Investigation of the semiochemicals of confused flour beetle *Tribolium confusum* Jaquelin du Val and grain weevil *Sitophilus granarius* (L.) in stored wheat grain and flour

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DOI: 10.5073/jka.2010.425.089

Abstract

This investigation sets out to identify specific volatile compounds from both flour infested with the confused flour beetle, *Tribolium confusum* and wheat grain infested with the grain weevil, Sitophilus granarius. These volatiles could help to aid the early detection of infestation by these pests. Volatiles by the infestation of these insect pests were entrained and analysed using Solid-Phase Micro-Extraction (SPME) coupled with gas chromatography-mass spectrometry (GCMS). Several volatile compounds were identified specific to *T. confusum* and *S. granarius*, including the known semiochemicals of *T. confusum*. The *T. confusum* larvae specifically emitted the volatiles 1-octen-3-one, benzeneacetaldehyde and decanal, whilst the adults specifically emitted the volatiles 2-methyl and 2-ethyl-1,3-benzenediols, the known semiochemicals 1-pentadecene, 2-methyl and 2-ethyl-1,4-benzoquinones and a series of yet to be fully identified unsaturated hydrocarbons. Both *T. confusum* adults and larvae emitted 2-methylbutanal and 2-butanone. Furthermore, four volatiles were identified unique to flour infested by *T. confusum*, 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol. The *S. granarius* adults specifically emitted the volatiles 2-methylpropanoic acid and 3-methylbutanoic acid, whilst infested wheat grain produced the following volatile organic compounds, 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine. We believe these specific volatiles may act as semiochemicals for these insects and could aid in semiochemical monitoring for the early detection of infestation by these insects.

Keywords: *Tribolium confusum*, *Sitophilus granarius*, GC-MS, SPME, Semiochemicals.

1. Introduction

Grain and food products are attacked by pests such as insects, mites and microorganisms during storage. The resulting post-harvest losses are approximately 10-15% worldwide annually (Hodges et al., 1996; Rajendran, 2002; Neethirajan et al., 2007). Infestation by insects encourages growth of fungi including those that produce mycotoxins, and results in contamination of commodities with insect bodies and waste products etc. Some of which are toxic, repulsive or allergenic (Freeman, 1976). Thus, insect infestation detection is very important to ensure the provision of healthy food to consumers, to evaluate the efficiency of pesticide treatment and to work as an early warning for taking suitable control measures.

This investigation sets out to identify volatile organic compounds (VOCs) as markers for the early detection of Confused Flour Beetle, *Tribolium confusum* Jaquelin du Val and grain weevil, *Sitophilus granarius* (L.) infestation in flour and wheat grain. Previous studies have identified VOCs in the headspace above *Tribolium* spp. (Villavarde et al., 2007) and in the headspace above the lesser grain borer, *Rhyzopertha dominica* (L.) (Seitz and Ram, 2004). This investigation was undertaken to discover new volatile compounds and to confirm the presence of compounds previously reported using solid-phase micro-extraction (SPME) to collect and concentrate the headspace volatiles above the samples, with the subsequent analysis by gas chromatography-mass spectrometry (GCMS).

2. Materials and methods

2.1. Insect identification and rearing

Insects were obtained from an organic farm which had a problem with infested grain. The insects were identified at the University of the West of England (UWE, Bristol, UK) and the identification confirmed by the Natural History Museum (London, UK).

Tribolium confusum beetles were reared on wheat flour and wholemeal flour and S. granarius weevils were reared on wheat grain. Moisture content of the grain was estimated using a "Digital Grain Master" moisture meter (Protimeter, Marlow, UK). Glass jars (200 mL), covered with nylon gauze, were used as insect containers and incubated at 25°C ± 2°C under a 14 h light: 10 h dark photoperiod at 70 ± 5% relative humidity (r.h.).

2.2. Collection of VOCs

2.2.1. SPME Fibre Conditioning

SPME fibres (75 µm carboxen/polydimethylsiloxane, Sigma Aldrich, Dorset, UK) were conditioned prior to first use according to the manufacturer instructions (300ºC for 3 h) and reconditioned in between sampling sets (280ºC for 12 min) by heating in the GCMS injector port (Clarus 500, Perkin Elmer, Beaconsfield, UK).

2.2.2. Collection of VOCs from T. confusum

Identification and comparison of the VOCs from *T. confusum* adults and larvae was undertaken using 100 adult beetles and 100 larvae, in two headspace vials (10 ml, Supelco, Poole, UK). VOCs were also collected from flour infested with *T. confusum* beetles (3 g) and non-infested flour (3 g) in headspace vials (10 mL). The SPME fibres were exposed for 16 h to the static headspace of the samples heated at 28°C. Each sample collection of VOCs was repeated three times with new samples. Periodic blank vials were used as controls to ascertain system impurities.

2.2.3. Collection of VOCs from S. granarius

VOCs released by *S. granarius* weevils (100 insects), infested wheat grain (3 g) and non-infested wheat grain (3 g) were collected from 10 ml headspace sample vials (10 ml) using the SPME fibres. The SPME fibres were exposed for 16 h to the static headspace of the samples heated at 28°C. Each sample collection of VOCs was repeated three times with new samples. Periodic blank vials were used as controls to ascertain system impurities.

2.3. GCMS analysis of the SPME fibres

VOCs were analysed using the GCMS system fitted with a split/splitless injector (250˚C, purge off 1.0 min) and separated in the GC using a Zebron-624 column (60 m length x 0.32 mm I.D., 1.40 µm film thickness, Phenomenex, Macclessfield, UK) using the GC oven temperature program (35°C for 5 min, ramped at 7°C min-1 to 250°C for 12 min, run time 47.71 min). The VOC analytes were identified through the MS in full scan mode (17-350 m/z, electron impact ionisation at 70 eV) using the NIST database library (reserves fit hits and Kovat's indices, NIST 2002) and by the comparison of known standard retention times.

3. Results

3.1. Analysis of headspace VOCs from T. confusum

The analysis of the headspace from *T. confusum* showed several of VOCs which were unique to adults compared to the larvae and infested flour samples and were found in all repeat experiments; 2-methyl-1,4-benzoquinone, 2-ethyl-1,4-benzoquinone, 2-methyl-1,3-benzenediol and 4-ethyl-1,3-benzenediol. Two further VOCs, 1-pentadecene and hexadecane, were repeatedly found in both adult and flour infested headspace samples, whilst 2-methylbutanal and 2-butanone were found in both the headspace samples of adults and larvae. A typical chromatogram illustrating the VOCs extracted from the headspace of adults of T. confusum by SPME fibre is shown in Fig. 1.

Analysis of the headspace of wheat flour infested with *T. confusum* showed the presence of four unique compounds; 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol. These were absent in the headspace samples of non-infested flour. The analysis of the headspace of *T. confusum* larvae samples contained the unique VOCs, 1-octen-3-one, benzeneacetaldehyde and decanal.

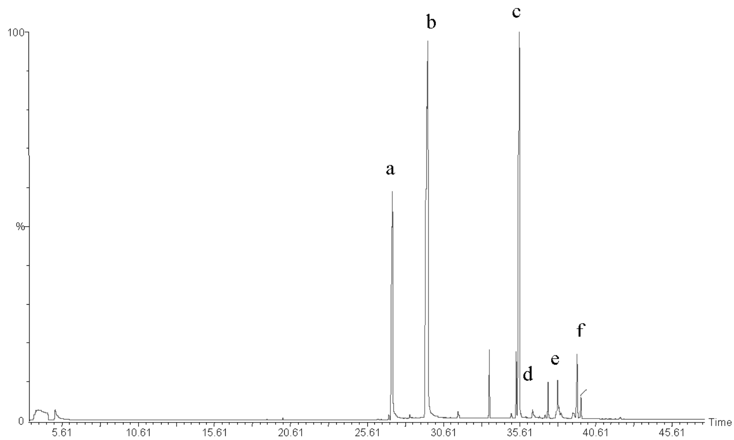

Figure 1 Typical chromatogram illustrating the major VOCs extracted from the headspace above a hundred beetles of *Tribolium confusum*. a = 2-methyl-1,4-benzoquinone, b = 2-ethyl-1,4-benzoquinone, c = 1-pentadecene, d = 2-methyl-1,3-benzeediol, e = hexadecane and f = 4-ethyl-1,3-benzenediol.

3.2. Analysis of headspace VOCs from S. granarius

Two VOCs 2-methylpropanoic acid and 3-methylbutanoic acid were unique to the headspace above *S. granarius* and were repeatedly identified (see Fig. 2).

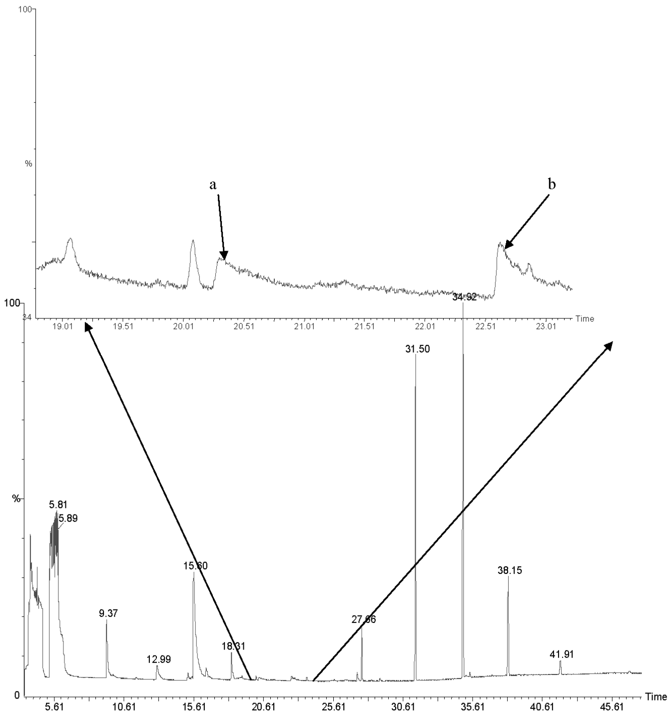

Figure 2 Typical chromatogram illustrating the VOCs extracted from the headspace above a hundred beetles of *Sitophilus granarius*.

Other volatiles were found only in the headspace of wheat grain infested by *S. granarius*; 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine. These were absent in the headspace samples of non-infested wheat grain.

4. Discussion

4.1. Analysis of headspace VOCs from T. confusum

The analysis of the headspace from *T. confusum* adults showed 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone were present and where found in all the repeat experiments. These quinones were unique to adult headspace samples, but surprisingly where not identified from the flour infested headspace samples. These odorous benzoquinones have been well documented previously (Engelhardt, et al., 1965, Pappas et al., 1996 and Villaverde et al., 2007) and can make infested flour unsuitable for human consumption (Phillips et al., 1984) and even toxic (El-Mofty, et al., 1992). These quinones act as defence secretions for *Tribolium* spp. (Tschinkel, et al., 1975) and are known to be released during overcrowding stressful conditions (Faustini et al., 1986).

1-Pentadecene and hexadecane were found in both adults of *T. confusum* and wheat flour infested with *T. confusum* beetles. 1-Pentadecene is associated with insect odour (Seitz et al., 1996) and is hypothesized as an epideictic (spacing) pheromone (Arnuad et al., 2002). The known conspecific aggregation pheromone of *Tribolium* spp. 2, 4-dimethyldecanal (Arnuad et al., 2002) was not identified in this study. Previously unreported VOCs, 2-methyl-1, 3-benzenediol and 4-ethyl-1,3-benzenediol, were unique to the headspace of *T. confusum* adults. Consequently these VOCs which were produced by adults might be used as biomarkers for detection of *T. confusum* in flour or grain. VOCs unique to flour infested by *T. confusum*, 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol, might also be potential biomarkers for infestation.

According to the available literature there has not been a detailed study of the VOCs produced by *T. confusum* larvae and only methyl fatty acid esters have been reported (Tebayashi et al., 2003). It is therefore significant, that 1-octen-3-one, benzeneacetaldehyde and decanal VOCs, were found in larvae samples and not in adults or laboratory air samples. These too might also be potential biomarkers.

4.2. Analysis of headspace VOCs from S. granarius

The analysis of the headspace from *S. granarius* adults showed 2-methylpropanoic acid and 3-methylbutanoic acid were unique to these samples but not present in the wheat grain infested with S. granarius. The known *S. granarius* attractant 3-methylbutan-1-ol (Germinara et al., 2008) was not identified by this study, however 3-methylbutanoic acid could be the precursor to this and may exhibit a semiochemical response.

VOCs unique to the headspace of the infested wheat grain were 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine and might also be potential biomarkers.

Some studies confirm that the *S. granarius* produces (2S, 3R)-1-ethylpropyl-1,2-methyl-3-hydroxypentanoate as an aggregation pheromone (Chambers et al., 1996). However the present study did not identify this pheromone, due to possible overcrowding during the sampling.

In summary, this study has identified some unique volatiles previously not identified from these species. Bioassays would be required to confirm if these volatiles act as semiochemicals.

Acknowledgments

This study was funded by the Higher Education Department of the Libyan Government.

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