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Abstract

Plants and pests communicate their physio-chemical status to their surroundings by emitting volatile organic compounds (VOCs). Emission of these compounds are affected by abiotic stresses, such as temperature, light, draught or waterlogging, but also by biotic stresses, like pest attack. It is well known that the Cotton Bollworm *Helicoverpa armigera* releases species-specific VOCs on their eggs and in their larval frass to deter oviposition of conspecifics, so called oviposition deterrent pheromones (ODPs). As such ODPs are species-specific VOCs, detectable also in absence of the adult pest insect and identified for *H. armigera*, these compounds might be potential candidates for reliable detection of the Cotton Bollworm.

In this task, we performed Cotton Bollworm related volatile collections followed by chemical analyses of healthy plants and with plants infested with eggs and larval frass. We worked with different host plants of *H. armigera* (tomato, sunflower, pea) with and without insect eggs or frass attached to the host plants. The aim was to find volatile marker compounds, which are robustly associated with our target pest, independent of plant species and other types of stress like the attack of other pests or environmental stresses.

The study revealed several ODP specific compounds (egg + larval frass, respectively) for the Cotton Bollworm on the three different plant species tested. However, looking for ODPs, which are detectable independent of plant species and independent of environmental stresses reduced the number of reliable candidate compounds to one, namely 1-dodecanol.

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1 INTRODUCTION

It is known that both plants and pests communicate their physio-chemical status to their surroundings by emitting volatile organic compounds (VOCs). Emission of these compounds are affected by abiotic stresses, such as temperature, light, draught or waterlogging, but also by biotic stresses, like pest attack. It is also well known that the Cotton Bollworm *Helicoverpa armigera* releases species-specific VOCs on their eggs and in their larval frass to deter oviposition of conspecifics, so called oviposition deterrent pheromones (ODPs).

Research on *H. armigera* VOCs has started in the 1970s with the identification of sex pheromones in *H. armigera,* namely (*Z*)-9-hexadecenal, (*Z*)-11-hexedecenal, hexadecanol, (*Z*)-11-hexadecenol and (*Z*)-9-tetradecenal. We want to highlight the most promising pheromones for our purpose, the oviposition marking pheromones (OMPs) or oviposition deterring pheromones (ODPs). ODPs have been identified for *H. armigera* around the turn of the millennium. ODPs are deposited by many parasitic and phytophagous insects associated with egg-laying, aiming for modification of the oviposition behaviour of conspecifics such that subsequent eggs are not deposited into an already utilized resource. After behavioural observations on *H. armigera* indicated the existence of oviposition-deterrent compounds, the three compounds 4-hydroxy-4-methyl-2-pentanone, hexadecanoic acid (palmitic acid) and (*Z*)-9-octadecenoic acid (oleic acid) have been identified from the tarsi of female *H. armigera* as oviposition-deterrent compounds. In further studies on ODPs in larval frass of *H. armigera*, a blend of fatty acid and corresponding methyl esters was found in the larval frass. Some compounds were found independent of the diet of the larvae, while others seem to be dependent on the food source. All compounds elicited responses in *H. armigera* moth antennae using electroantennography (EAG) analyses. Moreover, it was found that laid eggs resulted in similar EAG responses. Compounds identified from the laid eggs were the 4-oviposition deterring fatty acids myristic, palmitic, stearic, and oleic acid and their corresponding methyl esters.
As ODPs are species-specific VOCs, detectable also in absence of the adult pest insect and identified for *H. armigera*, these compounds might be potential candidates for reliable detection of *H. armigera.*

In Purpest we aim to develop a Cotton Bollworm-specific volatile-based sensor platform to detect the pest on imported plants. For that purpose, we performed volatile collections followed by chemical analyses of healthy and infested plants. We worked with different host plants of the moth (tomato, sunflower, pea) and we looked for differences in the volatile profile of healthy plants versus plants infected by eggs or larvae of the Cotton Bollworm. The aim was to find marker volatiles, which are robustly associated with our target pest, independent of plant species and other types of stress like the attack of other pests or environmental stressors (e.g. temperature, water logging, drought or complete darkness).

In this stage of the project, we have focused on the ODPs of *H. armigera*. We were investigating and comparing VOC profiles of the three different host plants with and without environmental stress, with and without ODPs from eggs and in larval frass, and the combination of both types of stresses (environmental stresses + ODPs).

2 MATERIAL AND METHODS

The VOCs of three plant species (pea, sunflower, tomato), which were cultivated in standard pots filled with standard growing substrate, were collected using the custom-made fragrance collector (Flusys Type FSP-2246F 2023). The plants were grown under greenhouse conditions at 22 ± 2 °C, 60-70% rH and 14:10 L:D until four to six leaves were fully developed and thereafter used for volatile collection (24 ± 2 °C, 60-70% rH and 14:10 L:D). For volatile collection, each plant was placed into a separate 2000 mL glass chamber. The bottoms of the chambers were closed by a collar fitted around the stems. The stem of the plant was wrapped in aluminum foil to seal the gap between the plant and the collar. Charcoal filtered air was pumped into the top of the chamber through an inlet port of Teflon tubing, across the plant and pumped out of the bottom of the chamber through a Tenax TA35/60 (Markes) desorption filter to collect the volatiles. The air stream was regulated automatically to ensure that a total volume of 100 ln was moved across the plants at a steady rate of 0.8 ln/min. for approx. 2 hours. At each collection time, 6 collections were made in parallel, one from a blank control (empty glass chamber on an empty pot without substrate) and five plants of the same species and treatment. To prevent scent contamination, all glassware was heated to 300 °C for 7 h before use.

In a first experiment (Experiment 1) we tested each plant species separately, without any stress, grown under standard conditions (22 ± 2 °C, 60-70% rH and 14:10 L:D) and under abiotic stress (*Dry*: 5 days without water; *Waterlogging*: 5 days; *Cold*: 8°C for 2 (sunflower, tomato) or 5 (pea) days; *Hot*: 35°C for 2 (pea) or 3 (sunflower, tomato) days; *Dark*: no light for 5 days).

In a second experiment (Experiment 2) we tested plants of each plant species respectively, grown under standard conditions (see Experiment 1), but infested with eggs of CBW, and the combination of abiotic and biotic stress (plants with eggs of CBW AND under suboptimal growing conditions; *Dry*: 5 days without water; *Waterlogging*: 5 days; *Dark*: no light for 5 days). Temperature stress as mentioned in Experiment 1 were not tested with plants infested with CBW eggs, as the eggs were not surviving the temperature stress.

In Experiment 3 we tested plants of all three plant species respectively, grown under standard conditions (see Experiment 1), but under the biotic stress of larval frass of CBW, and the combination of abiotic and biotic stress (plants with larval frass of CBW AND under suboptimal growing conditions (see Experiment 2). Temperature stress as mentioned in Experiment 1 were not tested with plants infested with CBW larval frass, as the larvae were not surviving the temperature stress.

After volatile collection the Tenax tubes were desorbed using a thermal desorption gas chromatograph coupled to a mass spectrometer (Agilent 5977B/8890). The identification and quantification of volatile compounds were performed based on comparing with NIST library spectra and for some compounds with the mass spectra of commercial standards in addition. Kovats index was calculated by injecting alkanes C8 to C40 and used for identification as well. The ODP specific VOCs were chosen based on their consistent presence in headspace from plants (with and without abiotic stress) with CBW eggs (ODP $_{egg}$) and with CBW larval frass (ODP $_{frass}$) and their consistent absence in headspace from uninfected plants (i.e. without CBW eggs or larval frass), with and without abiotic stress.

3 RESULTS

The ODPs stated in the literature were found also in our study, but they were not found reliable enough to be potential candidates for robust detection of *H. armigera.*
In total, we found 79-91 abiotic stress specific VOCs, depending on the host plant species (Table 1, Exp.1). For biotic stress, we found 123 ODP_{egg} specific VOCs for pea, 131 for sunflower and 118 for tomato, no ODP_{egg} specific VOCs which were reliable produced by all three host plants (Table 1, Exp.2). The ODP_{egg} specific VOCs were found in very low amounts and not reliable in all replicates. Thus, we see them not as promising candidate compounds for robust detection of our pest.

Table 1. Number of VOCs present in samples from plants (pea, sunflower, tomato) with and without abiotic stress (Exp.1); plants infested with eggs of CBW, with and without abiotic stress (Exp.2); and plants infested with CBW larval frass, with and without abiotic stress (Exp. 3). These VOCs were not present in the control samples.

			# VOCs found in headspace of plants		
Exp.	Type of stress		Pea	Sunflower	Tomato
1	No stress, standard conditions*	Total VOC profile, plants under common growing conditions*	36	46	57
	Abiotic stressors** (dry, waterlogging, cold, hot, dark)	Total VOC profile, plants under abiotic stress**	117	137	136
		Abiotic stress **specific VOCs, NOT found under optimal condition	81	91	79
2	***Biotic stress, eggs of CBW (ODP $_{egg}$)***	Total VOC profile ODP $_{egg}$ ± abiotic stress***	240	268	254
		ODP $_{egg}$ specific VOCs, NOT found in plants ± abiotic stress *** without eggs	123	131	118
	Common ODP $_{egg}$ specific VOCs for all 3 plant species, found in all ODP $_{egg}$ treatments, ± abiotic stress ***, but NOT found in uninfected plants ± abiotic stress**		**0**		
3	***Biotic stress, larval frass of CBW (ODP $_{frass}$)***	Total VOC profile ODP $_{frass}$ ± abiotic stress***	279	242	260
		ODP $_{frass}$ specific VOCs, found in all ODP $_{frass}$ treatments, ± abiotic stress ***, NOT found in plants ± abiotic stress *** without frass	18	21	6
	Common ODP $_{frass}$ specific VOC for all 3 plant species, found in all ODP $_{frass}$ treatments, ± abiotic stress ***, but NOT found in uninfected plants ± abiotic stress**		**1-dodecanol (CAS 112-53-8)**		

*greenhouse, 24°C, 60-70% rH, 14:10 L:D
**Dry: 5 days without water; Waterlogging: 5 days; Cold: 8°C for 2 (sunflower, tomato) or 5 (pea) days; Hot: 35°C for 2 (pea) or 3 (sunflower, tomato) days; Dark: no light for 5 days.
***Dry: 5 days without water; Waterlogging: 5 days; Dark: no light for 5 days.

However, for biotic stress ODP_{frass}, we found, reliable in all replicates, 18 ODP_{frass} specific VOCs for pea, 21 for sunflower and 6 for tomato (Table 1, Exp.3; Table 2), and one compound, 1-dodecanol (CAS 112-53-8), which was ODP_{frass} specific VOC and reliable produced by all three host plants (Table 1, Exp.3).

Table 2. List of ODP $_{frass}$ specific VOCs, found in all ODP $_{frass}$ treatments, with and without abiotic stress, but neither found in plants with and without abiotic stress without frass nor in the control samples, related to plant species the VOCs were found in.

Compound name	CAS number	Plant species
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	sunflower
.alpha.-Phellandrene	99-83-2	sunflower
1(3H)-Isobenzofuranone	87-41-2	pea
1,2-Propanediol, 1-acetate	627-69-0	pea
1,3,5-Trioxane	110-88-3	pea, sunflower
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	sunflower, tomato
1-Dodecanol	112-53-8	pea, sunflower, tomato
1-Hexanol	111-27-3	pea
2-Hexenal, (E)-	6728-26-3	pea
2-Propanol, 1-methoxy-	107-98-2	pea, sunflower
3-Carene	13466-78-9	pea
3-Hexen-1-ol, acetate, (Z)-	3681-71-8	pea
9H-Fluorene, 9-methylene-	4425-82-5	pea
Benzene, propyl-	103-65-1	sunflower
Benzeneacetaldehyde	122-78-1	sunflower, tomato
Benzoic acid	65-85-0	pea
Benzyl alcohol	100-51-6	pea, sunflower
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	3387-41-5	sunflower
Cyclic octaatomic sulfur	10544-50-0	sunflower
Ethyl Acetate	141-78-6	sunflower, tomato
Hexadecane	544-76-3	pea, sunflower
Neophytadiene	504-96-1	sunflower
Nonane, 4,5-dimethyl-	17302-23-7	pea
Phenylethyl Alcohol	60-12-8	sunflower
Phthalic anhydride	85-44-9	sunflower
Pyridine	110-86-1	pea
Succinimide	123-56-8	pea
Sulfur dioxide	7446-09-5	sunflower
Tetrachloroethylene	127-18-4	pea, sunflower
Tetradecane	629-59-4	pea
Tetrahydrofuran	109-99-9	sunflower
Trichloroethylene	79-01-6	sunflower, tomato
Trichloromethane	67-66-3	tomato
Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	488-97-1	sunflower

4 CONCLUSION

Our results showed that several ODP specific compounds (particularly larval frass) can be reliably detected for CBW on the three different plant species tested. This supports our hypothesis, that ODPs are species-specific VOCs, detectable also in absence of the adult pest insect and detectable for *H. armigera*, making them promising candidates for reliable detection of *H. armigera.* However, looking for ODPs, which are detectable independent of plant species and independent of environmental stresses (temperature, water logging, drought or complete darkness) reduced the number of reliable candidate compounds to one, namely **1-dodecanol**.
In the second phase of the project, we will analyze plant volatiles (HIPV) induced by CBW under the same criteria as we did here for the ODPs; aiming for VOCs, which are robustly associated with our target pest, and at the same time independent of plant species and independent environmental stress.