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Elicitors of tansy volatiles from cotton leafworm larval oral secretion

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ABSTRACT

The feeding of *Spodoptera littoralis* and *Autographa gamma* caterpillars on tansy leaves led to a complete different release of volatile monoterpenes, sesquiterpenes, and hexenyl alkanoates. Volatiles were collected from *S. littoralis* and *A. gamma* larvae damaged, mechanically wounded, and excised tansy leaves by closed loop stripping analysis. The qualitative and quantitative determination of the volatiles were done by GC–MS- and GC-measurements. The oligosaccharides sucrose, raffinose, stachyose, and verbascose have been detected in oral secretion of the caterpillars of the cotton leafworm *S. littoralis.* When applied to damaged leaves of tansy plants, these oligosaccharides induce the tansy leaves to emit a similar volatile blend as the feeding of *S. littoralis* larvae.

1. Introduction

Plants have developed different defense mechanisms against biotic and abiotic factors. The defenses can be classified into constitutive and inducible mechanisms. Cuticles, thorns and trichomes as well as constitutively formed defensive secondary metabolites represent the constitutive defenses (Mithöfer et al., 2005). Inducible defenses are only activated under conditions of actual threat, for example in reaction to pathogen or herbivore attack and can be divided into direct and indirect responses (Gatehouse, 2002). The accumulation of pathogenesis-related (PR) proteins after insect feeding or phytoalexins after infestation with potentially pathogenic microorganisms is a direct defense. An indirect defense is the formation and emission of volatile organic compounds (VOCs) after feeding of herbivorous insect larvae. VOCs attract carnivorous insects which search for the larvae (Walling, 2000; Kessler and Baldwin, 2002). Several plant species as maize (Turlings et al., 1990), cotton (Röse et al., 1996), lima bean (Dicke et al., 1990; Ozawa et al., 2000), and tobacco (Kessler and Baldwin, 2001) emit VOCs after insect feeding.

Herbivore-specific elicitors have been obtained from oral secretions of feeding insect larvae (Bonaventure et al., 2011). These elicitors are certain enzymes as Glc oxidase (Felton and Eichenseer, 1999), β-glucosidase (Mattiaci et al., 1995), and alkaline phosphatase (Funk, 2001) or *N*-acyl-glutamines such as volicitin (Alborn et al., 1997), *N*-linolenoyl-L-glutamine, and *N*-linolenoyl-L-glutamate (Halitschke et al., 2001). The *N*-acylglutamines and *N*-acylglutamates possess significant activity in inducing mechanically wounded plants to produce and release VOCs. The disulfide-bridged peptide inceptine from fall armyworm (*Spodoptera frugiperda*) larval oral secretion promotes in the cowpea (*Vigna unguiculata*) the ethylene production, increases the contents of the phytohormones salicylic acid and jasmonic acid and leads to the emission of VOCs (Schmelz et al., 2006). Saturated and monounsaturated disulfooxy fatty acids and their glycine conjugates (caeliferins) from the oral secretion of the nonlepidopteran American bird grasshopper (*Schistocerca americana*) induce the release of VOCs from damaged leaves of corn seedlings (Alborn et al., 2007). The *N*-acyl-glutamine volicitin is not generally active as elicitor for the emission of plant VOCs after application to wounded plants such as lima bean and cotton (Spiteller et al., 2001).

Tansy (*Tanacetum vulgare* L.) is a perennial, herbaceous plant which is native in Asia and Europe. The leaves of tansy contain and release terpenes (Rohloff et al., 2004). Tansy is the host plant for the larvae of *Cucullia tanaceti* Denis & Schiffermüller, 1775 (Lepidoptera, Noctuidae), *Antonechloris smaragdaria* Fabricius, 1787 (Lepidoptera, Geometridae) and some other larvae of Noctuidae and Geometridae (Beccaloni et al., 2013). The caterpillars of the cotton leafworm *Spodoptera littoralis* Boisd. and the Silver Y moth *Autographa gamma* Linnaeus belong also to the Noctuidae. They are generalists feeding also on leaves of *T. vulgare*. *S. littoralis* larvae were used for the investigation of the response of tansy leaves after their feeding, because the plant volatile elicitor volicitin occurs in their oral secretion (Spiteller et al., 2001). *A. gamma* larvae use the same feeding behaviour as *S. littoralis* larvae. We were interested in the question whether the feeding of *S. littoralis* and *A.*

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gamma larvae on tansy leaves leads to a similar or different tansy volatile blend. The herbivore-specific elicitors should be isolated from oral secretions of *S. littoralis* and *A. gamma* larvae.

2. Results

2.1. Induction of volatiles by lepidopteran larvae

Tansy is a terpene rich plant and its content was investigated before by GC–MS (Rohloff et al., 2004). Volatiles were collected from *S. littoralis* and *A. gamma* larvae damaged, mechanically wounded, and excised tansy leaves by closed loop stripping analysis (CLSA). The CLSA method is described in detail in Section 4.4. The qualitative and quantitative determination of the main fifteen monoterpenes, three sesquiterpenes and two hexenyl alkanoates were done by GC–MS- and GC-measurements (Table 1), whereby the following monoterpenes and hexenyl alkanoates can only be detected together due to very similar or identical GC retention times: *m*-cymene (**3**)/*p*-cymene (**4**), cuminaldehyde (**8**)/(*Z*)-3-hexenyl pentanoate (**9**), *cis*-carveol (**10**)/(*Z*)-3-hexenyl 3-methylbutanoate (**11**), *cis*-chrysanthenol (**13**)/umbellulone (**14**), *trans*-sabinol (**15**)/camphor (**16**) and, limonene (**19**)/1,8-cineol (**20**).

The released tansy volatiles can be divided into three groups. After feeding of *S. littoralis* larvae on tansy leaves the monoterpenes **1**–**4** and the sesquiterpenes **5**–**7** were found in higher concentrations compared to the feeding of *A. gamma* larvae (Table 2, Figs. 1 and 2). A stronger formation and emission of cuminaldehyde (**8**)/(*Z*)-3-hexenyl pentanoate (**9**) and *cis*-carveol (**10**)/(*Z*)-3-hexenyl 3-methylbutanoate (**11**) were observed after feeding of *A. gamma* in comparison with *S. littoralis* larvae (Table 2, Fig. 3). The third group consists of tansy released monoterpenes **12**–**20** with low statistically significant differences after feeding of *S. littoralis* and *A. gamma* larvae (Table 2, Figs. 4 and 5). Especially at the first day the monoterpenes **1**, **2** (Fig. 1), **17** (Fig. 5) and the sesquiterpenes **5**, **6** (Fig. 2) were released after mechanical wounding in higher amounts compared with those after feeding of *A. gamma* larvae. The reason is probably that the caterpillars need more feeding time to achieve a comparable damage area as after mechanical wounding. As expected, this correlation was only found at a very low release of volatiles after feeding of *A. gamma* larvae.

Table 2
Release of tansy volatiles. Higher concentrations of volatiles after feeding of *S. littoralis* larvae on tansy leaves compared to *A. gamma* larvae (S). Higher concentrations of volatiles after feeding of *A. gamma* larvae on tansy leaves compared to *S. littoralis* larvae (A). Volatiles were released with low statistically significant differences after feeding of *S. littoralis* and *A. gamma* larvae (SA).

Release	S	A	SA
Volatiles			
trans-Sabinene hydrate (**1**)	+		
cis-Sabinene hydrate (**2**)	+		
m-Cymene (**3**)/*p*-Cymene (**4**)	+		
Germacrene D (**5**)	+		
Bicyclogermacrene (**6**)	+		
trans-β-Caryophyllene (**7**)	+		
Cuminaldehyde (**8**)/(*Z*)-3-Hexenyl pentanoate (**9**)		+	
cis-Carveol (**10**)/(*Z*)-3-Hexenyl 3-methylbutanoate (**11**)		+	
Chrysanthenone (**12**)			+
cis-Chrysanthenol (**13**)/Umbellulone (**14**)			+
trans-Sabinol (**15**)/Camphor (**16**)			+
Borneol (**17**)			+
α-Pinene (**18**)			+
Limonene (**19**)/1,8-Cineol (**20**)			+

During 4 days the emission of *trans*-sabinene hydrate (**1**) from tansy leaves after *S. littoralis* feeding alternated between 8.8 and 16.8 ng/g fresh weight (fr. wt) and after *A. gamma* feeding between 0.9 and 6.7 ng/g fr. wt. The other three monoterpenes **2**–**4** and three sesquiterpenes **5**–**7** of this group show a similar trend. Cuminaldehyde (**8**)/(*Z*)-3-hexenyl pentanoate (**9**) belong to the second group and were released in amounts of 1.4–31.4 ng/g fr. wt after *A. gamma* feeding and 0.03–2.2 ng/g fr. wt after *S. littoralis* feeding. This trend was also observed for *cis*-carveol (**10**)/(*Z*)-3-hexenyl 3-methylbutanoate (**11**) of the second group.

The feeding of *S. littoralis* and *A. gamma* caterpillars on tansy leaves led to a different release of volatile monoterpenes, sesquiterpenes, and hexenyl alkanoates.

2.2. Oral secretions of lepidopteran larvae

The oral secretions of *S. littoralis* and *A. gamma* caterpillars were collected by squeezing the larvae, causing them to regurgitate. The

Table 1
Detected volatiles of *Tanacetum vulgare*.

Volatile	KI	MS (EI, 70 eV), *m*/*z* (%)
trans-Sabinene hydrate (**1**)	1092.0	154 (3) [M]$^+$, 139 (9), 121 (15), 111 (17), 93 (41), 81 (26), 71 (31), 55 (22), 43 (100)
cis-Sabinene hydrate (**2**)	1066.0	154 (2) [M]$^+$, 139 (8), 121 (18), 111 (19), 93 (31), 81 (25), 71 (27), 55 (22), 43 (100)
m-Cymene (**3**)/*p*-Cymene (**4**)	1017.3	134 (37) [M]$^+$, 119 (100), 105 (7), 91 (14), 77 (6), 65 (8), 51 (4), 41 (8)
	1024.0	134 (31) [M]$^+$, 119 (100), 105 (3), 91 (19), 77 (4), 65 (4), 53 (2), 41 (10)
Germacrene D (**5**)	1470.0	204 (21) [M]$^+$, 161 (100), 147 (10), 133 (22), 119 (47), 105 (85), 91 (70), 81 (44), 67 (24), 53 (18), 41 (79)
Bicyclogermacrene (**6**)	1482.0	204 (9) [M]$^+$, 189 (7), 175 (2), 161 (38), 147 (9), 133 (14), 121 (100), 107 (51), 93 (87), 79 (57), 67 (52), 53 (40), 41 (92)
trans-β-Caryophyllene (**7**)	1403.6	204 (2) [M]$^+$, 189 (9), 175 (5), 161 (11), 147 (14), 133 (35), 119 (16), 105 (41), 91 (64), 79 (49), 69 (32), 55 (21), 41 (100)
Cuminaldehyde (**8**)/(*Z*)-3-Hexenyl pentanoate (**9**)	1235.7	148 (35) [M]$^+$, 133 (81), 119 (40), 105 (100), 91 (38), 77 (48), 63 (12), 51 (35), 41 (20)
	1237.0	134 (2), 119 (3), 103 (6), 91 (9), 82 (89), 67 (100), 57 (64), 41 (54)
cis-Carveol (**10**)/(*Z*)-3-Hexenyl 3-methylbutanoate (**11**)	1228.0	152 (1) [M]$^+$, 134 (27), 119 (38), 109 (30), 105 (38), 91 (100), 84 (38), 77 (39), 67 (22), 55 (25), 41 (59)
	1232.0	85 (25), 82 (84), 67 (100), 57 (77), 41 (47)
Chrysanthenone (**12**)	1114.7	150 (7) [M]$^+$, 135 (7), 122 (21), 107 (93), 91 (100), 79 (51), 73 (8), 65 (22), 51 (24), 41 (51)
cis-Chrysanthenol (**13**)/Umbellulone (**14**)	1158.9	152 (3) [M]$^+$, 135 (20), 121 (52), 109 (57), 93 (69), 81 (94), 67 (71), 55 (40), 41 (100)
	1160.0	150 (8) [M]$^+$, 135 (18), 122 (5), 108 (100), 91 (74), 79 (52), 65 (20), 51 (26), 41 (42)
trans-Sabinol (**15**)/Camphor (**16**)	1130.0	152 (1) [M]$^+$, 134 (22), 119 (58), 105 (26), 91 (100), 79 (48), 65 (15), 55 (28), 43 (19)
	1134.0	152 (3) [M$^+$], 137 (3), 108 (36), 95 (100), 81 (67), 76 (54), 55 (51), 41 (97)
Borneol (**17**)	1159.0	136 (7), 121 (21), 110 (16), 95 (100), 79 (12), 67 (8), 55 (11), 41 (20)
α-Pinene (**18**)	925.0	136 (10) [M]$^+$, 121 (9), 105 (12), 93 (100), 79 (26), 67 (9), 53 (7), 41 (14)
Limonene (**19**)/1,8-Cineol (**20**)	1021.5	136 (9) [M]$^+$, 121 (11), 107 (12), 93 (89), 79 (46), 67 (100), 53 (28), 41 (32)
	1037.0	154 (3) [M]$^+$, 139 (12), 125 (4), 108 (17), 93 (22), 81 (30), 67 (21), 55 (23), 43 (100)