Phytochemistry Letters 16 (2016) 70–74

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Short communication

Herbivore-induced volatile organic compounds emitted by maize: Electrophysiological responses in *Spodoptera frugiperda* females

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ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 1 March 2016
Accepted 15 March 2016
Available online xxx

Keywords:
Herbivory
VOCs
Induced defenses
EAG-GC
Pest management

ABSTRACT

Herbivory induces the emission of volatile organic compounds (VOCs) which are involved in the attraction of predators and parasitoids. In spite of the large number of studies that have addressed the electrophysiological and behavioral responses of natural enemies to herbivore-induced VOCs, whether herbivores perceive these compounds remains largely unknown. By coupling chemical and electrophysiological (Gas Chromatography–Mass Spectrometry GC–MS and Gas Chromatography–Electroantennography GC–EAG) techniques we identified individual compounds emitted by maize upon feeding by *Spodoptera frugiperda* Walker (Lepidoptera: Noctuidae) that elicit a response in conspecific females. Herbivory induced significantly the emission of β-myrcene, (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, β-linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene [DMNT], indole, geranyl acetate, α-zingiberene, β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, β-sesquiphellandrene, (*E*)-nerolidol, and 4,8,12-trimethyl-1,3,7,11-tridecatetraene [TMTT]. From these, eleven compounds elicited a response in the antennae of *S. frugiperda* females (virgin and mated). The antennae of virgin and mated females responded consistently to (*Z*)-3-hexenyl acetate, β-linalool, indole, (*E*)-β-farnesene and TMTT. In addition the sesquiterpenes ylangene/(+)-cycloisosativene evoked a response in the antennae even though these compounds were not significantly increased upon herbivory. The numbers of antennae of virgin or mated females responding to individual compounds were similar even though a higher percentage of the antennae of gravid female tested responded to geranyl acetate. The results show that *S. frugiperda* detects several herbivore-induced VOCs emitted by maize plants. These compounds may be responsible for repellence previously observed in this species by volatiles induced by conspecifics.

1. Introduction

Plants have evolved an array of mechanisms to respond against harsh environments. Against herbivory, these induced responses include increases in the concentration of secondary metabolites with toxic or deterrent effect against insects (Mithöfer and Boland, 2012 for a review) and the emission of a novel blend of volatile organic compounds (VOCs). Herbivore-induced VOCs attract natural enemies e.g. predators and parasitoids of the herbivore that exert biological control (Clavijo McCormick et al., 2012), a phenomenon regarded to be an induced indirect defense in plants (Kant et al., 2009). Herbivore-induced VOCs-mediated interactions between plants and insects from the third trophic level have been largely studied (Clavijo McCormick et al., 2012). Qualitative and quantitative variations in the blends of undamaged and herbivore-damaged plants allow odor discrimination by foraging carnivores (Kappers et al., 2010) and can further offer information about the identity (De Boer et al., 2008) and density (Girling et al., 2011) of the attacking herbivore. They can also alert about co-occurrence of the prey and a non-prey species in the same plant (Zhang et al., 2009). Knowledge on VOC-mediated tritrophic interactions at the molecular and ecological level has opened new possibilities to develop sustainable strategies to improve the biological control of pests (Rodriguez-Saona et al., 2012; Pinto-Zevallos et al., 2013).

In addition to natural enemies, phytophagous insects perceive and respond to herbivore-induced VOCs. Some species, mainly moths and aphids, are repelled (Dicke and Baldwin, 2010 for a review) by these compounds, and avoid ovipositing on previously infested plants (Zakir et al., 2013), while others are more attracted to injured plants compared to uninfested plants (Dicke and Baldwin, 2010 for a review). In a general view, plant VOCs are of great importance for vital activities of herbivorous insects such as location of suitable oviposition or hibernation sites, food sources as

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http://dx.doi.org/10.1016/j.phytol.2016.03.005
1874-3900/

well as mate partners. Bioactive compounds that either attract or repel herbivores can be exploited to develop novel pest management strategies based on behavior manipulation (Rodriguez-Saona and Stelinski, 2009).

The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is one of the most important generalist pests in the American continent where it is widely distributed (Batista-Pereira et al., 2006). As for other destructive *Spodoptera* species, many studies in the area of chemical ecology have focused on the elucidation of the sex pheromone of this species with the aim of developing pest management strategies (Guerrero et al., 2014). Therefore, the electrophysiological responses of males' antennae to these compounds or the combined effect of pheromone and plant VOCs has been more studied compared to those of females to plant volatiles alone. Recently, it was found that *S. frugiperda* females discriminate between undamaged and herbivore-induced plants in a Y-tube olfactometer, and that they prefer the odors of undamaged plants (Signoretti et al., 2012). However, which compounds are perceived in the VOC blend by females remains unknown. By combining chemical and electrophysiological methods, we assessed the response of virgin and gravid females to VOCs emitted by maize plants upon herbivory by conspecifics to identify which compounds conspecific females detect.

2. Results and discussion

2.1. Emissions of plant volatile organic compounds

Twenty-four compounds were identified in the headspace of herbivore-damage plants. Most of them were not detected in the headspace of undamaged plants. Herbivory significantly increased the emissions of β-linalool ($P < 0.001$) and induced the emissions of β-myrcene ($P = 0.018$); (*Z*)-3-hexenyl acetate ($P = 0.003$) (only detected in one replicate in undamaged plants), (*E*)-β-ocimene ($P = 0.007$), (*E*)-4,8-dimethyl-1,3,7-nonatriene [DMNT] ($P = 0.003$), indole ($P = 0.003$), geranyl acetate ($P = 0.004$), α-zingiberene ($P = 0.016$), β-caryophyllene ($P < 0.001$), (*E*)-α-bergamotene ($P = 0.05$), (*E*)-β-farnesene ($P < 0.001$), β-sesquiphellandrene ($P = 0.007$) and 4,8,12-trimethyl-1,3,7,11-tridecatetraene [TMTT] ($P = 0.006$) (Table 1). These results are in agreement with previous studies (Degen et al., 2004; de Lange et al., 2016), who found several terpenoids (mono-, sesqui- and homoterpenes), green leaf volatiles as well as indole upon herbivory by *Spodoptera littoralis* and *S. frugiperda* in maize plants. Variations between our results and those reported by other studies may owe to genotype (Degen et al., 2004).

2.2. GC-EAG responses

Considering that, the response of the antennae of insects to odor molecules can be significantly modulated by the physiological status of the insect (Kromann et al., 2015) we tested the response of both, virgin and mated females to herbivore-induced VOC extracts. The antennae of virgin and mated *S. frugiperda* females responded to eleven compounds, most of them induced upon herbivory by conspecifics (Fig. 1 and Table 2). Based on Zakir et al. (2013) we set as threshold for considering active compounds with a consistent response when at least half of the antennae responded to the compound. The green leaf volatile (*Z*)-3-hexenyl acetate, the terpenoids β-linalool and TMTT (except for one antenna of mated females) as well as indole elicited a consistent response in the antennae tested regardless the mating status. *Spodoptera* species are known to respond physiologically and behaviorally to green leaf volatiles (Guerrero et al., 2014). In the related species *S. exigua* odor receptors exclusively and sensitively tuned to (*Z*)-3-hexenyl acetate have been identified (Zhang et al., 2013). The response of the antennae of *S. frugiperda* (males and females) has already been shown to be higher to several C6 alcohols and esters in addition to the monoterpenes α-pinene and linalool (Malo et al., 2004), which is consistent to our observations. The related species *S. littoralis* is

Table 1
Volatile organic compounds emitted by undamaged and *Spodoptera frugiperda*-damaged maize plants in ng g DW^{-1} 24 h^{-1} (Mean ± SEM).[a]

	Ret. Time	$KI_{(c)}$[b]	Undamaged plants	*S. frugiperda*-damaged plants	Statistical Significance
(*E*)-2-hexenal	5.578	n.c.	n.d.	0.66 ± 0.48	n.s.
(*Z*)-3-hexen-1-ol	5.642	n.c.	n.d.	0.91 ± 0.60	n.s.
β-myrcene	8.500	n.c.	n.d.	2.29 ± 0.90	0.018
(*Z*)-3-hexenyl acetate	8.848	1007	0.06[c]	4.93 ± 2.19	0.003
(*E*)-β-ocimene	9.758	1046	n.d.	0.14 ± 0.09	0.007
β-linalool	10.888	1105	1.25 ± 0.36	76.62 ± 18.25	<0.001
Nonanal	10.968	1109	1.36 ± 0.43	1.39 ± 0.66	n.s.
DMNT	11.245	1122	0.30[c]	33.70 ± 14.32	0.003
Decanal	13.100	1212	1.82 ± 0.81	0.94 ± 0.32	n.s.
Anthranilic acid	14.100	1259	n.d.	0.34 ± 0.15	n.s.
Indole	14.999	1307	0.16[c]	61.97 ± 19.22	0.003
(+) cycloisosativene	16.438	1379	1.58 ± 0.65	1.16 ± 0.56	n.s.
Ylangene	16.488	1382	0.66 ± 0.35	0.58 ± 0.38	n.s.
geranyl acetate	16.566	1386	n.d.	3.22 ± 0.85	0.004
α-zingiberene	16.783	1397	n.d.	0.10 ± 0.03	0.016
β-caryophyllene	17.417	1428	n.d.	1.93 ± 0.71	<0.001
(*E*)-α-bergamotene	17.623	1445	n.d.	32.52 ± 6.35	0.05
(*E*)-β-farnesene	17.927	1462	n.d.	62.44 ± 16.00	<0.001
α-caryophyllene	18.025	1469	0.04 ± 0.02	0.30 ± 0.12	n.s.
α-muurulene	18.800	1514	0.14 ± 0.07	0.18 ± 0.10	n.s.
β-bisabolene	18.892	1519	0.55 ± 0.29	1.75 ± 0.48	n.s.
β-sesquiphellandrene	19.174	1534	n.d.	4.15 ± 1.31	0.007
(*E*)-nerolidol	19.786	1569	n.d.	2.53 ± 1.18	n.s.
TMTT	20.032	1584	n.d.	7.90 ± 3.47	0.006

n.c. = not calculated.
n.d. = not detected.
n.s. = not significant.
[a] DW = dry weight; SEM = standard error of the mean.
[b] $KI_{(c)}$ = calculated Kovats Index.
[c] Emitted by only one plant, therefore SEM was not calculated.