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The detection and mapping of the spatial distribution of insect defense compounds by desorption atmospheric pressure photoionization Orbitrap mass spectrometry

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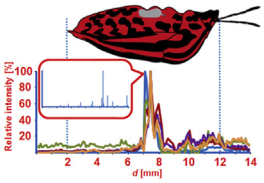
HIGHLIGHTS

- First use of DAPPI with Orbitrap MS to analyze insect defense compounds is shown.
- Defense secretion components were detected in termite and stink bug body extracts.
- Spatial distribution of the defense compounds was mapped on insect body surface.

GRAPHICAL ABSTRACT



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ABSTRACT

Many insects use chemicals synthesized in exocrine glands and stored in reservoirs to protect themselves. Two chemically defended insects were used as models for the development of a new rapid analytical method based on desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS). The distribution of defensive chemicals on the insect body surface was studied. Since these chemicals are predominantly nonpolar, DAPPI was a suitable analytical method. Repeatability of DAPPI-MS signals and effects related to non-planarity and roughness of samples were investigated using acrylic sheets uniformly covered with an analyte. After that, analytical figures of merit of the technique were determined. The spatial distribution of (*E*)-1-nitropentadec-1-ene, a toxic nitro compound synthesized by soldiers of the termite *Prorhinotermes simplex*, was investigated. Then, the spatial distribution of the unsaturated aldehydes (*E*)-hex-2-enal, (*E*)-4-oxohex-2-enal, (*E*)-oct-2-enal, (*E*,*E*)-deca-2,4-dienal and (*E*)-dec-2-enal was monitored in the stink bug *Graphosoma lineatum*. Chemicals present on the body surface were scanned along the median line of the insect from the head to the abdomen and vice versa, employing either the MS or MS^2 mode. In this fast and simple way, the opening of the frontal gland on the frons of termite soldiers and the position of the frontal gland reservoir, extending deep into the abdominal cavity, were localized. In the stink bug, the opening of the metathoracic scent glands (ostiole) on the ventral side of the thorax as well as the gland reservoir in the median position under the ventral surface of the anterior abdomen were detected and localized. The developed method has future prospects in routine laboratory use in life sciences.

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1. Introduction

Many insects use chemicals synthesized in exocrine glands and stored in reservoirs to protect themselves from predation or infections. When threatened or irritated, they release their defensive secretions to discourage or immobilize potential predators or pathogens. Insect chemical warfare is based on structurally diverse compounds, usually low-molecular-weight organic compounds like aldehydes, esters, terpenes, phenols, quinones, various nitrogen and sulfur-containing compounds, or peptides and proteins [1,2]. With the exception of the peptides and proteins used by Hymenopterans in sting venoms, insect defense compounds are commonly analyzed by gas chromatography-mass spectrometry. The sample preparation methods are based on gland dissections followed by extraction with organic solvents, or sampling with solid-phase microextraction fibers [3,4]. When studying new insect species, the production site of the defensive chemicals may not be obvious. In such cases, mass spectrometry imaging (MSI) can be a useful tool to localize the gland opening on the insect body and characterize the defensive compounds. MSI encompasses several techniques potentially suitable for this particular application, including MALDI-MSI and emerging techniques based on ambient ionization. MALDI-MSI featuring high lateral resolution [5,6] has become a mature technology for visualizing the spatial distribution of compounds in flat samples, typically tissue sections [7]. Unlike to planar objects, MALDI-MSI of non-planar samples (e.g. insects) still remains a challenging approach [8,9]. Vacuum in the ion source causes dehydration, which may induce deformation and morphology changes of insect specimen, and volatile compounds are easily lost. Variable thickness of the sample leads to different electric charging and discharging effects associated with dissipation of photoelectrons, which induces mass shifts on axial instruments [9]. Non-laser ambient ionization MSI methods have lower lateral resolutions, but they offer other attractive features for imaging of insects. Atmospheric pressure maintained during the ionization process ensures morphological stability of the samples, and low spontaneous vaporization of volatile compounds. Sample preparation is faster, because time-consuming matrix deposition is not needed.

Among the ambient ionization mass spectrometry techniques desorption atmospheric pressure photoionization (DAPPI) [10] provides high ionization efficiency not only for polar, but also for nonpolar compounds, whereas the most commonly used desorption electrospray ionization is suitable only for polar and ionic compounds. Considering the nonpolar characters of the insect defense compounds, their distribution on the insect cuticle can be followed by DAPPI mass spectrometry. The key part of the DAPPI setup is an electrically heated nebulizer microchip [11], which is used as a source for a hot confined jet of solvent vapor. The mechanisms of desorption and ionization of the analytes are considered to be thermal desorption and photoionization [12,13]. Thermal desorption is promoted by the high temperature of the jet [14]. Photoionization is enhanced by the presence of a solvent with ionization energy below the energy of the photons emitted by a vacuum ultraviolet lamp. DAPPI has previously been employed in the analyses of pesticides [15], polyaromatic hydrocarbons [15], street market confiscated drugs [16] and cannabis samples [17]. DAPPI has also been applied for MSI of parched leaves of *Salvia* [18]. The spatial distribution of small molecules like tocopherol and carnosol was visualized using high resolution mass spectrometry with lateral resolution of about 1000 μm.

Termites and stink bugs are good models for the development of the DAPPI-MSI method for the localization of defensive glands. Termite soldiers have evolved a unique exocrine organ, the frontal gland, storing the defensive secretion [19]. This is also the case of *Prorhinotermes simplex* (Hagen, 1858) (Isoptera, Rhinotermitidae), a species with a very populous soldier caste [20]. The frontal gland is a sac-like organ opening through the frontal pore (fontanelle) on the frons of the soldiers. Its reservoir is among the largest in all termites and extends up to the posterior abdomen. The gland produces and stores (*E*)-1-nitropentadec-1-ene and other components. The anatomy and chemistry of the frontal gland of this species and the biosynthesis of the nitro compounds in the soldiers have been described previously [19–24]. The striped stink bug *Graphosoma lineatum* (Linnaeus, 1758) (Heteroptera, Pentatomidae) is a cosmopolitan species that feeds on umbelliferous plants such as parsley and coriander. The metathoracic scent glands and their reservoirs are located in the posterior part of the thorax and anterior abdomen [25,26]. They produce a complex multifunctional secretion used in defense as well as in intraspecific communication, i.e. as alarm, aggregation and sex pheromones [27]. Scent gland secretion consists of alkenals, alkadienals, oxoalkenals, acetates, alkanes, alkenes, furanes and other components [4]. The chemical composition has been previously studied by means of GC-FID [3] and GC–MS [4,25,28,29].

The aim of this study is to develop a rapid analytical technique which would make it possible to localize secretory gland openings on the insect body surface and characterize the compounds of the secretion using a method combining DAPPI-MS with a software-controlled moveable motorized sample holder. For this purpose, we used termite soldiers and adult stink bugs to map the spatial distribution of selected analytes, known to be produced by these insects. The analytical figures of merit were determined for defense compounds and effects of the surface morphology on the signals were studied.

2. Experimental

2.1. Chemicals and materials

The solvents and the standards of defensive compounds, i.e. acetone (≥99.8% Chromasolv® for HPLC, gradient grade), methanol (LC-MS Chromasolv®, gradient grade), (*E*)-hex-2-enal, (*E*)-oct-2-enal, (*E*,*E*)-deca-2,4-dienal, (*E*)-dec-2-enal, (*E*)-undec-2-enal and verapamil hydrochloride (99%), were purchased from Sigma–Aldrich (St. Louis, MO, USA). (*E*)-1-Nitropentadec-1-ene was synthesized according to the previously published procedure [24]. The standards, extracts and isolated gland secretions were desorbed from a PTFE film (a thickness of 0.25 mm) supplied by Vink Finland (Kerava, Finland). The experiments studying signal repeatability and effects of the sample roughness were performed with 4-mm thick acrylic sheets Perspex XT Extruded (Perspex, Blackburn, Great Britain), either plain or modified by engraving regularly spaced channels (Fig. S11). The channels were created using a Laser engraver 8015 Rayjet 50 (Trotec Laser GmbH, Marchtrenk, Austria; for 300 μm-channels) and a TOS FN 20 milling machine (TOS, Čelákovice, Czech Republic; for 400, 800, 1200 and 1600 μm-channels). Insect specimens were fixed on glass microscope slides (Waldemar Knittel Glasbearbeitungs, Braunschweig, Germany) using a Kores Aqua correction fluid (Kores, Vienna, Austria), see Fig. S1.

2.2. Samples

The soldiers of the termite *Prorhinotermes simplex* (Isoptera, Rhinotermitidae) originated from a mature laboratory colony (collected in Soroa, Piñar del Rio, Cuba, in 1964); they were kept in permanent darkness at 26 ± 1 °C and elevated humidity (>85%). The adult stink bugs *Graphosoma lineatum* (Hemiptera, Pentatomidae) were collected near Prague, Czech Republic. They were