New Phytologist Supporting Information Notes S1

Article title: Covariation and phenotypic integration in chemical communication displays: biosynthetic constraints and eco-evolutionary implications


Article acceptance date: 29 January 2017
Note S1 Scent samples and methods to identify chemical communication displays.

Species sampled, references to the original studies and a brief summary of the sampling methods and the analysis of scent bouquets are given.
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<th>Author</th>
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Tab. S1-1 Species and samples included in the study. For each sample, the first author, the species, the organ as well as the treatment are given. Additionally, sample size, the number of compounds detected, the mean phenotypic integration, the number of modules, the number of modules with more than one compound as well as the mean phenotypic integration of modules are listed. Methodological details and additional information are given below NA, information not applicable.
Species: Achillea millefolium, Asteraceae; Cirsium arvense, Asteraceae

Organism / organ: flowers

Authors / contributors: Anne-Amélie C. Larue-Kontic¹, Robert A. Raguso² & Robert R. Junker¹

Affiliation: ¹University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria
²Department of Neurobiology and Behavior, Cornell University, Ithaca, USA


Location: fallow land, Salzburg, Austria

Method sketch / description of data:

- Experimental setup: Flower-visitor observations and scent manipulation of living flowers from A. millefolium and C. arvense plants.

- Scent sampling / analyses: Volatiles were collected by using dynamic headspace sampling. Flowers during anthesis from living plants were enclosed within scentless polyethylene tetrathalate (PET) oven bags (Toppits® Cofresco Fischhalteprodukte GmbH & Co. KG, Minden, Germany), headspace was enriched for 90 min and scented air was sucked through volatile traps for 2 min with a flow rate of 200 ml min⁻¹. Volatile traps contained a mixture of 1.5 mg Tenax-TA (mesh 60-80; Supelco, Germany) and 1.5 mg Carbotrap B (mesh 20-40; Supelco, Germany). Flower tissues were dried for at least 48 h at 40°C to obtain the dry mass. Volatiles were desorbed from traps using an automatic TD (thermal desorption) system (model TD-20, Shimadzu, Japan) coupled with a GC–MS (model QP2010 Ultra EI, Shimadzu, Japan). GC was equipped with a 60 m long column (Zebron ZB-5, Newport Beach, USA) with a diameter of 0.25 mm and a film thickness of 0.25 µm. The column flow (helium) had a rate of 1.5 ml min⁻¹ and the oven temperature was kept constant for 1 min at 40°C before it increased with 6°C min⁻¹ until the maximum of 250°C. The MS interface was set to 260°C and the ion source was at 200°C. For identification of the scent compounds, we used the GCMSsolutions Software (Version 2.72, Shimadzu Corporation) and mass spectra of authentic standards as well as spectral libraries (ADAMS, ESSENTIALOILS-23P, FFNSC 2, W9N11) and Kovat’s indices generated using n-alkanes.

- Funding: This work was funded by the Deutsche Forschungsgemeinschaft (DFG, JU2856/1-1) to RRJ and by NSF Grant DEB-0746106 to RAR.
(2) **Species**: *Arabidopsis thaliana*, Brassicaceae

*Organism / organ*: plant / flowers

*Authors / contributors*: Dorothea Tholl

*Affiliation*: 1Department of Biological Sciences, Virginia Tech, USA


*Location*: Max Planck Institute for Chemical Ecology, Jena, Germany

*Method sketch / description of data:*

- Experimental setup: Seventy inflorescences were detached each (n = 3–6) from flowering plants of 37 different *Arabidopsis* accessions. Plants were grown in chambers under controlled temperature and light conditions (22°C, 160 µmol m\(^{-2}\) s\(^{-1}\) PAR, 16 h : 8 h, light : dark cycle). Inflorescences of most accessions had an average of four to five open flowers.

- Scent sampling / analyses: Detached inflorescences were placed in a small glass beaker filled with tap water and transferred to 1 l bell jars. Emitted volatiles were collected for 8 h on 25 mg Super Q (Supelco; Sigma-Aldrich, St Louis, MO, USA) traps in a closed loop stripping procedure according to Donath & Boland (1995). Volatiles were eluted from the traps with 100 µl CH\(_2\)Cl\(_2\), and 120 ng of nonyl acetate were added as a standard. Samples from volatile collections were analyzed on a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass selective detector. Separation was performed on (5%-phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) of 30 m × 0.25 mm i.d. × 0.25 m thickness. Helium was the carrier gas (flow rate of 2 ml min\(^{-1}\)), a splitless injection (injection volume of 2 µl) was used, and a temperature gradient of 5°C min\(^{-1}\) from 40°C (3-min hold) to 240°C was applied. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards and with reference spectra in the NIST and Wiley libraries (Agilent Technologies, Palo Alto, CA, USA). Mass spectrometry was performed with a transfer line temperature of 230°C, source temperature of 230°C, quadrupole temperature of 150°C, ionization potential of 70 eV, and scan range of 50 to 400 atomic mass units. For quantification, primary ion peaks of each compound were integrated (single ion method) and the amounts were calculated in relation to the response of nonyl acetate at mass-to-charge ratio (m/z) 69. Response curves for the quantified compounds relative to the internal standard were generated by injecting a mixture of equal amounts of authentic standards and internal standard. Emissions were determined in ng h\(^{-1}\) per 70 inflorescences.


*Funding*: National Science Foundation Grant IBN-0211697 to Eran Pichersky and funds of the Max Planck Society to Jonathan Gershenzon
(3–5, 7, 28, 29, 33-38) **Species:**

- *Sinapis arvensis* L., Brassicaceae
- *Brassica nigra* L., Brassicaceae
- *Brassica juncea* L., Brassicaceae
- *Raphanus raphanistrum* L., Brassicaceae

**Organism / organ:** plant / flowers

**Authors / contributors:** Mathias Hoffmeister\(^1,2\) & Robert R. Junker\(^2\)

**Affiliation:**

- \(^1\)Heinrich-Heine-University, Institute of Sensory Ecology, Düsseldorf, Germany
- \(^2\)University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria


**Location:** University of Düsseldorf, Germany

**Method sketch / description of data:**

- Experimental setup: Experiment to test the effect of leaf herbivory on floral scent emission in different Brassicaceaes (\(n = 4\)). For herbivory treatment, larvae of the Brassicaceae specialist *Phaedon cochleariae* were allowed to feed inside of clip cages on single leafs of potted plants for 2 d 3–4 wk after germination; control plants were equipped with clip cages containing no larva. When flowering scent was sampled in control and herbivore-treated plants (\(n_{\text{plant}} = 7–9\) per species and treatment).

- Scent sampling / analyses: Scent was sampled from unpicked wrapped inflorescences (oven bags, Toppits, Minden, Germany) of control and herbivore-treated plants as well as from surrounding air (empty oven bags) to determine flower-specific compounds. After scent accumulation for 30 min, volatiles were collected in traps filled with a mix (1 : 1) of Tenax-TA (mesh 60-80) and Carbotrap B (mesh 20-40; Supelco, Bellafonte, USA), where air was pulled from flower bags (flow rate: 200 ml min\(^{-1}\), sampling: 2 min). Traps were analysed using an automatic thermal desorption system (TD-20, Shimadzu, Japan; ZB-5 fused silica column, Phenomenex, Aschaffenburg, Germany) coupled with a GC-MS (model QP2010 Ultra EI, Shimadzu, Japan). Column flow was set to 1.5 ml min\(^{-1}\), GC oven temperature was increased to 250°C (6°C min\(^{-1}\)) and held for 1 min and the MS interface worked at 220°C. We processed GC/MS data with the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012). For compound identification we compared compounds to mass spectra and retention times of commercially available standard substances or to compounds listed in mass spectral libraries Wiley Registry 9th Edition, NIST 2011 and FFNSC2.

**Funding:** Deutsche Forschungsgemeinschaft (DFG JU 2856/2-2).
(6, 65-70) **Species:** *Brassica nigra* L., Brassicaceae

**Organism / organ:** plant / leaves and flowers

**Authors / contributors:** Dani Lucas-Barbosa / Marcel Dicke

**Affiliation:** Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands.


**Location:** Glasshouse of Wageningen University, Wageningen, The Netherlands

**Method sketch / description of data:**

Experimental setup: Glasshouse experiment with potted *Brassica nigra* individuals (*n* = 20). Emission rates were quantified for compounds that were detected in at least 50% of the samples of one of the treatments.

Scent sampling and analyses: To investigate whether flowering *B. nigra* plants respond locally and systemically to herbivore infestation, we collected volatiles emitted by leaves and flowers of infested and non-infested control plants. Each *B. nigra* plant was infested with 100 second instar *P. brassicae* larvae, spread over two leaves, and allowed to feed for 48 h. To prevent caterpillars from moving to flowers, cotton wool was placed around the petiole leaf stalk or the caterpillars were confined to clip cages. When plants were tested, caterpillars had consumed about 60 % of the leaves where they were placed. Control plants were of the same batch of plants, of the same age and similar in height and number of flowers, but were not infested with herbivores. Either leaves or flowers of infested and control plants were enclosed in an oven bag (Toppits® Brat-Schlauch, polyester; 32 cm × 32 cm × 70 cm; Toppits, Minden, Germany). A strip of bag material wrapped around the stem and above or below the inflorescence was used to close the bag. Synthetic air was flushed through the bag at a flow rate of 300 ml min\(^{-1}\) (224-PCMTX8, air-sampling pump Deluxe, Dorset, UK; equipped with an inlet protection filter) by inserting Teflon tubing through an opening in the upper part of the bag. Air was sucked out and headspace volatiles were collected in the glass tube filled with Tenax for 1.5 h at a flow rate of 250 ml min\(^{-1}\) through a second Teflon tube at the opening of each bag, and connecting it to a glass tube filled with about 90 mg of Tenax-TA 25/30 mesh (Grace-Alltech; USA). Headspace samples were analysed in a gas chromatograph with a thermodesorption unit (GC) (6890 series, Agilent, Santa Clara, USA) connected to a mass spectrometer (MS) (5973 series, Agilent, Santa Clara, USA). Compounds were identified by comparison of mass spectra with those of NIST, Wiley libraries and the Wageningen Mass Spectral Database of Natural Products. Identity was confirmed by comparison of retention index described in the literature and the respective index calculated during this study.

**Funding:** The Earth and Life Sciences council of The Netherlands Organisation for Scientific Research (NWO-ALW).
(12) **Species:** Corydalis gotlandica Lidén, Papaveraceae

*Organism / organ:* plant / flowers

*Authors / contributors:* Jette T. Knudsen

*Affiliation:* 1Dept. of Biology, Lund University, Lund, Sweden, 2Nattaro Labs AB, Medicon Village, 223 81 Lund, Sweden.

*Acknowledgement:* I acknowledge Lotta Hallenfur for help with collection of scent samples.

*Reference:* NA

*Location:* Gotland and Stora and Lilla Karlsö

**Method sketch / description of data:**

- **Experimental setup:** Inflorescences on rooted plants (N=43) in natural settings.

- **Scent sampling / analyses:** Dynamic headspace samples were collected in situ from one inflorescence per plant. Each inflorescence was covered with a polyacetate oven bag and an absorbent tube, filled with 20 mg Tenax Gr (mesh size 60-80) was inserted into the bag and the air within the bag was sucked through the absorbent tube with a battery driven membrane pump at a flow rate of 90–110 ml min\(^{-1}\). In parallel blank samples were collected from leaves or air. Samples were collected for 4-6 h between 10:00 - 16:00 h and were extracted with 250 \(\mu\)l of hexane. The samples were analysed by coupled GC-MS on a HP 6890 GC connected to a HP 5973 mass selective detector. Emission was standardized to %/inflorescence. Most compounds were identified by comparison of mass spectra and retention times with standard compounds. Alternatively, compounds were identified using the mass spectral libraries NIST 2011 and Adams 2007.

*Funding:* Länsstyrelsen, Gotland
**Species:** *Dieffenbachia aurantiaca* Engl., Araceae

Organism / organ: plant / flowers

Authors / contributors: Florian Etl¹,²

Affiliation: ¹University of Vienna, Department of Botany and Biodiversity Research, Vienna, Austria ²University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria


Journal of Chemical Ecology, accepted.

Location: Tropenstation la Gamba, Costa Rica

Method sketch / description of data:

Experimental setup: Dynamic head space collection with *Dieffenbachia aurantiaca* individuals (*n* = 8) in their natural habitat which is a tropical lowland rainforest in the southernmost part of Costa Rica. Collections were made during the period of strong scent emission (pistillate phase; ca. 18:30; as determined by human nose and indicated by attraction of large numbers of mirid bugs during preliminary observations).

Scent sampling / analyses: Dynamic headspace samples were collected from one inflorescence per plant. Inflorescences were bagged with polyethylene oven bags (10 × 30 cm; Toppits, Germany) and scent was trapped for 2 min (either directly after bagging (*n* = 6) or 10 min after bagging (*n* = 2)) on adsorbent tubes (quartz glass tube: length 25 mm; inner diameter 2 mm) filled with 1.5 mg each of Carbotrap B (mesh 20-40, Supelco, Germany) and Tenax TA (mesh 60-80; Supelco, Germany). For scent collection, a membrane pump (Gardner Denver, Germany) was used and the flow was set at 200 ml min⁻¹. To obtain negative controls, we conducted the same procedure but with empty oven bags (*n* = 3).

The samples were analyzed by GC/MS (QP2010Ultra, Shimadzu Corporation, Japan) coupled to a thermal desorption unit (TD-20, Shimadzu, Japan) and equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m long, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex, USA). Samples were run at a column flow (carrier gas: helium) of 1.5 ml min⁻¹. GC oven temperature started at 40°C, then increased by 6°C per min to 250°C and held for 1 min. The MS interface worked at 260°C and the ion source at 200°C. Mass spectra were taken at 70 eV (in El mode) from *m/z* 30 to 350. The GC/MS data were processed using the GCMSolution Version 4.11 (Shimadzu Corporation, Japan). Compounds were identified by the NIST 11, Wiley 9, FFNSC 2, Essential Oils and Adams 2007 mass spectral data bases and confirmed by comparison of mass spectra and retention times with those of authentic standards.

Funding: FE was supported by a KWA scholarship of the University of Vienna and by a scholarship of the ‘Verein zur Förderung der Tropenstation La Gamba’.

Species: *Ficus racemosa* L. (Section Sycomorus), Moraceae

Organism / organ: plant / inflorescence

Authors / contributors: Renee M. Borges¹, Jean-Marie Bessière² & Yuvaraj Ranganathan¹

Affiliation: ¹Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India ²Ecole Nationale Supérieure de Chimie de Montpellier, Montpellier Cedex 5, France


Location: Indian Institute of Science Campus, Bangalore

Method sketch / description of data:
- Trees were sampled in situ.

Scent sampling / analyses: Volatiles of *F. racemosa* were collected from A–C2 phases from 10 trees, one fig bunch per tree. Two samples per phase from the same syconia were collected (11:00 h and 23:00 h on the same day) by dynamic headspace adsorption in situ. Figs were enclosed in a polyethylene terephthalate (NalophanTM) bag (Kalle Nalo GmbH, Wursthullen, Germany) through which a constant airflow over an Alltech Super Q® volatile collection trap (ARS Inc., Gainesville, FL, USA) was maintained for 1 h per sample at incoming and outgoing flow rates of 111 ml min⁻¹ and 94 ml min⁻¹, respectively, using glass flowmeters (Aalborg Instruments and Controls, Orangeburg, NY, USA) controlled by a micropump (KNF Neuberger GmbH, Germany; model number NMP50KNDC 12VDC). Incoming air was cleaned using activated charcoal filters (Sigma-Aldrich). VOC traps were eluted with 150 µl of dichloromethane, and the eluate concentrated by solvent evaporation at room temperature to 10 µl, to which 0.5 µl of the internal standard cumene was added at a concentration of 200 ng ml⁻¹.

Funding: Ministry of Environment and Forests, Department of Biotechnology, Government of India
Species: Ipomoea purpurea L. Roth, Convolvulaceae:

Organism / organ: plant / flowers

Authors / contributors: Cassie Majetic\textsuperscript{1} & Robert A. Raguso\textsuperscript{2}

Affiliation: \textsuperscript{1}Department of Biology, Saint Mary’s College, Notre Dame, IN, USA \textsuperscript{2}Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA


Location: Saint Mary’s College Glasshouse, Notre Dame, IN, USA

Method sketch / description of data:

- Experimental setup: Common garden experiment with inbred mutant or wildtype genetic lines ($n = 51$).

- Scent sampling / analyses: Dynamic headspace samples were collected from two detached flowers per plant. The flowers were enclosed with an oven bag and the emitted volatiles were then trapped on 10 mg SuperQ between quartz wool plugs in a glass Pasteur pipette for 1 h using a pump (flowrate c. 250 ml min\textsuperscript{-1}). Samples were collected between 7:30 - 10:30 h. Samples were eluted with 300 µl hexane immediately following collection and stored at -20°C until analysis. Before analysis, samples were blown down to a volume of 75 µl and an internal standard of 5 µl of 0.03% toluene was added. Samples were analyzed using a Shimadzu GC17A gas chromatograph (EC-wax column) with a QP mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). Compounds were identified by comparing the mass fragment signatures of peaks to NIST and Wiley electronic spectral libraries. We also compared our volatile compound retention times and $m/z$ ratios to the compounds identified using authentic terpenoid standards or Kovat's indices. Emission rates of ng compound g\textsuperscript{-1} fresh flower mass/h were calculated using external sesquiterpene standard curves.

Funding: CJM was supported by the Eli Lilly/Saint Mary's College New Faculty Scholars program; RAR was supported by US National Science Foundation grant DEB-0746106.
Species: Penstemon digitalis Nutt. ex Sims, Plantaginaceae

Organism / organ: plant / flowers

Authors / contributors: Amy L. Parachnowitsch¹ & André Kessler²

Affiliation: ¹Plant Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. ²Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA


Location: Thompkins County, NY, USA (42º29'249''N 77º25'671''W)

Method sketch / description of data:

• Experimental setup: Common garden experiment with Penstemon digitalis individuals from three source populations (n = 88).

• Scent sampling / analyses: Dynamic headspace samples were collected from the whole inflorescence. The inflorescence was enclosed with 500 ml polyethylene cup and continuously sampled for 8 h and the emitted volatiles were trapped on activated charcoal absorbent vials (ORBO-32, SIGMA-Aldrich) using a pump (flow rate 450-500 ml min⁻¹). Samples were collected between 10:30 - 6:30 h and taken over 4 d. ORBO-32 absorbent vials were each eluted with 350 ml of dimethylchloride (SIGMA®) with 430 ng tetralin added for an internal standard. Samples were analyzed using a Varian 2200 GC/MS equipped with an EC WAX-column (Alltech Associates, USA). Compounds were identified by comparison of mass spectra and retention times with standard compounds or using the mass spectral library Nist 2011.

Funding: Ecology and Evolutionary Biology Department at Cornell (ALP), NSF grants DEB 0746106 (RAR) and DEB 0717139 (AK)
(20–27) **Species:** All *Phlox* and *Polemonium* species, Polemoniaceae:

*Polemonium caeruleum* ‘Bressingham Purple’ and ‘Album’ (n=9)
*Phlox bifida* ‘Betty Blake’ and ‘Alba’ (n=8)
*Phlox carolina* ‘Bill Baker’ and ‘Miss Lingard’ (n=8)
*Phlox drummondii* ‘21st Century Mix’ (n=18)
*Phlox drummondii* Hook. Wild (n=31)
*Phlox paniculata* ‘David’s Lavender’, ‘Tall Tenor’, and ‘David’ (n=9)
*Phlox stolonifera* ‘Pink Ridge’ and ‘Bruce’s White’ (n=12)

**Organism / organ:** plant / flowers

**Authors / contributors:** Cassie Majetic\(^1\) & Robert A. Raguso\(^2\)

**Affiliation:** 1Department of Biology, Saint Mary’s College, Notre Dame, IN, USA  2Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA


**Location:** Saint Mary’s College Glasshouse, Notre Dame, IN, USA

**Method sketch / description of data:**

- **Experimental setup:** Common garden experiment with inbred horticultural lines of Polemoniaceae species (see complete list of sample sizes above).

- **Scent sampling / analyses:** Dynamic headspace samples were collected from one inflorescence per plant. The inflorescence was enclosed with an oven bag and the emitted volatiles were then trapped on 10 mg SuperQ between quartz wool plugs in a glass Pasteur pipette for 1 h using a pump (flowrate c. 250 ml min\(^{-1}\)). Samples were collected between 10:00 - 14:00 h. Samples were eluted with 300 µl hexane immediately following collection and stored at \(-20^\circ\)C until analysis. Before analysis, samples blown down to a volume of 75 µl and an internal standard of 5 µl of 0.03% toluene was added. Samples were analysed using a Shimadzu GC17A gas chromatograph (EC-wax fused column) with a QP mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). Compounds were identified by comparing the mass fragment signatures of peaks to NIST and Wiley electronic spectral libraries. We also calculated Kovat’s indices for all compounds and used this information to further verify compound identity where possible. Emission rates of ng compound g\(^{-1}\) fresh flower mass h\(^{-1}\) were calculated using the internal standard.

**Funding:** CJM was supported by the Eli Lilly/Saint Mary’s College New Faculty Scholars program; RAR was supported by US National Science Foundation grant DEB-0746106.
**Species**: Silene latifolia Poiret, Caryophyllaceae

**Organism / organ**: flowers

**Authors / contributors**: Stefan Dötterl

**Affiliation**: University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria


**Location**: University of Bayreuth, Germany

**Method sketch / description of data**:

- Experimental setup: Common garden experiment with potted individuals ($n = 98$).

- Scent sampling / analyses: Dynamic head-space samples were collected from single, newly opened flowers. A flower was enclosed within a polyethylene oven bag (Toppits®) and the emitted volatiles were trapped for 2 min in an adsorbent tube through the use of a membrane pump (ASF Thomas, Inc.). We took ChromatoProbe quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), cut the closed end, filled them with a mixture (1 : 1) of 3 mg Tenax-TA (mesh 60-80) and Carbotrap (mesh 20-40), and used them as adsorbent tubes. The samples were analysed by thermal desorption GC/MS on a Varian Saturn 2000 System using a 1079 injector, that had been fitted with the ChromatoProbe kit. A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m long, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex). Component identification was carried out using the NIST 02 mass spectral data base (NIST algorithm), or MassFinder 2.3, and confirmed by comparison of retention times with published data (Adams, 1995; Davies, 1990). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards. For quantification of compounds known amounts of lilac aldehydes, trans-β-ocimene, cis-3-hexenyl acetate, benzaldehyde, phenylacetaldehyde, and veratrole were injected, and the mean response of these compounds was used for quantification.


**Funding**: German Research Foundation (Research Training Group 678)
(31) **Species:** *Silene latifolia* Poiret, Caryophyllaceae

*Organism / organ:* flowers

*Authors / contributors:* Florian Schiestl

*Affiliation:* Institute of Systematic Botany, University of Zürich, Zollikerstrasse 107, 8008 Zürich


*Location:* Switzerland

*Method sketch / description of data:*

s. reference

*Funding:* SNF Swiss national science foundation
(32) **Species:** *Sinapis alba*, Brassicaceae

**Organism / organ:** inflorescence

**Authors / contributors:** Robert Glinwood

**Affiliation:** Dept Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, S750 07 Uppsala, Sweden

**Reference:** unpublished

**Location:** Controlled environment chamber, SLU, Sweden

**Method sketch / description of data:**
- Experimental setup: Laboratory experiment with potted plants. 10 replicates

Scent sampling / analyses: Inflorescences were enclosed in a glass vessel (450 ml), closed using an aluminum manifold with a hole for the stem. Charcoal filtered air was pumped into the vessel (push flow 600 ml min⁻¹) through a Teflon tube. Volatiles were trapped in glass tubes containing Tenax TA (50 mg) by letting air flowing through it (pull flow 300 ml min⁻¹). Volatiles were collected for 24 h.

After volatile collection, internal standards (nonane and dodecanal) were injected onto the Tenax tubes. Quantification was made by GC using internal standards and compounds tentatively identified by GC/MS using NIST (08) and commercial standards where available.

- **Funding:** The Swedish Foundation For Strategic Environmental Research (Mistra) and Carl Tryggers Stiftelsen.
(39) Species: Sinapis arvensis L., Brassicaceae

Organism / organ: plant / flowers

Authors / contributors: Jonas Kuppler¹,² & Robert R. Junker²

Affiliation: ¹Heinrich-Heine-University, Institute of Sensory Ecology, Düsseldorf, Germany
²University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria


Location: Botanical Garden of the University of Salzburg, Austria

Method sketch / description of data:

- Experimental setup: Common garden experiment with potted Sinapis arvensis individuals (n = 94). Plants were covered with net at least c. 12 h before sampling.

- Scent sampling / analyses: Dynamic headspace samples were collected from one inflorescence per plant. The inflorescence was enclosed with a polyester oven bag for 10 min and the emitted volatiles were then trapped on 1.5 mg Tenax (mesh 60-80) and 1.5 mg Carbotrap B (mesh 20–40) in a quartz vial for 2 min using a pump (flowrate 200 ml min⁻¹). Samples were collected between 8:00 - 12:00 h. They were analysed using an automatic thermal desorption system (TD-20, Shimadzu, Japan; ZB-5 fused silica column, Phenomenex, Aschaffenburg, Germany) coupled with a GC-MS (model QP2010 Ultra El, Shimadzu, Japan). Emission was standardized to ng/h/flower. Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 2011, FFNSC 2, Essential oils and Adams 2007 as well as the database available in MassFinder 3.

Funding: Graduate School ‘Evolutionary Networks: Organisms, Reactions, Molecules’ (E-Norm) of the Heinrich-Heine-University, Düsseldorf, Germany, and the Deutsche Forschungsgemeinschaft (DFG JU 2856/2-2).
Species: *Vicia faba* L., Fabaceae

Organism / organ: plant / inflorescences including flowers, leaves and EFNs

Authors / contributors: Mathias Hoffmeister¹,² & Robert R. Junker²

Affiliation: ¹Heinrich-Heine-University, Institute of Sensory Ecology, Düsseldorf, Germany ²University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria

Reference:

Location: University of Düsseldorf, Germany

Method sketch / description of data:

- Experimental setup: Experiment to test the effect of different herbivory treatments on floral scent emission. We tested four herbivory treatments, plants were either subjected (1) to a single jasmonic acid treatment (spraying of leaves until drip-off, 1 d before scent sampling, \( n_{\text{plants}} = 7 \)), (2) to multiple jasmonic acid treatments (7 d and 1 d before scent sampling, \( n_{\text{plants}} = 14 \)), (3) to *Spodoptera exigua* (2 larvae feeding on leaves in clip cages for 7 d until sampling, \( n_{\text{plants}} = 12 \)) or (4) to *Aphis fabae* (80 aphids feeding on leaves in clip cages for 7 d until sampling, \( n_{\text{plants}} = 15 \)). Control plants (C) were either sprayed with a solution containing no jasmonic acid for treatment 1 (\( n_{\text{plants}} = 9 \)) and 2 (\( n_{\text{plants}} = 7 \)), or equipped with empty clip cages for treatment 3 (\( n_{\text{plants}} = 12 \)) and 4 (\( n_{\text{plants}} = 13 \)).

- Scent sampling / analyses: Scent was sampled from unpicked wrapped inflorescences (oven bags, Toppits, Minden, Germany) as well as from surrounding air (empty oven bags) to determine plant-specific compounds. Volatiles were collected into traps filled with Poropak-Q (ARS, Inc, Gainesville, USA, flow rate: 1000 ml min⁻¹, sampling: 60 min). Subsequently volatiles were eluted with 150 µl Dichloromethane containing Nonylacacetate (10 ng µl⁻¹) as internal standard. By means of a AOC-20i autoinjector (Shimadzu, Tokyo, Japan) 1 µl of the eluate was injected at 220°C (split ratio 1 : 1) into a Shimadzu GC-MS-QP2010 Ultra (Shimadzu, Tokyo, Japan, ZB-5 fused silica column, Phenomenex, Aschaffenburg, Germany). Column flow (carrier gas: helium) was set to 3 ml min⁻¹. The GC oven temperature started at 40°C, was then increased to 220°C (10°C min⁻¹) and held for 2 min. The MS interface worked at 250°C and the ion source at 200°C. Mass spectra were taken at 70 eV (in El mode) from m/z 30 to 530. We processed GC/MS data with the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012). Compounds were identified, comparing them to mass spectra and retention indices of commercially available standard substances or to compounds listed in mass spectral libraries Wiley Registry 9th Edition, NIST 2011 and FFNSC2. Using the internal standard we determined the emission rates of the identified volatiles.

Funding: Deutsche Forschungsgemeinschaft (DFG JU 2856/2-2).
Species: Vigna unguiculata (L.) Walp. ssp. unguiculata var. spontanea (Schweinf.) Pasquet, Fabaceae/Leguminosae

Organism / organ: plant / flowers

Authors / contributors: Jette T. Knudsen¹² & Rémy S. Pasquet³.

Affiliation: ¹Dept. of Biology, Lund University, Lund, Sweden, ²Nattaro Labs AB, Medicon Village, 223 81 Lund, Sweden, ³IRD, Dept ECOBIO, 44 Bd de Dunkerque, 13572 Marseille cedex 02, France.

We acknowledge Beatrice Elesani for growing the plants and help with floral scent collection

Reference: NA

Location: Muhaka Field Station, 32 km S-SE of Mombasa, Kenya

Method sketch / description of data:

- Experimental setup: Screen house grown cowpea plants from seeds collected at 19 different locations mainly in coastal Kenya (n=177). Plants were screened off from pollinator visitation.

- Scent sampling / analyses: Dynamic headspace samples were collected from one flower per plant. Each flower was covered with a polyacetate oven bag and an absorbent tube, filled with 20 mg Tenax Gr (mesh size 60-80) was inserted into the bag and the air within the bag was sucked through the absorbent tube with a battery driven membrane pump at a flow rate of 90–110 ml min⁻¹. In parallel blank samples were collected from leaves or air. Floral scent collection started within 10 min of flower opening (c. 05:45-06:30 h local time) and lasted for about 120 min. Samples were extracted with 250 μl of hexane. The samples were analysed by coupled GC-MS on a HP 6890 GC connected to a HP 5973 mass selective detector. Emission was standardized to %/flower. Almost all compounds were identified by comparison of mass spectra and retention times with commercially available standard compounds. Alternatively, compounds were identified using the mass spectral libraries NIST 2011 and Adams 2007.

Funding: SIDA/SAREC Grant (SWE-2005-340) awarded to JTK
(50–57) **Species:** Fragaria × ananassa Duch., Rosaceae

**Organism / organ:** plant / fruit

**Authors / contributors:** Anne Plotto¹ & Celine Jouquand²

**Affiliation:** ¹USDA, ARS, US Horticultural Research Laboratory, Fort Pierce, Florida, U.S.A. ²Institut Polytechnique Lasalle, Beauvais, France


**Location:** Plant City, Florida, U.S.A.

**Method sketch / description of data:**
- Experimental setup: Fruit harvested at commercial maturity from commercial fields in Plant City, Florida, on multiple harvests at monthly intervals.

- Scent sampling / analyses: About ten fruit per replication were homogenized for 20 s in a blender, and an equal volume of saturated aqueous CaCl₂ was added to stop enzymatic activity. Five millilitres of the mix + 3-hexanone internal standard were placed in a 20 mL vial. Volatiles were extracted using a 2-cm SPME fiber (50/30 mm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA). After a sample equilibration time of 10 min at 40°C, the fiber was exposed to the headspace for 30 min at 40°C, then introduced into the injector of the GC-MS for 5 min at 250°C for desorption of volatiles. Identification of volatile compounds was carried out using an Agilent 6890 GC coupled with a 5973N MS detector and equipped with a DB5ms capillary column (60 m · 250 μm · 1.00 μm) (Agilent Technologies, Santa Clara, CA). Data were collected using the Chemstation G1701 AA (Agilent Technologies). Compounds were first identified by matching mass spectra with library entries (NIST/EPA/NIH Mass Spectral Library, Version 2.0d; National Institute of Standards and Technology, Gaithersburg, MD), then confirmed using retention indices calculated using retention time data from a series of alkane standards (C5–C17) run under the same chromatographic conditions. The relative concentration of volatile compounds was determined by normalizing the peak area of each compound to the peak area of internal standard.

**Funding:** University of Florida and USDA, ARS internal funds.
(58) **Species**: Tangarine, *Citrus reticulata* Blanco, Rutaceae

**Organism / organ**: plant / fruit

**Authors / contributors**: Anne Plotto¹ & Fred G Gmitter²

**Affiliation**: ¹USDA, ARS, US Horticultural Research Laboratory, Fort Pierce, Florida, U.S.A. ²University of Florida, Citrus Research and Education Center, Lake Alfred, Florida, U.S.A.


**Location**: Citrus Research and Education Center, Lake Alfred, Florida, U.S.A.

**Method sketch / description of data**:
- Experimental setup: Fifty to sixty fruit harvested from the University of Florida breeding population (single tree per genotype) *(n = 25)*.

- Scent sampling / analyses: Fruit were juiced and 2.5 ml juice + 2.5 ml saturated calcium chloride were introduced to 20 mL sample vials. Volatiles were extracted using a 2-cm SPME fiber (50/30 mm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA). After a sample equilibration time of 30 min at 40°C, the fiber was exposed to the headspace for 60 min at 40°C, then introduced into the injector of the GC-MS for 3 min at 250°C for desorption of volatiles. Identification of volatile compounds was carried out using an Agilent 6890 GC coupled with a 5973N MS detector and equipped with a DB5ms capillary column (60 m · 250 μm · 1.00 μm) or a DB-Wax (60 m · 250 μm · 0.50 μm) (Agilent Technologies, Santa Clara, CA). Data were collected using the Chemstation G1701 AA (Agilent Technologies). Compounds were first identified by matching mass spectra with library entries (NIST/EPA/NIH Mass Spectral Library, Version 2.0d; National Institute of Standards and Technology, Gaithersburg, MD), then confirmed using retention indices calculated using retention time data from a series of alkane standards (C5–C15) run under the same chromatographic conditions. The relative concentration of volatile compounds was determined by normalizing the peak area of each compound to the peak area of internal standard.

**Funding**: University of Florida and USDA, ARS Internal funds
(59, 60) **Species:** Beta vulgaris L., Amaranthaceae

**Organism / organ:** plant / whole, non-flowering plant

**Authors / contributors:** Robert Glinwood

**Affiliation:** Dept Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, S750 07 Uppsala, Sweden

**Reference:** unpublished

**Location:** Controlled environment chamber, SLU, Sweden

**Method sketch / description of data:**
- Experimental setup: Laboratory experiment with potted plants (4–5 wk old at vegetative growth stage, eight to 10 leaves per plant). Ten replicates induced (infested with aphids), 19 replicates uninfested

Scent sampling / analyses: The plant was covered by a polyester baking bag (Melitta Scandinavia AB, Toppits 60 × 55 cm). Charcoal filtered air was pumped into the bag (push flow 600 ml min⁻¹) from the lower part of the bag through a Teflon tube. Volatiles were trapped in glass tubes containing Porapak Q (mesh 50/80) by letting air flowing through it (pull flow 450 ml min⁻¹). Induced plants were infested with 300 Aphid fabae (Hemiptera: Aphididae) for 72 h before volatile collection. Control plants were uninfested.

After 72 h the collected volatiles compounds were rinsed out with 750 µl redistilled Dicloromethane and internal standard added (2-tridecanone). The sample was then reduced to 50 µl and analyzed on GC-MS. Quantification was made using internal standards and compounds tentatively identified using NIST (08) and commercial standards where available.

- **Funding:** The Swedish Research Council (Formas), Carl Tryggers Stiftelse för Vetenskaplig Forskning and the Faculty of Natural Resources and Agricultural Sciences, SLU.
**Species**: Arabidopsis thaliana, Brassica oleracea & Brassica nigra.

organ: plant

Organism: Brevicoryne brassicae, Rhizobacteria, Pieris rapae, Eggs of Pieris brassicae, Eggs of Mamestra brassicae, Cotesia glomerata, Cotesia rubecula.

**Author / contributor**: Berhane T. Weldegergis

**Affiliation**: Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands

**Reference**:


**Location**: Laboratory of Entomology, Wageningen University, Wageningen, the Netherlands

**Method sketch / description of data**:

- Experimental setup: Glasshouse experiment with potted individual plants ranging 5 – 10 replicates for each treatment group.

Plant volatiles were collected from the headspace of each sample. Before volatile collection, the pots were removed and the plant roots and soil were carefully wrapped with aluminium foil. The plants were then placed in glass jars and sealed with a Viton-lined glass lid with an inlet and outlet. Compressed air was filtered by passing through charcoal before entering the sampling container. Volatiles were trapped by drawing air out of the jars through a stainless steel tube filled with 200 mg Tenax TA (20/35 mesh). Thermo Trace Ultra GC coupled with Thermo Trace DSQ MS (Thermo Fisher Scientific Waltham, USA) were used for separation and detection of plant volatiles. The collected volatiles were released thermally from the Tenax...
TA cartridges using thermal desorption unit (Ultra 50:50, Markes, Llantrisant, UK), while re-collecting them in a thermally cooled universal solvent trap (Unity, Markes). Once the desorption process was completed, the cold trap was ballistically heated, while the volatiles being transferred to a ZB-5MSi capillary column: 30 m L × 0.25 mm I.D. × 1.00 µm F.T. (Phenomenex, Torrance, CA, USA) for further separation in a programmed GC oven temperature. The DSQ MS was operated in a scan mode and ionisation was performed in EI at 70 eV. The MS transfer line and ion source were set at 275 and 250°C, respectively. Identification of compounds was based on comparison of mass spectra with the NIST 2005 and Wageningen Mass Spectral Database of Natural Products libraries as well as linear retention indices (LRI). Quantitation (peak area measurements) was performed using a single (target) ion in selected ion monitoring (SIM) mode. Individual peak areas were corrected for the aerial fresh weight of each plant sample (peak area unit g⁻¹ plant FW). For details on the methodologies please refer to references 1 – 5 above.

_Funding:_ The Netherlands Organization for Scientific Research (NWO).
(74–76) **Species**: *Hordeum vulgare* L., Poaceae

**Organism / organ**: plant / whole, non-flowering plant

**Authors / contributors**: Robert Glinwood

**Affiliation**: Dept Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, S750 07 Uppsala, Sweden

**Reference**: unpublished

**Location**: Controlled environment chamber, SLU, Sweden

**Method sketch / description of data**:
- Experimental setup: Laboratory experiment with potted plants. Eight pots of 20 plats (1 pot = 1 replicate) for each of three cultivars Alva, Barke and Frieda

Scent sampling / analyses: A pot of 20 barley plants (two leaf stage) was covered by a polyester baking bag (Melitta Scandinavia AB, Toppits 60 × 55 cm). Charcoal filtered air was pumped into the bag (push flow 600 ml min⁻¹) from the lower part of the bag through a Teflon tube. Volatiles were trapped in glass tubes containing Porapak Q (mesh 50/80) by letting air flowing through it (pull flow 450 ml min⁻¹).

After 72 h the collected volatiles compounds were rinsed out with 750 µl redistilled Dicloromethane and internal standard added (1-nonene). The sample was then reduced to 50 µl and analyzed on GC-MS. Quantification was made using internal standards and compounds tentatively identified using NIST (08) and commercial standards where available.

- **Funding**: The Swedish Research Council (Formas), The Swedish Foundation For Strategic Environmental Research (Mistra) and the Faculty of Natural Resources and Agricultural Sciences, SLU.
**Species:** *Malus silvestris* Miller (variety De Costa), Rosaceae

**Organism / Organ:** plant / leaves

**Authors / contributors:** Luisa Amo¹² & Marcel Visser¹

**Affiliation:** ¹ Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands ² Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain


**Location:** Heteren, The Netherlands

**Method sketch / description of data:**

Experimental setup: Common garden experiment with potted *Malus silvestris* individuals (*n* = 32). Sixteen individuals were uninfested and 16 individuals were infested with 30 winter moth *Operopthera brumata* caterpillars (L5 stage) during 3 d. Caterpillars were located in clip-cages, so they could only eat one leaf. The day of chemical measurements, we removed caterpillars and cut the damaged parts of the leaves around 3 h before the chemical measurements. In the control uninfested trees, we also cut a similar number of leaves 3 h before the chemical measurements.

**Scent sampling / analyses:** Dynamic headspace samples were collected from one branch per tree (16 infested and 16 uninfested) between 16:30 and 19:00 h. The branch was enclosed with a polyethylene oven bag and the volatiles were trapped on 150 mg Tenax TA and 150 mg Carbopack B (Markes International Limited, Llantrisant, UK) tubes for 2 h using a vacuum pump (flowrate 200 ml min⁻¹). Traps were stored at 4°C for 10–11 wk until analysis. Volatiles were desorbed from the traps using an automated thermodesorption unit (model Unity, Markes, Llantrisant, UK) coupled with a GC-MS (model Trace, ThermoFinnigan, Austin, Texas). The volatiles were detected by the MS operating at 70 eV in EI mode. Mass spectra were acquired in full scan mode (33–300 amu, 0.4 scan s⁻¹). Compounds were identified by their mass spectra using deconvolution software (AMDIS; NIST (International Institute of Standards and Technology, MD, USA) and US DOD (Department of Defense, DC, USA)) in combination with Nist 98 and Wiley seventh edition spectral libraries and by comparing their linear retention indices. In addition, mass spectra and/or linear retention indices of chromatographic peaks were compared with values reported in the literature. Additional confirmation for compound identification was obtained by interpolating retention indices of homologous series, or by comparing analytical data with those of reference substances. The integrated signals generated by the AMDIS software from the MS chromatograms were used for comparison between the treatments. Peak areas in each sample were divided by the total volume in ml that was sampled over the trap, to correct for small differences in flow rates over individual traps.

**Funding:** MEC postdoctoral programme to L.A. and NWO-VICI grant.
Species: Phaseolus lunatus, Fabaceae

Organism / organ: leaves

Authors / contributors: Christian Kost¹,² & Martin Heil²

Affiliation: ¹Max Planck Institute for Chemical Ecology, Research Group Experimental Ecology and Evolution, Jena, Germany, ²University of Osnabrück, Department of Ecology and Evolution, School of Biology/ Chemistry, Osnabrück, Germany, ³CINVESTAV – Irapuato, Departamento de Ingeniería Genética, Irapuato, México.


Location: coastal area, dirt roads leading to extensively used pastures or plantations, Puerto Escondido, Mexico.

Method sketch / description of data:
- Experimental setup: Use of laboratory-grown plants to test the effect of feeding of a specialised leaf herbivore on the emission of volatile organic compounds. The five youngest leaves of two potted Lima bean plants were exposed to five adult Epilachna varivestis (Mexican bean beetle) for 2 d at room temperature, while two control plants were left undamaged. Volatiles were collected from the five youngest leaves of all four plants for the next 24 h. This experiment was replicated nine times.

- Scent sampling / analyses: Tendrils were bagged in a PET foil (‘Bratenschlauch’, Toppits, Minden, Germany) that does not itself emit detectable amounts of volatiles. The emitted VOCs were collected continuously over 24 h on charcoal traps (1.5 mg char-coal, CLSA-Filters, Le Ruissseau de Montbrun, France) using air circulation. After 24 h, leaves were dried for dry mass determination and volatiles were eluted from the carbon trap with dichloromethane (40 μl) containing 1-bromodecane (200 ng μl⁻¹) as an internal standard. Samples were analysed on a GC-Trace mass spectrometer (Thermo Finnigan, www.thermofinnigan.com). The extracts were directly analyzed by GC-MS using fused-silica capillary tubes (15 m, 0.25 mm; Alltech, Unterhaching, Germany) coated with DB 1 (0.1 m). Helium at 60 kPa served as the carrier gas. Separation of the compounds was achieved under programmed conditions (40°C for 2 min, then at 10°C min 1 to 200°C). MS analysis was performed (model MD800, Fisons, Bellevue, WA) with the GC interface at 260°C and the scan range at 35 to 300 D. Individual compounds (peak areas) were quantified with respect to the peak area of the internal standard and related to the dry weight of the measured tendril.

- Funding: This study was financially supported by the German Research Foundation (DFG grants HE 3169/2–1,2,3,4) to MH.
(81, 82) **Species**: *Pinus sylvestris* L., Pinaceae

**Organism / organ**: plant / leaves (needles)

**Authors / contributors**: James D. Blande

**Affiliation**: Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland.


**Location**: Research garden of the Kuopio campus of the University of Eastern Finland, Finland.

**Method sketch / description of data**:

- Experimental setup: Field study with potted 2-yr-old Scots pine saplings. A total of 17 saplings were each infested with two large pine weevils, which were enclosed in mesh bags around the stem; 16 saplings were fitted with an empty mesh bag and were used as controls.

- Samples were collected from the foliage of the saplings by dynamic headspace analysis after 3 d of herbivore-feeding. The main shoot and side branches above the site of damage were enclosed in a polyethylene terephthalate (PET) bag (size 45 cm × 55 cm). Clean filtered air was pumped into the bag and the headspace was drawn out through a stainless steel tube filled with Tenax TA adsorbent (150 mg) (Supelco, mesh 60/80) at a flow rate of 200 ml min⁻¹. Samples were analysed by GC-MS (Hewlett-Packard GC 6890, MSD 5973) with trapped compounds thermally desorbed with an automated thermal desorption unit (Perkin-Elmer ATD400). Emissions were calculated in units of ng g (DW)⁻¹ h⁻¹. Compounds were identified by comparing mass spectra and retention times with a series of commercially available terpene and green-leaf volatile standards. Compounds for which there was not an available standard were identified by comparison of mass spectra with the Wiley library.

**Funding**: Academy of Finland (decision numbers 111543, 256050, 251898 and 141053).
(83–88) Species: *Populus nigra*, Salicaceae

Organism / organ: Leaves

Authors / contributors:
Andrea Clavijo McCormick¹, Sandra Irmisch¹, Andreas Reinecke², G. Andreas Boeckler¹, Daniel Veit³, Michael Reichelt¹, Bill Hansson², Jonathan Gershenzon¹, Tobias G. Köllner¹ & Sybille B. Unsicker¹*

¹ Max Planck Institute for Chemical Ecology, Department of Biochemistry
² Max Planck Institute for Chemical Ecology, Department of Neuroethology
³ Max Planck Institute for Chemical Ecology, Department of Scientific Instrumentation and Utilities Management
⁴ Current address: Max Planck Institute for Ornithology, Department of Behavioural Ecology and Evolutionary Genetics

Reference:

Location: Glasshouse, climate chamber, Jena, Germany

Method sketch / description of data:
To investigate the emission of volatiles from young *P. nigra* after *Lymantria dispar* caterpillar feeding, two individual trees (c. 1.20 m in height) of each of 20 different genotypes were selected. Twenty trees were infested with gypsy moth caterpillars and the other 20 functioned as controls. Thus each treatment contained 20 tree genotypes as replicates. During the experiment, trees were kept in a climate chamber. The foliage of each tree enclosed with PET foil (Toppits® Bratschlauch, Minden, Germany) Seven fourth instar *L. dispar* caterpillars were released in the PET bags covering the basal section of the black poplar foliage and allowed to feed for 41 h.

Volatile compounds were collected for 4 h the second day after herbivore release on the plants. Volatile compounds were desorbed by eluting the filter twice with 200 µl dichloromethane containing nonyl acetate as an internal standard (10 ng µl⁻¹).

Analysis of black poplar volatiles was carried out using GC-MS/FID analysis an Agilent 6890 series gas chromatograph (Agilent, Santa Clara, CA, USA) coupled either to an Agilent 5973 quadrupole mass selective detector (interface temp. 270 °C, quadrupole temp. 150°C, source temp. 230°C; electron energy 70 eV) or a flame ionization detector (FID, temp. 300°C). The constituents were separated with a DB-5MS column (30 m × 0.25 mm × 0.25 µm) and He
(mass detector) or H₂ (FID) as carrier gases. One microliter of the sample was injected without splitting at an initial oven temperature of 40°C (2 min hold) followed by a ramp to 155°C (1°C min⁻¹), a ramp to 300°C (60°C min⁻¹), and a hold for 3 min. To confirm identification of coeluting compounds, samples were also run on an HP Innowax column (Agilent, 30 m × 0.25 mm × 0.25 μm) under the same conditions, except the final temperature gradient ended at 260°C.

Compounds were identified by comparison of retention times and mass spectra to those of authentic standards obtained from Fluka (Seelze, Germany), Roth (Karlsruhe, Germany), Sigma (St. Louis, MO, USA) or Bedoukian (Danbury, CT, USA) or to reference spectra in the Wiley and National Institute of Standards and Technology libraries and in the literature (Joulain & König, 1998). Standards not commercially available were provided by Wilfried A. König, University of Hamburg (essential oils of Oreodaphne porosa and Aloysia sellowii) and DMNT was kindly synthesized by Stefan Bartram (MPI-ICE). The quantity of each compound was determined from its peak area in the FID trace in relation to the area of the internal standard using the effective carbon number concept (Scanlon & Willis, 1985). Chiral analysis was performed using a Rt™-ßDEXsm column (Restek, Bad Homburg, Germany) with a temperature program from 50°C (2 min hold) to 220°C (1 min hold) with a gradient of 2°C min⁻¹.


**Funding:** This project was funded by the Max Planck Society.
**Species:** *Salix viminalis* L., Salicaceae

*Organism / organ:* plant / whole, non-flowering plant

**Authors / contributors:** Robert Glinwood

**Affiliation:** Dept Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, S750 07 Uppsala, Sweden

**Reference:** unpublished

**Location:** Controlled environment chamber, SLU, Sweden

**Method sketch / description of data:**

- **Experimental setup:** Laboratory experiment with potted plants. Eight induced (infested) and eight control (uninfested) plants

Scent sampling / analyses: The plant (c. 30–40 cm high with 2–3 ‘twigs’) was covered by a polyester baking bag (Melitta Scandinavia AB, Toppits 60 x 55 cm). Induced plants were infested by 10 *Anthocoris nemorum* (Hemiptera: Anthocoridae) for 24 h. Charcoal filtered air was pumped into the bag (push flow 600 ml min⁻¹) from the lower part of the bag through a Teflon tube. Volatiles were trapped in glass tubes containing Porapak Q (mesh 50/80) by letting air flowing through it (pull flow 450 ml min⁻¹).

After 24 h the collected volatiles compounds were rinsed out with 750 µl redistilled Dicloromethane and internal standards added (1-nonene and Dodecanal). The sample was then reduced to 50 µl and analyzed on GC-MS. Quantification was made using internal standards and compounds tentatively identified using NIST (08) and commercial standards where available.

- **Funding:** The Swedish Research Council (Formas) and the Faculty of Natural Resources and Agricultural Sciences, SLU.
**Species:** Austroplebeia australis F., Apidae

*Organism / organ:* animal / cuticular surface

**Authors / contributors:** Sara D. Leonhardt¹ & Thomas Schmitt¹

**Affiliation:** ¹Würzburg University, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


**Locations:** Australia: Sydney, Elonora, Dalby

**Method sketch / description of data:**

- Specimen sampling / analyses: Five to 10 specimens were obtained from the entrance of a bee colony by capturing leaving foragers in a clean clear plastic bag, killed by quick freezing and extracted in hexane for 5 min. Cuticular extracts were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) on a DB-5 fused silica capillary column (30 m _ 0.25 mm ID; d.f. ¼ 0.25 mm; J & W, Folsom, CA, USA) with helium used as carrier gas (constant flow of 1 ml/ min). Injection was carried out at 250°C in the splitless mode for 1 min. Temperature was raised from 60°C to 300°C with a 5°C min⁻¹ heating rate and held at 300°C for 10 min. Electron impact mass spectra were recorded at 70 eV.

Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 98 and Adams EO Library 2205.

**Funding:** Deutsche Forschungs-Gemeinschaft (DFG project: LE 2750/1-1) and a grant of the German Excellence Initiative to the Graduate School of Life Science, University of Würzburg.
(93, 94) **Species:** Crematogaster scutellaris, Formicidae, Hymenoptera

**Organism / organ:** animal / body surface

**Authors / contributors:** Florian Menzel

**Affiliation:** University of Mainz, Institute of Zoology, Mainz, Germany


**Location:** Esterel massif, (Côte d’Azur, France) and Dolceacqua, Liguria, Italy.

**Method sketch / description of data:**

- Cuticular extracts were obtained by immersing between four and 12 freeze-killed ants in hexane for 10 min (as a pooled sample), and analysed using GC-MS with a Hewlett Packard 6890 series gas chromatograph coupled to a HP 5973 Mass Selective Detector. The GC was equipped with a J&W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm). Temperature was kept at 60°C for 2 min, then increased by 60°C min⁻¹ up to 200°C and subsequently by 4°C min⁻¹ to 320°C, where it remained constant for 10 min. Helium was used as carrier gas with a constant flow of 1 ml min⁻¹. The samples were injected in splitless mode for 30 s at 250°C. The electron impact mass spectra (EI-MS) were recorded with an ionisation voltage of 70 eV, a source temperature of 230°C, and an interface temperature of 325°C. The cuticular hydrocarbons were identified based on diagnostic ions and the Kovat’s retention index.

**Funding:** none
(95–98) **Species**: *Dibrachys cavus*, Hymenoptera: Pteromalidae

**Organism / organ**: whole body extract

**Authors / contributors**: Joachim Ruther

**Affiliation**: University of Regensburg, Institute of Zoology, Regensburg, Germany


**Location**: Lab strain, originally collected from a bird’s nest near Hamburg, Germany.

**Method sketch / description of data**:

- Experimental setup: Parasitic wasps were reared on freeze-killed pupae of the green bottle fly *Lucilia Caesar* L. (Diptera: Calliphoridae) at 25°C and 55% r.h. at a L16 : D8 photoperiod. Male and female wasps were excised from the host 1-2 d before the expected eclosion date and extracted immediately or 1-2 d after emergence.

- Scent sampling / analyses: Newly emerged and 1- to 2-d-old males and females were extracted for 30 min with 10 µl dichloromethane containing 10 ng µl⁻¹ of n-tetracosane as an internal standard (n = 10 for each sex and age class). Extracts (1 µl per sample) were analysed by coupled gas chromatography–mass spectrometry (GC/MS) on a Fisons 8060 GC coupled to a Fisons MD 800 quadrupole MS (Thermo Finnigan, Egelsbach, Germany). Analytical conditions were as follows. Injector temperature: 280°C, column: 30 m x 0.32 mm inner diameter DB-5ms, film thickness 0.25 lm (J & W, Scientific, Folsom, CA, USA), carrier gas: helium, inlet pressure 10 kPa. The temperature program started at 150°C and increased at 3°C per min to 280°C. Methyl-branched hydrocarbons were identified using diagnostic ions resulting from the favoured fragmentation at the branching points and by comparing linear retention indices with literature data.

- **Funding**: Deutsche Forschungsgemeinschaft (DFG Ru 717/8-2).
Species: Heliothis virescens Fabricius and Heliothis subflexa Guenée, Noctuidae

Organism/organ: moth, female sex pheromone gland

Authors/contributors: Astrid T. Groot\textsuperscript{1,2}

Affiliation: \textsuperscript{1}University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics (IBED), Amsterdam, the Netherlands
\textsuperscript{2}Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany


Location: North Carolina State University

Method sketch/description of data:

- (Copied from Groot \textit{et al.} 2009): Eggs of Hv and larvae of Hv and Hs were collected in the field, as described in Groot \textit{et al.} 2009 (reference above). Were reared to adults on artificial diet at North Carolina State University (NCSU). Hs larvae were given \textit{Physalis angulata} fruits in addition to the artificial diet; pupae were separated by sex and checked daily for emergence. Pheromone glands were extracted from 2 to 5 d old virgin females that emerged from the field-collected larvae and/or from their female offspring (i.e. females that were reared in the lab for one generation). Glands were extracted and analyzed as described for \textit{M. brassicae}.

Funding: National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (CSREES), grant # 2005–00896, the W.M. Keck Center for Behavioral Biology, and the Blanton J. Whitmire Endowment at North Carolina State University.
**Species:** Homo sapiens

**Organism / organ:** NA

**Authors / contributors:** Niels O. Verhulst

**Affiliation:** Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, the Netherlands


**Location:** Wageningen University, The Netherlands

**Method sketch / description of data:**

- **Experimental setup:** Samples were collected from nine individuals that were highly attractive to malaria mosquitoes and seven individuals that were poorly attractive to these malaria mosquitoes. Individuals were asked to refrain from eating spicy food, taking a shower and using perfumed cosmetics for 24 h before sampling.

- **Scent sampling / analyses:** Volatiles were collected by rubbing the sole of the foot over 100 glass beads for 10 min. The beads were transferred to steel cartridges and emanations analyzed by thermodesorption followed by GC–MS (Trace GC Ultra, quadruple mass detector, DSQ, Thermo Scientific, USA). Analytes were split to 1/6 of the total amount and separated on an RTX-5ms GC column (Restek, USA). Compounds were identified by comparing their mass spectra and retention times with those of authentic reference compounds. Relative quantification of the compounds was done based on characteristic mass ions for each compound using the software package Xcalibur (Version 2.07, Thermo Scientific, USA). Integration settings for each compound were inserted in a processing setup, batch processed and evaluated in the Quan browser.

**Funding:** The Foundation for the National Institutes of Health through the Grand Challenges in Global Health Initiative (GCGH#121), and a grant from the Earth and Life Science Foundation of the Netherlands Organization for Scientific Research (820.01.019).
Species: Mamestra brassicae L., Noctuidae

Organism / organ: moth, female sex pheromone gland

Authors / contributors: Astrid T. Groot

Affiliation: 1University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics (IBED), Amsterdam, the Netherlands
2Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany


Location: Wageningen University and University of Amsterdam

Method sketch / description of data:

- (see Van Geffen et al. 2015; summary:) In a glasshouse, 20 open-top compartments of 50 × 50 × 60 cm³ were constructed. Each compartment was randomly assigned to one of four artificial light treatments: green-rich, warm white, red-rich or no artificial light at night. Treatments were divided over five randomised blocks. Each compartment contained five pupae, resulting in a nested design. Pupae were checked for emergence every day and freshly emerged moths were provided with sugar water. Each night, all three-night-old females were collected for gland extraction. Glands of 24, 22, 22, and 24 females from green, white, red and dark control treatments, respectively, were successfully extracted.

- Scent sampling / analyses: (full description in Groot et al. 2010): Each gland was extracted separately in a glass conical vial in 50 µl of n-hexane (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 25 ng of the internal standard pentadecane. After 30–40 min, the glands were removed and the extract stored at -20 °C until GC analysis. For GC analysis, the extracts were reduced to 1–2 µl under a gentle stream of nitrogen and taken up with a 10 µl syringe (701SN 26S GA 2 needle; Hamilton, Reno, NV, USA) together with 1 µl of octane (Fluka, St Louis, MO, USA) to avoid evaporation. The sample was injected with a 7683 automatic injector into the splitless inlet of an HP7890 GC, which was coupled with a high resolution polar capillary column (DB-WAXetr (extended temperature range); 30 m · 0.25 mm · 0.5 lm) and a flame-ionization detector (FID) using the following program: 60°C (hold for 2 min) to 180°C (30°C min), followed by an increase of temperature to 230°C (5°C min). The column was then heated to 245°C at 20°C min) for 15 min. The FID was kept at 250°C. To identify the particular compounds, a multicomponent blend (compounds from Pherobank, Wageningen) containing all the pheromone compounds was injected into the GC before or after each daily series of injections. For quantitative analysis, the GC signal was integrated with the software CHEMSTATION (Agilent, Technologies Deutschland GmbH, Böblingen, Germany).

Funding: NWO-STW grant 11110, Philips Lighting, and the Nederlandse Aardolie Maatschappij
Species: *Myrmica rubra*, Formicidae, Hymenoptera

Organism / organ: animal / body surface

Authors / contributors: Florian Menzel

Affiliation: University of Mainz, Institute of Zoology, Mainz, Germany


Location: Ober-Olmer forest near Mainz, Rhineland-Palatinate, Germany

Method sketch / description of data:

- Cuticular extracts were obtained by immersing individual freeze-killed ants in hexane for 10 min, and analysed using GC-MS with a Hewlett Packard 7890A series gas chromatograph coupled to a HP 5975C Mass Selective Detector. The GC was equipped with an Agilent HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm). Temperature was kept at 60°C for 2 min, then increased by 60°C min⁻¹ up to 200°C and subsequently by 4°C min⁻¹ to 320°C, where it remained constant for 10 min. Helium was used as carrier gas with a constant flow of 1 ml min⁻¹. The samples were injected in splitless mode for 30 s at 250°C. The electron impact mass spectra (EI-MS) were recorded with an ionisation voltage of 70 eV, a source temperature of 230°C, and an interface temperature of 325°C. The cuticular hydrocarbons were identified based on diagnostic ions and the Kovat’s retention index.

Funding: none
Species: Odynerus spinipes (Vespidae), Pseudospinolia neglecta, Chrysis mediata (Chrysididae)

Organism / organ: insects

Authors / contributors: Thomas Schmitt

Affiliation: University of Würzburg, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


Location: Kaiserstuhl, Baden-Württemberg, Germany

Method sketch / description of data:

- Experimental setup: Comparative study on chemical mimicry of a vespid host and its chrysid brood parasitoids.

- Scent sampling / analyses: CHCs were extracted by submerging the freeze-killed wasps for 10 min in n-pentane. In all extracts, the volume of n-pentane was adjusted to 200 µl, and all extracts were stored at -20°C before chemical analysis. The CHC extracts were analyzed with a gas chromatograph (GC) coupled with a Mass Selective (MS) Detector. The CHC extracts were analyzed with a HP 6890 gas chromatograph (GC) coupled with a HP 5973 Mass Selective (MS) Detector (Hewlett Packard, Waldbronn, Germany). The GC (split/splitless injector in splitless mode for 1 min, injected volume: 1 µl at 250°C) was equipped with a DB-5 Fused Silica capillary column (30 m × 0.25 mm ID, 0.25 m²; J&W Scientific, Folsom, USA). Helium served as a carrier gas at a constant flow of 1 ml min⁻¹. The following temperature program was used: start temperature 60°C, temperature increase by 5°C min⁻¹ up to 300°C, isotherm at 300°C for 10 min. The electron ionization mass spectra (EI-MS) were acquired at an ionization voltage of 70 eV (source temperature: 230°C). Chromatograms and mass spectra were recorded and quantified (via integrated peak areas; initial threshold: 16, manually corrected) with the software HP Enhanced ChemStation G1701AA (version A.03.00; Hewlett Packard). Individual CHC compounds were chemically identified by considering the MS data base Wiley275 (John Wiley & Sons, New York, USA), the compound-specific retention time, Kovat's indices, and the detected diagnostic ions (Carlson et al., 1998). Given that some substances could not be accurately separated with the above instrument and settings, we calculated the combined quantity by integrating over all substances within a peak in these cases. We additionally applied dimethyldisulfide (DMDS) derivatization before the gas chromatography-mass spectrometry (GC-MS) to identify the position of double bonds within unsaturated hydrocarbons. Note that it was not possible to reliably identify the position of double bonds in some alkenes and in most alkadienes due to low concentration of these compounds.


- Funding: This work was funded by the Deutsche Forschungsgemeinschaft (DFG, SCHM 2645/2-1 and SCHM2645/3-1).
(123) **Species:** *Plutella xylostella* L., Plutellidae

*Organism /organ:* moth, female sex pheromone gland

**Authors / contributors:** Astrid T. Groot¹,²

**Affiliation:** ¹University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics (IBED), Amsterdam, the Netherlands
²Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany


**Location:** University of Amsterdam

**Method sketch / description of data:**

- The moths were collected from the cages in their late instar/pupae stage and reared individually in 5 cm × 1.5 cm glass tubes and checked for emergence every day. The sex of the insects was determined after emergence by checking the abdomen of the adult moths. All adults were fed with honey-agar (50 ml water, 5 ml honey + 1 g agar-agar). One- to five-days-old females were used for the pheromone analysis. Glands were extracted and analysed as described for *M. brassicae*.

**Funding:** University of Amsterdam
**Species:** Scaptotrigona pectoralis D., Apidae

**Organism / organ:** animal / cuticular surface

**Authors / contributors:** Sara D. Leonhardt¹ & Thomas Schmitt¹

**Affiliation:** ¹Würzburg University, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


**Locations:** Costa Rica: Monte Alto, Hojancha, Santa Cruz, Atenas

**Method sketch / description of data:**

- Specimen sampling / analyses: 5–10 specimens were obtained from the entrance of a bee colony by capturing leaving foragers in a clean clear plastic bag, killed by quick freezing and extracted in hexane for 5 min.

Cuticular extracts were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) on a DB-5 fused silica capillary column (30 m _ 0.25 mm ID; d.f. ¼ 0.25 mm; J & W, Folsom, CA, USA) with helium used as carrier gas (constant flow of 1 ml min⁻¹). Injection was carried out at 250°C in the splitless mode for 1 min. Temperature was raised from 60°C to 300°C with a 5°C min⁻¹ heating rate and held at 300°C for 10 min. Electron impact mass spectra were recorded at 70 eV.

Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 98 and Adams EO Library 2205.

**Funding:** Carlsberg foundation, Denmark; Deutsche Forschungs-Gemeinschaft (DFG project: LE 2750/1-1) and a grant of the German Excellence Initiative to the Graduate School of Life Science, University of Würzburg.
**Species:** Tetragonilla collina S., Apidae

*Organism / organ:* animal / cuticular surface

*Authors / contributors:* Sara D. Leonhardt¹ & Thomas Schmitt¹

*Affiliation:* ¹Würzburg University, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


*Locations:* Malaysia/Borneo: Sepilok, Danum Valley

*Method sketch / description of data:*

- Specimen sampling / analyses: 5–10 specimens were obtained from the entrance of a bee colony by capturing leaving foragers in a clean clear plastic bag, killed by quick freezing and extracted in hexane for 5 min. Cuticular extracts were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) on a DB-5 fused silica capillary column (30 m _ 0.25 mm ID; d.f. ¹/₄ 0.25 mm; J & W, Folsom, CA, USA) with helium used as carrier gas (constant flow of 1 ml/ min). Injection was carried out at 250°C in the splitless mode for 1 min. Temperature was raised from 60°C to 300°C with a 5°C min⁻¹ heating rate and held at 300°C for 10 min. Electron impact mass spectra were recorded at 70 eV.

- Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 98 and Adams EO Library 2205.

*Funding:* Carlsberg foundation, Denmark; Deutsche Forschungs-Gemeinschaft (DFG project: LE 2750/1-1) and a grant of the German Excellence Initiative to the Graduate School of Life Science, University of Würzburg.
Species: Tetragonula carbonaria S., Apidae

Organism / organ: animal / cuticular surface

Authors / contributors: Sara D. Leonhardt¹ & Thomas Schmitt¹
Affiliation: ¹Würzburg University, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


Locations: Australia: Elonora, Dalby, Brisbane, Shiptonsflat

Method sketch / description of data:
- Specimen sampling / analyses: 5–10 specimens were obtained from the entrance of a bee colony by capturing leaving foragers in a clean clear plastic bag, killed by quick freezing and extracted in hexane for 5 min. Cuticular extracts were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) on a DB-5 fused silica capillary column (30 m _ 0.25 mm ID; d.f. ¼ 0.25 mm; J & W, Folsom, CA, USA) with helium used as carrier gas (constant flow of 1 ml min⁻¹). Injection was carried out at 250°C in the splitless mode for 1 min. Temperature was raised from 60°C to 300°C with a 5°C min⁻¹ heating rate and held at 300°C for 10 min. Electron impact mass spectra were recorded at 70 eV. Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 98 and Adams EO Library 2205.

Funding: Carlsberg foundation, Denmark; Deutsche Forschungs-Gemeinschaft (DFG project: LE 2750/1-1) and a grant of the German Excellence Initiative to the Graduate School of Life Science, University of Würzburg.
(128) **Species:** *Tetragonula melanocephala* G., Apidae

*Organism / organ:* animal / cuticular surface

**Authors / contributors:** Sara D. Leonhardt¹ & Thomas Schmitt¹

**Affiliation:** ¹Würzburg University, Department of Animal Ecology and Tropical Biology, Würzburg, Germany


**Locations:** Malaysia/Borneo: Sepilok, Danum Valley

**Method sketch / description of data:**

- Specimen sampling / analyses: 5–10 specimens were obtained from the entrance of a bee colony by capturing leaving foragers in a clean clear plastic bag, killed by quick freezing and extracted in hexane for 5 min. Cuticular extracts were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) on a DB-5 fused silica capillary column (30 m _ 0.25 mm ID; d.f. ¼ 0.25 mm; J & W, Folsom, CA, USA) with helium used as carrier gas (constant flow of 1 ml/ min). Injection was carried out at 250°C in the splitless mode for 1 min. Temperature was raised from 60°C to 300°C with a 5°C min⁻¹ heating rate and held at 300°C for 10 min. Electron impact mass spectra were recorded at 70 eV.

Compounds were identified by comparison of mass spectra and retention times with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 98 and Adams EO Library 2205.

**Funding:** Carlsberg foundation, Denmark; Deutsche Forschungs-Gemeinschaft (DFG project: LE 2750/1-1) and a grant of the German Excellence Initiative to the Graduate School of Life Science, University of Würzburg.
Species: *Trigona corvina* Cockerell, 1913 (Hymenoptera, Apidae, Meliponini)

Organism / organ: animal / cephalic labial glands

Authors / contributors: Stefan Jarau & Lena John

Affiliation: Ulm University, Institute for Neurobiology, Helmholtzstr. 10/1, 89081 Ulm, Germany


Location: Costa Rica; nests from three different populations: five nests from Heredia (Heredia Province), three nests from La Gamba (Puntarenas Province), one nest from Pozo Azul de Abangares (Guanacaste Province).

Method sketch / description of data:
- Experimental setup: Cephalic labial gland extracts prepared from foraging bees collected from nine different colonies at three different locations were chemically analysed. Comparisons of the composition from these glands were carried out in order to test whether the trail pheromones produced by them show any nest-specific signal and whether there are differences between colonies within a certain population or between colonies from different populations (for details see John et al., 2012).

- Scent sampling / analyses: Cephalic labial glands were dissected from the heads of 117 foragers and individually placed in 100 μl hexane for 24 h at room temperature. The resulting extracts were reduced to volumes of 50 μl before gas chromatographic analyses. One microliter per sample was injected into a HP 5890 GC equipped with a 30 m DB-5MS column and with hydrogen (2 ml min⁻¹ constant linear flow rate) as carrier gas. The GC was operated splitless at 50°C for 1 min, followed by a temperature increase to 310°C at a rate of 10°C min⁻¹. The resulting gas chromatograms were used for quantitative analyses of the single compounds detected in the individual extracts by calculating the relative proportion of each compound within the total extract. Identification of the single compounds in the extracts was done by comparing their retention times with the retention times of pure reference compounds. Structure elucidation of these compounds by means of GC-MS analyses was carried out in an earlier study published by Jarau et al. (2010).

Funding: Deutscher Akademischer Austauschdienst (DAAD) and Deutsche Forschungsgemeinschaft (DFG, JA 1715/3-1).