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# OLFACTORY RESPONSES OF *PLODIA INTERPUNCTELLA* (HÜBNER, 1813) (LEPIDOPTERA: PYRALIDAE) TO DRIED APRICOT VOLATILES

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## ABSTRACT

Dried apricot is one of the stored fruit most endangered to be infested by the Indianmeal moth, *Plodia interpunctella* (Hübner). The aim of the present study was to identify volatile compounds from dried apricot that elicit an electrophysiological response in *P. interpunctella* adults. The volatiles were collected from the headspace of a glass vial with dried apricots using closed-loop-stripping analysis. Measurement by coupled gas chromatography-mass spectrometry together with an electroantennographic detector (GC-MS/EAD) revealed that ten volatile compounds were consistently EAG-active. They were representatives of six different groups of organic compounds. These were four alcohols (1-butanol, 1-pentanol, 1-hexanol, 3-methyl-1-butanol), two esters (ethyl benzoate and 3-methyl-1-butanol acetate), one acid (acetic acid), one ketone (3-hydroxy-2-butanone), one pyrazine derivative (trimethylpyrazine) and one benzenoid compound (benzyl alcohol). In general, antennae of females responded more strongly than those of males. These EAG-active compounds can be considered as olfactory cues for *P. interpunctella*. They will be used in behavioral bioassays in order to determine whether they are attractive, or repellent towards the moth.

**Key words**: Volatiles, electroantennography, dried apricot, *Plodia interpunctella*

## INTRODUCTION

Due to the damages caused by the larvae which feed on a wide variety of foods (stored cereals, beans, nuts, dried fruits, dried flowers, dried vegetables and some spices), the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is considered the most important insect pest in the food and feed processing industry worldwide (Reichmuth et al., 2007). Since larvae develop within the stored foods, the early control of adults is required for an effective control of the population to avoid egg laying. Insect attractants can play an important part in the management of insect pests. Their use, especially attractant food volatiles, is now receiving great attention (Olsson et al., 2006; Uechi et al., 2007; Germinara et al., 2008) since certain products are able to produce hundreds of volatile compounds. Moths use volatiles from food materials to locate suitable partners and oviposition sites (Olsson et al., 2006; Uechi et al., 2007). For instance, it has been shown that extracts from chocolate as well as specific components attract *P. interpunctella* and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Olsson et al., 2005; Olsson et al., 2006; Uechi et al., 2007). *Plodia interpunctella* adults also orientate to volatile blends emitted by grains of cereals (Uechi et al., 2007).

Despite this behavioral evidence and the identification of many volatiles emitted by grains of several cereals little attention has been given to the response of *P. interpunctella* to dried fruit volatiles. The identification of electrophysiologically active volatile compounds emanating from food can be made by coupled gas-chromatography with two detectors: mass spectrometry and an electroantennographic detector (GC-MS/EAD) as described by Weissbecker et al. (2004). This technique presents two advantages, determining simultaneously which volatile in a complex mix an insect is able to perceive and the identification of the compound responsible for the induced EAG response (Weissbecker et al., 2004). Using the same technique, we identified in the present study active volatile compounds in dried apricot, which elicit an electrophysiological response in *Plodia interpunctella* adults.

## MATERIALS AND METHODS

### Insects

Adults of *P. interpunctella* were obtained from the insect culture of the institute. They were kept at 25±1°C temperature and 65±5% relative humidity (r.h.) in the dark. Insects were sexed during the last instar larval stage, when the male gonad is dorsally visible as a dark spot in fifth abdominal segment, while there is no spot visible in females (Reichmuth et al., 2007). Males and females were kept in different glass jars and chambers until the emergence of new adults. Twenty adult moths (10 males and 10 females) of 2 d-old were used for bioassays.

### Fruit sample

Dried apricot fruits (*Prunus armeniaca* L.), used in the present study was originated from Malatya, Turkey. The pitted samples were purchased from a retail organic food market in Berlin, Germany. They were mixed varieties of "Hacihaliloglu", Cöloglu", "Soganci", "Hasanbey", "Kabaasi" and "Cataloglu". Dried apricots were kept in original packages at +5°C until analysis.

### Sampling of volatiles

Dried apricots were cut by hand into small pieces (approx. 10 x 10 mm) and batches of 100 g were immediately introduced into a 250 ml glass flask with neck outlet (30 mm ID) and closed with a custom made polytetrafluoroethylene (PTFE) stopper. Volatiles were sampled from the headspace using the closed-loop stripping analysis (CLSA) method (Boland et al., 1984). Miniature pumps were used to pump air at a flow rate of 1 l/min from the flasks to CLSA tubes loaded with 1.5 mg activated charcoal in which the volatiles were trapped. The sampling time was 45 min. Odor was then eluted from the charcoal with a mixture of dichloromethane and methanol (2:1).

**Gas chromatography mass spectrometry / electroantennographic detection (GC MS/EAD) of odor sample**

Odor samples collected from dried apricot were analyzed by gas chromatography (GC) coupled to mass spectrometry (MS) and electroantennographic detection (EAD) as described by Weissbecker et al. (2004). The unit used consisted of a 6890N gas chromatograph and a 5973N quadrupole mass spectrometer (both Agilent, Santa Clara, USA). One µl of the odor extract was automatically injected into the injector port. The GC employed the following temperature program: start: 50°C, hold for 1.5 min, ramp 7.5 °C/min to 200°C, hold for 5 min. It was equipped with a split/splitless injector operated at 250°C in a pulsed-splitless mode and an INNOWAX column (30 m length, 0.25 mm ID and 0.25 µm film thickness). Helium was used as carrier gas at a constant flow of 1 ml/min. The effluent of the column was splitted between the quadrupole mass spectrometer and a modified "olfactory detection port" (ODP-2, Gerstel, Mülheim, Germany). The ODP was used to mix the effluent from the GC with the humidified air. The air flow carrying the odors was directed to the insect antenna which was fixed in a special antenna holder (Färbert et al., 1997) within the sensor containment. Signals from the antenna were amplified by a factor of 100 and recorded using the A/D-convertor (Agilent) and the HP ChemStation software. Peaks of the chromatogram were identified by using the NIST mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Mass Finder 3.0 software (Hochmuth, 2004) together with the library "Terpenoids and related constituents of Essential Oils" (König et al., 2004).

In addition a non-polar column, HP-5MS (Agilent Technologies, Santa Clara, USA), 30 m x 0.25 mm ID., 0.25 µm film thickness using the GC-MS method described above and the same analytical conditions parameters as the HP-INNOWAX column was used to ensure the identification of all (polar and non-polar) volatiles present in the substrates.

The identity of volatile compounds that elicited an EAG response was confirmed by matching their mass spectra and retention time to those of authentic standards on the two different columns used. The linear retention indices were calculated according to the formula of Van Den Pool and Kratz (1963).

## RESULTS

The present results show that dried apricot (mixed variety of Hacihaliloglu", Cöloglu", "Soganci", "Hasanbey", "Kabaasi" and "Cataloglu", emitted 36 different volatiles detectible by GC-MS. Out of the 36 volatiles, ten were able to elicit EAG responses in 17 of the 20 insects tested (Fig. 1). They belonged to the chemical classes of esters, alcohols, ketones, pyrazine derivatives and acids. Two constituents distinctly elicited higher EAG responses in both male and female *P. interpunctella*, 3-methyl-1-butanol [1] and 1-hexanol [6] (Fig. 1). The identity of the compounds was confirmed by injection of purchased authentic compounds. A positive verification of the identity of EAG-active compounds made by comparing the spectra with those of corresponding synthetic compounds in the NIST library was assumed when approximately the same retention time and mass spectrum could be found.

Table 1. List of electrophysiologically active substances found in the headspace of dried apricot

| N° | Component | Linear retention indices (LRI)[a] | Chemical class |
|---|---|---|---|
| 1 | 3-methyl-1-butanol acetate | 1119 | Esters |
| 2 | 1-butanol | 1136 | Alcohols |
| 3 | 3-methyl-1-butanol | 1202 | Alcohols |
| 4 | 1-pentanol | 1241 | Alcohols |
| 5 | 3-hydroxy-2-butanone | 1288 | Ketones |
| 6 | 1-hexanol | 1341 | Alcohols |
| 7 | trimethyl pyrazine | 1396 | Pyrazine derivatives |
| 8 | acetic acid | 1439 | Acids |
| 9 | ethyl benzoate | 1664 | Esters |
| 10 | benzyl alcohol | 1869 | Alcohols |

[a] Linear retention indices (LRI) were calculated from the chromatograms obtained with a polar column; the identity of the respective compounds was confirmed by comparing its mass spectra and retention indices with that of its authentic standard. Peak number correspond to the number in Fig.1

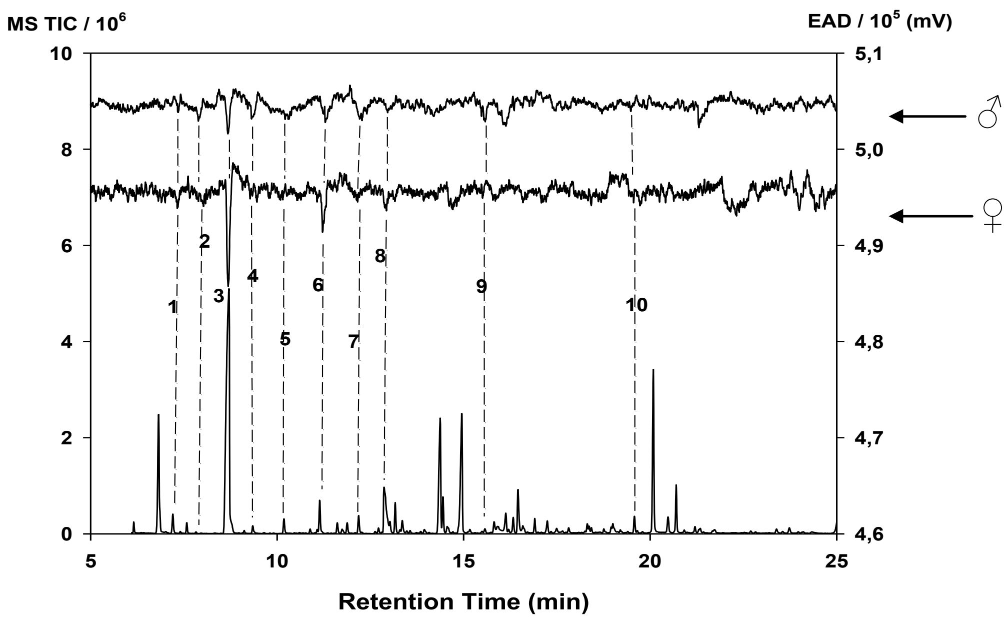


Fig.1- GC-MS/EAD recording of *P. interpunctella* in response to volatiles sampled from dried apricot headspace. Upper inverted trace: EAD signal (/$10^5$ + 0.07 mV) of the *P. interpunctella* male; lower inverted trace: EAD signal (/$10^5$ mV) of female; bottom line: MS-TIC (total ion current). Compounds labelled are as in table 2: [1] 3-methyl-1-butanol acetate; [2] 1-butanol; [3] 3-methyl-1-butanol; [4] 1-pentanol; [5] 3-hydroxy-2-butanone; [6] 1-hexanol; [7] trimethylpyrazine; [8] acetic acid; [9] ethyl benzoate; [10] benzyl alcohol.

## DISCUSSION

The antenna of *P. interpunctella* adults responded electrophysiologically to 10 volatile compounds found in dried apricot headspace. This obviously means that primary receptor cells located on the insect's antenna were stimulated by these volatiles. However, these compounds might be attractive, repellent or deterrent to *P. interpunctella* adults. Some of the EAG-active volatiles have earlier been identified. The alcolhols 1-pentanol and 1-hexanol are found in wheat flour (Uechi et al., 2007), in roasted almonds and dried apple (Ndomo, unpubl. data) and in cereals (Germinara et al., 2008). Sexual dimorphism in the perception of volatiles has been observed (Reinecke et al., 2005). The EAD peaks obtained with females were mostly stronger than those of males (Fig. 1). Biologically, this could make sense as females need to find suitable substrate for oviposition (Uechi et al., 2007; Olsson et al., 2006) while males may react to food volatiles just with the intention of finding females as mating partners because adult moths do not feed. In pitfall olfactometers, only mated females of *P. interpunctella* were attracted to volatiles of wheat flour (Uechi et al., 2007). In addition, alkanals ($C_6$-$C_{10}$) and 2*E*-alkenals ($C_7$-$C_{11}$) were as attractive as individual aldehydes or mixtures, as well as the mixture of many alcohols with aldehydes (Uechi et al., 2007). Isoamyl alcohol and acetic acid found in the present study in dried apricot were used to trap *P. interpunctella* female in mixture with acetic acid (1:1) (Toth et al., 2002). In their study on chocolate volatile attractants for *P. interpunctella* and *E. kuehniella,* Olsson et al. (2006) found that benzyl alcohol, nonanal and phenylacetaldehyde stimulated oviposition in the females of *E. kuehniella*. Therefore, benzyl alcohol [10] (Table 1) found in the present study as EAG-active compound could be attractive to *P. interpunctella.* However, depending on the concentration, some volatile compounds may act as attractant or repellent. 1-pentanol found in our sample, acted as attractant at low concentrations and repellent at higher ones towards *Sitophilus granarius* (L.) (Germinara et al., 2008). The EAG-active compounds found in dried apricot could be considered as olfactory cues which orientate the moth in the direction of stored dried apricots. However, this statement can be confirmed only after performing behavioral bioassays with different concentrations of pure compounds. Since olfaction in insects is mediated by receptors present on the antennal sensilla, the present study represents an important step towards the development of lures baited with attractant food volatiles for the monitoring or control of *P. interpunctella*.

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