Identification of Volatile Compounds Emitted by *Artemisia ordosica* (*Artemisia*, Asteraceae) and Changes due to Mechanical Damage and Weevil Infestation

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Volatiles emitted by healthy, mechanically damaged, and weevil-infested *Artemisia ordosica* (Asteraceae) were obtained through a dynamic headspace method and analysed by automatic thermal desorption/gas chromatography/mass spectrometry (ATD/GC/MS). Twenty-eight compounds in all were identified, and the qualitative as well as quantitative differences were compared. The green leaf volatiles 2-hexenal, (*Z*)-3-hexen-1-ol, 2-hexen-1-ol, 1-hexanol, and (*Z*)-3-hexen-1-ol acetate were present in all of the damaged plants, but in relatively lower portions when plants were infested by the weevil *Adosopius* sp., while the terpenoids *α*-copaene, *β*-cedrene, and (*E*,*E*)-*α*-farnesene and the ester methyl salicylate were only present in weevil-damaged plants. The volatiles from healthy and weevil-infested leaves were dominated by D-limonene, whereas mechanically damaged leaves emitted *β*-pinene as the dominant compound.

Key words: Artemisia ordosica, Mechanically and Weevil-Damaged, Volatile Compounds

Introduction

Artemisia ordosica (Asteraceae) is a Chinese unique, perennial, deciduous subshrub of 60 – 100 cm height consisting of short-lived shoots which bear plumose, linearly lobate leaves. It is mainly distributed in the arid and semi-arid areas of Inner Mongolia Autonomous Region, Ningxia Hui Autonomous Region, Gansu and Shanxi Provinces of China (Xu *et al*., 2007). Due to its prosperous root system, *A. ordosica* is one of the most important plants for desertification control. A major pest of *A. ordosica* is the weevil *Adosopius* sp. (Curculionidae, Coleoptera). The larvae mainly cause damage to the roots while adults feed on the leaves as supplemental source of nutrition, causing a serious decline of *A. ordosica* (Ma *et al*., 2009). The mite *Pyemotes tritici* and a kind of spider (the species has not been identified) have been shown to be predators of *Adosopius* sp. larvae and adults, respectively (Wang, 2011).

In the course of co-evolution, olfactory organs in herbivores and their predators have adapted to identify volatile gradients, so plant volatiles play a crucial role in the host selection of insects. Insects can detect them from a longer distance and then make host orientation, oviposition, aggregation, and pollination (Harrewijn *et al*., 1994; Jones, 1944; Lou and Cheng, 2000). On the other hand, plants defend themselves from herbivore feeding by producing chemical volatiles called herbivore-induced plant volatiles (HIPVs) (Zakir, 2011). Thus studies on volatiles as well as HIPVs are imperative.

Qualitative and quantitative differences among volatiles emitted by healthy, mechanically damaged, and pest-infested plants have received significant attention in recent years (Hare, 2011; Ramadan *et al*., 2011). Although a number of papers have been published concerning the general area of *A. ordosica*, little work has been carried out on this aspect. On the basis of existing literature data, we carried out studies in an effort to increase knowledge on such volatiles, and we intend to use this knowledge to provide clues how to control the weevils.

Material and Methods

Plant material

Sixty individuals of healthy *A. ordosica* from Gaoshawo county, Ningxia, China were transplanted into plastic pots before the blooming stage in mid-June. The pots were deposited in the experimental base in Gaoshawo county and watered regularly. All plants were two to three years old with an average of 15 – 18 branches; the crown diameters were 28 – 30 cm. The material was identified by Duanzheng Lu, the taxonomist of Beijing Forestry University, Beijing, China. A voucher specimen (No. 071054) was deposited in the specimen room of Haba Lake National Nature Reserve Administration Bureau, Ningxia, China.

Herbivore material

The pupae of *Adosopius* sp. were collected in the field and put in individual plastic containers at room temperature for eclosing to adults. In order to avoid any possible disturbance by sex pheromone, only virgin female adults were chosen.

General experimental procedures

Twenty days later, pre-sampling preparations were started. Thirty potted plants were chosen and divided into three equal groups. Plants of group 1 (undamaged) were controls; from group 2 (clipped) 50% of the foliage was clipped from the plants with sterilized scissors; plants of group 3 (weevil-damaged) were infested by 4 virgin female adults which had been fasting for 10 h. After 3 h, at 10 a.m. (the feeding peak of *Adosopius* sp. is between 7 a.m. and 10 a.m.) (Wang, 2011), the weevils were removed, and the volatiles were collected simultaneously from the 30 potted plants using a dynamic headspace method. Each aerial part was covered with an oven bag (48.26 cm x 23 cm x 1.27 cm; Reynolds, Richmond, CA, USA). The inner air was exhausted first and then the bag refilled with air percolated through an activated charcoal filter. Sampling then began. Gas was cycled through the oven bag, which was connected to a stainless steel tube (length, 8.89 cm; diameter, 0.635 cm) filled with 200 mg TenaxTA (60/80 mesh; Supelco, Bellefonte, DE, USA); the flow rate was kept at 100 ml/min, and volatiles were sampled for 15 min. Prior to headspace collection, Tenax tubes were cleaned in a thermal desorption oven (TP-2040; BFTP, Beijing, China) at 270 °C for 3 h. After sampling, all roots were split to make sure that they were healthy. Six replicates of every treatment were chosen for the next analysis.

Automatic thermal desorption/ gas chromatography/mass spectrometry (ATD/GC/MS)

The samples were analysed via an automatic thermal desorber (ATD650 TurboMatrix; Perkin Elmer, Fremont, CA, USA) directly connected to a gas chromatograph (Clarus600; Perkin Elmer) and a mass spectrometer (Clarus600; Perkin Elmer). All analytical conditions are summarized in Table I.

Identification of the volatile compounds

Retention indices (RI) were calculated using retention times of a series of *n*-alkanes (C_4–C_{26}) that were injected after the volatiles at the same conditions. Relative contents were calculated based on GC peak areas without using correction factors. The volatile compounds were identified by comparison of their mass spectra with those stored in the GC/MS database (TurboMass Ver5.4.2, NIST 08) or with those of authentic samples purchased from Anpel (Shanghai, China) and confirmed by comparison of their RI with data published in the literature (Davies, 1990; Ruther, 2000; Adams, 2007).

Results

Compounds representing at least 0.1% of the total amount of the volatile blend, including ter-

Table I. Analytical conditions for ATD/GC/MS.

ATD	First desorption	260 °C (10 min)
	Second desorption	–25 °C → 40 °C/s → 300 °C (5 min)
GC	Column	DB-5 MS (30.0 m × 0.25 mm × 0.25 μm) (J & W, Santa Clara, CA, USA)
	Carrier gas	He (1.5 ml/min)
	Temperature program	40 °C (2 min) → 4 °C/min → 160 °C (3 min) → 20 °C/min → 270 °C (3 min)
MS	Analytical mode	Full-scan
	Mass range	*m/z* 29 – 600

penoids, alcohols, aldehydes, carboxylic acids, and esters, are listed in Table II. The volatiles from healthy and weevil-infested leaves were both dominated by D-limonene (32.1% and 25.7%), whereas mechanical damage evoked β-pinene (22.0%) as the dominant compound. The green leaf volatiles 2-hexenal, (*Z*)-3-hexen-1-ol, 2-hexen-1-ol, 1-hexanol, and (*Z*)-3-hexen-1-ol acetate were present in all of the damaged plants, but in relatively lower portions when plants were infested by *Adosopius* sp., while the terpenoids α-copaene, β-cedrene, and (*E*,*E*)-α-farnesene and the ester methyl salicylate were only present in weevil-damaged plants. Additionally, the most noticeable feature was the induced variation of terpenoids: the relative contents of α-pinene and 4-carene increased in all of the damaged plants while β-myrcene, D-limonene, β-phellandrene, and (*E*)-β-caryophyllene showed a decreasing trend. Besides, mechanical damage and weevil-afflicted damage induced a distinct quantitative variation in camphene, sabinene, β-pinene, (*E*)-β-ocimene, and β-patchoulene. Specifically, the relative content of β-patchoulene from mechanically damaged leaves was significantly different from that of the weevil-damaged ones; the former was about 10 times that of the latter.

Discussion

Terpenoids and methyl salicylate, which were also determined in our experiments, are very

Table II. Contents of compounds identified in healthy (H), mechanically damaged (MD), and weevil-damaged (WD) *Artemisia ordosica* and the standard deviations (s), n = 6.

No.	RI[a]	Compound	Relative content (%)					
			H	s	MD	s	WD	s
1[b]	851	2-Hexenal	–[c]	–[c]	2.8	0.3	0.1	0.1
2[b]	853	(*Z*)-3-Hexen-1-ol	–[c]	–[c]	5.0	0.6	0.9	0.5
3[b]	867	2-Hexen-1-ol	–[c]	–[c]	0.7	0.2	0.1	0.2
4[b]	870	1-Hexanol	–[c]	–[c]	3.2	0.3	0.4	0.2
5	927	α-Thujene	0.5	0.3	0.4	0.2	0.5	0.3
6[b]	939	α-Pinene	3.7	0.5	13.0	0.5	5.7	0.4
7[b]	947	Camphene	0.1	0.1	0.5	0.2	0.1	0.2
8	971	Sabinene	12.9	0.6	11.7	0.4	14.7	0.4
9[b]	975	β-Pinene	16.6	0.6	22.0	0.4	15.1	0.4
10[b]	989	β-Myrcene	6.9	0.6	5.1	0.5	3.4	0.5
11	994	(*Z*)-3-Hexen-1-ol acetate	–[c]	–[c]	3.0	0.5	1.1	0.3
12[b]	997	4-Carene	0.4	0.1	1.1	0.5	1.3	0.3
13	1024	*p*-Cymene	2.2	0.3	0.7	0.4	0.5	0.1
14[b]	1027	D-Limonene	32.1	0.1	14.3	0.3	25.7	0.3
15[b]	1031	β-Phellandrene	16.1	0.4	7.1	0.2	6.4	0.3
16	1036[d]	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	0.5	0.1	0.6	0.1	19.3	0.6
17[b]	1041	(*E*)-β-Ocimene	2.8	0.2	4.2	0.3	1.3	0.3
18	1186[d]	Butanoic acid 3-hexenyl ester	0.2	0.1	0.2	0.1	0.4	0.1
19[b]	1189	Methyl salicylate	–[c]	–[c]	–[c]	–[c]	0.2	0.1
20	1193	(1*R*)-(–)-Myrtenal	0.7	0.1	0.9	0.3	0.6	0.3
21	1217	*cis*-3-Hexenyl isovalerate	0.3	0.1	0.4	0.1	0.5	0.2
22	1261	(*E*)-2-Decenal	0.8	0.3	0.5	0.1	0.2	0.6
23	1311	2-Butyl-1-octanol	1.3	0.1	0.4	0.2	0.1	0.1
24	1378	β-Patchoulene	1.5	0.5	1.9	0.5	0.2	0.1
25[b]	1391	α-Copaene	–[c]	–[c]	–[c]	–[c]	0.2	0.2
26[b]	1418	β-Cedrene	–[c]	–[c]	–[c]	–[c]	0.6	0.1
27[b]	1434	(*E*)-β-Caryophyllene	0.5	0.4	0.3	0.2	0.1	0.3
28[b]	1508	(*E*,*E*)-α-Farnesene	–[c]	–[c]	–[c]	–[c]	0.1	0.5

[a] Observed retention index.
[b] Compounds identified by comparison with authentic samples.
[c] Not detected.
[d] Compounds for which RI values have not been previously reported in the literature.

important in plants' direct or indirect defences against herbivore attack (Batovska *et al*., 2008; Todorova *et al*., 2010; Ament *et al*., 2010). Some terpenoids have also been reported in the study of healthy *A. ordosica* (Liu *et al*., 2010; Yang *et al*., 2012), but compared to our study, there are considerable quantitative differences probably because different methods were used. In our study, we used dynamic headspace to analyse compounds emitted by the plant, while Liu *et al*. (2010) and Yang *et al*. (2012) used steam distillation and thus analysed endogenous compounds. In addition, age, crown diameter, etc. of the plants analysed differed in the various studies.

Numerous studies demonstrated that herbivores display a conspicuous preference or avoidance behaviour for feeding and oviposition in plants infested by their own species (Turlings and Tumlinson, 1991; Kessler and Baldwin, 2001; Addesso *et al*., 2011). These cases raise the question if the induced volatiles will attract the herbivores' natural enemies, and which behaviour *Adosopius* sp. will display when plants are infested by its own species.

On the basis of this study and previous reports (Ma *et al*., 2009), a chemical ecological control of weevil infestation may be feasible. In this case, the effective compounds and their optimal concentrations within the volatile blend must be identified to exploit attractive and repellent functions of individual compounds and to use them for effective protection of the desert plant *A. ordosica* from weevils.

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