Visible Near Infrared Hyperspectral (VNIH) technique to differentiate Trogoderma variabile reared on different commodities

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Abstract

Under Trogoderma sp, some comes major stored grain pest, which are of economic concern and most of the times accurate identification becomes very difficult. Under this study a new diagnostic system using visible near-infrared hyperspectral (VNIH) imaging methods is developed to address identification gaps for T. variabile. This technique is useful because different materials have unique reflectance spectra, and this difference in reflectance spectra can be used to identify various constituents in an image. For this study both larvae and adult were studied for T. variabile fed on wheat, maize, canola, barley, oats and rice for more than 4 generations. Each individual insects killed by ethanol were scanned using a hyperspectral imaging system from 400 to 1000nm. Matlab 2016b was used to develop predictive model for hyperspectral image classification. Deep neural network approach gave more than 90% accuracy for both larvae and adult stages fed on different commodities. From this result we can say that T. variabile on the different host can lead to difference in VNIH reflectance spectra. This is one of most fundamental factors for development of robust VNIH technique as diagnostic tool.

In search of a new attractant for monitoring *Stegobium paniceum* L. (Coleoptera: Anobiidae)

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Abstract

Stegobium paniceum (L.) is a major pest for several stored products worldwide. Monitoring methods for this species, based on pheromone traps, are affected by the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2*S*,3*R*,1'*R*)-Stegobinone] and/or by its lost of efficacy after two weeks at room temperature. So other semiochemicals that can be exploited for monitoring this species are highly desirable. In this study was tested the behavioral response in two-choice olfactometer of *S. paniceum* adults to Volatile Organic Compounds (VOCs) collected from colonized substrate. The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes. The GC-MS chemical analysis of the extract indicated the presence of several alkanes, alcohols, aldehydes and fatty acids, some of them already reported to attract other stored product coleopteran pests and promising candidates for further studies to test their attractiveness on *S. paniceum*.

keywords: drugstore beetle, monitoring, attractant, volatile organic compounds, headspace

1. Introduction

The drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Anobiidae), , is among the major pests for a wide variety of dry and durable stored agricultural products (Edde et al., 2012). Drugstore beetle females produce a sex pheromone that induces attraction behavior of males, with the highest responses 5–12 days after adult emergence (Kuwahara et al., 1975; Kodama et al., 1987).

However, the response to this synthetic pheromone in trapping experiments often had unsatisfactory results (Mahroof and Phillips, 2007). This can be due to the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2*S*,3*R*,1'*R*)-Stegobinone] at high purity grade and/or by its lost of efficacy after two weeks at room temperature (Kodama et al., 1987). For these reasons, it is important to investigate alternative attractants or pheromone synergists for monitoring and/or mass trapping *S. paniceum* adults. The *Volatile Organic Compounds* (VOCs) produced from the insect colonies of *S. paniceum* could be useful in order to develop an efficient and economically sustainable attractant for the drugstore beetle as positively exploited for *Lasioderma serricorne*, a related anobiid species (Buchelos and Trematerra, 1998; Mahroof and Phillips, 2007). To date, the olfactory responses of *S. paniceum* to VOCs of its colonies and their volatile chemical composition have never been investigated. In this study, the behavioral responses of *S. paniceum* adults to VOCs from colonized substrate were evaluated in a two-choice olfactometer and analyzed by gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

Colony VOCs collection

VOCs from a *S. paniceum* colony (insects plus rearing substrate) were collected by a dynamic headspace method (pull system). To collect volatiles, 90 grams of the rearing substrate were placed into a cylindrical plastic container. About 200 unsexed adults of *S. paniceum* were added to the rearing media in the plastic cylinder. Volatiles were collected for 24 hours on adsorbent tubes filled with 40 mg of Tenax-TA and 40 mg of Carbotrap B, both Supelco (Bellefonte, PA, USA). The adsorbent materials were fixed in the adsorbent tubes using glass wool. To generate a flow rate of charcoal-filtered air (200 ml/min) a vacuum pump was used. Headspace samples from empty cylindrical plastic boxes and oven bags were used as controls. Volatiles trapped in the tubes were eluted with 500 µl of cyclohexane. All samples were stored in screw cap vials at -20 °C until chemical and behavioral studies.

Chemical analysis

GC-MS analysis of the colony headspace samples were performed on an Agilent 5890 GC system, equipped with a DB-5ms column, interfaced with a 5973 quadruple mass spectrometer. The GC oven temperature was set at 40°C for 5 min, then, increased by 10°C/min to 250°C. Identification of compounds was carried out by comparison of mass spectra and retention times with standard compounds purchased from Sigma Aldrich (Milan, Italy). When synthetic standard was missing, the identification was made by using the NIST 98 library and by Kovats retention indices (Adams, 2007).

Two-choice olfactometer bioassays

To validate the two-choice olfactometer used in our behavioural experiments, the sex pheromone [(2*S*,3*R*,1'*R*)-Stegobinone] was tested in preliminary experiments on *S. paniceum* adults. Subsequently, 200 µl of headspace elution was tested versus a blank headspace collection. The olfactometer consisted of a glass chamber (cm: 26 long × 17 wide × 13 high) covered by a glass lid and illuminated by a lamp positioned 1 m above the instrument. Each side of the chamber was covered outside with white printer paper to eliminate potential distractions to beetles and to diffuse light. Two pairs of white plastic cups (0.2 l and 0.3 l) were used as olfactometer arms. For each bioassay, one beetle was released inside the chamber through the entry-hole on the long side of the chamber. The presence of the beetle inside the olfactometer arm was verified after 24 hours. The position of the stimuli in the arms was switched after each replication to avoid the influence of the olfactometer position on beetle choices. Before each replication, to prevent the accumulation of odors, the collection cups and the ramps were changed while the glass chamber was cleaned with acetone and dried by paper towel and a hair-dryer.

3. Results

Chemical analysis

Overall, twenty-four VOCs were found in the GC-MS analysis of the elutions from the colony of *S. paniceum*. Among them, nineteen compounds were identified whereas five were unknown. The most abundant compounds were hexanoic acid (12.4%), heptanoic acid (9.5%), decane (9.1%), 4-methyldecane (8.5%), and nonanal (8%), contributing to 47.5% of the total composition. Other compounds detected were heptanal, 1-octen-3-ol, octanal, limonene, 3,6-methyl decane, 2-phenylethanol, tridecane, tetradecane and hexadecane. Stegobinone comprised 0.3 % of the total volatile profile.

Olfactometer bioassays

The results of the behavioral experiments are summarized in Fig. 1. The olfactometer was validated by the response elicited by the sex pheromone that attracted males ($\chi^2 = 6.3$, df = 1, $P = 0.01$, N= 30) but not females ($\chi^2 = 2.4$, df = 1, $P = 0.1$). The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes (males: $\chi^2 = 11.6$, df = 1, $P = 0.0007$, N = 30; females: $\chi^2 = 5.1$, df = 1, $P = 0.02$, N = 35).

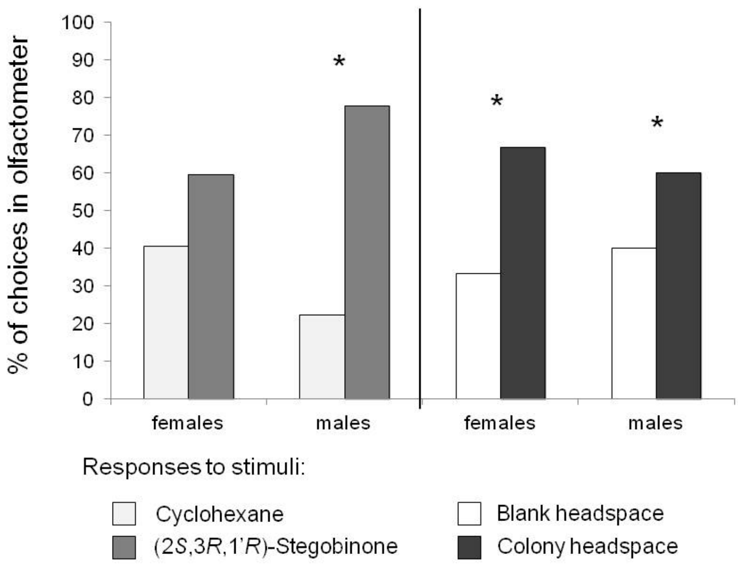

Fig 1. Percentage of choices of *S. paniceum* females and males to sex pheromone and to VOCs of the colony. * P ≤ 0.01

Discussion

Our behavioural results showed that the elution from the dynamic headspace collection of the colony of *S. paniceum* elicited attraction for both adult sexes (Figure 1). Among the compounds identified in the chemical analyses of the colony VOC, several alkanes, alcohols, aldehydes and fatty acids have been already reported to attract other stored product coleopteran pests as *Oryzaephilus* spp., *Trogoderma* spp. and *Cryptolestes ferrugineus* (Levinson, 1978; Pierce et al., 1990, 1991). Interestingly, some of the chemicals detected in our analysis also co-occur in volatiles emitted from Chinese medicinal plant materials that elicit attraction toward *S. paniceum* adults (Cao et al. 2018).

Since *S. paniceum* sex pheromone attracts only males and loses its attractive capacity after two weeks at room temperature (Kodama et al., 1987), it is desirable to exploit alternative attractants for monitoring this pest. In anobiid beetles that feed on stored products, the use of food volatiles that, acting as kairomones, synergize the pheromone lures have been successful tested on *L. serricorne* (i.e. VOCs from *Capsicum* spp.) (Mahroof and Phillips, 2007). Similarly, the results of our study showed that the headspace elution, containing the VOCs from *S. paniceum* colony, is an attractant for both sexes of this pest species. In this context, our study gave the basis for the development of a new alternative and sustainable attractant for trapping the drugstore beetle. Further investigations are in progress aimed to identify which are the behavioral-active VOCs of the entire chemical composition involved in the attraction of *S. paniceum* adults.

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Field trials on attractiveness of the synthetic sex pheromone of the four-spotted bean weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae).

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Abstract

Quarantine pests of legumes pose a threat to many countries of the world including Russia. Pests that can enter the country as a result of the transportation of regulated articles (by sea, air, road, rail, etc.) pose a particular danger (Shutova, 1970; Dankvert *et al*., 2009). Monitoring and identification of legume pests is complicated by the fact that small beetles have a hidden mode of life. One of the most dangerous quarantine pest is the four-spotted bean weevil *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), which is widespread throughout the world and can cause serious economic losses in agriculture of Russia. Research work on the identification, synthesis and laboratory evaluation of the synthetic sex pheromone of *Callosobruchus maculatus* was carried out at the All-Russia Plant Quarantine Center (Bykovo, Moscow region). Tests have shown that synthesized sex pheromone of *C. maculatus* has a high attractiveness for males. An effective dose of pheromone that attracts males of the four-spotted bean weevil has been found at the laboratory and is equal to 0.5 µg per