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HEADSPACE VOLATILES OF WHOLE PLANTS AND MACERATED PLANT PARTS OF *BRASSICA* AND *SINAPIS*

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Key Word Index—*Brassica napus*, *B. campestris*, *B. carinata*, *B. juncea*, *B. nigra*, *Sinapis alba*; Cruciferae; adsorption; GC/MS; plant volatiles; macerated buds; macerated leaves.

Abstract—Headspace volatiles collected from six Crucifer species of the two genera *Brassica* and *Sinapis* were investigated by GC/MS. A total of 34 compounds were identified from both whole plants and macerated plant parts. Typical cell-degradation compounds including alcohols, aldehydes and glucosinolate breakdown products were primarily found in macerate samples, while terpenes were detected almost exclusively in whole plants. Macerated buds generally contained higher amounts of nitriles and isothiocyanates than did macerated leaves. Several compounds here identified have, to our knowledge, not been previously reported in *Brassica* and *Sinapis*.

INTRODUCTION

Glucosinolates and their volatile breakdown products are characteristic of the Cruciferae and are known to play an important role in interactions between plants and phytophagous insects [1–3]. They may function both in insect attraction and in plant defence against insects [1]. *Brassica* and *Sinapis* species have been much studied because of their use and importance as vegetables, fodder and oilseeds. The mechanisms of glucosinolate degradation have been investigated [4, 5]. In several studies volatiles were collected by rather 'rough' methods, such as solvent extraction and distillation of fresh or boiled plant material [4, 6, 7], and reports of volatiles from intact or whole plants are few [8–10].

In *Brassica* and *Sinapis*, plant volatiles have been suggested to be one possible reason for reported differences in the suitability of the host species to the Brassica pod midge *Dasineura brassicae* Winn. (Diptera: Cecidomyiidae) [11–13], as well as in the differing attractivity of separate plant parts to the pollen beetle *Meligethes aeneus* F. (Coleoptera: Nitidulidae) [14].

An increased understanding of the chemical communication between Crucifers and their insect pests would be useful in breeding insect-resistant plants and in decreasing the use of insecticides, by development of pest control methods. In the present study, we examined headspace volatiles released from both whole plants and macerated buds and leaves of six species of *Brassica* and *Sinapis*. The purpose was to investigate if the composition of plant volatiles in different species could explain previously reported differences in insect reactions on Crucifer odours [11–14]. The chemical data reported here are also intended as a base for further studies on plant–insect interactions.

RESULTS AND DISCUSSION

In the headspace of whole-plant samples we identified a total of 34 volatiles (Table 1), with a composition differing between species. In the headspace of macerated plant parts we also identified 34 volatiles (Table 2), representing mainly compounds other than those present in whole-plant samples. In addition to the above compounds, we detected some unknowns in all samples (mainly isoprenoids and fatty-acid derivatives).

Isoprenoids

In four of the species, terpenes were the major compounds of whole-plant samples. They were the monoterpenes *trans*-β-ocimene in *Brassica juncea*, verbenone in *B. nigra*, and the sesquiterpene α-farnesene in both *B. napus* and *B. campestris*. Other prominent monoterpenes found were β-pinene, sabinene, myrcene, limonene and β-phellandrene. These terpenes are flower-fragrance components, being mainly released from undamaged inflorescences. They were not detected in macerates (Table 2). Limonene and some terpenoid alcohols (e.g. linalool, citronellol, geraniol and nerol), are reported earlier from *Brassica* [7, 15]. Other isoprenoids not previously reported are indicated in Table 1.

Fatty acid derivatives

Volatile leaf alcohols and aldehydes are known to be present in several plant families and are mainly degradation products from leaf lipids [16]. *cis*-Hex-3-en-1-ol, *trans*-hex-2-enal and *cis*-hex-3-en-1-yl acetate are known to be present in volatiles of both whole and macerated *Brassica* [8, 9, 17], which was confirmed here with the exception of the aldehyde which we did not find in whole-plant samples. Other leaf volatiles identified in macerates were hexanal, *trans*-hex-2-enal, pentan-1-ol and hexan-1-ol. Many of the leaf volatiles present here are known to be attractants for phytophagous insects [3].

Benzenoids

Benzenoids previously reported in plant odours by several investigators [7, 15, 18], were here detected from

Table 1. Relative* amounts of volatiles identified from whole-plant samples of five *Brassica* and *Sinapis* species

Compound	Method†	*B. napus*	*B. campestris*	*B. juncea*	*B. nigra*	*S. alba*
Isoprenoids						
α-Pinene‡	RT, MS	1.9				
β-Pinene‡	RT, MS	0.8	2.9		4.5	
Sabinene‡	RT, MS	9.7		3.3		
Myrcene‡	RT, MS	11.6	8.6	0.7	7.0	0.1
Limonene	RT, MS	14.8	4.4		5.1	
β-Phellandrene‡	RT, MS				5.7	
1,8-Cineole‡	RT, MS	3.2			tr§	0.7
cis-β-Ocimene‡	RT, MS			3.3	0.4	0.1
trans-β-Ocimene‡	RT, MS	2.3		78.9	7.4	4.5
Perillene‡	MS	0.9		0.3		
α-Cedrene‡	MS	3.0				
Linalool	MS	3.2				
β-Elemene‡	MS	1.5				
Caryophyllene‡	MS	1.9			1.1	
trans-Verbenol‡	RT, MS				1.1	
Verbenone‡	RT, MS				25.8	
α-Farnesene‡	MS	38.9	48.0	3.4	6.0	9.9
Sesquiterpene	—				10.9	9.2
Fatty-acid derivatives						
cis-Hex-3-en-1-yl acetate	RT, MS	3.2	3.0	1.3	1.9	8.3
cis-Hex-3-en-1-ol	RT, MS		0.9	1.0		1.5
Benzenoids						
Benzaldehyde	RT, MS	0.6	5.8	2.2	3.3	40.4
Phenylacetaldehyde	RT, MS	0.6	13.8	0.5	0.8	
Naphthalene‡	RT, MS		tr	0.3	0.2	
2-Phenylethanol	MS	1.1	tr	tr	3.7	
4-Methoxybenzaldehyde‡	RT, MS	0.4			4.7	2.8
Nitrogen containing						
sec-Butyl-ITC‖	RT, MS		4.6	1.3		
Allyl-ITC	RT, MS			1.6	1.1	
Phenylacetonitrile	RT, MS					17.9
But-3-enyl-ITC	MS					0.1
Pent-4-enyl-ITC	MS		3.0			
Benzyl-ITC	RT, MS					3.3
Indole	RT, MS	tr	tr	0.7	3.3	1.1
2-Aminobenzaldehyde‡	RT, MS	tr	1.7	0.7	5.9	
Sulphides						
Dimethyl disulphide	RT, MS	0.4	2.4	0.5		
Dimethyl trisulphide	MS		0.9			

* Quantitative values are percentages of the total amount of volatiles in each species (column).

† Identity confirmed by reference compound GC-RT (retention time) or by comparison of mass spectral data with previously reported spectra (MS).

‡ Compounds, to our knowledge, not previously reported in *Brassica* and *Sinapis*.

§ Trace amount found by selective ion monitoring.

‖ Isothiocyanate.

both whole and macerated plants. Benzaldehyde was the main compound of *Sinapis alba* whole-plant samples. Phenylacetaldehyde, 2-phenylethanol and 4-methoxybenzaldehyde, were probably flower-fragrance components, since they were detected mainly in whole plants.

Nitrogen-containing compounds

Volatile nitrogen compounds in Cruciferae are formed through enzymatic degradation of different glucosinolates (GS″s) to give isothiocyanates (ITC″s), thiocyanates and nitriles [4, 5]. This process is pH dependent, with low pH favouring formation of nitriles and high pH ITC″s [19]. From alkenyl-GS″s, nitriles can be formed both as aliphatic nitriles and epithionitriles [4, 5]. In our macerate suspensions, the pH decreased from 5.8 (± 0.2) to 5.3 (±0.2) during the sampling period, suggesting the production of both nitriles and ITC″s.

Whole-plant samples showed mostly low amounts of nitrogen-containing volatiles. Indole, phenylacetonitrile, and 2-aminobenzaldehyde are floral-fragrance compounds, since they were detected mainly from whole

Table 2. Relative* amounts of volatiles identified from macerated bud and leaf samples of six *Brassica* and *Sinapis* species

Compounds	Method†	*B. napus*		*B. campestris*		*B. carinata*	
		Bud	Leaf	Bud	Leaf	Bud	Leaf
Isoprenoids							
Monoterpene	—					0.1	
β-Ionone‡	MS	0.1					0.2
Fatty-acid derivatives							
Hexanal	RT, MS			0.7	tr	0.8	0.2
trans-Hex-2-enal	RT, MS	2.2	3.9	8.8	12.3	3.5	7.3
Pentan-1-ol	RT, MS	0.4	0.3			0.3	
Pent-4-en-1-ol‡	RT, MS	0.2		0.8			
Pent-2-en-1-ol	MS	1.5	1.2	tr	1.9	tr	tr
cis-Hex-3-en-1-yl acetate	RT, MS	0.4	6.6	0.8			
Hexan-1-ol	RT, MS	3.0	3.7	6.7	0.5	1.0	0.1
trans-Hex-3-en-1-ol	MS		3.4				
cis-Hex-3-en-1-ol	RT, MS	36.4	79.4	45.1	41.1	16.4	4.7
trans, trans-Hepta-2,4-dienal	RT, MS	0.5	0.1		1.1		
Benzenoids							
Benzaldehyde	RT, MS						
Phenylacetaldehyde	RT, MS		0.5				
2-Phenylethanol	MS	0.1					
Benzothiazole	MS					0.6	
Isothiocyanates							
Isopropyl-ITC‖	RT, MS						
Butyl-ITC	RT, MS					2.9	0.2
sec-Butyl-ITC	RT, MS			9.0	9.8		
Allyl-ITC	RT, MS	0.2				65.1	86.2
But-3-enyl-ITC	MS	3.3		2.2	7.4	4.3	0.3
Pent-4-enyl-ITC	MS	2.7	0.1	8.2	24.6		
Benzyl-ITC	RT, MS						
2-Phenethyl-ITC	RT, MS	1.0		0.3	0.4	0.1	
Nitriles							
Pent-4-enonitrile	MS	2.4					
Hex-5-enonitrile	MS	15.8	tr	13.4	0.4		
1-Cyano-2,3-epithiopropane	MS					2.5	0.8
5-(methylthio) Pentanonitrile	MS	0.3					
Phenylacetonitrile	RT, MS						
2-Phenylpropionitrile	MS	0.4		0.9	0.4		
6-(methylthio) Hexanonitrile	MS	0.1		0.2			
Sulphides							
Dimethyl disulphide	RT, MS	1.1	0.7				
Methyl pentyl sulphide	MS	0.1					
Dimethyl trisulphide	MS	25.8		3.0		1.7	
Ethyl methyl trisulphide	MS	2.2					

* † ‡ § ‖ See Table 1.

plants. The ITC"s detected in whole-plant samples might in part be present as a result of some damage to the plants during sampling.

In contrast to whole-plant samples, many nitrogen compounds were present in macerates. Most prominent were allyl-ITC in *B. carinata*, *B. juncea* and *B. nigra* and benzyl-ITC in *S. alba*. Alkenyl-ITC"s were detected in various samples, often together with one of their two corresponding nitriles. Contrary to Cole [4], we detected pent-4-enonitrile and hex-5-enonitrile instead of the epithionitriles, probably because of the different pH in our macerate suspensions. We did not find ω-methylthioalkyl-ITC"s reported in some *Brassica* species [4, 7], but we did detect two of their corresponding nitriles in macerates of *B. napus* and *B. campestris*. No thiocyanates were detected in this present investigation. There are several investigations where nitrogen compounds are reported as insect attractants [2, 10–14].

Sulphides

Some volatile sulphides may act as attractants and oviposition stimulants to phytophagous insects [3]. In several whole-plant and macerate samples, we found dimethyl disulphide and dimethyl trisulphide, both previously reported in *Brassica* [6, 7]. Since saturated di-

and trisulphides are reported to be formed by heating of alkyl-alkenyl sulphides [20], some sulphides here identified in macerates might have been formed during sampling.

Comparison of whole plants and macerates

Many compounds detected in whole plants were missing in macerates, and *vice versa* (see Tables 1 and 2). In macerates, many cell-degradation compounds were present and we detected a total of eight alcohols/aldehydes and nine nitrogen compounds absent in whole plants, while 17 terpenes were detected only in whole-plant samples. The only terpenoid compounds in macerates were β-ionone and one monoterpene (probably α-pinene).

Comparison of different plant parts

Young shoots and seeds of some Crucifers are known to contain high amounts of GS"s [17], and plant parts of *B. juncea* are reported to differ primarily in their relative amounts of volatililes [15]. In macerated buds we detected nitrogen and sulphur compounds in greater number and larger quantity than in mature leaves (Table 2), while both plant parts contained high amounts of fatty-acid derivatives. Preliminary results on volatiles from macerated pods of *B. napus* indicate similarities to results obtained for macerated buds (Tollsten, L., unpublished data). The differences in volatiles released from different plant parts and growth stages might be of great importance for insect attraction to plants.

CONCLUSIONS

The method of sorption and analysis here used is accurate enough to identify whole-plant volatiles. Similar methods have been described and evaluated previously [9, 21]. The Crucifer species investigated produce characteristic odours, with interspecific differences evident in both flower fragrance (terpenes and benzenoids) and GS breakdown products (ITC"s and nitriles). High amounts of allyl-ITC in *B. carinata*, *B. nigra* and *B. juncea* might in part explain the lower suitability of these species as hosts for *Dasineura brassicae*, as compared with *B. napus* and *B. campestris* [11, 12]. Other GS breakdown products (*e.g.* benzyl-ITC in *S. alba*) may also play a role in host-plant selection. The generally greater amounts of ITC"s and nitriles in buds than in leaves, could act as cues for the pollen beetle *Meligethes aeneus*, and may explain the greater beetle attraction to buds and stamens of *B. carinata* and *B. napus* as compared to leaves [14]. Differences in volatile profiles between whole-plant and macerate samples point to the importance of examining both intact and macerated plant material in order to obtain a complete picture of volatile production in Crucifers.

EXPERIMENTAL

Plant material. Investigated plants were: *Brassica napus* L. ssp. *oleifera* DC. (summer rape), *B. campestris* L. ssp. *oleifera* DC. (summer turnip rape), *B. juncea* (L.) Czern. (brown mustard), *B. nigra* (L.) Koch. (black mustard), *B. carinata* A Braun. (Abyssinian mustard, only macerated plant material) and *Sinapis alba* L. (white mustard). Potted plants in growth stage 4.1–4.3 (see ref. [22]) were harvested from the greenhouse immediately before sampling started.

Collection of volatiles. Plants were cut at ground, and put in H_2O inside a glass vessel. Purified air was passed over the plants (200 ml/min), and volatiles were trapped on a column of 150 mg Porapak Q (mesh 80–100), at 20–24° for 24–96 hr. Samples were eluted with 2 ml distilled pentane and concd by evapn before analysis. Replicates ($n=3$) were only analysed from whole plants.

For macerates, aliquots of 5 to 10 g plant material were homogenized in 200 ml H_2O, in a Turmix blender and transferred to the sampling vessel. Volatiles were collected for 20–24 hr as described above. The pH of the suspension was measured at sampling start and end. Vessels containing distilled water were used as controls to all samples.

Chemical analysis. GC: Hewlett–Packard 5880 (splitless injection, FID and N_2 detector in parallel), Inj. and det. temp.: 220 and 250°, respectively, N_2 1.0 ml/min, Fused silica WCOT capillary columns (24 m long) coated with OV-351/Superox FA (i.d. 0.20 mm, film thickness (df) 0.6 μm), oven 60° for 2 min, 4°/min to 220° and then isothermal. GC-MS: Finnigan 4021 Quadropole, Inj. and det. temp. 210 and 220°, respectively, He 0.5 ml/min, Oven 50° for 4 min, 8°/min to 230° and then isothermal, fused silica WCOT (23 m long), OV-351 (i.d. 0.2 mm, df 0.6 μm).

Identification. Compounds were identified by comparison of mass spectra, with spectra earlier reported from Crucifers [23–25], or available in the computer library. Some identifications were confirmed by comparison of GC retention times, with those of known reference substances.

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