

Electrophysiologically-Active Maize Volatiles Attract Gravid Female European Corn Borer, *Ostrinia nubilalis*

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Abstract The European corn borer (ECB) is an important pest of maize in the northern hemisphere, but no reliable techniques exist for monitoring females during their reproductive period. In this study, we aimed to identify host-plant volatiles used by gravid Z-strain females in search for oviposition sites. Headspace of maize plants, to which gravid females orientated in a wind tunnel, was collected, and physiologically-active components were identified by using gas chromatography (GC) coupled with electroantennographic detection followed by GC-mass spectrometry. The antennae of female moths consistently responded to two maize volatiles, nonanal and decanal. Although these compounds are individually not characteristic for maize, a synthetic mix in a ratio found in maize headspace, 1:2.4 at $1 \mu\text{g } \mu\text{l}^{-1}$ induced source contact and landing responses similar to maize plants in the wind tunnel. However, fewer females took flight in response to the mix, and those that took flight did so with an increased latency. To our knowledge, this is the first blend of host-plant volatiles that has been found to be physiologically active and to be able to induce attraction of gravid ECB females under laboratory

conditions. Future tests will evaluate the attractiveness of the blend to the E-strain of ECB, the attractiveness of the blend in the field, and its potential in monitoring ECB populations.

Keywords *Ostrinia nubilalis* · Volatile collection · Kairomone · GC-EAD · Nonanal · Decanal

Introduction

Under the EU regulation (EU Directive 2009/128/ec (2009)) a roll back of the use of insecticides in agriculture is enforced, along with an initiative to support the implementation of integrated pest management (IPM). However, methods that would allow adopting IPM are not yet available for all pests. Host plants emit a wide range of volatile chemical compounds that herbivore pest species are able to use to locate their host and to find suitable oviposition sites (Pichersky et al. 2006; Reinecke and Hilker 2014). As monitoring using lures from the host plants should be directly linked to oviposition and damage, such techniques can be used for effective monitoring of various pest insects to aid precise timing of interventions (Lefebvre et al. 2014). Indeed, volatiles have been used in the monitoring and control of some Coleoptera pest species (Maurisse et al. 2008; Van Tol et al. 2012), and there also are some examples of successful identification of attractive plant and flower volatiles in Lepidoptera (Camelo et al. 2006; Gregg et al. 2010; Knudsen et al. 2008; Landolt et al. 2013). For instance, volatile-based traps (Trece Inc. Salinas CA, USA) became commercially available for monitoring populations of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) in North American orchards following the pioneering work of Knight et al. (2011).

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is found primarily on maize, *Zea*

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mays L. across the northern hemisphere. This species is native to Europe and is polyphagous, attacking more than 200 host plants (Ponsard et al. 2004). Although ECB can be found primarily on maize today, the ECB-maize association is very recent and followed the introduction of this crop into Europe and Asia in the 16th century (Rebourg et al. 2003). As larvae enter the stalk and are out of reach of insecticides, insecticide-based control must target vulnerable adults and eggs masses, which requires sensitive monitoring of adults in order to target control measures in effectively time and space. Currently, pheromone-based field trapping is used worldwide to forecast population dynamics of this species, but its sensitivity has been reported to be low in many places (Kennedy and Anderson 1980; Laurent and Frerot 2007; Oloumi-Sadeghi et al. 1975). This may be due partly to a natural pheromone polymorphism that occurs in this species. Two strains exist that use opposite ratios of two pheromone components (Klun et al. 1973), which has been subject of several recent studies on production and preference (Kárpáti et al. 2010, 2013; Koutroumpa et al. 2014; Lassance et al. 2010). Although both strains are pests in maize with a partially overlapping geographic distribution, in France the Z-strain almost exclusively feeds on maize, whereas the E-strain is found predominantly on putative ancestral hosts mugwort, *Artemisia vulgaris* L. and hop, *Humulus lupulus* L. (Malaus et al. 2007; Pelozuelo et al. 2004).

To complement pheromone-based monitoring, we set out to identify kairomones from maize. So far, no kairomone blend has been identified that could be used for attracting this species in the field, in spite of considerable research effort in the past decades. Several studies have identified maize and other host plant volatiles both chemically (Buttery and Ling 1984; Leppik and Frérot 2012) and electroantennographically (Bengtsson et al. 2006b; Solé et al. 2010). Furthermore, in a Y-tube, females were attracted to several of these maize volatiles when presented singly (Solé et al. 2010). However, no volatiles or blends induced upwind flight in the wind tunnel (Bengtsson et al. 2006b). Thus, while host plant preferences for oviposition are well documented, and several maize volatiles have been identified, none of the previous studies have successfully identified blends that induce odor-mediated behavioral responses in gravid female ECB.

In this study, we examined the behavioral response of gravid ECB Z-strain females to maize plants in a wind tunnel and collected volatiles from plants of the same batch. Using gas chromatography (GC) coupled with electroantennographic detection (GC-EAD), we tested which compounds in the headspace induced antennal responses, and identified these chemicals using GC-mass spectrometry (GC/MS). Finally, we examined behavioral responses of females to three different concentrations of the synthetic blend of identified active compounds in wind tunnel bioassays.

Methods and Materials

Insects A laboratory colony of Z-strain ECB was established from adults collected from a maize field in 2010 in Kéty town (46°26'41.2" N, 18°31'27.6" E), county of Tolna, Hungary. Moths were reared in the laboratory, and the culture was maintained on a semi-artificial diet (Nagy 1970) at 25 °C, 50 % RH under 18:6 L:D light conditions. The pheromone composition of Z-strain culture was monitored by GC/MS to verify the purity of the Z-strain. Genetic introgression of E-strain genes would be evident from a changes in the pheromone blend (Roelofs et al. 1987). Pupae were sexed and separated in glass containers. Adults were fed with 5 % honey in water, and mating pairs were placed in cylindrical plastic containers (10 × 5 cm) immediately after emerging. Two-d-old, presumed mated females, were used for all experiments.

Plants Maize (Pioneer P9578) plants used for wind tunnel bioassays and volatile collections were planted in regular potting soil (Biopakk, T-MIX-KER Ltd., Seregény, Hungary), watered once a day, and kept in the greenhouse at 25–28 °C and 18:6 L:D. For the wind tunnel bioassays and volatile collections, we used plants with four leaves with the soil covered by aluminum foil. Collections were made from three maize plants separately.

Volatile Collection After confirmation that gravid female ECB were attracted to the maize plants in the wind tunnel, we made headspace collections from plants ($N = 3$) that originated from the same batch, i.e., same variety, planted at the same time and kept under the same conditions, to obtain the attractive components. Each maize plant was enclosed in a plastic oven bag (Alufix GmbH, Wr. Neudorf, Austria). The bag was closed tightly with cable ties around the stem and charcoal filtered continuous airflow was drawn through at 0.8 l min^{-1} using a vacuum pump (Thomas G 12/02 EB, Garder Denver Thomas, Fürstenfeldbruck, Germany). Volatiles were collected continuously for 24 h using 50 mg SuperQ (80/100 mesh, Altech, Deerfield, IL, USA) packed in a glass column (ID: 4 mm). Before experiments, this filter was purified sequentially with methanol (purity: 99.8 %; Reanal, Budapest, Hungary), a mixture of methanol and chloroform (chloroform purity: 99.9 %; Reanal) (3:1), acetone (purity: 99.5 %; Reanal), and dichloromethane (purity: 99.9 %; Reanal). Filters were dried with N_2 flow and heated 2 h at 70 °C in an oven (Kunert et al. 2009). Adsorbed volatiles were eluted with 200 μl of dichloromethane. The extracts were concentrated to 40 μl under N_2 stream and kept at -40 °C. Extracts were used for electrophysiological recordings with GC-EAD and chemical identification with GC/MS. A system blank volatile collection also was made using the system described above without maize plant in order to exclude the possibility of contaminations.

Electrophysiology For GC-EAD recordings an Agilent 6890 N gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm, J&W Scientific, Folsom, CA, USA) with on-column injection port was used. The oven temperature was held at 50 °C for 1 min, and then increased at 10 °C min⁻¹ to 230 °C, using helium as carrier gas at 2.9 ml min⁻¹. The GC effluent as well as a 4 ml min⁻¹ make-up gas entered a low dead-volume glass four-way splitter (Graphpack 3D/2, Gerstel GmbH, Germany). The effluent was split equally into two deactivated fused silica capillary columns (100 cm × 0.25 mm), one leading to the FID (280 °C) and one into a heated EAD port (220 °C) (transfer line: Syntech, Kirchzarten, Germany). The EAD effluent was delivered into a stream of charcoal-filtered and humidified air (1 l min⁻¹) and led via a glass tube (ID 8 × 150 mm) over the antennal preparation.

The antenna of a 2-d-old, mated female ($N = 3$) was excised and inserted between two glass capillaries (ID 1.17 mm, Syntech) filled with Ringer solution (Beadle and Ephrussi 1936) and attached to a reference silver/silver chloride electrode held in a micromanipulator. The tip of the moth antenna was cut. The antennal signal was amplified 10 times, converted to a digital signal by a high input impedance DC amplifier interface (IDAC-232, Syntech), and recorded simultaneously with the FID signal on a computer using GC-EAD software (GC-EAD 2000, version 1.2.3, Syntech). Prior to each GC-EAD recording the mounted antenna was stimulated with phenylacetaldehyde (≥95 %, CAS: 122–78-1 Sigma-Aldrich, St. Louis, MO, USA) in a 10 ng μl⁻¹ dose to check the sensitivity. For every recording a new antennal preparation was used.

Chemical Identification Samples were analyzed on a GC/MS (5890GC and 5975MS, Agilent Technologies, Palo Alto, CA, USA) with electron impact (EI) ionization mode at 70 eV, scanning m/z 29–400, at 2 scans s⁻¹. The GC was equipped with Rxi®-5Sil MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm, Restek, Bellefonte, PA, USA). Carrier gas was helium at an average linear flow rate of 35 cm s⁻¹. Two μl of each sample were injected in splitless mode with split closed for 30 s at 220 °C. The oven temperature was held at 50 °C for 1 min and then programmed at 10 °C min⁻¹ to 230 °C and held for 1 min. Compounds were identified by comparison with MS libraries (NIST11 and Willey), published Kovat's index (K_i) values, and verified through injection of synthetics (Sigma-Aldrich). The physiologically active nonanal and decanal also were verified by GC-EAD analyzes on female antennae, injecting a dual mixture containing 100 ng of each authentic standard (Sigma-Aldrich) ($N = 6$).

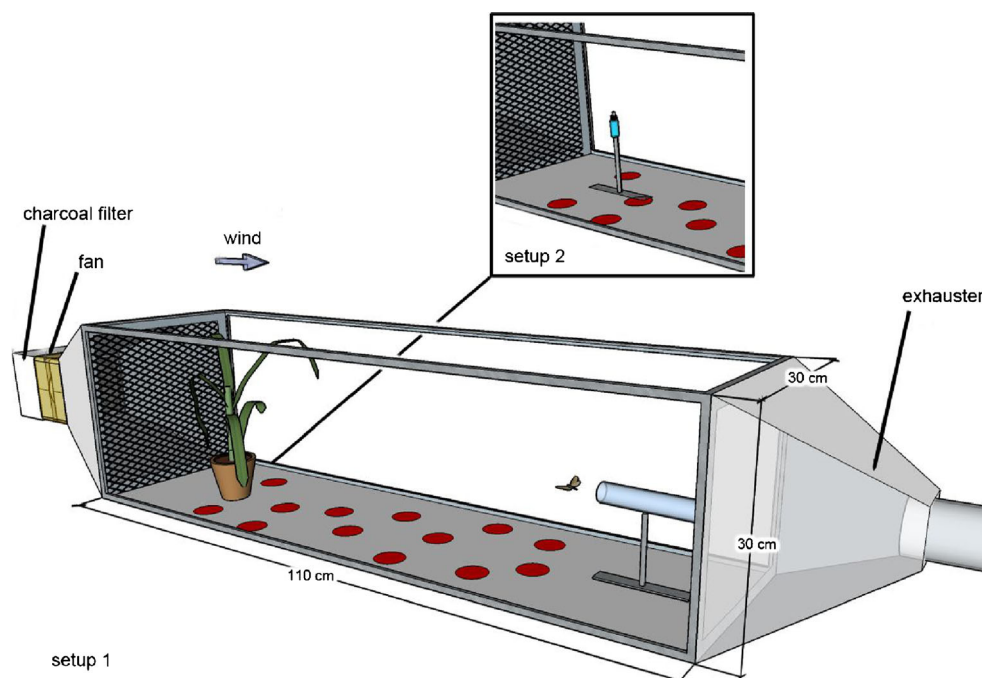
Wind-Tunnel Bioassay A laminar flow wind tunnel (110 cm long × 30 cm wide × 30 cm high) was used for the behavioral

assays (Fig. 1). Charcoal filtered air at 20–22 °C and 50–70 % RH was pushed into the wind tunnel through a fine-mesh screen at 11 cm sec⁻¹ airflow. An exhaust expelled wind tunnel air outside the building. The illumination of white LED room lighting was set to 0.6 lx, while an 11 lx red LED light source was used to observe the insects. The temperature and the relative humidity were 20–22 °C and 50–70 %, respectively. During the experiments, the ambient air pressure was 1015–1024 hPa. Two-d-old, mated females were individually placed into wire mesh capped glass cylinders (length: 10 cm, ID: 3 cm) and moved into the wind tunnel room 1 h before the experiment for acclimatization. Experiments were conducted between 2 and 4 h of the scotophase. Experiments started by placing a single glass tube into the wind tunnel on a 10 cm high release platform, 80 cm downwind from the source. During 5 min experimental time, the occurrence and latency of the following behavioral steps were scored: (1) wing fanning (WF): activation; (2) taking flight (TF): leaving the release cage; (3) upwind flight (UF): flying upwind toward the source in the center of the wind tunnel; (4) half way (HW): crossing the half way between the source and the releasing point; (5) 10 cm approach (AP): 10 cm approach to the source; (6) source contact (SC): moths that contacted the source; (7) landing (LA): moths that landed on the source. Ten to 15 females were tested per day, and each treatment was tested on 3 different days. In the plant treatment group, females were tested ($N = 32$) in the presence of a maize plant, whereas in the control group, responses of females were observed in the absence of an upwind odor source ($N = 32$). In the three synthetic blend treatment groups ($N = 35$, in each group), we used a 1:2.4 blend of nonanal and decanal (NAL/DAL henceforward) in three different concentrations: 10 ng μl⁻¹, 100 ng μl⁻¹, and 1000 ng μl⁻¹, respectively. Blends were prepared using synthetic nonanal (≥95 %, CAS: 124–19-6; Sigma-Aldrich) and decanal (≥98 %, CAS: 112–31-2; Sigma-Aldrich), which were diluted in mineral oil (CAS: 8042–47-5; Sigma-Aldrich). We used wick-type dispensers for releasing the synthetic mixture, because these can provide constant release rate over the experimental period (for detailed description of this method, see Zakir et al. (2013)). Glass vials (2 ml) were used as the dispenser body to store 1.5 ml of the mixture and a Teflon tube (ID 2 mm) with a cotton wick was inserted into the vial, through a hole in the lid and dipped into the synthetic mixture while the top was exposed to the air.

Statistical Analysis

Data collected during the behavioral assays were analyzed in R 3.1.1 (R Core Team 2014). First, we computed a score for each individual from the number of behavioral steps completed during the trial. A score of zero indicated that an individual did not show any response to the presence of the attractant

Fig. 1 Schematic drawing of the wind tunnel. The female releasing cage was 10 cm above the wind tunnel floor and 80 cm downwind from the odor sources, which was either a maize plant (setup 1) or the synthetic source (setup 2). Red circles were randomly placed on the floor of the wind tunnel providing visual cues for female orientation



during the trial, whereas a score of 7 implied that individual undertook all steps and successfully landed on the odor source within 5 min. We used a Kruskal-Wallis test to investigate whether individual scores differed between treatment groups (i.e., various types of attractants), and applied Games-Howell *post-hoc* tests following the recommendations of Ruxton and Beauchamp (2008) to examine differences between treatments. Second, the proportion of females taking flight, proportion of landing females, time to taking flight and time to landing also were compared between those treatment groups where at least one individual landed on the odor source; for that, we used proportion tests and Wilcoxon rank sum tests. All tests were two-tailed at $\alpha = 0.05$.

Results

Electrophysiological Recordings and Identification Volatiles After confirmation that Z-strain females oriented to maize plants, we analyzed the volatile profile of these plants by GC/MS. Collections contained 29 organic compounds in detectable amounts, which fell into 7 different categories: aldehydes, ketones, monoterpene alcohols, monoterpenes, sesquiterpenes, hydrocarbons, aromatic hydrocarbons (Table 1). By using GC-EAD experiments with gravid female antennae, we found that only two peaks (retention times 7.56 min and 9.26 min) elicited reliable and detectable responses in the gravid female ECB antenna (Fig. 2), and these were identified as nonanal and decanal (mean \pm SE: 0.535 mV \pm 0.08 and 0.427 mV \pm 0.07, respectively). Co-injection of 100–100 ng of synthetic nonanal and decanal into the GC-EAD confirmed that these components

elicited strong EAD responses in the female antennae. By using GC/MS analyzes, the ratio of nonanal and decanal in the headspace was 1:2.4 (calculated released amount: nonanal 0.455 \pm 0.007, decanal 1.096 \pm 0.045 ng hour⁻¹, $N = 3$). Additionally, we identified the physiologically non-active compounds among which *p*-xylene, 6-methyl-5-hepten-2-one, octanal, pentadecane, and geranylacetone, were the most abundant (> 3 %, Table 1).

Wind-Tunnel Bioassay We found that behavioral scores differed significantly among the applied attractants during the trials ($\chi^2_4 = 61.55$, $P < 0.001$). Females had higher overall value, i.e., undertook more behavioral steps in general, in the presence of maize compared to any dose of the synthetic attractant (Fig. 3a). All pairwise comparisons were significant except for three cases: there was no significant difference between the control and the 10 ng μl^{-1} dose, between the 10 ng μl^{-1} and the 100 ng μl^{-1} doses, and between the 100 ng μl^{-1} and 1000 ng μl^{-1} doses (Table 2). However, source contact and landing on the odor source was observed only in the presence of maize and the 1000 ng μl^{-1} dose mixture (Fig. 3b). Whereas the proportion of activation (i.e., proportion of females that took flight) was higher (maize: 97 %, 1000 ng μl^{-1} dose attractant: 69 %; $\chi^2_1 = 7.28$, $P = 0.007$) and the latency to activation shorter with maize compared to the 1000 ng μl^{-1} dose blend ($W = 63$, $N_1 = 31$, $N_2 = 24$, $P < 0.001$; Fig. 3c), we found no significant difference in the proportion of females that landed on the source (maize: 34 %, 1000 ng μl^{-1} dose attractant: 29 %; $\chi^2_1 = 0.06$, $P = 0.804$) nor in the latency to reach the source ($W = 45$, $N_1 = 10$, $N_2 = 10$, $P = 0.734$; Fig. 3d) between these two

Table 1 Volatile organic compounds (VOCs) identified in headspace collections from maize plants of variety Pioneer P9578. Values are expressed as percentage relative to total VOCS

#	RI ^a	VOCs	Mean ± SE (N = 3)	Identification method ^b
1	857	ethylbenzene	1.93 ± 0.52	MS
2	873	<i>o</i> -xylene	2.32 ± 0.01	MS
3	888	<i>p</i> -xylene	3.47 ± 0.60	MS
4	900	Nonane	1.68 ± 0.14	MS
5	935	α-pinene	2.83 ± 0.94	MS, Rt ^c
6	936	propyl-benzene	1.22 ± 0.03	MS
7	958	1-ethyl-3-methyl-benzene	2.32 ± 0.93	MS
8	985	6-methyl-5-hepten-2-one	6.37 ± 0.13	MS, Rt
9	1004	1,2,4-trimethyl benzene	2.90 ± 0.32	MS
10	1008	octanal	4.27 ± 0.51	MS
11	1013	3-carene	0.71 ± 0.01	MS, Rt
12	1030	limonene	1.30 ± 0.17	MS, Rt
13	1049	<i>cis</i> -β-ocimene	1.75 ± 0.09	MS, Rt
14	1100	undecane	1.28 ± 0.06	MS
15	1106	nonanal	12.30 ± 0.39	MS, Rt
16	1190	naphthalene	1.57 ± 0.25	MS, Rt
17	1200	dodecane	1.06 ± 0.08	MS
18	1204	decanal	30.08 ± 0.94	MS, Rt
19	1260	(<i>E</i>)-2-decenal	0.57 ± 0.02	MS
20	1305	undecanal	2.52 ± 0.09	MS
21	1340	2-undecenal	1.57 ± 0.04	MS
22	1371	α-copaene	0.19 ± 0.06	MS, Rt
23	1400	tetradecane	1.12 ± 0.10	MS
24	1406	dodecanal	2.03 ± 0.21	MS
25	1420	β-caryophyllene	0.22 ± 0.02	MS, Rt
26	1460	geranylacetone	3.34 ± 0.05	MS
27	1500	pentadecane	4.10 ± 0.94	MS
28	1600	hexadecane	1.48 ± 0.04	MS
29	1700	heptadecane	2.37 ± 0.02	MS

^a retention index (RI)^b identified by mass spectrum (MS)^c identified by co-injection of synthetic standards

treatment groups. Thus, difference in behavioral score of females between the maize and the 1000 ng μl⁻¹ dose attractant originated from the higher activation by the plant, but not the higher attraction to the plant.

Discussion

In this study, we investigated which maize volatiles play a role in host plant localization in gravid female ECB. First, we confirmed that gravid Z-strain female ECB were attracted to maize in a wind tunnel. Second, our GC-EAD and GC/MS analysis identified two maize volatiles, nonanal and decanal, that are consistently detected by the antenna of female ECB. Third, we found that a blend of these two compounds in the ratio found in maize was highly attractive in wind tunnel

assays. Whereas undoubtedly other volatiles also may contribute to host finding in this species, the fact that orientation to the two-component blend at 1000 ng μl⁻¹ blend was similar to that to maize itself, underlines the significance of these two compounds in this host plant-pest species system.

Nonanal and decanal have been identified from maize in earlier studies (Huang et al. 2009), although none of them reported these compounds as potential attractants (Bengtsson et al. 2006a; Buttery and Ling 1984; Leppik and Frérot 2012; Leppik et al. 2014). Nonanal was found to be physiologically active on the antennae of females of Asian corn borer, *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae) (Huang et al. 2009), but appeared to inhibit oviposition of gravid females. Solé et al. (2010) also found nonanal and decanal in maize headspace, although when tested singly in behavioral assays neither compounds induced a behavioral response from the

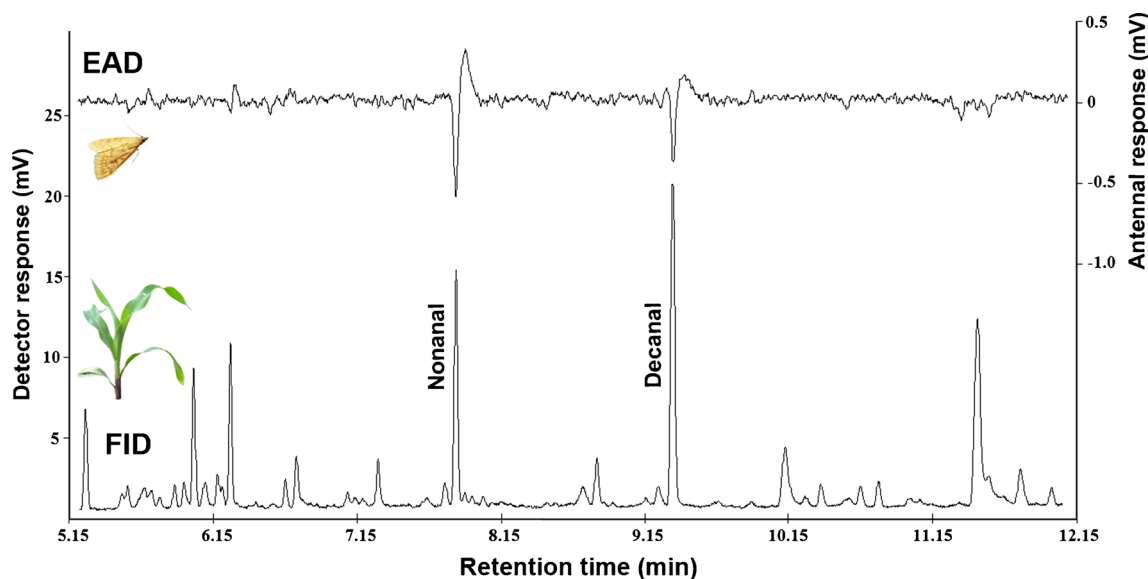


Fig. 2 Typical responses of gravid European corn borer (ECB) female to volatile compounds from maize plant (Pioneer P9578) (antennal responses (mean \pm SE): nonanal $0.535 \text{ mV} \pm 0.08$, decanal

$0.427 \text{ mV} \pm 0.07$, $N = 3$). Analysis were carried out using gas chromatograph coupled with electroantennographic detection (GC-EAD). Top, antennal signal (EAD); bottom, GC trace (FID)

tested individuals. Currently, we have no clear explanation why some of the previous studies failed to detect nonanal and decanal from the headspace of maize at all, or found no evidence for their attractiveness. Whereas differences in the identified volatiles may be due to differences in varieties and/or in collection methods, the lack of behavioral attraction to nonanal or decanal in previous studies may be due to the significance of mixtures over single compounds in orientation, as has been shown in numerous cases (Birkett et al. 2004; Bruce et al. 2005; Bruce and Pickett 2011; Fraser et al. 2003; Natale et al. 2003; Visser and Ave 1978; Webster et al. 2010). In addition, the fact that we used the active volatiles in a ratio found in the host plant may have further augmented attractiveness to the ECB. Other potential causes of differences compared to previous studies may include variation in ratio and/or concentration due to abiotic factors (Gouinguene and Turlings 2002), although these are less likely explanations under laboratory conditions.

We found that the highest dose of the applied mixture of nonanal and decanal attracted gravid females similar to maize in the wind-tunnel bioassays. However, activation was higher with the plant compared to any dose of the synthetic mixture. This latter result could be due to difference in the release rate of the volatile components between maize plant and the dispenser used. Alternatively, other volatile components emitted by the maize can play a role in host finding. We argue that the latter is the most likely explanation as orientation towards a host plant is rarely based on only two volatile chemicals (Cha et al. 2008; Riffell et al. 2009; Zakir et al. 2013). In addition, visual cues of the maize plants also may have been important in eliciting upwind flight and locating suitable oviposition sites. In the complex natural environment, odors do not exist

as individual molecules, but rather consist of mixtures of different compounds. Nonanal and decanal are common volatiles that are released by different plant species (El-Sayed et al. 2013) and appear attractive for different insect species. For instance, Cha et al. (2008) identified nonanal and decanal as two of the 11 antennally-active compounds from grape shoots a blend of which successfully attracted females of the grape berry moth, *Paralobesia viteana* Clemens (Lepidoptera: Tortricidae). Both compounds also were found to synergize the response of codling moth, *Cydia pomonella* to pear ester and increase trapping success of both males and females (El-Sayed et al. 2013). Similarly, nonanal and decanal were part of an 8-compound blend that attracted female sphinx moths, *Manduca sexta* L. (Lepidoptera: Sphingidae) in a wind tunnel, an important pest of Solanaceae crops (Fraser et al. 2003). In southern house mosquitoes, *Culex quinquefasciatus* Say (Diptera: Culicidae), nonanal combined with CO_2 increased the attraction of females when compared to CO_2 alone (Syed and Leal 2009). Siderhurst and Jang (2010) found that nonanal and decanal, which are parts of the cucumber volatile blend, attracted female melon flies, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae). All of these studies suggest that nonanal and decanal are important kairomones that can enhance the attractiveness of a blend; on the other hand, these compounds themselves rarely have been shown to attract pest species. In apple fruit moths, *Argyresthia conjugella* Zeller (Lepidoptera: Yponomeutidae), Bengtsson et al. (2006a) found that decanal, as a single compound, attracted females in field-trapping tests. It remains to be seen whether a combination of nonanal and decanal can attract ECB in the field or that trapping under field conditions will require a more complex blend. For that, extensive field experiments are needed to

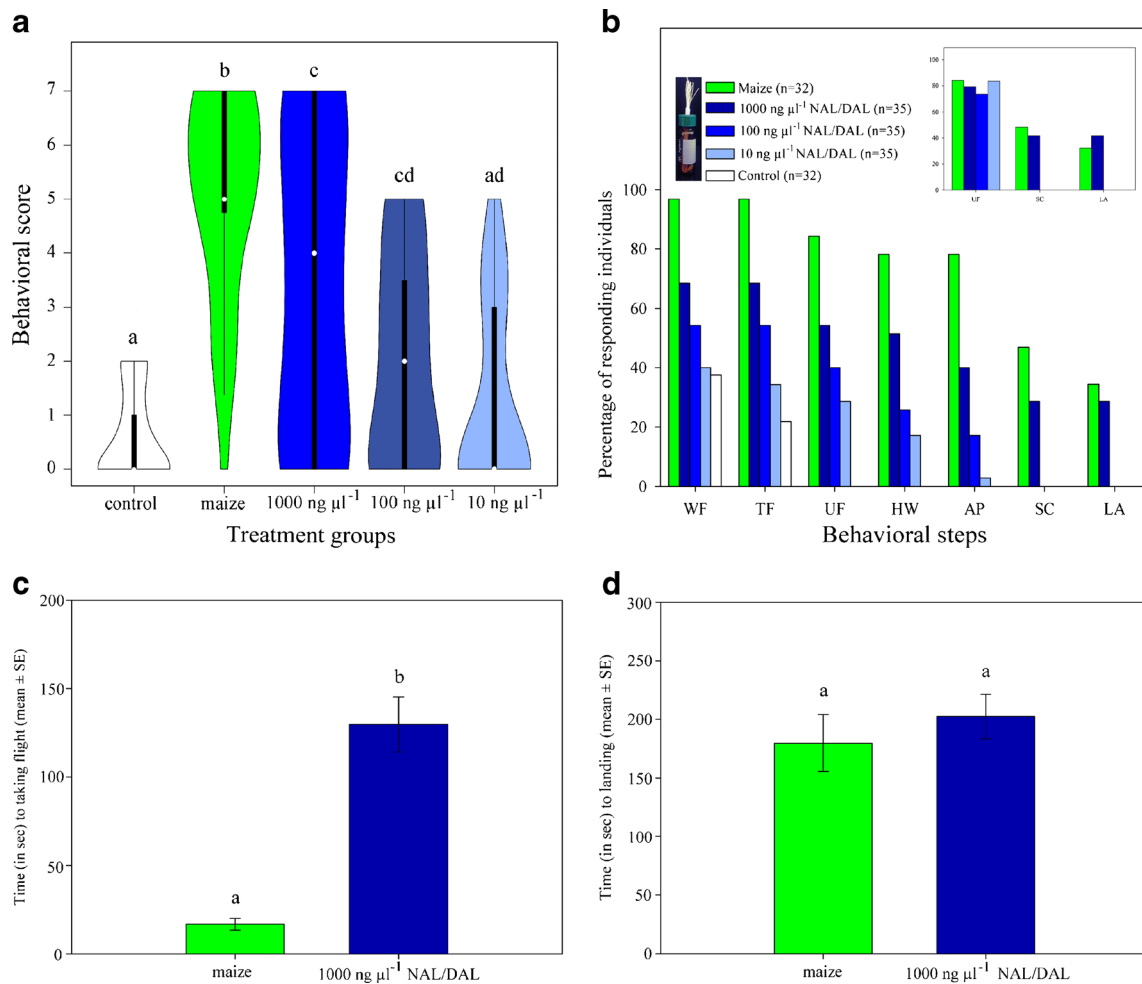


Fig. 3 Behavioral responses of gravid European corn borer (ECB) females during the wind-tunnel bioassays. **a** Behavioral scores of individuals in the different treatment groups, **b** percentage of individuals which undertook given behavioral steps (WF – wing fanning, TF – taking flight, UF – upwind flight, HW – half way, AP – approach, SC – source contact, LA – landing; NAL/DAL – mixture of nonanal and decanal in the ratio of 1:2.4; inset in the upper right corner – comparison of the proportion of individuals undertaking the three highlighted behavioral steps if the number of individuals which took flight in given group was taken as 100 %), **c**

comparison of time to taking flight and **d** time to landing in the maize and the 1000 ng μl^{-1} dose of NAL/DAL treatments. Lowercase letters indicate similarities between treatment groups according to the result of the applied Games-Howell *post-hoc* test in **(a)** and the Wilcoxon rank sum tests in **(c)** and **(d)**. A violin plot **(a)** is a combination of a box plot and a kernel density plot, in which the white circles denotes the medians, whereas the black bars the interquartile range. Behavioral score for each individual was calculated by summing those steps that the given individual performed during the trial

Table 2 Results of the applied Games-Howell *post-hoc* test for comparing behavioral scores between different treatment groups in the wind tunnel bioassay. NAL/DAL denotes the synthetic mixture of nonanal and decanal in a ratio of 1:2.4

Pairwise Comparisons	<i>t</i>	<i>df</i>	<i>P</i>
Control – Maize	12.13	42.33	<0.001
Control – 1000 ng μl^{-1} NAL/DAL	5.55	40.27	<0.001
Control – 100 ng μl^{-1} NAL/DAL	3.82	47.02	0.003
Control – 10 ng μl^{-1} NAL/DAL	1.96	50.54	0.298
Maize – 1000 ng μl^{-1} NAL/DAL	2.86	59.87	0.044
Maize – 100 ng μl^{-1} NAL/DAL	6.60	64.60	<0.001
Maize – 10 ng μl^{-1} NAL/DAL	8.68	62.16	<0.001
1000 ng μl^{-1} NAL/DAL - 100 ng μl^{-1} NAL/DAL	2.44	59.94	0.118
1000 ng μl^{-1} NAL/DAL - 10 ng μl^{-1} NAL/DAL	3.86	55.33	0.003
100 ng μl^{-1} NAL/DAL - 10 ng μl^{-1} NAL/DAL	1.70	66.77	0.441

estimate the efficacy and specificity of this volatile blend, using different concentrations and trap designs.

Although the ECB is a major pest of maize in northern hemisphere, currently there are no techniques available for monitoring females of this species. Whereas phenylacetaldehyde, a common flower odorant, appeared to attract ECB females in the field, the capture rates were too low for its use in monitoring (1.05 females per trap per week), and the compound also attracted many non-target species (Laurent and Frerot 2007; Maini and Burgio 1990). The identification of a mixture of nonanal and decanal attracts female ECB could, however, complement and potentially increase the accuracy of pheromone-based monitoring (see e.g., Yu et al. 2014), perhaps even in a combination, as host plant volatiles and sex pheromones have been found to interact both behaviorally (Deisig et al. 2012), and physiologically (Bengtsson et al. 2014; Christensen and Hildebrand 2002; Trona et al. 2013) in other moth species. Further studies should evaluate whether the E-strain of ECB also is attracted to this mixture, as this strain is also a pest of maize (Sorenson et al. 2005), but has a reportedly different host preference in at least part of its range (Malausa et al. 2007; Pelozuelo et al. 2004).

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