 1970 and is now a cause for serious concern over much of sugarcane growing areas of South Africa (Webster et al., 2009). As part of the integrative pest management strategy against *E. saccharina* in sugarcane (Conlong and Rutherford, 2009), the authors are investigating an

approach that involves the use of push-pull plants (Kasl, 2004; Barker *et al.*, 2006) and endophytic fungi (McFarlane and Rutherford, 2005, 2006) to protect the sugarcane crop from this pest. Following on from these studies, Smith *et al.* (2006) provided the first indications that chemical ecology was important in the tritrophic relations of *E. saccharina* by showing that volatiles emitted by sugarcane and *Cyperus papyrus* damaged by *E. saccharina* were very different, and that an indigenous parasitoid, *Goniozus indicus* Ashmead (Hymenoptera: Pyralidae), responded only to the volatiles emitted by damaged *C. papyrus*. The present study aims to develop the chemical ecology aspect further, by identifying the actual volatiles involved in influencing insect behaviour, especially that of *E. saccharina*, and determining how behaviour is influenced.

### Materials and Methods

The grasses *Melinis minutiflora* and *Brachiaria humidicola* were grown in 2 L pots filled with potting mix and maintained under the glasshouse conditions (20-27°C) of the Natural Resources Institute (University of Greenwich) in Kent. Volatiles were collected from 2 month-old plants. An oven bag was secured over leaves and stems of each grass. Charcoal filtered air passing through Tygon tubing (internal diameter 3 mm) entered the base of the bag at a rate of 700 ml/min. Porapak Q filters were prepared by first breaking off a 40 mm tip from a 150 mm long glass Pasteur pipette. Glass wool was inserted into the base of the pipette, which was then filled with 0.2 g of Porapak Q granules. Glass wool was also inserted into the body of the pipette to keep the Porapak Q granules in place. Plant volatiles were collected over a 24 h period onto Porapak Q filters that were inserted on the top two ends of the bag (Figure 1). The flow rate measured over the Porapak Q filters were 300 ml/min for each filter. A plant entrainment kit developed by Rothamsted International was used for the collection of volatiles from plants in the glasshouse.

Moistened maize kernels (100 g kernels and 70 ml distilled water) were autoclaved (121°C, 1.5 kg/cm<sup>2</sup>). Maize kernels were inoculated with cultures of *Fusarium pseudonygamai* (SC17) and *Fusarium sacchari* (PNG40) and incubated in the dark (27°C) for two weeks. Fungal cultures were grown in 250 ml conical flasks. A condenser filled with charcoal was inserted into the mouth of the flask. The Porapak Q filter was inserted into a side-arm extension that was connected to the flask. A low vacuum pump was connected to the filter and used to draw air (300 ml/min) through the charcoal filled condenser over the fungal culture in the flask and out through the Porapak Q filter. Fungal volatiles were collected onto Porapak Q filters over a 24 h period.

The plant and fungal volatiles were extracted from the Porapak Q filter using 1 ml of dichloromethane. An Agilent Mass Spectrometer (MS) with a non-polar column (J&W DB5) and a Varian MS with a polar column (J&W DBWAX) were used to identify the plant and fungal volatiles. The film thickness (30 m x 0.25 mm internal diameter x 250 µm) for both the polar and non-polar columns was the same.

### Results and Discussion

The plant volatiles DMNT; (Z)-3-Hexenyl acetate; β-Caryophyllene; Methyl salicylate and TMTT were common to both *M. minutiflora* and *B. humidicola* (Table 1). These volatiles are normally emitted by a plant when it is under herbivorous attack by insects or when it

undergoes mechanical damage (James, 2003; Kost and Heil, 2006). In this study it was determined that these herbivore induced plant volatiles (HIPVs) are emitted by undamaged *M. minutiflora* and *B. humidicola* plants, and confirmed the findings of Khan *et al.* (1997) for the former grass. These push plants that are not under herbivorous attack are producing HIPVs. It is suggested that most sugarcane varieties have lost the ability to produce HIPVs, or ‘SOS volatiles’, and appear unable to repel *E. saccharina* or attract parasitoids. Barker *et al.* (2006) showed that intercropping sugarcane fields with *M. minutiflora* resulted in a 50% reduction in *E. saccharina* populations and a 57% decrease in stalk damage. In other work, Gohole *et al.* (2003) demonstrated that the cereal stem borer parasitoid, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) is attracted by the volatiles emitted by *M. minutiflora*. Conlong and Kasl (2001) demonstrated that parasitism of *E. saccharina* pupae by the parasitoid *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) was doubled in cages where sugarcane plants seeded with pupae were in the presence of *M. minutiflora* compared to control sugarcane cages. If the push-plant *M. minutiflora* can effectively repel *E. saccharina* from sugarcane while also attracting parasitoids, then the use of this push plant would be extremely beneficial to the sugar industry for the control of *E. saccharina*. Identification of plant volatiles that are either attractive or repulsive to *E. saccharina* is important, as synthetic blends of these volatiles can be used in traps to reduce borer populations or to repel this pest from the sugarcane crop.



**Figure 1. Collection of volatiles from (A) undamaged *Melinis minutiflora* plants, and (B) maize kernels that were inoculated with *Fusarium pseudonygamai* (SC17) and *Fusarium sacchari* (PNG40).**

Some endophytic fungi produce volatiles and secondary metabolites that are attractive to insects, which in turn vector the fungi into other plants when it feeds. For example, volatiles from *Fusarium verticilloides* are attractive to the Nitidulid beetle (Coleoptera) (Bartlett and Wicklow, 1999). A similar mechanism appears to be evident for *E. saccharina* as it is strongly attracted to *Fusarium pseudonygamai*, an endophytic fungus present in sugarcane (McFarlane *et al.*, 2009). Furthermore, in that study a second *Fusarium* strain (*F. sacchari*) was identified that produced volatiles that were repellent to *E. saccharina* first instar larvae. Volatiles have been identified from both *F. pseudonygamai* and *F. sacchari* in this study and include 4-heptanone; 1,2,3-trimethylbenzene; 2-pentyl furan; t-butyl isobutyl ketone; 4-ethyl-

3-methyl phenol; 6-methyl-3,5-heptadiene-2-one; 4-ethyl-1,2-dimethoxy benzene; spiro[4.5]dec-7-ene; 1H-3a,7-methanoazulene; 3-nonanone and styrene.

**Table 1. List of plant volatiles collected from *Melinis minutiflora* and *Brachiaria humidicola*. Plants were entrained for 24 h and a Gas Chromatograph (GC) linked to a Mass Spectrometer (MS) was used to identify the volatiles.**

Retention time (min)	Kovats Index	<i>Melinis minutiflora</i>	<i>Brachiaria humidicola</i>
8.87	1075	Acetic acid	ND*
9.59	1108	4,8-Dimethyl-1,3,7-nonatriene (DMNT)	4,8-Dimethyl-1,3,7-nonatriene (DMNT)
9.8	1118	(Z)-3-Hexenyl acetate	(Z)-3-Hexenyl acetate
11.27	1185	3-Hexen-1-ol	ND
11.47	1194	2-Nonen-1-ol	ND
14.66	1348	1,6-Octadien-3-ol	ND
15.48	1387	Bergamotene	ND
15.75	1400	$\beta$ -Caryophyllene	$\beta$ -Caryophyllene
17.03	1467	$\alpha$ -Farnesene	ND
17.79	1507	ND	Benzaldehyde
18.00	1518	4-Ethylbenzaldehyde	ND
19.07	1575	Methyl salicylate	Methyl salicylate
19.73	1610	4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT)	4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT)
20.09	1631	ND	4-Ethylbenzoic acid
20.75	1669	ND	Benzoic acid
22.50	1770	ND	4-(1-Hydroxyethyl) benzaldehyde
22.85	1790	ND	phenol

\*Not detected

### Conclusion

The identification of HIPVs from the push grasses and compounds released by endophytic fungi is a major step in the larger study on the impact of odorant volatiles on *E. saccharina* behaviour. Work can now proceed to establish which volatiles, or group of volatiles, are attractive or repulsive to *E. saccharina* using electrophysiological studies (EAG) and behavioural experiments (olfactometer, wind tunnel and field trials).

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