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ORIGINAL ARTICLE

Growth form rather than phylogenetic relationship predicts broad volatile emission patterns in the Brassicaceae

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Abstract Volatile organic compounds (VOCs) released from plants are known to mediate indirect defense against herbivores and trigger intra- and interplant signaling. While systemic defense response can be mediated both via volatile and vascular signals, it is not clear whether common ancestry and/or plant growth forms influence the choice of either mode *in planta*. We hypothesize that larger woody plants with a complex anatomy should rely more on volatile-mediated signaling, apparently to circumvent vascular restrictions that slow down the communication over a large distance. On the other hand, in smaller herbaceous plants faster systemic response can be achieved via vascular signaling. To investigate whether plant VOCs emission is related to plant phylogeny or growth form, we studied the composition of herbivory-induced plant volatiles in 13 Brassicaceae species representing all four evolutionary lineages, because this family is characterized by both a well-resolved phylogeny and highly diverse growth forms. Our results revealed that woody species consistently emitted a more complex blend of volatiles than herbaceous species. However, phylogenetic relatedness of the species did not explain the observed volatile emission patterns. This emphasizes the influence of growth form, rather than phylogenetic relationships on the variation in plant volatile emissions. Our findings suggest that woody, perennial plant species emit diverse VOCs, likely because these compounds comprise a more efficient mode of defense response in these large, anatomically complex plants.

Keywords Brassicaceae · Herbivore-induced volatiles · Induced resistance · Life form · Phylogeny · Plant habit

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Introduction

Most plant species are sessile during a large portion of their life histories and therefore have evolved a sophisticated network of chemical defense systems to deal with the host of harmful environmental conditions they encounter. Resistance strategies of plants against biotic enemies (e.g., herbivore) are very diverse and include constitutive defenses, which are already present in the absence of an attack, as well as induced responses, which are triggered only when signals that indicate the presence of an attacker are noticed (Baldwin et al. 2006; Agrawal 2007; Heil and Karban 2010).

While induced defense production in remote plant parts is triggered by systemic wound signaling via the vascular system with assimilated transport (Davis et al. 1991), the signal conveyance via the vascular traces does not reach

the systemic regions (Frost et al. 2007). Orthostichous (connected) regions can communicate well among themselves to receive vascular wound signals from herbivore-damaged leaves, while non-orthostichous (unconnected) regions cannot (Watson and Casper 1984; Van Dam et al. 2001). Thus, isolated vascular traces constrain systemic responses to herbivores in space and time, which causes variability in the systemic induction of foliar defenses (Davis et al. 1991; Arnold and Schultz 2002; Schittko and Baldwin 2003) even among leaves of basal rosettes (Kiefer and Slusarenko 2003). Therefore, different plant forms, herbaceous (with simple, orthostichous vascular connection) and woody (with more complex and often non-orthostichous vascular network), might adapt different strategies for signaling and communication for their defense.

Over the last few decades, it has been established that the plants can "talk" among themselves via chemical cues (Baldwin and Schultz 1983; Heil and Adame-Álvarez 2010) and trigger resistance induction in a perceiver plant that is exposed to the volatile organic compounds (VOCs) released from an infested or infected emitter. Plants respond to damage inflicted by herbivores or infection by pathogens with the emission of a diverse array of VOCs that trigger multiple resistance-related effects (Unsicker et al. 2009; García-Guzmán and Heil 2014). Moreover, within-plant signaling via volatiles overcomes vascular constraints on systemic signaling (Frost et al. 2007) to prime responses against herbivores (Howe and Jander 2008; Wu and Baldwin 2010; Heil and Karban 2010; Farmer et al. 2014).

It is well established that plant–plant signaling does not require genetic relatedness between the emitter and receiver and can even cross species borders (Karban et al. 2003, 2004; Heil and Bueno 2007). However, it is less studied and often controversial whether the evolution of plant secondary chemistry, including volatiles, in response to insect herbivory remains a classic example of coevolution and therefore conserved in the taxa with common ancestry (Agrawal and Weber 2015).

The well-resolved molecular phylogeny of the family Brassicaceae has the potential to resolve the above-mentioned dilemma (Franzke et al. 2011) as this plant family comprises taxa with highly diverse growth forms. In this study, we selected 13 species of the family Brassicaceae representing eight genera and all main Brassicaceae lineages (Aethionemeae, lineage I–III), as well as two different growth forms: herbaceous species (non-woody, annual herb) and woody species (woody, perennial subshrub/shrub). We analyzed the chemical composition of volatiles, with state-of-the-art GC–MS techniques, emitted by the species before and after stimulated herbivory to test the hypothesis that the spectrum of emitted VOCs by plant species primarily depend on their growth forms rather than their phylogenetic relationships. In other words, we tried to find out whether (1) woody and anatomically complex species produce more diverse VOCs compared to their herbaceous relatives and alternatively, and (2) the diversity of VOCs is more similar in phylogenetically closely related plant species. The scope of this study does not provide a critical, quantitative estimation of the plant volatile communication within and among species, but addresses the fundamental question whether volatile communication machinery is prompted by the evolutionary status of the species or guided by the practical aspects of feasible communication method.

Materials and methods

Plants and insects

We analyzed 13 Brassicaceae species representing eight genera and all main Brassicaceae lineages (Aethionemeae, lineage I–III) sensu Franzke et al. (2011) (*Aethionema spinosum* (Boiss.) Prantl, *Mummenhoff 2368*; *Arabidopsis thaliana* (L.) Heynh., ecotype Columbia-O; *Dontostemon elegans* Maxim., OSBU 14740; *D. micranthus* C.A.Mey., OSBU 20612; *Erysimum cheiri* (L.) Crantz, BG Osnabrück 12-40-0051-10; *Iberis sempervirens* L., BG Osnabrück, cultivated; *Lepidium bidentatum* Montin, *Mummenhoff 2386*; *L. foliosum* Desv., *Mummenhoff 2383*; *L. leptopetalum* (F.Muell.) F.Muell., *Mummenhoff 244*; *L. sativum* L., Kiepenkerl Saatgut, Krause Kresse; *Sinapidendron frutescens* (Sol.) Lowe, *Gomez-Campo 5471-79*; *Vella bourgaeana* (Coss.) S.I.Warwick & Al-Shehbaz, *Gomez-Campo 1543-68*; *V. pseudocytisus* L., *Gomez-Campo 5302-79*). Morphologically, these species could clearly be assigned to two different growth forms: herbaceous species (non-woody, annual herb) and woody species (woody, perennial subshrub/shrub) (see Table 1; Fig. 2).

Perennial woody species are permanently cultivated in the Botanical Garden of the University of Osnabrück (Germany). Herbaceous plant species were grown from seeds in the greenhouse as described in Mühlhausen et al. (2013). Plants were transported to the Max-Planck Institute for Chemical Ecology in Jena (Germany) where they were acclimatized for 2–3 days before the onset of the experiment. Throughout the experiment, plants were kept under sodium lights and ambient conditions in the laboratory (12-h/12-h day/night cycle) and were watered once daily.

Plant treatments and volatile collection

No damage or disturbance was inflicted to the control set of plants before the volatile emission measurements. To

Table 1 Volatile organic compounds (VOCs) emitted from 13 species representing herbaceous and woody growth forms and Brassicaceae main lineages Aethionemeae, lineage I–III

Species	Aethion-emeae	Lineage I						Lineage II / expanded II			Lineage III		
	Aethionema spinosum	Erysimum cheiri	Lepidium bidentatum	Lepidium foliosum	Lepidium leptopetalum	Lepidium sativum	Arabidopsis thaliana	Iberis sempervirens	Vella bourgaeana	Vella pseudocytisus	Sinapidendron frutescens	Dontostemon elegans	Dontostemon micranthus
Growth form													
VOCS													
Sesquiterpenoids (ST)													
α-Bergamoten	-	-	-	X	-	-	-	-	-	-	-	-	-
α-Copaen	-	-	-	-	-	-	-	X	-	-	-	-	-
α-Elemen	-	-	-	-	-	-	-	X	-	-	-	-	-
α-Humulen	-	-	-	-	-	-	-	X	X	-	-	-	-
α-Amorphen	-	-	-	-	-	-	-	X	-	-	-	-	-
α-Gurjunen	-	-	-	-	-	-	-	X	-	-	-	-	-
β-Bisabolen	-	-	-	-	-	-	-	X	-	-	-	-	-
β-Caryophyllen	-	-	-	-	-	-	X	X	X	X	-	-	-
β-Cubeben	-	-	-	-	-	-	-	X	-	-	-	-	-
δ-Cadinen	-	-	-	-	-	-	-	X	-	-	-	-	-
E,E α-Farnesen	-	X	-	-	-	-	-	X	-	-	-	-	-
γ-Cadinen	-	-	-	-	-	-	-	X	-	-	-	-	-
Germacren D	-	-	-	-	-	-	-	X	X	-	-	-	-
Naphtalen	-	-	-	-	-	-	-	X	-	-	-	-	-
Homoterpenoids (HT)													
Dimethylnonatrien	-	-	X	-	-	-	X	-	X	-	-	-	-
Monoterpenoids (MT)													
α-Pinen	-	-	-	-	-	-	-	-	-	-	-	X	-
Eucalyptol	-	-	-	-	-	-	-	-	-	-	-	X	X
Geranial	-	X	-	-	-	-	-	-	-	-	-	-	-
Myrcen	-	-	-	-	-	-	-	-	-	-	-	X	-
Sabinen	-	-	-	-	-	-	-	-	-	-	-	X	-
Trans-β-2-Ocimen	-	-	-	-	-	-	-	-	-	-	-	X	-
Octatrien	-	-	-	-	-	-	-	-	-	X	X	-	-
Glucosinolate-metabolite (GD)													
3-Butenyl-isothiocyanat	X	-	-	X	X	-	X	-	X	X	-	-	-
4-Pentenyl-isothiocyanat	-	-	-	-	-	-	X	-	-	-	-	-	-
Benzylcyanid	-	-	-	X	-	-	-	X	-	-	-	X	-
Benzyliso-thiocyanat	-	-	X	-	-	X	-	-	-	-	-	-	-
Methylethyl-isothiocyanat	-	-	-	-	-	-	-	-	-	-	-	X	-
Methylpropyl-isocyanat	-	-	-	-	-	-	-	-	-	-	-	X	-
Green-Leaf-Volatiles (GLV)													
2-Hexene	-	-	-	-	-	-	-	-	-	-	-	X	-
3-Hexene-1-ol	-	-	-	-	-	-	-	-	-	-	-	X	-
Z-3-hexenyl-acetate	-	X	-	-	-	-	-	-	-	-	-	-	-
Cis-3-Hexenylacetate	-	-	-	-	-	-	-	-	X	-	-	-	-
Long-chain Hydrocarbons (LCH)													
5-Heptene-2-one	-	-	-	-	-	-	-	-	-	-	-	X	-
Diacyclododecan	-	-	-	-	-	-	-	-	-	-	X	-	-
Decan	-	-	-	-	-	-	-	-	-	-	-	X	-
Dodecan	-	X	-	-	-	-	-	-	-	-	X	-	-
Pentadecan	-	X	-	-	-	-	-	-	-	-	-	X	-
Undecan	-	-	-	-	-	-	-	-	-	-	X	X	-
Aromatic Hydrocarbons (AH)													
Ethylbenzaldehyd	-	-	-	-	-	-	X	-	-	-	-	-	-
Benzylacetat	-	-	-	-	-	-	-	X	-	-	-	-	-
Phenetylacetat	-	-	-	-	-	-	-	X	-	-	-	-	-
Benzaldehyd	-	-	-	-	-	X	X	-	-	-	-	-	-
Benzylaldehyd	X	-	-	-	-	-	-	-	-	-	-	-	-
Ethylbenzoat	X	-	-	-	-	-	-	-	-	-	-	-	-
Methylbenzoat	-	-	X	-	-	-	-	-	-	-	-	-	-
Methyl Salicylate	-	X	X	X	-	-	-	X	-	-	-	-	-
Benzen	-	-	-	-	-	-	-	-	-	-	-	X	-
Diphenyl-nitroethan	-	-	-	-	-	-	-	X	-	-	-	-	-
Phenyl-ethylalcohol	-	-	-	-	-	-	-	X	-	-	-	-	-

Table 1 continued

Species	Aethion-emeae	Lineage I						Lineage II / expanded II			Lineage III		
	Aethionema spinosum	Erysimum cheiri	Lepidium bidentatum	Lepidium foliosum	Lepidium leptopetalum	Lepidium sativum	Arabidopsis thaliana	Iberis sempervirens	Vella bourgaeana	Vella pseudocytisus	Sinapidendron frutescens	Dontostemon elegans	Dontostemon micranthus
Growth form													
Nitrogenous volatiles (NV)													
3-Methyl-butyl-aldoxime	-	-	-	-	X	-	-	-	-	-	-	X	-
Butyl-aldoxime	-	-	-	-	-	-	-	-	-	-	-	X	-
Indol	X	-	X	-	-	-	-	X	-	-	-	X	-
N-Methylanilin	X	X	-	-	-	-	-	-	-	-	-	-	X
Total number of detected VOCs	5	7	5	3	3	2	6	20	6	3	4	18	2

Assignment of species to Brassicaceae main lineages Aethionemeae, lineage I, II/expanded II, and III follows Franzke et al. (2011). Growth forms: herbaceous species (non-woody, annual herb) and woody species (woody, perennial subshrub/shrub) . The presence and absence of VOCs are indicated by X and –, respectively. Volatile organic compounds (VOCs) are classified into eight different substance classes shown in bold print with abbreviations in parenthesis

accumulate volatile emission from bigger woody plant species, only one branch was selected and enclosed in a PET bag (polyethylene terephthalate cooking bag that releases very few volatiles) by fixing the openings of the branch with a cable binder to the branches. Smaller herbaceous plants were completely enclosed in PET bags for volatile collections. The biomass enclosed in PET bags from the different species was similar (visual estimation). However, the size of the bags was not equal and differed at maximum by a factor of 2. For the volatile collections from the plants, a portable system was constructed which consist of a Lock & Lock ® plastic box attached to two vacuum pumps with emission-free Teflon tubes (Thomas, Gardner Denver) and powered by a 12 V battery. During volatile collections, one pump pushed charcoal purified air at a flow rate of 1 L min^{-1}, while the other pump sucked the air out of the bag at a rate of 0.5 L min^{-1}. Volatiles were collected for 3 h with 20 mg Super-Q filters (Alltech, Gainsville, FL, USA) during the daytime.

Herbivore-induced volatile emission was collected following stimulated herbivory by infesting plant species with third instar caterpillars (Spodoptera littoralis Boisd.; Lepidoptera, Noctuidae). Caterpillars were hatched from eggs (supplied by Syngenta, Switzerland) and reared on artificial diet in a growth chamber at 23–25 °C with 16/8 h L/D cycle until they reached the third instar (for further details see: Fontana et al. 2009) in controlled conditions. Caterpillars were starved for 24 h before they were released to feed on the plants for 16 h (5 pm to 9 am). As the investigated plant species substantially differed in size and biomass, adapted numbers of caterpillars were released on them, ranging from one to four individuals (four caterpillars: A. thaliana, S. frutescens; three caterpillars: L. bidentatum, E. cheiri; two caterpillars: A. spinosum, D. elegans, D. micranthus, I. sempervirens, L. foliosum, L.

sativum, V. pseudocytisus; and one caterpillar: L. leptopetalum, V. bourgaeana). The foliage was enclosed within a cellophane bag (205 × 380 mm, Unipack, Germany) to prevent caterpillars from escaping. Experimentally inflicted caterpillar herbivory caused 5–15% loss of total leaf area. Prior to volatile collections, caterpillars and cellophane bags were removed from the plants.

To mimic the herbivory-induced volatile in a controlled way, leaves were treated with 1 μM methyl jasmonate (MeJA) by enclosing them in PET bags containing MeJA moistened filter tissue (of ∼5 cm^2) for 24 h. Volatile emission was collected directly after the removal of filter tissue.

Volatile analysis

After volatile collections, the filters containing the volatiles were eluted with 200 μl dichloromethane containing 10 ng/μl nonyl acetate as an internal standard. Samples were stored at −20 °C until further analysis. Individual analytes were analyzed by an Agilent 6890 Series ion trap MS connected to a ZB5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA); 1 μL of samples was splitlessly injected onto the column. The GC was programmed as follows: injector held at 220 °C, initial column temperature at 40 °C held for 2 min, then ramped at 7 °C min^{-1} to 155 °C and finally at 60 °C min^{-1} to 300 °C, held for 2 min. Helium carrier gas was used and the column flow set to 1 mL min^{-1}. Compounds eluted from the GC column were transferred to a mass spectrometer (Hewlett-Packard model 5973) with a quadrupole mass selective detector for the MS analysis. The MS was programmed as follows: transfer line at 270 °C, trap temperature 270 °C, manifold temperature 40 °C, source heater 150 °C, ionization potential 70 eV

and scan range from 40 to 350 m/z. As coelution of some compounds occurred with the DB-5 MS column, all samples were additionally analyzed on the GC–MS with a DB-wax column (30 m × 0.25 mm, 0.25 μm film, J&W Scientific). The GC for DB-wax column was programmed as follows: injector held at 230 °C, initial column temperature at 45 °C held for 2 min, then ramped at 7 °C min^{-1} to 90 °C, at 20 °C min^{-1} to 150 °C, and finally at 60 °C min^{-1} to 250 °C, held for 2 min.

Compounds eluted from the GC column were transferred to a FID for further analysis. Quantification was accomplished by comparing the peak areas in the FID traces with that of the internal standard (nonyl acetate) calculated according to the effective carbon number concept (Scanion and Willis 1985). For further technical details, see also Kigathi et al. (2013). Individual volatile compounds were determined by comparing the retention time and mass spectra and matching it with the internal database for volatiles emitted from Brassicaceae of the Max-Planck Institute for Chemical Ecology (Jena, Germany) and reference spectra in the Wiley and National Institute of Standards and Technology (NIST) libraries. The accordance of the compounds detected in the GC and the database was given in percentages; only database matches above 80% were named and used for further analysis. Quantitative data of volatiles are not considered for further analyses as it is flawed in terms of cooperativity of measured biomass between the species. However, quantitative data are available as Online Resource 1. All compounds collected were considered as plant volatiles due to prior removal of caterpillars and filter plastic disk contaminated with MeJA before the volatile collection and the use of emission-free Teflon tubes and PET bags.

Neighbor-joining analysis

To clarify whether the volatile spectrum of the Brassicaceae taxa included in this study mirrors a phylogenetic pattern similar to the molecular phylogeny of the family, the neighbor-joining algorithm of Saitou and Nei (1987) was used to construct the phylogenetic tree from the presence and absence of volatiles detected in all the studied species (Table 1). A distance matrix was computed from the presence/absence of data matrix and used as input for this bottom-up clustering algorithm (Fig. 2b) to reconstruct the phylogenetic relationships among the species. Support for the reliability of the branching pattern was evaluated by bootstrap analysis (1000 replicates). All analyses were performed in R statistical programming language (v. 3.3.1, http://www.R-project.org). Neighbor-joining analysis was performed using the library package "Analyses of Phylogenetics and Evolution" (ape) (Paradis et al. 2004).

Results

Plant volatiles are more diverse in woody species

Altogether, the 13 plant species emitted 53 different volatile compounds of eight chemical groups. Terpenoids [sesquiterpenoids (ST, 14 VOCs), homoterpenoids (HT, 1), monoterpenoids (MT, 7)] and the aromatic hydrocarbons (AH, 11) were the most abundant volatiles among all the species (Table 1). *Iberis sempervirens* emitted the most diverse blend of 18 VOCs of the above four groups. Twenty other volatile chemicals from the remaining four groups (glucosinolate-derived metabolites, GD; green leaf volatiles, GLV; long-chain hydrocarbons, LCH; nitrogenous volatiles, NV) were also detected in less abundance in the studied species. *Lepidium sativum* and *D. micranthus,* both with herbaceous growth form, produced only two volatiles each, while *A. thaliana* (another herbaceous species) emitted six VOCs. On the other hand, every woody plant species produced a cocktail of 3–20 chemicals.

In control plants, where no damage was inflicted, more diverse volatiles (29 VOCs) were detected from the six species of lineage II compared to the others (lineage I, 18; lineage III, 11; and Aethionemeae, 5). A similar trend of a more complex blend of volatiles was detected in members of lineage II (30 VOCs) after stimulated herbivory by infesting plant species with third instar caterpillars (*S. littoralis*) and following the application of MeJA that mimics the plant defense response to herbivory.

The control plants of the woody species emitted 61 volatiles, while the herbaceous species studied emitted only two volatiles (Fig. 1). After simulated herbivory or MeJA treatment that mimics herbivory, the similarly high number of the volatile compounds was detected in woody species (57–71 VOCs) compared to the herbaceous species (5–7 VOCs) that highlights at least tenfold enhanced volatile signal in woody species. The number of detected VOCs from 13 species, irrespective of the treatment, significantly differ among the eight different chemical groups (ANOVA, $F_{7, 304} = 2.623$, $P < 0.05$, Fig. 1, Table 1). While more variety of long-chain hydrocarbons (LCH) are emitted after herbivory/MeJA treatment in woody species, dimethylnonatrien (HT), monoterpenoids (MT) and glucosinolate metabolites (GD) were the specific groups of volatiles released after similar treatment in herbaceous species (Fig. 1).

Reconstructed neighbor-joining tree from emitted VOCs does not corroborate with the conventional phylogenetic tree of Brassicaceae species studied

A data matrix with the presence/absence of data of detected volatiles in the 13 studied Brassicaceae species (Table 1)
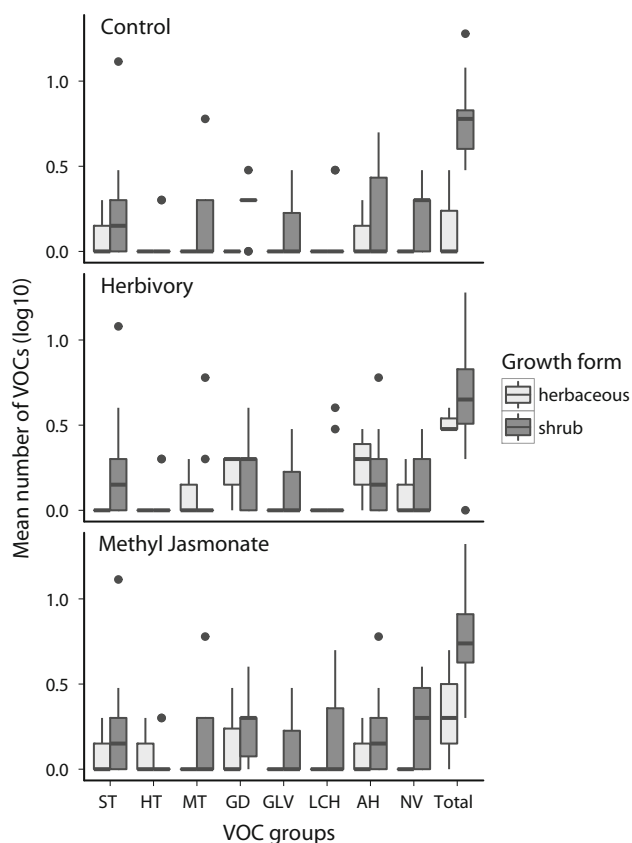
Fig. 1 Woody species emit more complex volatile bouquet than the herbaceous species. *Box* and *whisker plots* showing numbers of emitted volatile organic compounds (VOCs) (log10-transformed) from 13 Brassicaceae species representing all main Brassicaceae lineages and woody and herbaceous growth forms. The *box* delimits the first and third quartiles of the data; the *solid line* within the *box* represents the second quartile, whiskers, *upper* and *lower* fence (95th and 5th percentile), respectively, and *dots*, outliers. VOC groups: *ST* sesquiterpenoids; *HT* homoterpenoids; *MT* monoterpenoids; *GD* glucosinolate-derived metabolites; *GLV* green leaf volatiles; *LCH* long-chain hydrocarbons; *AH* aromatic hydrocarbons; *NV* nitrogenous volatiles. For distribution of emitted VOCs in Brassicaceae species studied, see Table 1

was used to construct neighbor-joining tree from the distance matrix (Saitou and Nei 1987). The network topology is not strongly supported by the bootstrap confidence and is less than 50% (Fig. 2b). Only the node *Aethionema/Dontostemon* is supported by 100% bootstrap support. Considerable confidence should be attributed to nodes or groups supported by bootstrap values of 75% or better higher; conversely, branches supported by less than 75% should be treated with great caution (Van de Peer 2009) and nodes below 50% should be collapsed. This indicates that the distribution of volatile patterns in Brassicaceae does not appear to reflect phylogenetic relationships of Brassicaceae species studied. Furthermore, the network topology does not agree at all with the accepted phylogenetic family concepts based on multi-gene approaches (see Franzke et al. 2011 and compare Fig. 2a and b).

Discussion

In this study, we investigated the composition of herbivory-induced plant volatiles in 13 Brassicaceae species representing all four evolutionary lineages because this family is characterized by both a well-resolved phylogeny and herbaceous and woody growth forms. Our results provide the first evidence that plant growth form is more important for the composition of constitutive and induced volatile emission than phylogenetic relatedness. To our knowledge, this is the first study investigating whether VOC emission pattern reflects the phylogenetic relationship of the taxa or patterns are rather determined by plant traits such as growth form.

Based on the assumption that many secondary metabolites (including VOCs) are important for plant fitness, it is expected that the traits have been subjected to natural selection during evolution and therefore significant to interpret plant taxonomy and phylogeny (Abbott 1886). While chemotaxonomy is a well-accepted genre of plant systematics (Dahlgren 1980), failure to a) strictly attribute a given class of secondary metabolites to a certain clad and b) ubiquitous occurrence of structurally diverse chemicals in close relatives severely thwarted their reliability as an evolutionary marker or to deduce phylogeny (Wink 2003). In this study, we detected that VOCs from eight structurally diverse chemical classes are omnipresent in the members of three lineages of Brassicaceae (I–III), while Aethionemeae (*A. spinosum*) emitted VOCs belonging to three chemical classes (glucosinolate metabolite, GD; aromatic hydrocarbons, AH; nitrogenous volatiles, NV; Table 1). Moreover, similar diversity of chemical classes was observed for the emitted VOCs from sister species of the same genus (*D. elegans* and *D. micranthus*; *L. bidentatum, L. leptopetalum, L. foliosum* and *L. sativum, V. bourgaeana* and *V. pseudocytisus*). These altogether compounded the phylogenetic reconstruction among the studied members of Brassicaceae and eventually led to a poorly supported neighbor-joining tree with bootstrap values less than 50% (Fig. 2b). Phylogenetic relationships based on neighbor-joining analysis of the presence/absence of 84 VOCs do not corroborate the accepted molecular phylogenetic positions based on multi-gene approaches (see Franzke et al. 2011 and Fig. 2a). Furthermore, the weak bootstrap support for the neighbor-joining topology poses the question of the relevance of VOCs to infer/reflect phylogenetic conclusions.

The lack of equivalence between specific chemistry and phylogeny may be triggered by several different phenomena. One causal factor is convergent evolution by which the same or similar traits originate independently in taxa that are not necessarily closely related, as exemplified by
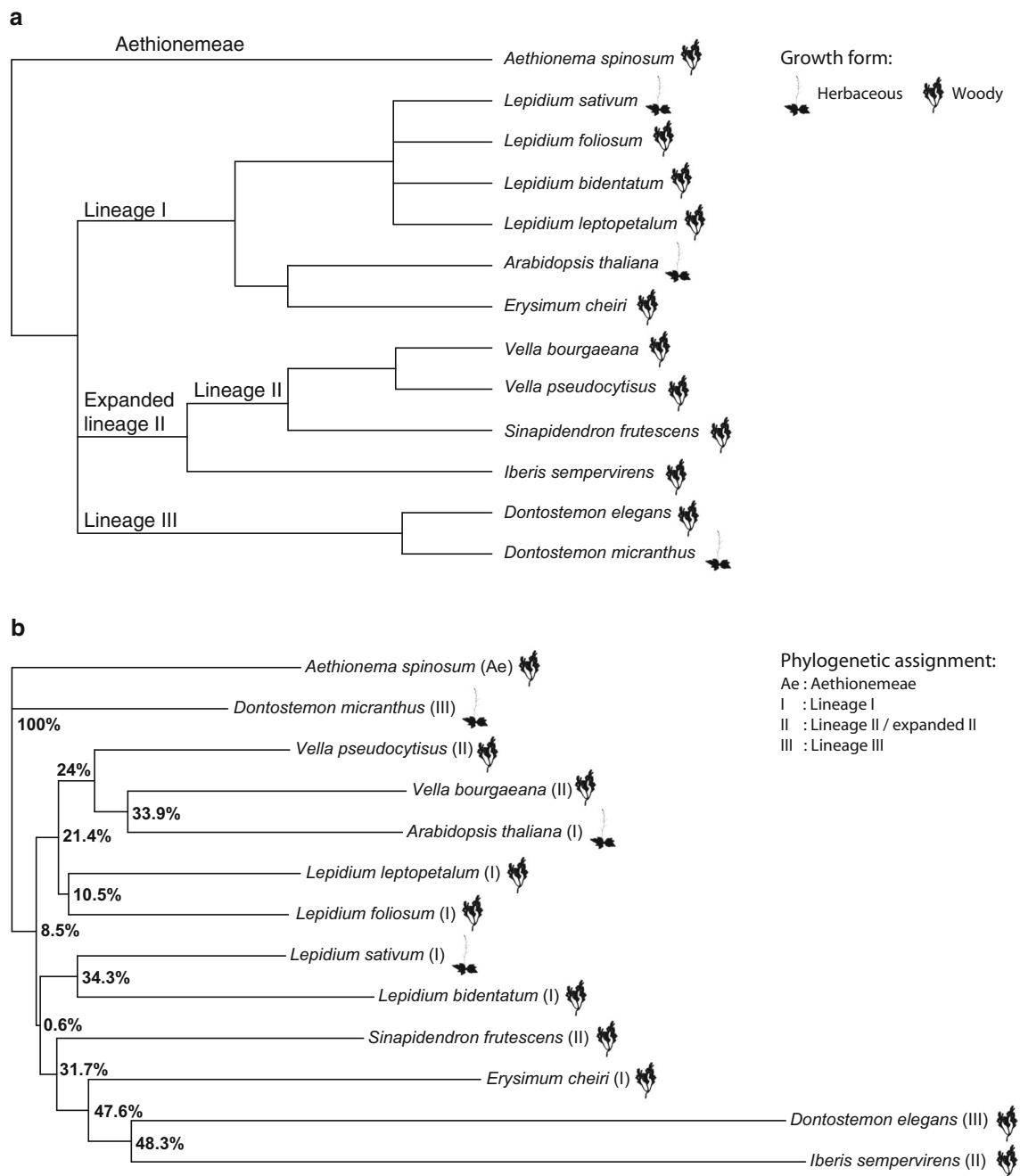
Fig. 2 Neighbor-joining tree computed from the emitted VOCs do not corroborate with the classical phylogeny of the 13 Brassicaceae species. **a** Classical phylogenetic relationships among the 13 selected Brassicaceae taxa based on a multi-gene approach. **b** VOCs-based phenogram computed by the neighbor-joining network based on the presence/absence of 53 volatile organic compounds (VOCs) in 13 species representing two growth forms (woody vs herbaceous) and all main Brassicaceae lineages. Phylogenetic assignment of studied Brassicaceae species to main lineages Aethionemeae, lineage I–III based on a multi-gene approach (Franzke et al. 2011) is indicated in parenthesis after the species names. Numbers at nodes indicate bootstrap values. For distribution of emitted VOCs in the Brassicaceae species studied, see Table 1

the production of cyanogenic glycosides in diverse plant families (Pichersky and Lewinsohn 2011). On the contrary, dedicated classes of defense compounds were detected for the families Fabaceae (quinolizidine), Solanaceae (tropane) and Lamiaceae (essential oils) suggesting a strong phylogenetic and ecological component (Wink 2003). In Amaryllidaceae, phylogenetic prediction of chemical diversity and biosynthetic pathways were demonstrated (Rønsted et al. 2012) and signature chemical classes could be assigned to certain clades.

However, the reverse presumption that the defense compounds (including VOCs) are produced according to the phylogenetic relation of the species may not hold true and was rarely tested in a rigorous way due to empirical limitations (Heil and Karban 2010; Karban 2011). Specialized plant defense compounds are not continuously expressed, but may be produced as a response to herbivory, mechanical wounding or abiotic environmental factors (Gatehouse 2002). Plants often exhibit a combination of several of those defensive traits (Agrawal 2007, 2011). Moreover, ontogeny can also restrain the expression of resistance to herbivore damage via the production of secondary metabolites (Boege and Marquis 2005) that influence the induced defense (Barton and Koricheva 2010). Therefore, in this study, we carefully avoided such variables by acclimatizing all plant species to a normalized environment in glasshouse before and during the course of the experiment, and by comparing the VOCs spectra in control and herbivory-damaged plants of the comparable developmental stage (Fig. 1).

Plant functional traits such as growth form and biochemical features have high potential to explain ecological strategies to defend themselves against adversities (Violle et al. 2007; Pérez-Harguindeguy et al. 2013) and therefore are preferred for the interpretation of defense responses rather than the phylogenetic relationships of species (Díaz and Cabido 2001; Díaz et al. 2016). Due to the complex anatomy and non-orthostichous vascular regions, woody perennial plants require more reliable systemic response mode in the event of herbivory. One major defense strategy is to rely on secondary metabolites (Mauricio et al. 1997; Carmona et al. 2011) which can be perpetually generated in plant organs or can be transiently expressed when needed to maximize cost-effectiveness (Heil 2004; Heil et al. 2004; Boege and Marquis 2005). It is known that perennial woody plant species occupying niches with the highest probability of biotic stress produce greater quantity and diversity of secondary compounds (Coley and Barone 1996). Our results provide a first hint that the VOCs spectra of the woody species of Brassicaceae are composed of more diverse concoction of volatiles than the herbaceous ones in control (undamaged) or induced (by MeJA or stimulated herbivory) plants (Fig. 1). Such diversity in woody members, sharing a complex morphology, is observed both in the number of different substance classes emitted and in the higher number of VOCs present in the emitted bouquets than herbaceous species with a simple morphology. While limited spatial and temporal scales of induced responses make plants more variable from the herbivore's perspective (Schuman et al. 2009), recent work suggests that plant heterogeneity in terms of defense communication may be difficult for herbivores to cope with (Karban 2011; Schuman and Baldwin 2016). Our findings

underscore such diversity in woody species of Brassicaceae that probably contribute to their readiness and unpredictable chemical response to startle the herbivores with a novel concoction of defense metabolites.

The scope of this study did not provide a critical, quantitative estimation of the plant volatile communication within and among species, in other words, if herbivory and induced herbivory by methyl jasmonate treatments on species A induce a defense response in a related, neighboring species B. For instance, neither comparative intra-specific quantitative/qualitative variations in VOC emissions among different parts of a plant's metabolism nor the function of VOCs as intra-plant systemic signals was tested. Similarly, the imbalance in species/growth-form sampling size should be avoided in future studies to rule out a bias in the compositional diversity of VOC estimation. However, in this pilot study, we empirically addressed the fundamental question whether the volatile communication machinery has evolved following the phylogeny or is developed to circumvent the limitation of communications in different plant growth forms.

Conclusion

Our results indicate that the emission of VOCs, the primary signals in successful plant communication, does not depend on the phylogenetic relatedness of plant taxa but on plant functional traits such as growth and life forms. Woody plant species within the Brassicaceae exhibited a more complex blend of VOCs than their herbaceous relatives. This was true for both the constitutive and the damage-induced volatiles. Whether woody plants with larger size and higher architectural complexity rely more on the volatiles as signals in plant defense than their herbaceous relatives requires further investigation. Also, future studies have to test whether species within other plant families exhibit similar growth-form-related volatile emission patterns as the Brassicaceae tested in our study. Our results open up the possibility of more rigorous biochemical quantitation of the VOCs emitted by different life forms to estimate the extent of volatile communication. We hope that our findings stimulate plant taxonomist and ecologists to explore the evolutionary and ecological relevance of such divergence.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Quantity of detected volatile organic compounds (VOCs) emitted from 13 species representing herbaceous and woody growth forms and Brassicaceae main lineages Aethionemeae, lineage I – III.

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