Electrophysiological and behavioral responses of female African rice gall midge, Orseolia oryzivora Harris and Gagné, to host plant volatiles

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Abstract African rice gall midge, Orseolia oryzivora Harris and Gagné, is a major pest of rice in Africa. Depsite its economic importance, its chemical ecology is not well understood. Here, we assessed behavioral and electrophysiological responses of O. oryzivora to host plant volatiles. In olfactometer bioassays, mated female O. oryzivora were attracted to volatiles emitted from intact rice plants but were repelled by volatiles collected from plants infested by conspecifics. In a choice test, there was a preference for volatiles from uninfested plants over those from infested plants. Coupled gas chromatography-electroantennography analyses of pani-cle volatiles isolated four electrophysiologically active components: (S)-linalool, 4,8-dimethyl-1,3,7-nonatriene, (E)-caryophyllene, and (R/S)-(E)-nerolidol. A synthetic blend of volatiles at the same concentration and ratio as that from an intact plant was attractive to mated females, whereas a blend based on the ratio of volatiles from an infested plant was repellent. This suggests that O. oryzivora uses olfaction for host plant recognition. The identification of blends of volatiles emitted by plants that can both attract and repel O. oryzivora may aid the development of sustainable control measures.

Keywords Insect-plant interactions · Plant volatile · Semiochemicals · Olfactometer · Host location · Pest management · Diptera · Cecidomyiidae

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Introduction

The indigenous African rice gall midge, Orseolia oryzivora Harris and Gagné (Diptera: Cecidomyiidae), is one of the most destructive insect pests of rice in Africa (Ogah and Nwilene 2014). The cryptic nature of the larvae, hidden within galls, makes both the use of insecticides and the monitoring of pest populations challenging. However, monitoring could be enhanced if attractive semiochemicals such as host-plant vol-atiles or sex pheromones could be identified. Despite the eco-nomic importance of O. oryzivora, little is known about its host-plant interaction, particularly host-finding behavior. Plant-feeding cecidomyiids typically are specialists, being found only on a single plant species or on a limited number of species within a single genus (Gagne 1989). Ovipositing females play a crucial role in selecting suitable hosts due to the limited mobility of the larvae. A few studies have revealed that plant volatiles play a significant role in cecidomyiid host-plant location (e.g., Birkett et al. 2004; Foster and Harris 1992). Research has also shown that gravid female African rice gall midges prefer certain rice varieties to others for egg laying (Ogah et al. 2012), suggesting that there may be plant chemicals that explain this preference.

Host-plant volatiles that attract female O. oryzivora have not been reported. In the present study, we evaluated the re-sponse of female O. oryzivora to rice plant volatiles through EAG-coupled gas chromatography, chemical analysis, and ol-factometer studies.

Methods and materials

Insect Rearing Rice gall midges (Biotype OSG-1) used for all the experiments were imported from the mass rearing facility of the African Rice Centre IITA, Ibadan, Nigeria. They were reared on ITA 306, a susceptible rice variety, as described by Omoloye (1998), in net cages (62 × 30 × 42 cm) in a controlled environment cabinet CMP6010 (Adaptis Conviron, Canada) at 25 °C, 12 L: 12D photoperiod and 80 % R.H.

Authentic Chemicals Chemical standards were authentic samples of (S)-linalool (95 % purity, Botanix Chem. Co., UK), 4,8-dimethyl-1,3,7-nonatriene (DMNT) (98 % purity, synthesized at Rothamsted), (E)-caryophyllene (97 % purity, Koch-Light Chem. Co., UK), and (R/S)- (E)-nerolidol (90 % purity, Aldrich Chem. Co., MO, US).

Volatile Collection Rice plant volatiles were collected using an air entrainment system following the procedures desribed by Birkett et al. (2004). Volatiles were sampled from whole rice seedlings (TOG7106, a popular African rice variety), with and without O. oryzivora infestation (N = 3 for each treat-ment). Each entrainment lasted for 48 h, after which the vol-atiles adsorbed on Porapak Q were eluted with 0.5 ml re-distilled diethyl ether. Samples were pooled and stored at −20 °C until required for behavioral, electrophysiological, or chemical analyses.

Gas Chromatography (GC) Volatiles were analyzed on a Hewlett-Packard 6890GC equipped with a cold on-column injector, flame ionization detector (FID), nonpolar HP-1 bond-ed-phase fused silica capillary column (50 m × 0.32 mm i.d., film thickness 0.52 μm), and a polar DB-WAX column (30 m × 0.32 mm i.d., film thickness 0.82 μm). The oven temperature was maintained at 30 °C for 1 min, and programmed at 5 °C min^{-1} to 150 °C and held for 0.1 min, then 10 °C min^{-1} to 230 °C. Four μl of each headspace sample were injected into the GC and quantified using known amounts of authentic standards. The stereochemistry of linalool was determined using an HP5890 GC fitted with a cool on-column injector and FID, and equipped with a β-cyclodextrin chiral capillary column (Supelco, 30 m × 0.25 mm i.d., 0.25 μm film thickness).

Coupled Gas Chromatography-Electroantennography (GC-EAG) EAGs from excised antennae of mated female O. oryzivora were recorded using Ag-AgCl glass electrodes filled with saline as described in Birkett et al. 2004. The coupled GC-EAG system has been described previously (Birkett et al. 2004). Four replicates were carried out.

Coupled Gas Chromatography/Mass Spectrometry (GC/ MS) Identification of electrophysiologically active FID peaks was carried out by GC/MS. A 2 μl aliquot of an air entrain-ment sample was injected into a capillary GC column (50 m × 0.32 mm i.d. HPI) coupled to a mass spectrometer (Thermo-Finnigan, MAT95, Bremen, Germany). Ionization was electron impact at 70 eV, 250 °C. The oven temperature was maintained at 30 °C for 5 min and then programmed at 5 °C min^{-1} to 250 °C. Tentative identification of the peaks was made by comparison of spectra with those in a database (NIST 2005). Identifications were confirmed by co-injection of an air entrainment sample with authentic standards using both HP-1 and DB-WAX columns, with peak enhancement indicating co-elution. Compounds were quantified by injecting known amounts of authentic standards.

Olfactometer Bioassays A Perspex four arm olfactometer (Birkett et al. 2004) was used to observe responses of O. oryzivora to infested and intact plant air entrainment samples, as well as to a blend of synthetic samples that comprised the electrophysiologically active compounds at the same concentrations and ratio as in the air entrainment sample. Ten μl of a test solution (air entrainment samples or synthetic blend) were applied to a piece of filter paper, and the solvent was allowed to evaporate for 30 s, before the paper was placed in an arm. The dose represented by 10 μl of headspace sample was approximately the amount of volatiles emitted by 7 rice plants over 8 min, and was, therefore, a physiologically relevant amount that an insect might be exposed to in an ecological setting with several rice plants. Eight replicates were carried out for each odor source tested and each bioassay lasted 8 min. In each replicate, the mean time in the three control arms was calculated, and the mean times spent in the treated and control regions were compared by a paired t-test (Genstat). A choice test between a 10 μl solution of intact plant volatiles and a 10 μl solution of infested plant volatiles also was carried out, with the two treatments assigned ran-domly to different arms in each replicate. The other two arms contained filter paper with 10 μl solvent (diethyl ether) and were used as controls. For the choice test, mean times spent in treated and control regions were compared using ANOVA and Fishers Least Significant Difference test (Genstat).

Results

Olfactometer Bioassays with Headspace Samples Mated O. oryzivora females were attracted to volatile samples collected from uninfested rice seedlings (P = 0.007, Fig. 1a). In contrast, they were repelled by volatile samples from infested plants (P = 0.009, Fig. 1b). In a choice test, volatiles from uninfested plants were preferred to volatiles from infested plants (P = 0.021, Fig. 1e).

Identification of Electrophysiologically Active Compounds EAG analyses revealed four electrophysiologically active compounds from rice headspace samples that consistently elicited antennal responses from female O. oryzivora. These compounds were identified as (S)-linalool, 4,8-dimethyl-

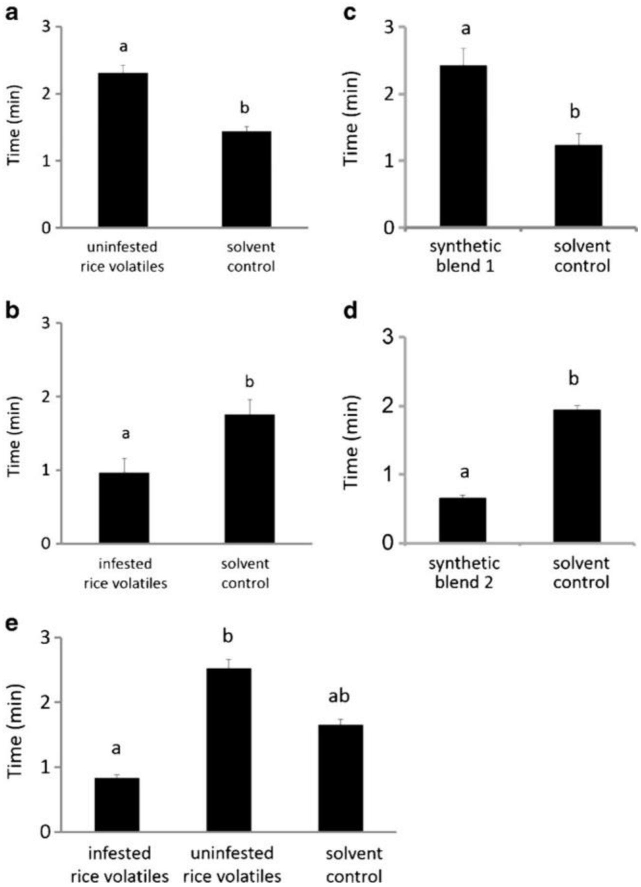

Fig. 1 Olfactometer responses of female African rice gall midge, Orseolia oryzeivora, to rice volatiles. a headspace volatiles from an uninfested plant, b headspace volatiles from an infested plant, c synthetic blend at the concentration and ratio of the compounds in an uninfested plant, d synthetic blend at the concentration and ratio of the compounds in an infested plant, and e choice test between headspace volatiles from an uninfested and from an infested plant. Note, that time in control areas is the mean/arm (there were 3 control arms in A and B and 2 in C). Different letters above a bar denote a difference ($P < 0.05$)

1,3,7-nonatriene (DMNT), (E)-caryophyllene, and (R/S)-(E)-nerolidol. Their quantities and ratio are presented in Table 1.

Olfactometer Bioassays with Synthetic Blends of Volatiles

Synthetic blends, in hexane, of compounds that elicited an EAG response were formulated and tested with mated O. oryzivora. When a synthetic blend at the same concentration and ratio of compounds as the natural uninfested headspace sample was tested, female O. oryzivora spent longer ($P = 0.010$) in the region treated with the synthetic blend than in the control region (Fig. 1c). Conversely, when tested with a synthetic blend with components at the same concentration and ratio as the infested plant sample, O. oryzivora females were ($P = 0.001$) repelled by the synthetic blend (Fig. 1d).

Discussion

In the present study, O. oryzivora olfactory responses to host-plant volatiles, as determined by behavioral and electrophysio-logical assays, are reported for the first time. Four host plant compounds, (S)-linalool, 4,8-dimethyl-1,3,7-nonatriene (DMNT), (E)-caryophyllene, and (R/S)-(E)-nerolidol, were identified from headspace samples of rice volatiles as influenc-ing gall midge behavior.

Previous studies have shown that plant volatiles play significant roles in insect host location and behavior in many insect species, including those in the family Cecidomyiidae (Birkett et al. 2004; Bruce and Pickett 2011). Cecidomyiid adults have a very short life span, and the larvae are monophagous or oligophgagous; therefore, chemical communication and host location by adult females is crucial for survival of the larvae (Hall et al. 2012). Many species of Cecidomyiidae show flight responses to host chemicals (Harris and Foster 1999), supporting the role of plant chemicals in host location. The similarity of behavioral responses of O. oryzivora to the synthetic blends and natural headspace samples demonstrated that the synthetic blends explained (at least most of) the activity of the natural samples. Our study showed that volatiles from uninfested plants were attractive, whereas volatiles from infested plants were repellent. This change in biological activity was due to a change in the concentration and ratio of the four key compounds, the main difference being an increase in the ratio of (E)-caryophyllene to the other three. This suggests

Table 1 Quantification and ratio of compounds in headspace samples of volatiles collected from infested and uninfested rice

Compound		Concentration (ng/μl)		Dose in bioassay (ng)		Ratio (to least abundant compound)	
		Infested	Uninfested	Infested	Uninfested	Infested	Uninfested
1	(S)-linalool	127	2.4	1270	24	1.0	2.0
2	4,8-dimethyl-1,3,7-nonatriene	622	3.7	6220	37	4.9	3.2
3	(E)-caryophyllene	2122	1.5	21220	15	16.7	1.3
4	(R/S)-(E)-nerolidol	162	1.2	1620	12	1.3	1.0

These are the amounts in the natural samples used for the bioassays in Fig. 1

that repellency is context-dependent, as seen previously in studies with the black bean aphid (Webster et al. 2010). It is well established that herbivore feeding can induce changes in the chemical composition of a plant, and this explains the different responses to the different blends in our study.

The cryptic nature of rice gall midges makes their detection and control difficult, and the current study provides a first step toward integrated pest management of O. oryzivora. Identification of semiochemicals that medi-ate host location by females opens up possibilities for a monitoring system for early detection of pest outbreak as well as the development of novel control interventions. For effective management of an insect pest of such eco-nomic importance, monitoring and forecasting are para-mount. While future studies should develop monitoring and control methods for rice gall midges, identification of the sex pheromone of O. oryzivora also would be of great benefit for population monitoring.

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