

Electronic Supplementary Material

Maternal food provisioning in relation to condition-dependent offspring odours in burrower bugs (*Sehirus cinctus*)

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Running head: Parental care and offspring odours

a) Volatile collection and exposure apparatus

The apparatus was set up such that air was blown at a flow-rate of five l/min from a tank with highly-purified compressed air (Fig. 1; (1); Zero Grade 1.0, <1ppm total hydrocarbons; Airgas, Inc.) through a humidifying chamber (Fig. 1; (2)) containing wetted glass-wool. The air was then split among four arms (1.25 l/min each), blown through the biosource chambers (Fig. 1a; (3); cylindrical glass separatory funnels; Pyrex[®]) containing either wetted filter paper (control), or wetted filter paper with either a low-condition or a high-condition clutch of nymphs. The biosource chambers never contained mint nutlets. A vacuum pump (Fig. 1; (5); Air Cadet[®], Cole-Palmer Instrument Co.) pulled air at a rate of approximately 0.6 l/min through a volatile collection trap (VCT) packed with 30 mg of the SuperQ[®] polymer (Fig. 1; (4); Analytical Research Systems, Inc.), and the remaining 0.65 l/min were passively blown into the assay arena (Fig. 1; (6)) containing the assay family where we quantified maternal provisioning. The material used in the apparatus consisted of Teflon[®] (PTFE) tubing, brass joints to connect Teflon[®] tubes and

glass separatory funnels (humidifying and biosource chambers). To calibrate the system, we measured both airflow into the assay arena (Fig. 1; (6)) and through the VCT (Fig. 1; (4)) before and after each experiment using RMA-14 flowmeters (Dwyer Instruments, Inc.). Measured average airflow through the VCT over the course of

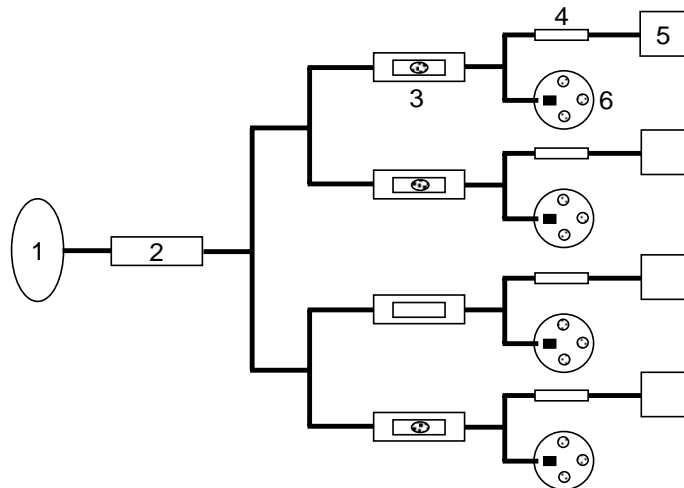


Fig. 1. Volatile collection/exposure apparatus used in the experiments for the simultaneous collection of and exposure to nymph odours. (1) tank containing highly-purified compressed air, (2) humidifying chamber, (3) biosource chambers containing either a wetted piece of filterpaper (control) or an biosource clutch on a wetted piece of fileterpaper, (4) SuperQ[®] volatile collection traps, (5) vacuum pumps and (6) assay family exposed to the air. See text for more details.

the experiments was 0.60 l/min (\pm 0.003 SE), and measured average airflow into the assay arena was 0.64 l/min (\pm 0.009 SE). A more detailed description of the construction and physical properties of the apparatus is described elsewhere (Chuckalovcak et al., submitted).

b) Chemical analysis using GC-MS

Gas Chromatography / Mass Spectrometry (GC/MS) was conducted using a HP 6890 GC and a HP 5793 MSD (electron impact 70 eV). The GC was equipped with a DB-WAX column (30 m / 60 m x 0.25 mm ID with 0.25 μ m film-thickness, J&W Scientific, Folsom, California).

The oven temperature started at 40°C for 2 min, and then increased to a final temperature of 230°C at 10°C/min. The oven was held at this temperature for 10 min. The flow rate of helium through the column was kept at 1 ml/min. The scan range of the MSD was set from 30 to 550 m/z. A few nymph collections that were not part of the experimental design were analyzed using a GC-column that was less polar (DB-5, 30 m x 0.25 mm ID, 0.25 μ m film-thickness, J&W Scientific). These analyses were done using an HP 5890 Series II GC linked with an HP 5972 MSD. Because this MSD was less sensitive, we used selected ion monitoring of characteristic ions on the monoterpenes (m/z 93, 121, and 136) and the internal standard (m/z 156). Other GC/MS operating conditions were identical to those described above.

Before running a sample on the GC/MS, we added 10 μ l of 0.5 ng / μ l (5ng) of an internal standard (Undecane; C₁₁H₂₄; Alltech Associates, Inc., Deerfield, IL, USA) to each sample and blew the sample down to approximately 2 μ l using a steady stream of N₂. The 2 μ l of sample were then injected into the GC.

c) Exposure to synthetic compounds

The synthetic camphene (camphene, 95%) and α -pinene (1R-(+)- α -pinene) were purchased from Sigma-Aldrich (6000 North Teutonia Ave., Milwaukee, WI 53209, USA). The delivery

rates of camphene and α -pinene were estimated by inducing the compounds on a piece of filter paper in our volatile collection and delivery apparatus (Fig. 1) and pulling air over the probes through a volatile collection trap over the duration of six hours. Delivery rate was estimated to be 60% and 30% for camphene and α -pinene, respectively. The synthetics were diluted in the solvent hexane to give 0.99 ng/ μ l (camphene, low-quantity), 5.26 ng/ μ l (camphene, high-quantity), 19.54 ng/ μ l (α -pinene, low-quantity) and 99.65 ng/ μ l (α -pinene, high-quantity). 1 μ l of the solution plus one μ l of hexane were applied on the filterpaper probe which was then entered in the apparatus. The control treatment consisted of 2 μ l of hexane applied on filterpaper. Sample sizes were 20 assay families in the control group, 17 in the camphene low-quantity treatment, 18 in the camphene high-quantity treatment, 19 in the α -pinene low-quantity treatment and 20 in the α -pinene high-quantity treatment. The provisioning was then quantified by counting every hour the remaining mint nutlets in the food dishes over the course of six hours.