Volatile Compounds of Healthy and Insect-Damaged *Hippophae rhamnoides sinensis* in Natural and Planted Forests

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Volatile compounds of healthy and insect-damaged stems of *Hippophae rhamnoides sinensis* were analysed using dynamic headspace and thermal-desorption cold-trap injector gas chromatography/mass spectroscopy (TCT-GC/MS). Sixteen compounds, belonging to alkanes, alcohols, aldehydes, esters, ketones, and ethers, were identified in the stems of healthy *H. rhamnoides sinensis*; the compounds in *H. rhamnoides sinensis* occurring naturally or cultivated in plantations were similar, but the relative contents were significantly different. In plants damaged by *Holcocerus hippophaecolus*, the nature and content of the volatile compounds were greatly changed. Butanedione and butyl glyoxylate were newly generated after damage by the pest, and the relative levels of pentanal, heptanal, eucalyptol, terpineol, and camphor were sharply increased in both naturally occurring and plantation-grown plants. *n*-Decane, *trans*-2-nonen-1-ol, and *n*-hexadecane levels increased in plants cultivated in the plantation and decreased in natural forests, whereas the levels of other types were reduced. Thus, both the nature and the content of volatile compounds of *H. rhamnoides sinensis* are affected by *H. hippophaecolus* damage, providing a theoretical basis to identify the mechanism of pest destruction.

Key words: Holcocerus hippophaecolus, *Hippophae rhamnoides sinensis*, Volatile Compounds

Introduction

Sea buckthorn (*Hippophae rhamnoides sinensis*) is a small baccate deciduous shrub or subarbor plant that belongs to the *Hippophae* genus of the Elaeagnaceae family. Sea buckthorn plants share the characteristics of being arid-resistant, cold-tolerant, barren-enduring, and light-degree salt-tolerant. They also have a strong ability to fix nitrogen. *H. rhamnoides sinensis* readily sends out tillers, or shoots growing from the base of the stem of the plant, so as to form dense shrubs with well developed root systems. These plants can rapidly improve adverse ecological conditions through effective conservation of soil and water resources and promotion of the growth of other plants. This species therefore has enormous potential for use on a large scale to control desertification, particularly in northern China, and is of tremendous industrial value in the development of the regional economy and the protection of biodiversity.

The seabuckthorn carpenterworm, *Holcocerus hippophaecolus*, belongs to the Lepidoptera order and, more specifically, the *Holcocerus* genus of the Cossidae family. The larva stage of *H. hippophaecolus* bores into the stems and roots of sea buckthorn. Most roots are bored to the point of being hollow, causing the whole tree to wither. In recent years, the seabuckthorn carpenterworm has broken out in the Inner Mongolia Autonomous region and the Ningxia Hui Autonomous region, as well as the Shaanxi, Shanxi, Liaoning, Hebei, and Gansu provinces. The entire area affected by *H. hippophaecolus* amounts to over 133,000 hectares, with 66,500 of these representing a total withering of sea buckthorn. Hence, the severity of *H. hippophaecolus* damage has yielded a catastrophic economic and industrial loss in China (Hua *et al.*, 1990; Luo *et al.*, 2003, 2007; Zong *et al.*, 2005, 2006a, b, 2011).

In the long-term co-evolution process, insects and plants have formed various interactions that are beneficial to one species or the other, or both. The behavioural response of herbivorous insects toward host plants is an important core issue in the study of such interspecies relationships. This

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response includes the preference of the insect for the plant host and/or its habitat and the selection of the particular feeding and/or spawning site. The regulatory control of the host plant itself on the feeding or spawning activities of the insect is also of consideration. Volatile compounds of the plant play a decisive role in the latter process (Bolter *et al*., 1997; Dicke, 1999; Du and Yan, 1994; Paré and Tumlinson, 1999). To date, the volatile components of *H. rhamnoides sinensis*, and especially the changes in the content and types of compounds produced, have not yet been reported. The main objective of the present study was to establish in detail the volatile constituents of healthy and insect-damaged *H. rhamnoides sinensis* in natural forests versus plantations. Identification of these compounds will be critical to understand the defences of *H. rhamnoides sinensis* against *H. hippophaecolus* and to effectively control the damage to this valuable plant caused by the insect.

Material and Methods

Sea buckthorn (H. rhamnoides sinensis)

Pengyang county of the Ningxia Hui Autonomous region, located east of the Liupan mountains, is the eastern part of the Northwest Loess Plateau hilly region. Pengyang county has a temperate, semi-arid, and typical continental monsoon climate. A large number of natural and planted sea buckthorn forests are found there. Three healthy and three damaged sea buckthorn plants were selected each from a natural and a planted sea buckthorn forest, respectively. The elevation, degree of slope, direction of slope, and other environmental factors were similar in the two sea buckthorn forests.

Collection of volatiles

The dynamic headspace method was used to collect volatile compounds. Briefly, tested sets of stems were covered with microwave bags (Reynolds, Richmond, VA, USA). Each bag was deflated and then filled with air previously percolated using activated carbon and the gas chromatographic stationary phase GDX101 (60/80 mesh). Sampling was then started. Gas was cycled through the bag connected to a quartz glass tube (length, 16 cm; inner diameter, 321 μm) filled with the absorbent TenaxTA (60/80 mesh, 200 mg), a polymer packing material that is particularly suited for trapping volatile compounds. The volatile compounds produced by the stems were collected during a collection time of 3 h using an exhaust flow rate of 100 ml/min.

Analysis of volatile compounds

The quality and quantity of volatiles produced by *H. rhamnoides sinensis* stems were analysed by thermal-desorption cold-trap injector gas chromatography/mass spectroscopy (TCT-GC/MS) (CP 4020-Trace 2000/Voyager; Finnigan, ThermoQuest, San Jose, CA, USA).

Thermal desorption conditions were as follows. The system pressure was 20 kPa. The tube carrying the adsorbates was heated to 250 °C within 10 min; the volatiles were thermally desorbed in turn, then cooled to –120 °C by liquid nitrogen, and next condensed on a short capillary column. The inlet temperature was 260 °C. After injection, the column was instantaneously heated from –120 °C to 260 °C, and the volatiles flowed to the GC column with a carrier gas.

GC conditions were as follows. A DB-5 Low Bleed/MS column (60 mm × 0.32 mm inner diameter, with 0.5 μm film thickness) was used with helium as the carrier gas. The injector temperature was kept at 250 °C. The GC oven temperature was started at 40 °C for 3 min and programmed to 270 °C at a rate of 6 °C/min. The post run was at 280 °C for 5 min.

MS conditions were as follows. Ion source was set at 70 eV. Interface temperature was 250 °C. Ion source temperature was set at 200 °C. Emission current was 150 μA. Detector voltage was 300 V. Full scan was run within the mass range of *m/z* 29–350 amu at a scan rate of 0.4 s/scan.

Identification of volatile compounds

The NIST98 mass spectral library and retention time database were analysed using xcalibur software (version 1.2) for identification of the volatile compounds produced by *H. rhamnoides sinensis*. The normalization method of peak area was used to quantify the various types and relative contents of the volatile substances, and the difference between the volatiles produced by different plant subgroups was analysed using the least significant difference (LSD) multiple comparison method.

Results

Volatile compounds isolated from stems of healthy H. rhamnoides sinensis

The results from gas chromatography/mass spectroscopy (GC/MS) analysis showed that the extract from stems of healthy *H. rhamnoides sinensis* comprised 16 different volatile compounds, including alkanes, alcohols, aldehydes, esters, ketones, and others (Table I). Among these, the main compounds were butyl cyclopropane, *n*-heptane, alkyl methallylether, *n*-octane, *n*-nonane, 5-methyl-5-hydroxyhexanoic acid lactone, *n*-decane, *n*-dodecane, and *n*-hexadecane. The volatile compounds released by *H. rhamnoides sinensis* occurring naturally or cultivated in plantations were similar, but the relative contents of the compounds were significantly different. The relative levels of butyl cyclopropane, *n*-heptane, alkyl methallylether, *n*-octane, 5-methyl-5-hydroxyhexanoic acid lactone, and eucalyptol were higher in plants grown in the plantation compared with plants grown in the natural forest. The relative levels of other compounds, for example, pentanal, *n*-nonane, and *trans*-2-nonen-1-ol, were higher in plants grown in the natural forest. The difference in the types and relative content of volatile compounds in *H. rhamnoides sinensis* found in the plantation and natural forests indicated that the chemical constituents were the product of the interaction of *H. rhamnoides sinensis* and environmental selective pressure.

Volatile compounds isolated from stems of insect-damaged H. rhamnoides sinensis

The nature and relative content of volatile compounds were greatly changed in *H. rhamnoides sinensis* damaged by *H. hippophaecolus* (Figs. 1 and 2). For instance, butanedione and butyl glyoxylate were newly released when the plants were infected with *H. hippophaecolus*, but their relative content was very low. The other volatile compounds were the same as those released from undamaged *H. rhamnoides sinensis*, but their relative levels varied between damaged and undamaged plants. In plants grown in either natural or planted forests, the relative levels of heptanal, eucalyptol, terpineol, and camphor were sharply increased and reached the level of significance ($p = 0.05$). *n*-Decane, *trans*-2-nonen-1-ol, and *n*-hexadecane levels increased in *H. rhamnoides*

Table I. Volatile compounds released from *H. rhamnoides sinensis* grown in natural and artificial forests.

No.	RT [min]	Compound	Undamaged		Damaged	
			Artificial forest	Natural forest	Artificial forest	Natural forest
1	6.76	Butanedione	-	-	0.14a	0.29 ± 0.04a
2	8.49	Pentanal	2.02 ± 0.23b	2.74 ± 0.37b	3.97 ± 0.12ab	5.72 ± 0.04ab
3	9.2	Butyl cyclopropane	9.82 ± 1.12a	3.95 ± 0.49b	5.40 ± 0.13b	3.93 ± 0.49b
4	9.36	*n*-Heptane	8.22 ± 0.002a	1.46 ± 0.017b	1.08 ± 0.002b	1.46 ± 0.001b
5	12.47	Alkyl methallylether	6.80 ± 0.05a	3.41 ± 0.003b	0.90 ± 0.002b	1.40 ± 0.003b
6	12.71	*n*-Octane	5.61 ± 0.04b	4.70 ± 0.000b	0.62 ± 0.001c	0.71 ± 0.001c
7	15.46	Butyl glyoxylate	-	-	0.14 ± 0.001a	0.17 ± 0.000a
8	16.19	*n*-Nonane	12.58 ± 0.002a	14.47 ± 0.33a	6.58 ± 0.005c	14.45 ± 0.32a
9	16.35	5-Methyl-5-hydroxyhexanoic acid lactone	6.80 ± 0.05a	2.41 ± 1.03b	4.90 ± 0.022a	1.40 ± 0.003b
10	16.51	Heptanal	1.08 ± 0.52a	3.35 ± 1.04b	4.59 ± 0.12b	7.37 ± 0.31c
11	19.16	*n*-Decane	13.45 ± 1.98b	16.81 ± 1.71b	14.39 ± 1.79b	8.73 ± 1.70a
12	21.12	Eucalyptol	2.19 ± 1.47a	1.16 ± 0.23a	7.55 ± 1.61b	5.08 ± 0.36b
13	21.31	Terpineol	3.19 ± 0.49b	3.85 ± 0.40b	9.04 ± 1.36c	8.92 ± 1.46c
14	22.74	*n*-Dodecane	12.65 ± 1.52a	14.58 ± 1.70a	11.23 ± 1.85a	12.51 ± 0.69a
15	23.22	*trans*-2-Nonen-1-ol	1.39 ± 0.001a	10.39 ± 2.22c	4.29 ± 0.001b	5.82 ± 0.003b
16	24.9	Camphor	3.92 ± 0.29a	4.29 ± 0.41a	7.55 ± 0.89c	10.26 ± 1.41d
17	26.19	*trans*-2-Decen-1-ol	1.39 ± 0.001a	3.82 ± 1.13b	0.29 ± 0.13a	4.82 ± 0.36b
18	33.2	*n*-Hexadecane	5.19 ± 0.14a	6.23 ± 0.04a	7.18 ± 0.31b	5.80 ± 0.36b

The total ion current strength of the volatile organic compounds was above 10^3. Means (± SD) from three replications were analysed using the LSD test. The same letters signify no significant difference ($p > 0.05$).

sinensis cultivated in the plantation and decreased in their counterparts found in natural forests. The relative levels of other volatiles were significantly reduced ($p = 0.05$) or stayed the same ($p > 0.05$) (see also Table I). These data indicated that *H. rhamnoides sinensis* plants chemically defend themselves against *H. hippophaecolus* damage by changing the composition and content of volatile compounds.

Discussion

The results of the field survey showed that high levels of esters, alcohols (*trans*-2-decen-1-ol, *trans*-2-nonen-1-ol), and aldehydes (heptanal) are found in *H. rhamnoides sinensis* grown in natural forests compared with plantations. These compounds may have different effects on the host selectivity of *H. hippophaecolus*, leading to more

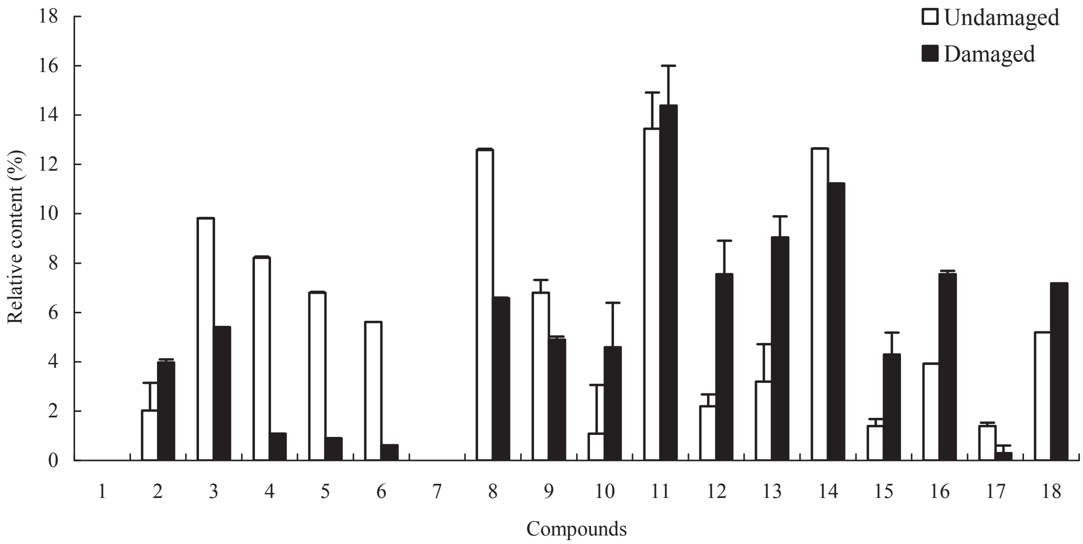

Fig. 1. Types and relative contents of volatile compounds from damaged and undamaged *Hippophae rhamnoides* in artificial forests. The numbers of the compounds match the numbers provided in Table I.

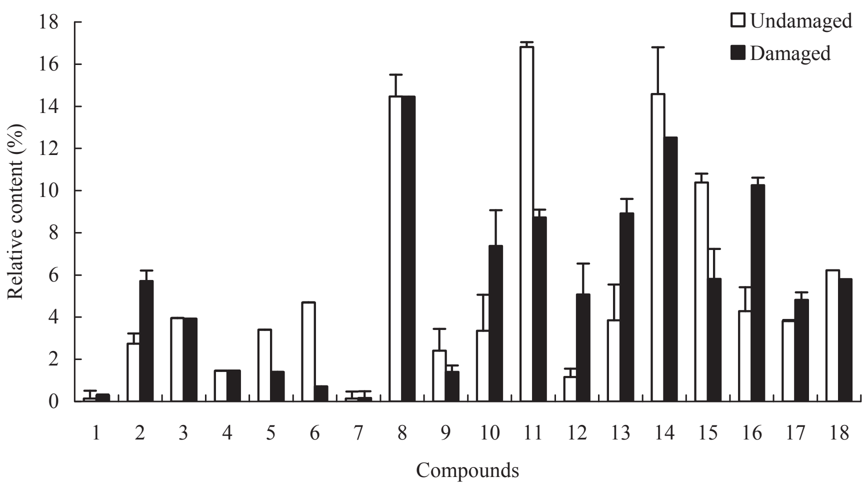

Fig. 2. Types and relative contents of volatile compounds from damaged and undamaged *Hippophae rhamnoides* in natural forests. The numbers of the compounds match the numbers provided in Table I.

deleterious effects of the adult pests in the plantation.

The feeding of *H. hippophaecolus* on the stems of *H. rhamnoides sinensis* leads to a significant increase in the relative levels of pentanal, heptanal, eucalyptol, terpineol, and camphor. Moreover, the levels of terpineol and camphor are significantly higher in plants found in infested forests compared with healthy forests. This is important because the release of terpineol is one of the most critical chemical defences of the host plant, directly influencing the behaviour of phytophagous insects (Paré and Tumlinson, 1999). Hence, sea buckthorn is not passive when facing insect invasion, but uses effective chemical strategies to increase its chance of survival.

Many reasons may be responsible for the disastrous effect of *H. hippophaecolus* in areas with high *H. rhamnoides sinensis* populations. These include consecutive years of drought, a population comprising a single plant species, and an advanced age of *H. rhamnoides sinensis*. The role that the major volatile substances of *H. rhamnoides sinensis* play in the mechanism of environment-specific host selection of *H. hippophaecolus*, especially the volatiles induced by pest damage, requires further study. In addition, a large number of studies have shown that plant volatiles can have a synergistic effect with insect sex pheromones (Landolt and Heath, 1989; Landolt *et al.*, 1994; Phillips *et al.*, 1993). The question of whether the specific volatile compounds of *H. rhamnoides sinensis* in the plantation environment can act in synergy with *H. hippophaecolus* sex pheromones to increase plant vulnerability also requires further study.

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