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DETECTION OF BED BUGS FROM VOLATILE ORGANIC COMPOUNDS IN DWELLINGS

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Abstract Bedbugs are blood-sucking insects which are harmful to human health. They cause bites that can lead to itching, allergies or hives. Their presence has a significant impact on mental health too. People who are infested can suffer from insomnia, anxiety or psychosis. Travels, second-hand markets and their acute resistance to insecticides are conducive to their spread. The best way to get rid of them is to conduct an Integrated Pest Management process. Moderate infestation levels trigger control measures. In that case, mechanical and thermal actions can be sufficient to eliminate bed bugs and if it is not efficient chemical treatment applied by a specialist could be necessary. When experiencing night-time bites, the first thing to do is make sure that bed bugs are responsible. However, they may be hidden and difficult to spot during the day. Bugs communicate with each other using pheromones. Some of them called VOC (Volatile Organic Compounds) are interesting chemical tracers. They can indicate the presence of bed bugs without searching for them. So, developing a tool to detect volatile chemical emission of bed bugs for non-experts represents a great interest. In this context, we developed a method of detection of bed bugs using their emitted VOCs indoors. An exhaustive VOC profile emitted in the air by bed bugs was first conducted in the laboratory. Measurements of these specific tracers were then realised in real dwellings to assess the analytical performance in living conditions. A detection index was developed, demonstrating promising performance in terms of both sensitivity (accurate bed bug detection) and specificity (accurate safety), which led to a patent application.

Key words *Cimex lectularius*, bed bugs, semiochemical substances, VOC, insect, infestation

INTRODUCTION

Bed bug detection or monitoring is mostly performed by visual inspection. In addition, traps and canine detection can be used by an expert as complementary tools to confirm their presence or even locate them. Like all insects, bed bugs have powerful olfactory receptors that allow them to capture semiochemical substances. Some of them are airborne and are referred to as volatile organic compounds (VOCs). Among these substances, some alarm and aggregation pheromones which are specific molecules of communication within the species are described in literature. (Siljander et al. 2008; Harraca et al. 2010; Mendki et al. 2014; Gries et al. 2015; Liu, Xiong, et Liu 2017)

VOCs produced by *Cimex lectularius* may be useful for detecting the presence of bed bugs. (Weeks et al. 2011; Kilpinen, Liu, et Adamsen 2012; Eom, Risticevic, et Pawliszyn 2012; Cannon, Stejskal, et Perrault 2020; Crawley et Borden 2021; Akhoundi et al. 2023). According to our past experience in indoor air quality (Langer et al., 2016; Achille et al., 2019; Sivanantham et al., 2021) these molecules are rarely found in indoor environments. However, they are not strictly specific to bed bugs, as some can also originate from a few building materials, decorative products, cleaning agents or other insects. Considering this, the current study was to test a method for detecting bed bugs *via* their VOC emissions. First, VOC profile of

Cimex lectularius was characterized in the laboratory. Next, infested dwellings were investigated to detect and identify target compounds in real-life conditions. These two approaches enabled us to develop a bed bug infestation index.

MATERIALS AND METHODS

Insects. *Cimex lectularius* specimens used in the laboratory experiments were obtained from the Mycology-Parasitology laboratory at Avicenne Hospital in Bobigny, France. They are sensitive to insecticides authorized in France. Insects were incubated at 24°C with 60% relative humidity and a 12-hour light/dark photoperiod. They were artificially fed twice a week with human blood through a polymer membrane.

Investigations. Investigations were carried out from October 2019 to April 2021 in bed bug-infested dwellings owned by eight social housing landlords in Paris and around. A questionnaire covering materials, ventilation systems, usage, and other characteristics were administered to the main occupant to account for any potential confounding factors.

Air sampling. Active air samples were collected in the laboratory using an experimental bench and in the dwellings with an SKC pocket pump. The sampling duration ranged from 30 minutes to 1 hour, with the flow rate adjusted accordingly between 100 and 200 cm^3/min. Sampling tubes containing Tenax® TA, Carbosieve® SIII and other home-made absorbent were used alone or in combination to fully characterize the VOC profile of insects.

Chemical analysis. Analysis was performed using an automatic thermodesorber (TD, Turbomatrix 650) coupled with a GC-MS system (Clarus 580-Clarus SQ8S) equipped with an ELITE 5ms column (Perkin Elmer). Analytical conditions followed the standards NF EN ISO 16000-6 (2012) and NF EN 16516 (2017). Identification of major VOCs were confirmed by external standards. Other VOCs were identified according to the NIST mass spectral database.

Machine learning method. VOC concentration profiles from 102 real dwellings either infested or not infested were used as a training dataset to feed a Classification and Regression Tree (CART) model in order to predict the presence or absence of bed bugs. The performance of the model was assessed through both its sensitivity (accurate presence of bed bugs) and its specificity (accurate absence of bed bugs) using the VOC data from 41 new dwellings.

RESULTS AND DISCUSSION

Experiments conducted in laboratory conditions allow for the characterization of the volatile chemical profile of *Cimex lectularius* using a sampling methodology with different adsorbents, combined with a TD-GC-MS analysis method. A total of 47 VOCs were identified, 21 of which had already been reported in the literature. (Siljander et al. 2008; Eom, Risticevic, et Pawliszyn 2012; Gries et al. 2015; Cannon, Stejskal, et Perrault 2020). This approach enabled the identification of 26 new compounds, improving the knowledge on VOCs profile of *Cimex lectularius*: acetone, propanal, acetic acid, 2-butanone, butanal, 2-pentanone, pentanal, 3-methylbutanal, hexane, 3-methyl-2-butenal, phenol, dimethyl disulfide, (2E,4E)-hexadienal, (2E)-hexenal, 3-hexenal, hexanal, (2E)-hexen-1-ol, benzaldehyde, benzyl alcohol, (2E,4E)-heptadienal, heptanal, 2-heptanone, 1-hexanol, acetophenone, (2E,4Z)-octadienal, (2E,4E)-octadienal, 2-n-butyl furan, 6-methyl-5-heptene-2-one, (2E)-octenal, 1-nonene, dimethyl trisulfide, octanal, 2-ethyl-1-hexanol, d-limonene, nonanal, 2-nonen-1-ol, 1-nonanol, benzyl acetate, decanal, dihydromyrcenol, 2-decen-1-ol, undecanal, E-geranylacetone, Z-geranylacetone, and 2,4,4-trimethyl-3-(3-methylbutyl)cyclohex-2-enone.

Some of these VOCs may also originate from other sources in indoor environments like other insects, microorganisms, construction and decoration products, cleaning products... For instance, (2E)-hexenal has been identified in *Blatta orientalis* and *Periplaneta americana* (Krivosheina & Shatov, 1980) and both can be found in dwellings. (2E)-octenal could also be emitted by molds (Lacaze 2016). These other potential sources, which may or may not have biological origins, are called confounding factors. VOCs have been categorized according to our past experience in indoor air quality, their relevance and experiments conducted in dwellings in this study.
Investigations in real dwellings were conducted between October 2019 and April 2021 in three main stages: assessing the infestation level, conducting VOC measurements in a room or resting area, and administering a questionnaire to the main occupant.

Following chemical analyses of air samples, a database of concentrations of VOCs of interest and infestation level was created. From these data a predictive model was then developed using a CART classification tree, trained on the concentration data from 40 target VOCs (continuous variables) measured in 102 dwellings, some of which are infested. This cost-effective model identifies the most relevant variables for classification. The performance of the decision tree applied on an independent panel of 41 dwellings, provides an accurate detection of bed bugs 93% of the time. A negative predictive value of 95% was achieved which means that the model is accurate in predicting the absence of bed bugs 95% of the time. Thus with minimal intrusive impact, simple implementation, an excellent prediction of bed bugs presence or absence can be obtained, by this index using VOC levels and decision tree modelling.

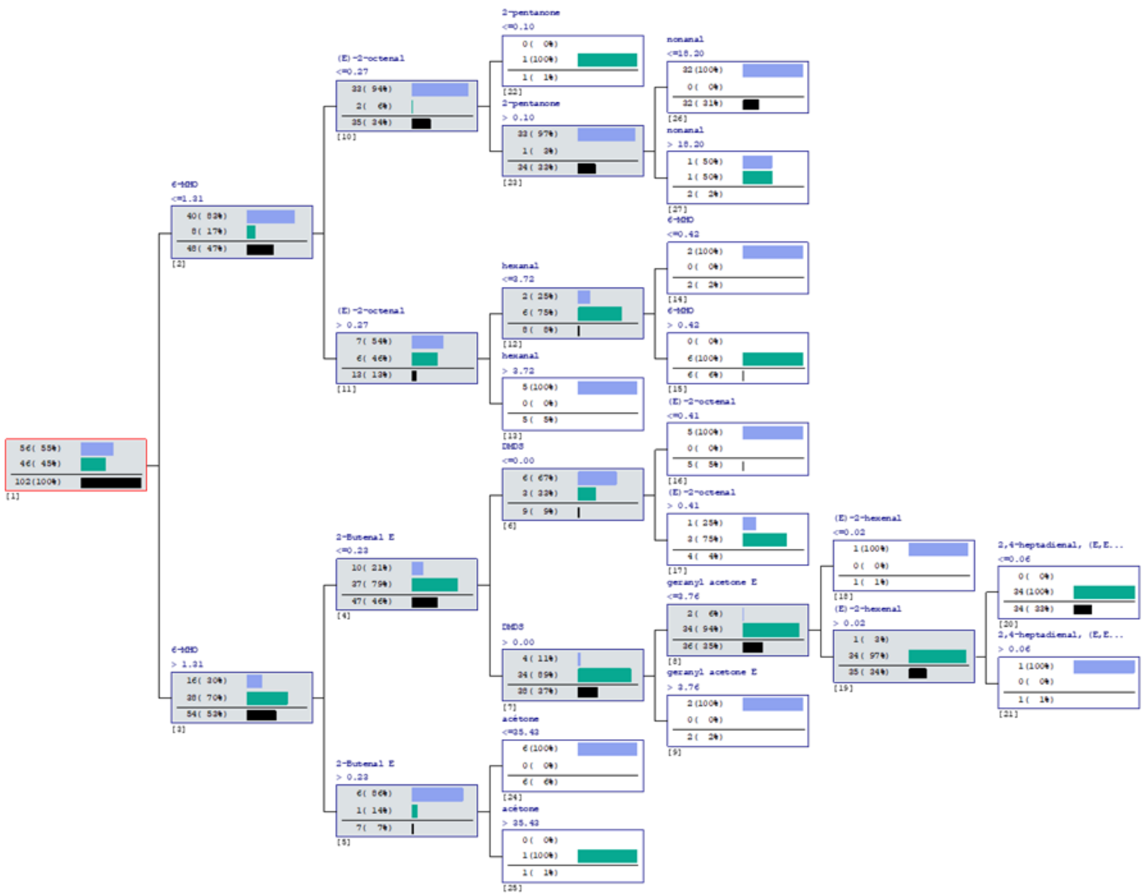

Figure 1 CART predictive model for predicting bed bug presence or absence based on specific VOCs concentration in 102 dwellings.

CONCLUSION

This study focuses on the most comprehensive VOCs profile of *Cimex lectularius* to date, independent of environmental influences. It consists of 47 VOCs, with carbon numbers ranging from C_2 to C_{14}, predominantly featuring aldehydes, alcohols, and ketones. The identification of VOCs tracers in realistic environmental context associated with an adapted sampling and analysis methods were proposed. Laboratory and field approaches led to the development of a bed bug infestation index, based on a statistical approach *via* a CART decision tree based on 102 dwellings tested in this study. The performance, in terms of sensitivity/specificity, was tested and validated on a sample of 41 independent dwellings. With minimal intrusive impact, a simple implementation, the bed bug index built in this study is an excellent predictive tool. This work resulted in a patent application, published internationally as WO 2024/069385 A1.

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