Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 1941–1955
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Volatile profiling of *Arabidopsis thaliana* – Putative olfactory compounds in plant communication

Jens Rohloff *, Atle M. Bones

Cell and Molecular Biology Group, Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway

Received 15 June 2005; accepted 16 June 2005
Available online 2 August 2005

Abstract

Arabidopsis thaliana from the Brassicaceae family has arisen as the model organism in plant biology research. The plant's genome has been characterized and worldwide studies are conducted at the genetic, protein and metabolic level to unravell the function of genes involved in growth, reproduction, biosynthesis, and plant communication. As part of the multidisciplinary project BIOEMIT at NTNU, metabolomic studies of *Arabidopsis* T-DNA knock-out mutants and ecotypes have been carried out. Volatile profiles of autolyzed, intact plants and single plant organs were obtained by solid-phase microextraction coupled with gas chromatography–mass spectrometry. The studies were aimed at the diversity of defense-related compounds from the glucosinolate–myrosinase system – the isothiocyanates and nitriles. Metabolites from methionine, leucine and phenylalanine-derived glucosinolates were most abundant (4-methylthiobutyl, 4-methylpentyl, 2-phenylethyl). In addition, 24 monoterpenes, 26 sesquiterpenes and 12 aromatic structures, predominantly observed in inflorescenses, are described. Excluding the vast group of straight chain aliphatic structures, a total of 102 volatile compounds were detected, of which 59 are reported in *Arabidopsis thaliana* for the first time, thus emphasizing the sensitivity and applicability of solid-phase microextraction for volatile profiling of plant secondary metabolites.

Keywords: *Arabidopsis thaliana* (L.) Heinh.; Brassicaceae; Volatile profiling; Solid-phase microextraction; Glucosinolate–myrosinase system; Isothiocyanates; Nitriles; Monoterpenes; Sesquiterpenes; Aromatics

1. Introduction

In the past five years, multidisciplinary attempts have been made to decipher the biological functions of single chemical compounds or structure groups belonging to the vast diversity of secondary metabolites, which play a major role in communication between plants and their environment. *Arabidopsis thaliana* from the Brassicaceae family has been established as an ideal plant model system for such investigations (Van Poecke and Dicke, 2004). The plant shows a high degree of self-pollination and seed production, has a short life-cycle and a small genome, which has been characterized recently. Worldwide, research studies are being carried out at the genetic, protein and metabolic level in order to unravell the function of genes with regard to growth and reproduction, biosynthesis and plant communication (Kliebenstein, 2004). The fact that *Arabidopsis thaliana* takes the pride of place of being the model plant-of-choice, makes it even more intrigueing to look into the details of functional genomics, since the metabolism of compounds involved in plant signaling is restricted to the plant's natural tool box of a limited set of genes and potential biosynthetical products on the protein level (Winkel, 2004; Davies and Schwinn, 2003; Vom Endt et al., 2002). Precise knowledge about the variety

* Corresponding author. Tel.: +47 73596093; fax: +47 73596100.
E-mail address: jens.rohloff@bio.ntnu.no (J. Rohloff).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.06.021

of secondary metabolites (D'Auria and Gershenzon, 2005), either produced under unstressed condition or induced by biotic/abiotic factors, is, therefore, crucial when conducting plant-environment studies.

Depending on the scientific problem and experimental design, one has to distinguish between the tools and techniques when considering and approaching a biological problem from either the genetic/molecular biology or the metabolic/biochemistry site. We present results focusing on the metabolism of volatile organic compounds involved in the defense systems in *Arabidopsis* with special respect to the glucosinolate–myrosinase system (GLS-MYR) (Rossiter et al., 2003; Husebye et al., 2002; Bridges et al., 2002). The studies were carried out as part of the cross-disciplinary project "BIOEMIT – *Prediction and modification in functional genomics: combining bioinformatical, bioethical, biomedical and biotechnological research*" conducted at NTNU (www.bioemit.math.ntnu.no). With respect to the research group's activities, the work was especially aimed at answering questions on metabolic patterns of four T-DNA knock-out mutants (TGG1, TGG2, Cyp83A1, Cyp83B1) showing deficiencies in the GLS pathway, and the morphological and ecotypical variation of *Arabidopsis thaliana*: Columbia (Col), Cape Verde Islands (Cvi), Landsberg *erecta* (L*er*) and Wassilewskija (Ws).

The main tasks of the study were the (1) establishment of headspace solid-phase mictroextraction (HS-SPME) methods for the volatile extraction from autolyzed samples and in vivo, (2) characterization of the vast diversity of plant volatiles being involved or potentially active in plant–insect interactions and defense, and (3) identification of metabolites from the GLS-MYR system. HS-SPME has been introduced and applied as a proven method at the authors' institute for metabolic profiling and semi-quantitative analyses of plant volatiles such as essential oils and fruit aroma (Rohloff, 1999, 2002a,b, 2004; Rohloff et al., 2000, 2004). Instead of classical headspace trapping (Jacobsson et al., 2004), solvent extraction (Lambrix et al., 2001), and steam distillation (Valette et al., 2003) for the analysis of GLS degradation products, HS-SPME was preferred as the analytical tool-of-choice due to its feasibility regarding minimal sample size and preparation, fast sample throughput, and very high sensitivity for the simultaneous extraction of a broad range of analytes belonging to the groups of terpenes, aromatics, hydrocarbons, isothiocyanates and nitriles. Newer reports recommend the application of HS-SPME sampling for volatile profiling purposes both in *Arabidopsis* research (Tholl et al., 2005; Meyer et al., 2003; Meija et al., 2002; Vercammen et al., 2001) and other fields of plant science (Di et al., 2004; Jelen, 2003; Kushalappa et al., 2002).

The presented results from volatile profiling studies of *Arabidopsis thaliana* describe possible metabolic products of both intact plants and autolyzed (wounded) plant material belonging to the following chemical groups: isothiocyanates, nitriles, sulfur-containing compounds, mono- and sesquiterpenes and aromatic structures. Straight chain aliphatic hydrocarbons, such as alkanes, aldehydes, ketones, acids, esters and alcohols, which are dominated by C_5- and the C_6-structures from the lipoxygenase pathway, are also known to be involved in plant–insect communication. The most abundant compounds detected in this study are being presented, but the vast diversity of straight chain aliphatics and in particular green leaf volatiles, will be discussed and published elsewhere. Although the character of the presented study is solely biochemical-descriptive, the extensive data on volatile secondary metabolites potentially involved in plant-environment communication provide important and useful information to the *Arabidopsis* science community.

2. Results and discussion

2.1. The "mustard bomb" – glucosinolate hydrolysis products

The hydrolysis (autolysis) of GLS in *Arabidopsis thaliana* through enzyme-mediated breakdown by MYR (or: β-thioglucosidase) leads to unstable aglycones (Bones and Rossiter, 1996). Depending on the biosynthetic origin of the GLS – namely methionine-, leucine-, tryptophan and phenyl alanine-derived chemical structures (Reichelt et al., 2002) – the presence of epithiospecifier protein (ESP), ferrous ions and the pH, the aglycones undergo a spontaneous rearrangement to yield a variety of toxic metabolites such as isothiocyanates (ITC), nitriles (CN), cyanoepithioalkanes and thiocyanates, which are reported to be involved in plant–insect interactions exerting distinct biological activity (Rossiter et al., 2003; Wittstock et al., 2003). In this study, 18 ITC, 9 CN and 6 sulfur containing structures were tentatively identified by the application of HS-SPME coupled with GC–MS (see Table 1). Among the detected GLS degradation products from our study, six structures, namely allyl ITC, 3-butenyl ITC, 3-methylthiopropyl ITC, 2-phenylethyl ITC, 2-phenylethyl CN, 4-methoxy indol-3-ylmethyl CN, are known to show activity in plant–insect communication (Wittstock et al., 2003). Although reports on the stress-induced volatilization of GLS-degradation products from *Arabidopsis* plants exist (Van Poecke et al., 2001; Vercammen et al., 2001), the detection of ITCs, CNs and other sulfur-containing compounds within this study was restricted to autolyzed plant material except these compounds were also detected from detached stems.

Apart from the vast group of straight chain aliphatic compounds, which were dominated by green leaf

Table 1
Main and trace volatile compounds in *Arabidopsis thaliana* wildtype and knock-out mutant plants detected by HS-SPME and GC–MS with reported and potential activity in plant–insect interactions and defense

Compound name	RT_{Sil}	RI_{Sil}	RT_{Wax}	RI_{Wax}	Column	Organ	Sample	Plant references
Isothiocyanate (ITC)								
Allyl ITC			12.92	*1259*	Wax	s	aut	*Arabidopsis thaliana* (Lambrix et al., 2001)
sec-Butyl ITC*	7.84	*925*			Sil	s,l,f	aut	Brassicaceae (Brown and Morra, 1995)
Isobutyl ITC*	8.10	*931*	13.77	*1274*	Sil,Wax	s,l,f	aut	Brassicaceae (Brown and Morra, 1995)
Butyl ITC*	9.56	*975*	15.12	*1308*	Sil,Wax	s,l,f	aut	Brassicaceae (Smolinska et al., 2003)
3-Butenyl ITC*	9.70	*982*	15.35	*1315*	Sil,Wax	s,l,f	aut	Brassicaceae (Mewis et al., 2002)
2-Methylbutyl ITC*			18.42	*1412*	Wax	l	aut	Brassicaceae (Jacobsson et al., 2004)
3-Methylbutyl ITC*	11.73	*1036*	18.60	*1418*	Sil,Wax	s,l,f	aut (iv)	Brassicaceae (Siegl et al., 1997)
Pentyl ITC	13.20	*1077*	20.33	*1473*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Vercammen et al., 2001)
4-Methylpentyl ITC	15.55	*1148*	21.93	*1529*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
Unidentified ITC*[a]	15.73	*1153*	22.05	*1533*	Sil,Wax	s,l,f	aut	
Hexyl ITC	16.92	*1185*	23.37	*1581*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
Heptyl ITC	19.22	*1253*	24.92	*1636*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
3-Methylthiopropyl ITC	20.60	*1291*	33.40	*1979*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
Octyl ITC*	22.87	*1354*	27.93	*1764*	Sil,Wax	s,l,f	aut	Brassicaceae (Di Cesare et al., 1999)
4-Methylthiobutyl ITC	24.52	*1403*	36.90	*2132*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
2-Phenylethyl ITC	25.65	*1430*	38.82	*2216*	Sil,Wax	s,l,f	aut (iv)	*Arabidopsis thaliana* (Vercammen et al., 2001)
Nonyl ITC*	26.23	*1458*	30.73	*1862*	Sil,Wax	s,l,f	aut	
Decyl ITC*	29.47	*1564*	33.52	*1984*	Sil,Wax	s,l,f	aut	Brassicaceae (Brown and Morra, 1995)
Nitrile (CN)								
5-Methylpentyl CN*	7.87	*929*	16.03	*1337*	Sil,Wax	s,l,f	aut	Brassicaceae (Di Cesare et al., 2001)
Hexyl CN*	11.28	*1024*	16.57	*1354*	Sil,Wax	s,l,f	aut	Brassicaceae (Krumbein et al., 2001)
4-Methylthiopropyl CN*	12.21	*1051*	29.28	*1806*	Sil,Wax	s,l,f	aut	Brassicaceae (Miyazawa et al., 2002)
Heptyl CN	15.05	*1131*	19.98	*1462*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Osswald et al., 2002)
5-Methylthiobutyl CN	16.40	*1174*	32.30	*1931*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Van Poecke et al., 2001)
2-Phenylethyl CN	18.22	*1227*	34.75	*2038*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Osswald et al., 2002)
Octyl CN*	18.73	*1240*	22.92	*1565*	Sil,Wax	s,l,f	aut	Naturally occurring volatile
Nonyl CN*	20.07	*1276*	25.95	*1677*	Sil,Wax	s,l,f	aut	Naturally occurring volatile
4-Methoxyindol-3-ylmethyl CN	33.40	*1752*			Sil	s,l,f	aut	Brassicaceae (Mewis et al., 2002)
Sulfur								
2-Ethyl thiophene*	5.85	*837*			Sil	s,l,f	aut	Brassicaceae (Krumbein et al., 2001)
Dimethyl disulfide			7.97	*1071*	Wax	s,l,f	aut	*Arabidopsis thaliana* (Van Poecke et al., 2001)
Dimethyl trisulfide	8.85	*964*	16.88	*1364*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Van Poecke et al., 2001)
Furfuryl methyldisulfide*			19.92	*1460*	Wax	s,l,f	aut	Naturally occurring volatile
Benzothiazole*	18.35	*1230*			Sil	s,l,f	aut	Naturally occurring volatile
Cyclooctasulfur*	41.10	*2151*			Sil	s,l,f	aut	Various species (Williams and Cooper, 2003)

(*continued on next page*)

Table 1 (*continued*)

Compound name	RT_{Sil}	RI_{Sil}	RT_{Wax}	RI_{Wax}	Column	Organ	Sample	Plant references
Monoterpene								
α-Thujene	8.02	*930*	7.42	*1028*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2004)
α-Pinene	8.23	*939*	7.33	*1021*	Sil,Wax	s,l,f	iv	*Arabidopsis thaliana* (Chen et al., 2004)
Camphene*	8.52	*954*	7.92	*1070*	Sil,Wax	s,l,f	iv	Naturally occurring volatile
Sabinene	9.47	*975*	8.65	*1124*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2004)
β-Pinene	9.57	*979*	8.51	*1113*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2004)
β-Myrcene	10.12	*991*	9.20	*1161*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Bohlmann et al., 2000)
α-Phellandrene*	10.53	*1003*	9.45	*1168*	Sil,Wax	s,l,f	aut,iv	Brassicaceae (Hashem and Saleh, 1999)
3-Carene*	10.83	*1013*	8.69	*1147*	Sil,Wax	s,l,f	iv	Brassicaceae (Jakobsen et al., 1994)
α-Terpinene*	10.95	*1017*			Sil	s,l,f	aut	Brassicaceae (Vuorinen et al., 2004)
p-Cymene*	11.07	*1025*	13.10	*1269*	Sil,Wax	s,l,f	aut,iv	Brassicaceae (Sefidkon et al., 2002)
Limonene	11.38	*1029*	11.62	*1196*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Van Poecke et al., 2001)
β-Phellandrene*	11.39	*1030*	11.89	*1207*	Sil,Wax	s,l,f	aut,iv	Brassicaceae (Tollsten and Bergstroem, 1988)
1,8-Cineole	11.45	*1031*	11.95	*1214*	Sil,Wax	s,l	aut	*Arabidopsis thaliana* (Steeghs et al., 2004)
(*Z*)-β-Ocimene*	11.76	*1037*			Sil	s,l	iv	Brassicaceae (Tollsten and Bergstroem, 1988)
(*E*)-β-Ocimene	12.16	*1050*	12.45	*1250*	Sil, Wax	s,l,f	iv	*Arabidopsis thaliana* (Bohlmann et al., 2000)
γ-Terpinene*	12.50	*1060*	12.38	*1245*	Sil,Wax	s,l,f	aut,iv	Brassicaceae (Vuorinen et al., 2004)
Terpinolene	13.60	*1089*	14.43	*1286*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2004)
Linalool	13.90	*1097*	22.22	*1549*	Sil,Wax	f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
Lilac aldehyde isomer A*	15.08	*1132*			Sil	f	iv	Natural flower volatile
Lilac aldehyde isomer B*	15.40	*1141*			Sil	f	iv	Natural flower volatile
p-Cymen-8-ol*	16.82	*1183*			Sil	f	iv	Naturally occurring volatile
α-Terpineol	17.13	*1189*			Sil	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2004)
Verbenone*	17.40	*1195*			Sil	s,l,f	iv	Brassicaceae (Tollsten and Bergstroem, 1988)
α-Terpinyl acetate*	22.62	*1349*			Sil	s,l,f	aut,iv	Naturally occurring volatile
Sesquiterpene								
α-Ylangene*	23.82	*1375*	20.85	*1491*	Sil,Wax	f	iv	Natural flower volatile
α-Copaene	23.90	*1377*	20.78	*1487*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2003b)
β-Elemene	24.30	*1403*	23.54	*1587*	Sil,Wax	s,l,f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
(*Z*)-β-Caryophyllene*	24.83	*1409*			Sil	s,f	aut,iv	Brassicaceae (Jirovetz et al., 2002)
α-Barbatene	24.97	*1410*	23.08	*1572*	Sil,Wax	s,f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
α-Cedrene*	25.08	*1412*	23.45	*1582*	Sil,Wax	s,f	aut,iv	Brassicaceae (Tollsten and Bergstroem, 1988)
(*E*)-β-Caryophyllene	25.25	*1419*	23.73	*1596*	Sil,Wax	s,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2003b)
(*Z*)-Thujopsene*			23.92	*1606*	Wax	f	iv	Naturally occurring volatile
(*E*)-Thujopsene	25.67	*1431*	24.32	*1630*	Sil,Wax	s,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2003b)
(*Z*)-β-Farnesene*			25.07	*1639*	Wax	f	iv	Naturally occurring volatile

Table 1 (*continued*)

Compound name	RT_{Sil}	RI_{Sil}	RT_{Wax}	RI_{Wax}	Column	Organ	Sample	Plant references
α-Himachalene*			25.42	*1649*	Wax	f	iv	Naturally occurring volatile
α-Caryophyllene	25.80	*1454*	25.72	*1668*	Sil,Wax	f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
(*E*)-β-Farnesene			25.67	*1667*	Wax	f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2003b)
α-Acoradiene*	26.71	*1463*	26.27	*1690*	Sil,Wax	s,f	iv	Naturally occurring volatile
β-Acoradiene	26.95	*1475*	25.93	*1677*	Sil,Wax	s,f	iv	Naturally occurring volatile
β-Chamigrene	27.03	*1478*	26.58	*1701*	Sil,Wax	s,f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
Germacrene D*	27.13	*1485*			Sil	f	iv	Brassicaceae (Geervliet et al., 1997)
α-Chamigrene			27.47	*1753*	Wax		iv	Naturally occurring volatile
Valencene*	27.55	*1496*			Sil	s,f	iv	Naturally occurring volatile
β-Himachalene*	27.73	*1505*	26.92	*1718*	Sil,Wax	s,f	iv	Naturally occurring volatile
Cuparene	27.77	*1505*	29.64	*1821*	Sil,Wax	s,f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
(*E*,*E*)-α-Farnesene	27.89	*1506*			Sil	f	iv	*Arabidopsis thaliana* (Aharoni et al., 2003)
β-Bisabolene	27.93	*1506*	27.05	*1729*	Sil,Wax	f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
δ-Cadinene*	28.40	*1523*	27.50	*1755*	Sil,Wax	s,f	iv	Brassicaceae (Jirovetz et al., 2002)
β-Sesquiphellandrene			28.62	*1776*	Wax	f	iv	*Arabidopsis thaliana* (Chen et al., 2003b)
Longiborneol*	30.50	*1599*			Sil	l,f	iv	Naturally occurring volatile
Aromatic								
Anisole*			15.85	*1327*	Wax	f	iv	Naturally occurring volatile
Benzaldehyde*	8.65	*960*	21.22	*1511*	Sil,Wax	f	iv	*Arabidopsis lyrata* (Peer and Murphy, 2003)
Benzyl alcohol*			30.73	*1862*	Wax	s,l,f	aut,iv	Brassicaceae (Jirovetz et al., 2002)
Phenylacetaldehyde*			25.32	*1645*	Wax	s,l,f	aut	*Arabidopsis lyrata* (Peer and Murphy, 2003)
Methyl benzoate	13.63	*1091*			Sil	s,l,f	aut,iv	*Arabidopsis thaliana* (Chen et al., 2003a)
2-Phenylethyl alcohol*			31.62	*1891*	Wax	s,l,f	aut	Brassicaceae (Tollsten and Bergstroem, 1988)
Methyl salicylate	17.13	*1192*			Sil	s,l,f	aut,iv	*Arabidopsis thaliana* (Van Poecke et al., 2001)
3,5-Dimethyl anisole*			22.05	*1533*	Wax	f	iv	Naturally occurring volatile
2,6-Dimethyl benzaldehyde*			25.12	*1640*	Wax	f	iv	Naturally occurring volatile
Ethyl *p*-ethoxybenzoate*	28.10	*1521*			Sil	f	iv	Naturally occurring volatile
Benzophenone*	31.00	*1628*			Sil	f	iv	Naturally occurring volatile
Benzyl benzoate*	33.55	*1760*			Sil	f	iv	Brassicaceae (Valette et al., 2003)
Misc.								
β-Cyclocitral	17.93	*1218*	24.28	*1629*	Sil,Wax	s,l,f	aut	*Arabidopsis thaliana* (Van Poecke et al., 2001)
(*E*)-α-Ionone*	25.10	*1413*			Sil	s,l,f	aut	Naturally occurring volatile
Geranyl acetone*	25.90	*1455*	30.57	*1855*	Sil,Wax	s,l,f	aut,iv	Naturally occurring volatile
(*E*)-β-Ionone	26.93	*1475*	32.67	*1947*	Sil,Wax	s,l,f	aut,iv	*Arabidopsis thaliana* (Van Poecke et al., 2001)
Methyl (*Z*)-dihydrojasmonate*	31.67	*1656*	40.43	*2287* Sil,Wax	s,l,f	aut,iv		Brassicaceae (Bodnaryk and Yoshihara, 1995)
Farnesyl acetaldehyde*	36.90	*1933*			Sil	s,l,f	aut	
Hexahydrofarnesyl acetone*	37.31	*1955*			Sil	s,l,f	aut	Brassicaceae (Hartleb and Seifert, 1995)

Compounds marked with * are being reported in *Arabidopsis thaliana* for the first time.

RT, retention time; RI, retention index; Sil, apolar column (CP-Sil 5); Wax, polar column (CP-Wax); s, stem; l, leaf; f, inflorescense (flowers); aut, autolyzed plants from in vitro cultivation; iv, intact plants or detached plant; organs, (in vivo) from the greenhouse; (iv), only detected in detached stems (greenhouse plants).

[a] *m/z* $[143]^+$ (7%), 128 (100%), 41 (95%), 72 (75%), 115 (40%).

volatiles (hexanol- and hexenol-type derivatives) and C_5-structures, a characteristic distribution of ITCs and CNs could be observed when analyzing 6-weeks old flowering in vitro cultivated *Arabidopsis thaliana* wildtype (ecotype Columbia) and T-DNA knock-out mutants (see Table 2). The methionine-derived 3-methylthiopropyl ITC (from glucoiberverin) and 4-methylthiobutyl ITC (from glucoerucin), the leucine-derived 3-methylbutyl ITC (from 3-methylbutyl GLS) and 4-methylpentyl ITC (from 4-methylpentyl GLS), and the phenylalanine-derived 2-phenylethyl ITC (from gluconasturtiin) were most abundant within the isothiocyanate group. Distinctly higher amounts of 4-methylpentyl ITC and 4-methylthiobutyl ITC along with increased CN levels

Table 2
Autolysis products of in vitro-cultivated *Arabidopsis thaliana* wildtype and knock-out mutants (ecotype Col) detected by HS-SPME (no. of sample plants = 10; $n = 3$)

No.	RT	RI	Compound	Peak area% (average values)				
				Col	TGG1	TGG2	Cyp83A1	Cyp83B1
1	8.10	931	Isobutyl ITC	0.01	0.26	*nd*	0.06	0.24
2	9.56	975	Butyl ITC	*tr*	0.02	*tr*	*tr*	0.03
3	9.70	982	3-Butenyl ITC	0.23	0.09	*nd*	0.52	0.12
4	11.73	1036	3-Methylbutyl ITC	0.93	0.83	0.45	0.30	3.56
5	13.20	1077	Pentyl ITC	0.25	0.21	0.03	*nd*	0.58
6	15.55	1148	4-Methylpentyl ITC	2.93	3.35	2.31	2.23	12.82
7	15.73	1153	*Unidentified* ITC[a]	0.13	0.05	0.04	0.08	0.37
8	16.92	1185	Hexyl ITC	0.52	0.48	0.34	0.28	1.18
9	19.22	1253	Heptyl ITC	0.48	0.17	0.22	0.60	0.34
10	20.60	1291	3-Methylthiopropyl ITC	1.03	1.19	0.50	0.54	3.15
11	22.87	1354	Octyl ITC	0.31	0.26	0.40	0.17	0.62
12	24.52	1403	4-Methylthiobutyl ITC	3.24	2.33	3.59	2.06	14.28
13	25.65	1430	2-Phenylethyl ITC	0.78	0.67	0.78	0.79	1.15
14	26.23	1458	Nonyl ITC	0.27	0.28	0.03	0.22	0.33
15	29.47	1564	Decyl ITC	*nd*	0.05	*nd*	0.04	*nd*
16	41.10	2151	Cyclooctasulfur	0.27	0.12	0.09	*nd*	0.33
17	7.87	929	5-Methylpentyl CN	0.21	0.20	*nd*	*nd*	0.46
18	11.28	1024	Hexyl CN	0.05	0.12	*nd*	0.09	0.20
19	15.05	1131	Heptyl CN	*nd*	0.06	*nd*	*nd*	0.06
20	16.40	1174	5-Methylthio butyl CN	*nd*	0.09	0.10	*nd*	0.35
21	18.73	1240	Octyl CN	0.12	0.33	0.06	0.07	0.10
22	2.53	673	1-Penten-3-ol	2.11	2.94	5.65	3.41	3.66
23	3.85	685	(*Z*)-2-Penten-1-ol	*nd*	0.77	1.26	0.64	0.65
24	5.72	770	(*Z*)-3-Hexen-1-ol	3.01	4.69	8.86	6.69	6.10
25	6.13	858	1-Hexanol	1.60	1.43	1.65	3.06	3.64
26	7.00	888	(*Z*)-3-Ethyl-1,5-octadiene	0.67	0.68	0.43	0.95	0.85
27	7.20	897	(*Z*)-2-Pentenyl acetate	14.08	5.84	9.93	3.38	1.48
28	8.57	957	(*E*)-3-Ethyl-1,5-octadiene	1.65	1.56	1.43	2.65	1.55
29	9.76	986	6-Methyl-5-hepten-2-one	0.74	0.79	0.65	0.56	0.73
30	10.23	999	Octanal	0.14	0.27	0.26	0.85	0.33
31	10.37	1001	(*Z*)-3-Hexenyl acetate	26.90	17.69	23.64	12.25	5.50
32	10.67	1009	Hexyl acetate	3.84	2.05	1.69	1.52	0.49
33	11.07	1025	*p*-Cymene	*nd*	0.13	0.08	0.16	0.06
34	11.38	1029	Limonene	0.76	0.25	0.31	2.11	1.53
35	12.50	1060	γ-Terpinene	*nd*	0.06	0.16	0.06	0.07
36	13.82	1095	Nonanal	0.68	1.09	0.31	0.88	0.91
37	17.72	1202	Decanal	1.83	1.86	0.96	2.51	2.53
38	17.93	1218	β-Cyclocitral	*nd*	0.08	0.07	*nd*	0.10
39	21.18	1296	Undecanal	0.21	0.15	0.09	0.31	0.12
40	24.58	1405	Dodecanal	0.56	0.24	0.99	0.30	0.33
41	25.90	1455	Geranyl acetone	1.26	0.66	0.46	0.85	0.59
42	26.93	1475	(*E*)-β-Ionone	0.41	0.05	0.19	0.12	0.09
43	27.65	1500	Pentadecane	*nd*	0.32	0.07	0.17	0.09
44	27.72	1503	Tridecanal	*nd*	0.32	0.23	0.34	0.15
45	30.77	1613	Tetradecanal	0.28	0.87	1.09	0.44	0.71
46	33.67	1761	Pentadecanal	0.32	0.48	0.17	0.21	0.47
47	36.90	1933	Farnesyl acetaldehyde	0.16	*nd*	0.05	0.11	0.05

RT, retention time (CP-Sil 5); RI, retention indices on an apolar column; *nd*, not detected; *tr*, traces.
[a] *m/z* $[143]^+$ (7%), 128 (100%), 41 (95%), 72 (75%), 115 (40%).

could be detected for the Cyp83B1 mutant, thus pointing at deficiencies in the GLS pathway leading to higher amounts of aliphatic GLS and their corresponding ITCs. The Cyp83A1 mutant showed lower levels of aliphatic ITCs compared to Cyp83B1 and the wildtype according to deficiencies in aliphatic GLS biosynthesis. Thus, the presented results underscore the depleted function of Cyp83A1 catalyzing the conversion of aliphatic aldoxime intermediates, in contrast to Cyp83B1, which selectively metabolizes tryptophan-derived aldoxime intermediates (Naur et al., 2003; Bak and Feyereisen, 2001). Although one might expect decreased ITC levels in the TGG1 and TGG2 samples compared to the wildtype, no obvious differences, also between mutant lines, could be detected with regard to the active myrosinases responsible for GLS degradation (Thangstad et al., 2004; Husebye et al., 2002; Zhang et al., 2002; Rask et al., 2000), and thus, underscores the similar MYR functionality. The amounts of (*Z*)-3-hexenol and 1-hexanol were distinctly increased in the mutants TGG2, Cyp83A1, and Cyp83B1 and simultaneously, levels of (*Z*)-3-hexenyl acetate were drastically decreased (Cyp83A1 and Cyp83B1). Although the detection of volatiles by HS-SPME is limited to semi-quantitative purposes, the enormous differences might not only be explained as an effect of shifted metabolite portions on the extraction fibre. To what extent the enzyme affinity and/or activated stress pathways play a role for varying levels of ITC and other secondary metabolites, has to be characterized by further metabolic studies of the investigated knock-out mutants.

In contrast to reports where short periods of hydrolysis time (5 min) coupled with solvent extraction (dichloromethane) were applied (Wittstock et al., 2003; Lambrix et al., 2001), GLS-MYR products such as methylsulfinylalkyl ITCs, hydroxyalkyl ITCs and epithiostructures could not be identified. This fact might be explained by the lower volatility of the compounds, and the applied autolysis time of 3 h, resulting in a further structural rearrangement of the primary hydrolysis products in the direction of aliphatic ITCs and CNs, as observed in our study. The authors want to point at the fact, that substantially ground tissue samples have been analyzed compared to other reports investigating herbivore damage and otherwise stressed plant samples. In spite of possible conversion of metabolites due to long extraction times, autolysis generates a set of potential volatile products released from damaged plant tissue, in addition to what stressed but still living plants are able to metabolize.

Limitations of HS-SPME when analyzing volatile organic sulfur compounds as reported by Murray (2001) might be overcome by long extraction times under equilibrium conditions as applied in this study. Albeit the described HS-SPME methods are based on sampling from "biological active" material, i.e., the composition of GLS-MYR-derived hydrolysis products and other volatile compounds shows time-dependent variation, thus leading to a continuous partitioning of volatiles between the sample matrix headspace and the headspace ↔ fiber coating, a stable distribution of the analytes of interest, namely the ITCs and CNs, was reached after an extraction time of 3 h. The concentration range of these compounds did not reach the saturation point of the fiber capacity, as detected in newer investigations of *Arabidopsis thaliana* ecotypes (data not shown). The estimated levels of one of the main autolysis products, namely 4-methylthiobutyl ITC, when adding phenyl ITC (30 ng) as internal standard to a 200 mg sample, averaged 18 ng/g f.w. with a relative standard deviation of up to 15% for the detected phenyl ITC (data not shown). It is not surprising that amounts of 4-methylthiobutyl ITC reported in in vivo experiments with *Arabidopsis thaliana* exposed to oxidative stress mediated by paraquat spraying, resulted in levels between 10 and 20 pg/g f.w. (Vercammen et al., 2001) – in contrast to fully hydrolyzed plant samples used in our study.

Trace amounts of sulfur-containing volatiles other than ITC were found in both wildtype and T-DNA knock-out mutant plants. The induced biosynthesis of sulfides, which has been reported earlier as an effect of insect herbivory (Van Poecke et al., 2001), could be confirmed by our study and thus, underscores the impact of mechanical wounding on sulfide release. The detection of cyclooctasulfur in hydrolyzed samples is noteworthy because of the compound's antimicrobial activity. Elemental sulfur is naturally occurring in plants and has been reported from various plant families (Williams and Cooper, 2003).

When switching to the selected ion mode by searching for the isothiocyanate-characteristic mass fragment *m/z* 72 (see Fig. 1), the whole range of 16 ITCs presented in Table 2 could be easily visualized in chromatograms obtained with both an apolar and polar column. In general, all hydrolysis products were tentatively identified by MS database search, retention index tables, and the use of relevant literature reporting MS data of the particular compounds (Al-Gendy and Lockwood, 2003; Spencer and Daxenbichler, 1980; Kjær et al., 1963), of which the majority has been either reported in *Arabidopsis* and/or other Brassicaceae species or are naturally occurring plant volatiles (see Table 1).

2.2. Differences of smell – terpene diversity in Arabidopsis

Although *Arabidopsis thaliana* is an almost self-pollinating plant, producing low levels of pollinator-attracting volatiles such as mono- and sesquiterpenes, outcrossing in wild populations occurs (Chen et al., 2003b). In the past five years, functional genomics studies have been carried out in order to characterize the

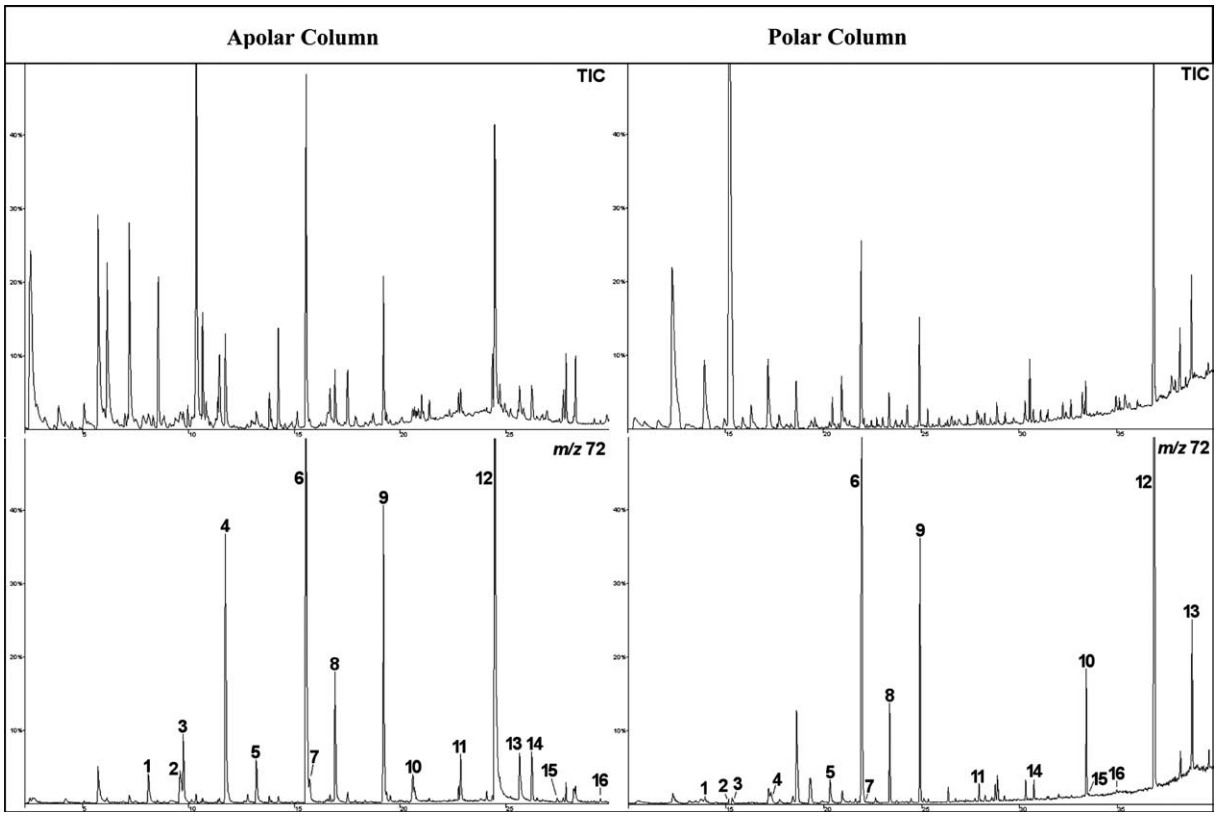

Fig. 1. Volatile profiles of 6-weeks old in vitro cultivated *Arabidopsis thaliana* (ecotype Col) detected by HS-SPME of autolyzed plant material. The figures show the total ion chromatograms obtained with an apolar and polar column. The selected ion mode was set to the characteristic mass ion *m*/*z* 72 in order to localize isothiocyanates in the chromatogram. Peak numbering refers to Table 2.

Arabidopsis terpene synthase (AtTPS) family and the range of possible metabolites derived from either the cytoplasmic mevalonate route (MVA) or the plasticidal 2-*C*-methyl-D-erythritol 4-phosphate pathway (MEP) (Chen et al., 2004; Steeghs et al., 2004; Aharoni et al., 2003; Chen et al., 2003b; Fäldt et al., 2003; Van Poecke et al., 2001; Bohlmann et al., 2000). Several of these reports describe volatile profiles of *Arabidopsis* flowers and other plant organs of living plants, comprising a total of 14 monoterpenic and 23 sesquiterpenic compounds, of which 25 structures were also tentatively identified within this study (Tholl et al., 2005; Aharoni et al., 2003; Chen et al., 2004, 2003b; Van Poecke et al., 2001). It was, therefore, surprising to detect such a vast diversity of 24 monoterpenes and 26 sesquiterpenes by HS-SPME with 25 "new" compounds being reported in *Arabidopsis thaliana* for the first time (see Table 1). The majority of the described monoterpenes have already been reported in *Arabidopsis* and/or other Brassicaceae species, thus, validating the potential occurrence of these compounds within this study.

It is noteworthy to mention the detection of traces of the toxic 1,8-cineole in both stems and leaves and its absence in inflorescences, together with limonene, one of the most abundant monoterpenes, detected in both intact and autolyzed plants. 1,8-Cineole, being synthesized in an *E. coli* assay by the enzyme AtTPS-Cin together with other monoterpenic structures, has just recently been identified in roots of *Arabidopsis thaliana* (Chen et al., 2004; Steeghs et al., 2004). The in vivo detection of traces of 1,8-cineole from in vitro cultivated plants additionally points at the main site of biosynthesis – namely the roots, whereas in all other HS-SPME analyses roots were excluded from sampling. In general, the whole range of monoterpene hydrocarbons with the characteristic mass fragment of *m*/*z* 93 could be located in chromatograms from both autolyzed material, plant organs and in volatile profiles of greenhouse cultivated plants at the stage right before bolting (see Fig. 2 and Table 3). No distinct differences in the monoterpene profiles between the investigated ecotypes (Col, Cvi, L*er*, Ws) could be observed. The monoterpene alcohol linalool could not be traced in these samples in contrast to flowering plants, thus emphasizing the restriction of linalool biosynthesis to flowers with regard to pollinator/predator attraction. In addition, two lilac aldehyde isomers tentatively identified as lilac aldehyde A and B based on their mass spectra are also known to be involved in plant–insect interactions and could be traced from the volatile matrix of *Arabidopsis* inflorescences (see Table 3). In contrast, the intermediate of 8-hydroxylinalool (beside linalool) in lilac aldehyde biosynthesis

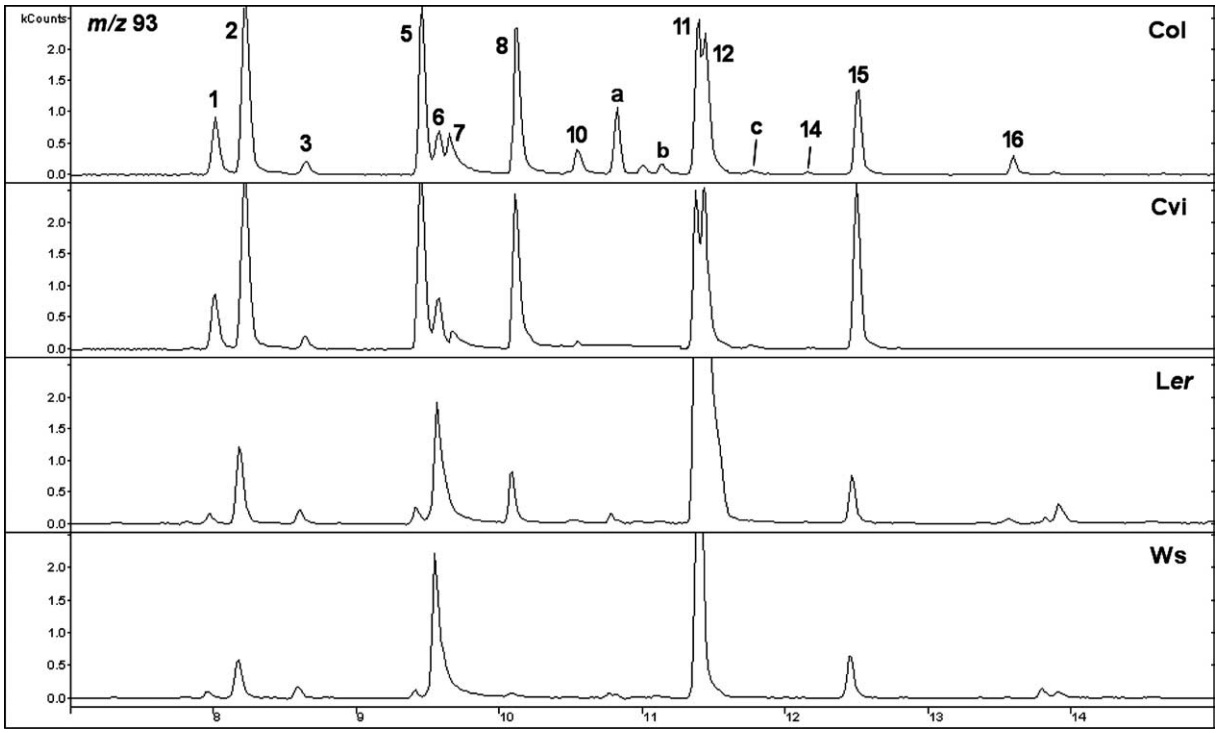

Fig. 2. Volatile profiles of 3–4-weeks old greenhouse-cultivated *Arabidopsis thaliana* ecotypes: Col, Cvi, Ler and Ws. The intact plants were analyzed by HS-SPME and GC–MS and chromatograms were obtained on an apolar column (selected ion mode *m*/*z* 93). Peak numbering refers to Table 3 (**a** 3-carene; **b** *p*-cymene; **c** (*Z*)-β-ocimene).

(Burkhardt and Mosandl, 2003) – as reported by Aharoni and co-workers (Aharoni et al., 2003) – was not detected.

Volatile profiles of flowering in vitro cultivated plants and single plant organs (leaf, stem, flower) from the greenhouse, revealed the high variability of sesquiterpenic structures in *Arabidopsis thaliana* (see Tables 3 and 4) (Tholl et al., 2005), which could be easily traced in different sample matrices when searching for their characteristic mass fragments of *m*/*z* 161 and *m*/*z* 204 (see Fig. 3). While many monoterpenes were observed in both autolyzed and intact plants or plant organs, many of the detected sesquiterpenes were exclusively found in inflorescences/flowers. This observation is supported by volatile profiles of bolting plants of different *Arabidopsis* ecotypes from greenhouse cultivation, producing trace amounts of characteristic compounds such as α-copaene, α-cedrene, (*E*)-thujopsene, β-chamigrene and δ-cadinene (data not shown). The flower-dominating sesquiterpene, (*E*)-β-caryophyllene, was almost absent in these samples, and only trace amounts could be detected in leaves and stems (see Table 3). Despite the fact, that the detaching of plant parts surely leads to the biosynthesis of wound-induced volatiles as in the case of β-cyclocitral, geranyl acetone, ionones and the farnesyl derivatives (see Table 1, Miscellaneous; Tables 2 and 3), the sesquiterpene patterns of single inflorescences and intact flowering plants (in vitro) showed quite good similarity. However, the presented diversity of sesquiterpenic structures has to be designated as ''tentative'' since mass spectra of many of these compounds show high similarity and thus, complicate their unambiguous identification only based on MS data.

2.3. Flower attraction and signaling – aromatic and pentacyclic structures

As already pointed out for the GLS-MYR-derived structures and the terpenes, aromatic compounds are also directly involved in plant–insect interactions due to their insecticidal and/or repellent/attractant activity. Two of the most important structures belonging to the salicylic acid and jasmonic acid signaling pathway investigated in *Arabidopsis thaliana* are the volatile hormone analogues methyl salicylate (Chen et al., 2003a; Van Poecke et al., 2001) and methyl jasmonate. Low levels of methyl salicylate could be detected in both autolyzed plant material and single plant organs in vivo, whereas methyl (*Z*)-dihydrojasmonate and not the reported methyl jasmonate (Meyer et al., 2003) could be traced (see Table 1). Three other aromatic structures – benzyl alcohol, 2-phenylethyl alcohol and benzyl benzoate, which have been reported from flower scent of many plant families, are also known to exert insect attractive properties in the Brassicaceae family (Smart and Blight, 1997). The detection of benzyl benzoate is in contrast to studies on the activity of the benzoyl-transferase like proteins in *Arabidopsis*, which are proposed to lead to

Table 3
Composition of headspace volatiles of detached leaves, stems and inflorescences of greenhouse cultivated *Arabidopsis thaliana* (ecotype Col) detected in vivo by HS-SPME (no. of plant organs per sample = 10; n = 3)

No.	RT	RI	Compound	Peak area% (average values)		
				Leaves	Stems	Flowers
1	8.02	930	α-Thujene	0.53	0.92	0.32
2	8.23	939	α-Pinene	0.53	1.61	0.71
3	8.52	954	Camphene	0.12	0.24	*nd*
4	8.65	960	Benzaldehyde	*nd*	*nd*	0.09
5	9.47	975	Sabinene	1.03	1.45	0.82
6	9.57	979	β-Pinene	*nd*	0.39	0.14
7	9.76	986	6-Methyl-5-hepten-2-one	8.23	6.42	6.57
8	10.12	991	β-Myrcene	0.70	1.16	1.87
9	10.23	999	Octanal	1.64	1.23	1.38
10	10.53	1003	α-Phellandrene	*nd*	0.08	*nd*
11	11.38	1029	Limonene	1.25	1.92	2.91
12	11.39	1030	β-Phellandrene	0.55	0.73	0.56
13	11.57	1033	2-Ethyl-1-hexanol	0.24	0.16	*nd*
14	12.16	1050	(*E*)-β-Ocimene	*nd*	*nd*	0.18
15	12.50	1060	γ-Terpinene	0.41	0.55	0.36
16	13.60	1089	Terpinolene	0.09	0.14	0.12
17	13.82	1095	Nonanal	3.47	1.43	2.47
18	13.90	1097	Linalool	*nd*	*nd*	0.54
19	15.08	1132	Lilac aldehyde isomer A	*nd*	*nd*	0.24
20	15.40	1141	Lilac aldehyde isomer B	*nd*	*nd*	0.17
21	17.60	1200	Dodecane	0.07	0.21	0.19
22	17.72	1202	Decanal	10.23	3.94	6.80
23	21.18	1296	Undecanal	0.96	0.42	0.77
24	21.47	1300	Tridecane	0.34	0.44	0.27
25	22.43	1341	γ-Nonalactone	0.21	0.20	0.36
26	23.82	1375	α-Ylangene	*nd*	0.17	0.04
27	23.90	1377	α-Copaene	*nd*	*nd*	0.19
28	24.00	1400	Tetradecane	0.11	0.29	0.11
29	24.58	1407	Dodecanal	1.61	1.44	1.32
30	24.83	1409	(*Z*)-β-Caryophyllene	*nd*	*nd*	0.13
31	24.97	1410	α-Barbatene	*nd*	*nd*	0.17
32	25.08	1412	α-Cedrene	*nd*	*nd*	0.04
33	25.25	1419	(*E*)-β-Caryophyllene	*Tr*	0.09	10.39
34	25.67	1431	(*E*)-Thujopsene	0.18	0.23	0.94
35	25.83	1454	α-Caryophyllene	*nd*	*nd*	0.64
36	25.90	1455	Geranyl acetone	13.59	4.85	9.03
37	26.95	1475	β-Acoradiene	*nd*	*nd*	0.42
38	27.03	1478	β-Chamigrene	*nd*	0.14	0.58
39	27.65	1500	Pentadecane	0.28	0.21	0.39
40	27.93	1506	β-Bisabolene	*nd*	*nd*	0.18
41	28.40	1523	δ-Cadinene	*nd*	*nd*	0.15
42	30.50	1599	Longiborneol	0.06	0.08	0.11
43	30.77	1613	Tetradecanal	8.13	2.30	6.63
44	31.67	1656	Methyl (*Z*)-dihydrojasmonate	*nd*	*nd*	*tr*
45	36.90	1933	Farnesyl acetaldehyde	0.92	0.28	0.32

RT, retention time (CP-Sil 5); RI, retention indices on an apolar column; *nd*, not detected; *tr*, traces.

the biosynthesis of green leaf volatiles (D'Auria et al., 2002). In sum, a total of 12 aromatic compounds with close structural similarities could be tentatively identified from mainly flower volatile mixtures of *Arabidopsis thaliana* by HS-SPME.

2.4. Volatile profiling of Arabidopsis – concluding remarks

The presented results from volatile profiling of the plant model organism *Arabidopsis thaliana*, based on HS-SPME methods coupled with GC–MS, emphasize the microextraction properties of SPME. A broad range of more than 100 volatile organic compounds, excluding the important chemical group of straight chain aliphatic structures, could be detected in one single GC–MS run, thus providing vast information about the biosynthetical products from different plant samples. Although no separation of enantiomers of the monoterpenes and lilac aldehydes has been undertaken, the majority of the detected structures is known to be involved in plant–insect interactions in general.

Table 4
Composition of headspace volatiles of in vitro cultivated *Arabidopsis thaliana* (ecotype Col) detected in situ by HS-SPME (no. of sample plants = 10; $n = 3$)

No.	RT	RI	Compound	Peak area% (average values)
1	9.20	1161	β-Myrcene	0.04
2	11.62	1196	Limonene	0.05
3	11.82	1200	Dodecane	0.26
4	15.85	1327	Anisole	0.06
5	15.91	1332	6-Methyl-5-hepten-2-one	0.46
6	17.78	1391	Nonanal	0.18
7	18.10	1400	Tetradecane	0.02
8	20.78	1487	α-Copaene	0.12
9	20.96	1495	Decanal	0.39
10	21.08	1500	Pentadecane	1.00
11	22.05	1533	3,5-Dimethyl anisole	0.02
12	22.22	1549	Linalool	0.13
13	23.08	1572	α-Barbatene	3.23
14	23.45	1582	α-Cedrene	0.12
15	23.73	1596	(*E*)-β-Caryophyllene	2.79
16	23.92	1606	(*Z*)-Thujopsene	0.47
17	24.32	1630	(*E*)-Thujopsene	3.57
18	25.07	1639	(*Z*)-β-Farnesene	0.83
19	25.12	1640	2,6-Dimethyl benzaldehyde	0.03
20	25.42	1649	α-Himachalene	0.33
21	25.67	1667	(*E*)-β-Farnesene	0.02
22	25.72	1668	α-Caryophyllene	0.07
23	25.93	1677	β-Acoradiene	1.08
24	26.27	1690	α-Acoradiene	0.58
25	26.58	1701	β-Chamigrene	2.07
26	26.79	1711	Dodecanal	1.04
27	27.05	1729	β-Bisabolene	0.06
28	27.47	1753	α-Chamigrene	0.27
29	28.62	1776	β-Sesquiphellandrene	0.20
30	30.73	1862	Benzyl alcohol	0.02
31	34.18	2000	Eicosane	0.06
32	34.43	2004	Pentadecanal	0.65

RT, retention time (CP-Wax); RI, retention indices on a polar column.

HS-SPME is a useful tool for both answering questions about the composition of volatile matrices and, simultaneously, distinct metabolites at the ng and pg-level, thus not only supplementing but also being capable of supporting results from transcriptional profiling and enzyme analyses (Verdonk et al., 2003; Aharoni et al., 2000). Distinct differences in the volatile profiles of knock-out mutants compared to the *Arabidopsis* wildtype could be shown with regard to the hydrolysis products from the GLS-MYR and the lipoxygenase pathway. Since in many cases quantitative measurements of plant metabolism are necessary with regard to the effect of abiotic and biotic factors, one has to make the right decision when choosing suitable analytical tools. Working with complex sample matrices as presented in this study, however, requires solvent extraction or headspace trapping methods other than HS-SPME for exact quantitative purposes.

3. Experimental

3.1. In vitro cultivation of Arabidopsis wildtype and mutant plants

Seeds were sterilized according to standard lab routines (EtOH, NaOCl/NaOH) prior to aseptical (in vitro) cultivation in 500 ml screw cap jars on MS medium (4.3 g/l; 50 ml/jar) containing Bacto- and Phytoagar (1:2; 6 g/l) and 30 g/l sucrose. Ten seeds were pipetted into each jar and plants grown for 6 weeks until flowering at a temperature of 20 °C under a 16/8 h day/night regime using fluorescent tubes (Osram Lumilux Plus Eco 36 W). Both *Arabidopsis thaliana* wildtype plants of ecotype Columbia-0 (**Col**) and 4 Col-derived T-DNA knock-out mutants (homozygous lines) showing deficiencies in the GLS biosynthesis pathway were used in this study (five parallels for wildtype and mutants): **TGG1** (Atg526000; Salk_130469), **TGG2** (At5g25980; Salk_038730), **Cyp83A1** (At4g13770) and **Cyp83B1** (At4g31500; Salk_028573).

3.2. Greenhouse-cultivation of Arabidopsis ecotypes

The following *Arabidopsis* ecotypes were used in the study: Columbia (**Col**), Cape Verde Islands (**Cvi**), Landsberg *erecta* (**L*er***) and Wassilewskija (**Ws**). Single plants were greenhouse-cultivated on fertilized soil (P-Jord; Emmaljunga Torvmull AB) in plug trays (9×6 cells) at a temperature of 20 °C (three parallels for each ecotype). Due to the 6-weeks growth period (November/December 2003), the plants were cultivated under a 16/8 h day/night regime using metal halide lamps (Osram HQI-T 400 W) placed 130 cm above the trays. Depending on the ecotypical plant development, whole plants were sampled after 3–4 weeks right before bolting for in vivo studies, while investigations of single plant organs (leaf, stem, inflorescence) were carried out after 5–6 weeks of cultivation.

3.3. Headspace solid-phase microextraction (HS-SPME)

All analyses were carried out by HS-SPME using a manual fiber holder (Supelco Inc.) and a 65 μm PDMS/DVB coated fiber (Supelco Inc.). Prior to analysis, each fiber was thermally conditioned according to the manufacturer's instructions. Additionally, fibers were inserted into the GC inlet for 3 min at 220 °C for thermal desorption of potential residues before headspace sampling at ambient temperature. Equilibrium tests on the MS response of ITCs were carried out by using standard samples (2 ml) with increasing concentrations (1, 5, 10, 25, 50, 100 and 200 μg) of butyl isothiocyanate and phenyl isothiocyanate (both Sigma–Aldrich Co.). The detector response showed very good linearity over the whole concentration range for the

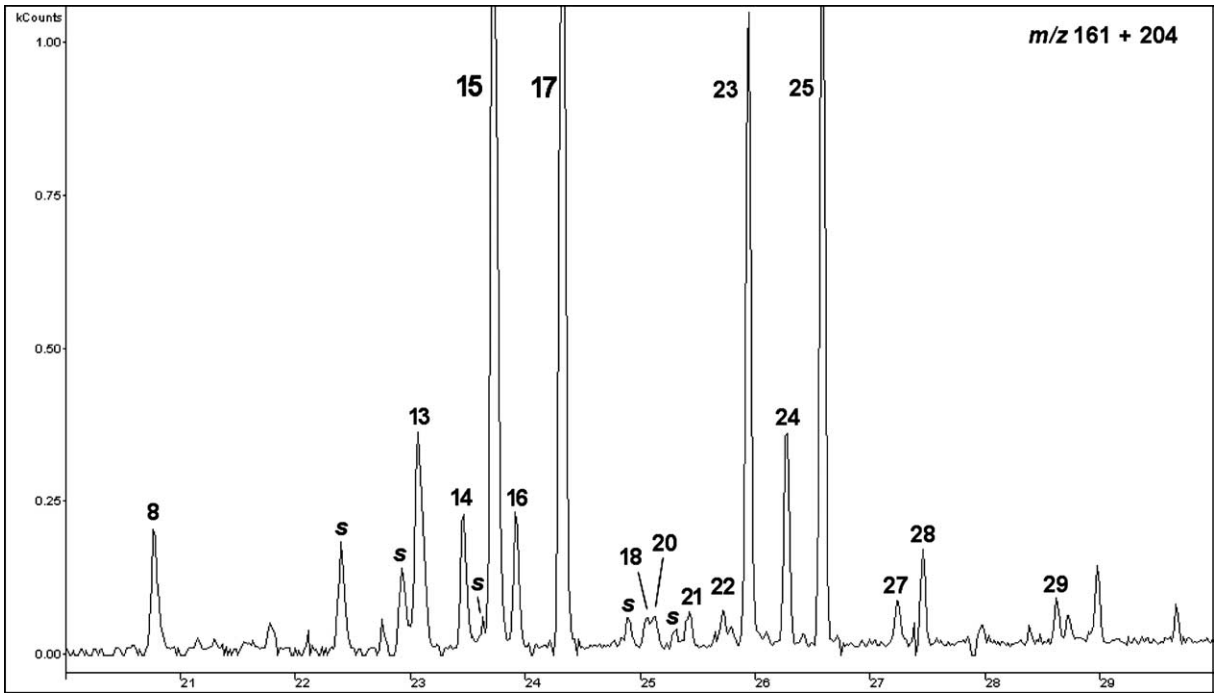

Fig. 3. Tentative identification of sesquiterpenes from flowering 6-weeks old in vitro cultivated *Arabidopsis thaliana* (ecotype: Col) by using the selected ion mode (*m/z* 161 and 204). Analyses were carried out on intact plants in situ by HS-SPME, and chromatograms were obtained with a polar column. Numbering refers to Table 4, while unidentified sesquiterpene structures are marked with ***s***.

corresponding compounds ($R^2 = 0.988$ and 0.983, respectively). Preliminary tests with varying fiber exposure times (1, 2, 3 and 6 h) were applied, in order to find a satisfactory extraction routine with regard to equilibrium of the distribution of the main volatile compounds and, simultaneously, the detection of trace compounds and semivolatiles. Although low-molecular weight compounds with high volatility might be discriminated under long fibre exposure times (Rohloff, 2004), a feasible extraction time of 3 h, when the ITCs started to show a stable distribution in the headspace, was chosen, which is in accordance with previous observations from Brassica species (Warton et al., 2001).

Depending on the experimental design, HS-SPME was applied under the following conditions using three parallels each. In vitro cultivation of Arabidopsis wildtype and mutant plants: Autolyzed samples were prepared by crushing 10 flowering sample plants (6-weeks old) 100 times with a flat-ended glass rod in a 15 ml screw top vial containing 2 ml H_2O, which was finally sealed with a phenolic cap and PTFE/silicone septum (Supelco Inc.). Volatile profiles of flowering 6-weeks old plants in vivo were obtained by covering the cultivation jar with a double-layer of aluminum foil, inserting and exposing the SPME fiber for 3 h (GC–MS analysis as described below). *Greenhouse cultivated plants* (*ecotypes*): Plant organs of 5–6-weeks old flowering *Arabidopsis* were harvested in daylight (between 9 and 11 a.m.) by detaching 10 leaves, stems or inflorescenses with scissors, respectively, placing them upright into a 20 ml beaker with 5 ml H_2O added, which again was put into a 250 ml Erlenmeyer flask. The flask was sealed with aluminum foil and HS-SPME was carried out for 3 h followed by GC–MS analysis as described below. Whole-plant studies of 3–4-weeks old plants (right before bolting) were carried out by carefully washing off soil residues from the roots with distilled water and placing five plants into a 25 ml Erlenmeyer flask with 5 ml H_2O added. The flask was sealed with aluminum foil and the SPME fiber was exposed to the sample for 3 h (GC–MS analysis as described below). Autolyzed samples were prepared and analyzed following a modified procedure as described above for the in vitro plants (five sample plants per analysis).

3.4. Gas chromatography and mass spectrometry analysis (GC–MS)

A Varian Star 3400 CX gas chromatograph coupled with a Varian Saturn 3 mass spectrometer was used for GC–MS analysis. The following capillary columns were used: an apolar Chrompack WCOT CP-SIL 5 (30 m × 0.25 × 0.25 μm) and a polar Chrompack CP-Wax 52CB (30 m × 0.32 × 0.25 μm). In addition, a polar J&W DB-Wax column (60 m × 0.32 mm × 0.25 μm) was applied, in order to ensure satisfactory separation and identification of closely eluting mono- and sesquiterpenes. The carrier gas flow (He) was held at 50 ml/min

(injector) and 30 cm/s (column). The injector, kept at 220 °C (splittless injection; 1 min), was fitted with an SPME inlet liner (Supelco Inc.). The GC temperature program was 40 °C for 1 min, followed by 40–220 °C at a rate of 4 °C/min.

The MS detector was set at 170 °C for all analyses, and a mass range of *m/z* 39–300 was recorded. All mass spectra were acquired in EI mode (70 eV). All volatile organic compounds were tentatively identified by the use of a combination of mass spectra database search (IMS Terpene Library, 1989; NIST MS Database, 1998), deconvolution software (AMDIS v.2.0, 1999), retention index tables (Acree and Arn, 2004; Adams, 2001; ESO 2000, 1999; Davies, 1990; Jennings and Shibamoto, 1980), and retention indices from accessible scientific literature as well as comparison of mass spectra from relevant literature.

Acknowledgment

Financial funding from the cross-disciplinary project "BIOEMIT – *Prediction and modification in functional genomics: combining bioinformatical, bioethical, biomedical and biotechnological research* (2003–2006)" at the Norwegian University of Science and Technology (NTNU) through a post-doctoral fellowship is greatly acknowledged. This work was supported by grant NFR 151991/S10 from the functional genomics (FUGE) program of the Norwegian Research Council.

References

Acree, T., Arn, H., 2004. Available from: <http://www.flavornet.org/flavornet.html>.

Adams, R.P., 2001. Identification of Essential Oil Components. Allured Publishing Corporation, Carol Stream.

Aharoni, A., Keizer, L.C.P., Bouwmeester, H.J., Sun, Z.K., Alvarez-Huerta, M., Verhoeven, H.A., Blaas, J., Van Houwelingen, A.M.M.L., De Vos, R.C.H., Van Der Voet, H., Jansen, R.C., Guis, M., Mol, J., Davis, R.W., Schena, M., Van Tunen, A.J., O'Connell, A.P., 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12, 647–661.

Aharoni, A., Giri, A.P., Deuerlein, S., Griepink, F., De Kogel, W.-J., Verstappen, F.W., Verhoeven, H.A., Jongsma, M.A., Schwab, W., Bouwmeester, H.J., 2003. Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. Plant Cell 15, 2866–2884.

Al-Gendy, A.A., Lockwood, G.B., 2003. GC–MS analysis of volatile hydrolysis products from glucosinolates in *Farsetia aegyptia* var. *ovalis*. Flavour Fragr. J. 18, 148–152.

Bak, S., Feyereisen, R., 2001. The involvement of two P450 enzymes, Cyp83B1 and Cyp83A1, in auxin homeostasis and glucosinolate biosynthesis. Plant Physiol. 127, 108–118.

Bodnaryk, R., Yoshihara, T.J., 1995. Structure–activity relationships of cyclopentane analogs of jasmonic acid for induced responses of canola seedlings, *Brassica napus* L. J. Chem. Ecol. 21, 1735–1743.

Bohlmann, J., Martin, D., Oldham, N.J., Gershenzon, J., 2000. Terpenoid secondary metabolism in *Arabidopsis thaliana*: cDNA cloning, characterization, and functional expression of a myrcene/(*E*)-β-ocimene synthase. Arch. Biochem. Biophys. 375, 261–269.

Bones, A.M., Rossiter, J.T., 1996. The myrosinase–glucosinolate system, its organisation and biochemistry. Physiol. Plant. 97, 194–208.

Bridges, M., Jones, A.M., Bones, A.M., Hodgson, C., Cole, R., Bartlet, E., Wallsgrove, R.M., Karapapa, V.K., Watts, N., Rossiter, J.T., 2002. Spatial organization of the glucosinolate–myrosinase system in *Brassica* specialist aphids is similar to that of the host plant. Proc. R. Soc. Lond. B 269, 187–191.

Brown, P.D., Morra, M.J., 1995. Glucosinolate-containing plant tissues as bioherbicides. J. Agric. Food Chem. 181, 307–316.

Burkhardt, D., Mosandl, A., 2003. Biogenetic studies in *Syringa vulgaris* L.: Bioconversion of O-18(H-2)-labeled precursors into lilac aldehydes and lilac alcohols. J. Agric. Food Chem. 51, 7391–7395.

Chen, F., D'Auria, J.C., Tholl, D., Ross, J.R., Gershenzon, J., Noel, J.P., Pichersky, E., 2003a. An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. Plant J. 36, 577–588.

Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E., Gershenzon, J., 2003b. Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. Plant Cell 15, 481–494.

Chen, F., Ro, D.-K., Petri, J., Gershenzon, J., Bohlmann, J., Pichersky, E., Tholl, D., 2004. Characterization of a root-specific *Arabidopsis* terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. Plant Physiol. 135, 1956–1966.

D'Auria, J.C., Chen, F., Pichersky, E., 2002. Characterization of an acyltransferase capable of synthesizing benzyl benzoate and other volatile esters in flowers and damaged leaves of *Clarkia breweri*. Plant Physiol. 130, 466–476.

D'Auria, J.C., Gershenzon, J., 2005. The secondary metabolism of *Arabidopsis thaliana* : growing like a weed. Curr. Opin. Plant Biol. 8, 308–316.

Davies, K.M., Schwinn, K.E., 2003. Transcriptional regulation of secondary metabolism. Funct. Plant Biol. 30, 913–925.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 503, 1–24.

Di Cesare, L.F., Proietti, M., Maestrelli, A., Giombelli, R., 1999. Aroma composition of cauliflower submitted to technological treatments. Ind. Aliment. 38, 132–135.

Di Cesare, L.F., Vitale, R., Acciarri, N., Branca, F., 2001. The characteristic volatile composition of some horticultural cultivars of broccoli, cauliflower and cabbage grown in Italy. Ind. Aliment. 40, 508–512.

Di, X., Shellie, R.A., Marriott, P.J., Huie, C.W., 2004. Application of headspace solid-phase microextraction (HS-SPME) and comprehensive two-dimensional gas chromatography (GC × GC) for the chemical profiling of volatile oils in complex herbal mixtures. J. Sep. Sci. 27, 451–458.

ESO 2000, 1999. Database of Essential Oils. Boelens Aroma Chemical Information Service, The Netherlands. Available from: <http://www.xs4all.nl/~bacis>.

Fäldt, J., Arimura, G., Gershenzon, J., Takabayashi, J., Bohlmann, J., 2003. Functional identification of AtTPS03 as (*E*)-β-ocimene synthase: a monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in *Arabidopsis thaliana*. Planta 216, 745–751.

Geervliet, J.B.F., Posthumus, M.A., Vet, L.E.M., Dicke, M., 1997. Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris species*. J. Chem. Ecol. 23, 2935–2954.

Hartleb, I., Seifert, K., 1995. Acid constituents from *Isatis tinctoria*. Planta Med. 61, 95–96.

Hashem, F.A., Saleh, M.M., 1999. Antimicrobial components of some Cruciferae plants (*Diplotaxis harra* Forsk. and *Erucaria microcarpa* Boiss). Phytother. Res. 13, 329–332.

Husebye, H., Chadchawan, S., Winge, P., Thangstad, O.P., Bones, A.M., 2002. Guard cell- and phloem idioblast-specific expression of thioglucoside glucohydrolase 1 (myrosinase) in *Arabidopsis*. Plant Physiol. 128, 1180–1188.

Jacobsson, A., Nielsen, T., Sjöholm, I., 2004. Influence of temperature, modified atmosphere packaging, and heat treatment on aroma compounds in broccoli. J. Agric. Food Chem. 52, 1607–1614.

Jakobsen, H.B., Friis, P., Nielsen, J.K., Olsen, C.E., 1994. Emission of volatiles from flowers and leaves of *Brassica napus* in situ. Phytochemistry 37, 695–699.

Jelen, H.H., 2003. Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. Lett. Appl. Microbiol. 36, 263–267.

Jennings, W., Shibamoto, T., 1980. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromtography. Academic Press, New York.

Jirovetz, L., Smith, D., Buchbauer, G., 2002. Aroma compound analysis of *Eruca sativa* (*Brassicaceae*) SPME headspace leaf samples using, GC, GC–MS, and olfactometry. J. Agric. Food Chem. 50, 4643–4646.

Kjær, A., Ohashi, M., Wilson, J.M., Djerassi, C., 1963. Mass spectra of isothiocyanates. Acta Chem. Scand. 17, 2143–2154.

Kliebenstein, D.J., 2004. Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant Cell Environ. 27, 675–684.

Krumbein, A., Schonhof, I., Ruhlmann, J., Widell, S., 2001. Influence of sulphur and nitrogen supply on flavour and health-affecting compounds in Brassicaceae. Dev. Plant Soil Sci. 92, 294–295.

Kushalappa, A.C., Lui, L.H., Chen, C.R., Lee, B., 2002. Volatile fingerprinting (SPME-GC-FID) to detect and discriminate diseases of potato tubers. Plant Dis. 86, 131–137.

Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D.J., Gershenzon, J., 2001. The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. Plant Cell 13, 2793–2807.

Meija, J., Montes-Bayon, M., Le Duc, D.L., Terry, N., Caruso, J.A., 2002. Simultaneous monitoring of volatile selenium and sulfur species from Se accumulating plants (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction. Anal. Chem. 74, 5837–5844.

Mewis, I., Ulrich, C., Schnitzler, W.H., 2002. The role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomol. Exp. Appl. 105, 129–139.

Meyer, R., Rautenbach, G.F., Dubery, I.A., 2003. Identification and quantification of methyl jasmonate in leaf volatiles of *Arabidopsis thaliana* using solid-phase microextraction in combination with gas chromatography and mass spectrometry. Phytochem. Anal. 14, 155–159.

Miyazawa, M., Maehara, T., Kurose, K., 2002. Composition of the essential oil from the leaves of *Eruca sativa*. Flavour Fragr. J. 17, 187–190.

Murray, R.A., 2001. Limitations to the use of solid-phase microextraction for quantitation of mixtures of volatile organic sulfur compounds. Anal. Chem. 73, 1646–1649.

Naur, P., Petersen, B.L., Mikkelsen, M.D., Bak, S., Rasmussen, H., Olsen, C.E., Halkier, B.A., 2003. Cyp83A1 and Cyp83B1, two nonredundant cytochrome P450 enzymes metabolizing oximes in the biosynthesis of glucosinolates in *Arabidopsis*. Plant. Physiol. 133, 63–72.

Osswald, S., Wajant, H., Effenberger, F., 2002. Characterization and synthetic applications of recombinant AtNIT1 from *Arabidopsis thaliana*. Eur. J. Biochem. 269, 680–687.

Peer, W.A., Murphy, A.S., 2003. Floral scent of *Arabidopsis lyrata* (Brassicaceae). Biochem. Syst. Ecol. 31, 1193–1195.

Rask, L., Andréasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B., Meijer, J., 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42, 93–113.

Reichelt, M., Brown, P.D., Schneider, B., Oldham, N.J., Stauber, E., Tokuhisa, J., Kliebenstein, D.J.;, Mitchell-Olds, T., Gershenzon, J., 2002. Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. Phytochemistry 59, 663–671.

Rohloff, J., 1999. Monoterpene composition of essential oil from peppermint (*Mentha × piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis. J. Agric. Food Chem. 47, 3782–3786.

Rohloff, J., 2004. Essential oil drugs – terpene composition of aromatic herbs. In: Dris, R., Jain, S.M. (Eds.), Production Practices and Quality Assessment of Food Crops, Quality Handling and Evaluation, vol. 3. Kluwer Academic Publishers, Dordrecht, pp. 73–128.

Rohloff, J., 2002a. Essential oil composition of sachalinmint from Norway detected by solid-phase microextraction and gas chromatography/mass spectrometry analysis. J. Agric. Food Chem. 50, 1543–1547.

Rohloff, J., 2002b. Volatiles from rhizomes of *Rhodiola rosea* L. Phytochemistry 59, 655–661.

Rohloff, J., Nestby, R., Folkestad, J.A., Iversen, T.-H., 2004. Influence of rain cover cultivation on taste and aroma quality of strawberries (*Fragaria ananassa* Duch.). J. Food Agric. Envir. 2, 74–82.

Rohloff, J., Skagen, E.B., Steen, A.H., Iversen, T.-H., 2000. Production of yarrow (*Achillea millefolium* L.) in Norway: essential oil content and quality. J. Agric. Food Chem. 48, 6205–6209.

Rossiter, J.T., Jones, A.M., Bones, A.M., 2003. A novel myrosinase–glucosinolate defense system in cruciferous specialist aphids. In: Romeo, J. (Ed.), From Ethnobotany to Molecular Ecology, Recent Advances in Phytochemistry, vol. 37. Elsevier Science, Oxford, pp. 127–142.

Sefidkon, F., Naderi Nik, A., Bagaii, P., Rezaee, M.B., 2002. Essential oil composition of the aerial parts of *Sinapis alba* L. J. Ess. Oil-Bearing Plants 5, 90–92.

Siegl, H., Hanke, A., Schnitzler, W.H., 1997. Cultivar differences of volatile aroma compounds in fresh Chinese cabbage. Gartenbauwissenschaft 62, 17–22.

Smart, L.E., Blight, M.M., 1997. Field discrimination of oilseed rape, *Brassica napus* volatiles by cabbage seed weevil, *Ceutorhynchus assimilis*. J. Chem. Ecol. 23, 2555–2567.

Smolinska, U., Morra, M.J., Knudsen, G.R., James, R.L., 2003. Isothiocyanates produced by Brassica species as inhibitos of *Fusarium oxisporum*. Plant Dis. 87, 407–412.

Spencer, G.F., Daxenbichler, M.E., 1980. Gas chromatography–mass spectrometry of nitriles, isothiocyanates and oxazolidinethiones derived from cruciferous glucosinolates. J. Sci. Food Agric. 31, 359–367.

Steeghs, M., Bais, H.P., De Gouw, J., Goldan, P., Kuster, W., Northway, M., Fall, R., Vivanco, J.M., 2004. Proton-transfer-reaction mass spectrometry as a new tool for real time analysis of root-secreted volatile organic compounds in *Arabidopsis*. Plant Physiol. 135, 47–58.

Thangstad, O.P., Gilde, B., Chadchawan, S., Seem, M., Husebye, H., Bradley, D., Bones, A.M., 2004. Cell-specific, cross-species expression of myrosinases in *Brassica napus*, *Arabidopsis thaliana* and *Nicotiana tabacum*. Plant Mol. Biol. 54, 597–611.

Tholl, D., Chen, F., Petri, J., Gershenzon, J., Pichersky, E., 2005. Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. Plant J. 42, 757–771.

Tollsten, L., Bergstroem, G., 1988. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. Phytochemistry 27, 2073–2077.

Valette, L., Fernandez, X., Poulain, S., Loiseau, A.-M., Lizzani-Cuvelier, L., Levieil, R., Restier, L., 2003. Volatile constituents from Romanesco cauliflower. Food Chem. 80, 353–358.

Van Poecke, R.M.P., Dicke, M., 2004. Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. Plant Biol. 6, 387–401.

Van Poecke, R.M.P., Posthumus, M.A., Dicke, M., 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesiarubecula*: chemical, behavioral, and gene-expression analysis. J. Chem. Ecol. 27, 1911–1928.

Vercammen, J., Pham-Tuan, H., Arickx, I., Van Der Straeten, D., Sandra, P., 2001. Monitoring of isothiocyanates emanating from *Arabidopsis thaliana* upon paraquat spraying. J. Chromatogr. A 912, 127–134.

Verdonk, J.C., De Vos, C.H.R., Verhoeven, H.A., Haring, M.A., Van Tunen, A.J., Schuurink, R.C., 2003. Regulation of floral scent production in petunia revealed by targeted metabolomics. Phytochemistry 62, 997–1008.

Vom Endt, D., Kijne, J.W., Memelink, J., 2002. Transcription factors controlling plant secondary metabolism: what regulates the regulators? Phytochemistry 61, 107–114.

Vuorinen, T., Reddy, G.V.P., Nerg, A.M., Holopainen, J.K., 2004. Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO_2 concentration. Atmos. Envir. 38, 675–682.

Warton, B., Matthiessen, J.N., Shackleton, M.A., 2001. Glucosinolate content and isothiocyanate evolution – two measures of the biofumigation potential of plants. J. Agric. Food Chem. 49, 5244–5250.

Williams, J.S., Cooper, R.M., 2003. Elemental sulphur is produced by diverse plant families as a component of defence against fungal and bacterial pathogens. Physiol. Mol. Plant Pathol. 63, 3–16.

Winkel, B.S.J., 2004. Metabolic channeling in plants. Annu. Rev. Plant Biol. 55, 85–107.

Wittstock, U., Kliebenstein, D.J., Lambrix, V., Reichelt, M., Gershenzon, J., 2003. Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. In: Romeo, J. (Ed.), From Ethnobotany to Molecular Ecology, Recent Advances in Phytochemistry, vol. 37. Elsevier, Oxford, pp. 101–125.

Zhang, J., Pontoppidan, B., Xue, J., Rask, L., Meijer, J., 2002. The third myrosinase gene TGG3 in *Arabidopsis thaliana* is a pseudogene but is spesifically expressed in stamen and petal. Physiol. Plant. 115, 25–34.