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Detection of *Tribolium castaneum* (Herbst) volatile defensive secretions by solid phase microextraction–capillary gas chromatography (SPME-CGC)

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Abstract

The solid phase microextraction (SPME) technique was used for the collection of the volatile defensive secretions released by *Tribolium castaneum* (Herbst), the red flour beetle. The detection of the major components methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ), together with 1-pentadecene (C15:1), was performed by capillary gas chromatography (CGC). SPME samples were identified by CGC coupled to mass spectrometry (MS). Volatile organic compounds (VOC) released from insects were compared employing different SPME fibers. The relative amounts of the major volatiles collected varied with the fiber coating. Quinones accounted for ~75% of the volatiles trapped by the carboxen/polydimethylsiloxane (CAR/PDMS) fiber, whereas they only represented ~45% of the volatiles trapped with the PDMS fiber. The PDMS/divinylbenzene (DVB) coating exhibited intermediate affinity. In a 15-min extraction period, the highest amounts of VOC extracted from disturbed insects with CAR/PDMS were 349 ± 107 ng/beetle of MBQ, 780 ± 290 ng/beetle of EBQ, and 144 ± 69 ng/beetle of C15:1. The total VOC amounts extracted by SPME were estimated by adding the disturb-released VOC to heat-released VOC of the same insects, resulting in 742 ± 93 ng/beetle of MBQ, 1672 ± 244 ng/beetle of EBQ, and 946 ± 237 ng/beetle of C15:1. SPME-CGC is a simple method to detect and estimate *T. castaneum* volatiles. This method has the potential to detect insect infestation in stored products.

Keywords: *Tribolium castaneum*; Benzoquinone; Volatile; Solid phase microextraction; Capillary gas chromatography

1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a major pest affecting the storage, handling, and processing of grain-based products. Tenebrionids use prothoracic and abdominal glands to produce specific quinone-containing defensive secretions with repellent and irritant properties against predators (Roth, 1943; Eisner, 1966; Tschinkel, 1975; Ruther et al., 2001). These secretions can also contaminate and confer unpleasant organoleptic properties to the food source. Methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ) are the major components (Blum, 1981; Attygalle et al., 1991, 1993; Eisner et al., 1998), and are usually present together with large amounts of 1-pentadecene (C15:1). The secretions of *T. castaneum* were reported as a mixture of benzoquinones (BQ = EBQ + MBQ) and alkenes (Alexander and Barton, 1943; Happ, 1968; Suzuki et al., 1975), together with the male aggregation pheromone 4,8-dimethyldecanal (Suzuki, 1980). Alkenes were proposed to behave as solvents for the uptake and spreading of the toxic quinones over the beetle's body (Peschke and Eisner, 1987).

Insect infestation can be determined either by visual inspection of a commodity or by detection of the amount and quantity of the quinones present (Hodges et al., 1996). A variety of sophisticated and complex methods formerly used to analyze *Tribolium* volatile secretions have been summarized by Unruh et al. (1998). Basically, their analyses required a large number of insects and shared common difficulties due to the instability of quinone

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solutions. The volatile secretion levels were reported to vary from almost undetectable amounts just after adult eclosion, to levels of increasing concentration as the adults aged. Thirty-day-old females of *Tribolium* spp. were reported to contain 35–46 μg of benzoquinone and 14–24 μg of 1-pentadecene (Wirtz et al., 1978). A large variation in quinone content was reported, partially due to different procedures employed, but also to lack of information on the age of the insects assayed. More recent studies employing genetically distinct strains of *T. confusum* quantified the amounts of the two major quinones secreted (external amounts), using high performance liquid chromatography (HPLC). The mean values detected were about 0.4 μg/beetle for MBQ and 2 μg/beetle for EBQ, whereas their internal, glandular amounts were close to 10 and 15 μg/beetle, respectively, with very large variations between "high-producing" and "low-producing" strains (Yezerski et al., 2004).

The objective of the present study was to investigate the potential of the solid phase microextraction (SPME) technique coupled with capillary gas chromatography (CGC) as a simple, fast, reliable, and solvent-free alternative to detect and estimate beetle volatile secretions.

2. Materials and methods

2.1. Insects

Tribolium castaneum were maintained under a 12L:12D photoperiod at 27 ± 2 °C and 70 ± 5% relative humidity (r.h.) and reared on wheat flour. Adult beetles used in the test were mixed-sex and 30-d-old.

2.2. Volatile organic compounds (VOC) collection

Five adult beetles were placed in a 4-ml glass vial sealed with a Teflon cover with rubber septum. Although one insect provided a detectable signal, insects were pooled for VOC measurement since preliminary assays showed high individual variability. After 24 h volatiles secreted were sampled from the head space (HS), corresponding to the gaseous phase in contact with the insect sample. Each vial was successively subjected to three different conditions: ambient temperature, 30-s agitation at ambient temperature, and finally vials were heated at 90 °C for 15 min in order to evaluate the amount of VOC remaining in the gland reservoir (internal VOC) that were detectable by this procedure, as described below. In each condition, volatiles were sampled by HS–SPME for 15 min employing either a carboxen/polydimethylsiloxane (CAR/PDMS) fiber (75 μm film thickness), a polydimethylsiloxane (PDMS) fiber (100 μm film thickness), or polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB, supplied by Supelco, Bellefonte, PA, USA) with a 65 μm film thickness. Selection of fibers was based on manufacturer's recommendations for sampling volatiles of low to intermediate polarity, and relatively low molecular weight. Fibers were previously conditioned according to manufacturer instructions, and systematically reconditioned before each analysis. Vials containing wheat flour but no beetles were used as controls.

2.3. Solid phase microextraction (SPME)–capillary gas chromatography (CGC)–mass spectrometry (MS)

Quantitative analysis by CGC was performed using a Hewlett Packard 6890 gas chromatograph employing a non-polar DB-5 capillary column (30 m length, 0.32 mm I.D., 0.25 μm film thickness) (J&W, Folsom, CA, USA). The injector was operated in the splitless mode at 250 °C and the oven temperature was programmed (40 °C for 3 min, 5 °C/min to 80 °C, 20 °C/min to 150 °C, and 30 °C/min to 250 °C, with a holding time of 10 min at the final temperature). The flame ionization detector temperature was set at 280 °C. VOC identification was performed by CGC–MS analysis with a Finnigan Polaris Q ion trap mass spectrometer with chromatographic conditions similar to the CGC; the ion source was set at 200 °C and the transfer line at 275 °C. VOC were tentatively identified by interpretation of their mass spectral fragmentation; spectra were also compared to data from MS libraries (NIST/EPA/NIH, NIST 98). Hydrocarbon chain length was confirmed by matching their retention times with those of hydrocarbon standards (Sigma-Aldrich, St. Louis, MO, USA).

To estimate benzoquinone and 1-pentadecene (C15:1) amounts released by disturbed insects during the 15-min sampling period, known amounts of 1-pentadecene (Sigma-Aldrich, St. Louis, MO, USA) and 1,4-benzoquinone (Sigma-Aldrich, St. Louis, MO, USA) in 1 μl hexane were separately spiked into a SPME vial. Sampling with the CAR/PDMS fiber, and GC analytical conditions, were identical to those described for the insect VOC analyses at ambient temperature.

Total amounts released from inert solid matrixes can be estimated by performing several consecutive HS–SPME extractions (multiple HS–SPME) from the same sample, the total area is independent of the matrix and can be estimated by adding the areas of individual extractions (Ezquerro et al., 2003; Ezquerro and Tena, 2005; Martinez-Urunuela et al., 2005). However, no method was available to estimate total VOC from a living organism; consecutive extractions did not seem appropriate because volatile synthesis very probably continues during the sampling period. Hence, multiple HS–SPME extraction was modified by replacing consecutive extractions by a heat-shock period. VOC amounts released by heating were calculated by placing replicates of different concentrations of benzoquinone and 1-pentadecene standard (1 μl each) into SPME vials, then volatiles were collected with a CAR/PDMS fiber by heating at 90 °C for 15 min in order to mimic heat-release VOC conditions. However, the extraction temperature has opposing effects on the SPME technique; with a temperature increase, diffusion coefficients are increased, although partition coefficients to the extraction phase are decreased, and hence the VOC