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Article

Studies on Volatile Organic Compounds of *Tuber borchii* and *T. asa-foetida*

Maurizio D'Auria[1]*, Gian Luigi Rana[2], Rocco Racioppi[1] and Alessandro Laurita[1]

[1]Dipartimento di Chimica "A.M. Tamburro," Università della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy, and
[2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy

*Author to whom correspondence should be addressed. Email: maurizio.dauria@unibas.it

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Ascomata of two truffle species, *Tuber borchii* and *T. asa-foetida*, both naturally growing in woodlands of the Basilicata region (southern Italy), were identified on the basis of ascospore morphology and compared under a volatile organic compound profile to determine the particular volatile organic compounds that characterize each taxon. Solid-phase microextraction–gas chromatography–mass spectrometry analysis of the samples showed the presence of 1-methyl-1,3-butadiene as a primary component in both truffles. *T. asa-foetida* showed a compound, toluene, not present in *T. borchii*, which creates the penetrating "solvent" smell of the truffle.

Introduction

Although studies on the biodiversity of hypogeous fungi naturally growing in Basilicata (southern Italy) began only 15 years ago, the number of truffles and false truffles thus far discovered in the region comprises approximately 65 taxa and puts Basilicata at the fourth position in the list of Italian regions that research this interesting field of mycology (1–3).

Among truffle species that grow and are collected in the region, *T. borchii* Vittad. follows only *T. aestivum* Vittad. in quantitative profile and *T. magnatum* Pico for qualitative features. In fact, its natural beds range from sea level to mountain (3) and its aroma is sweetish, of truffle, with a weak alliaceous component.

Its habitat is constituted of mixed forests or conifers and it is usually collected from October to April. Actually, *T. borchii* is considered as a complex species, comprising at least five other truffles; i.e., *T. gibbosum* Harkness, *T. puberulum* Berk and Br., *T. maculatum* Vittad., *T. foetidum* Vittad., and *T. dryophilum* Tul. and C. Tul., which have maturation periods and habitats or symbiotic plants (except for *T. gibbosum*) almost overlapping that of *T. borchii* (4).

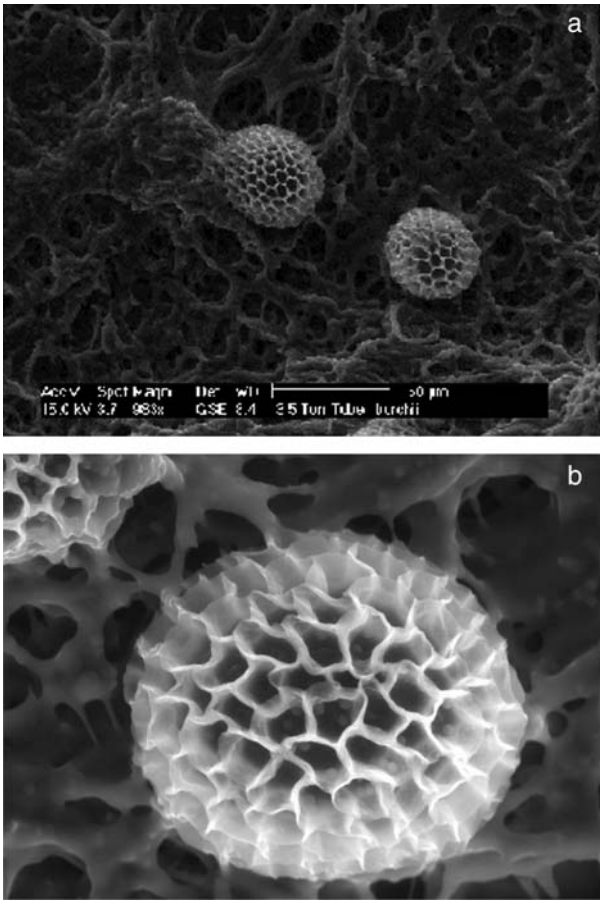

Figure 1. ESEM of *Tuber borchii*.

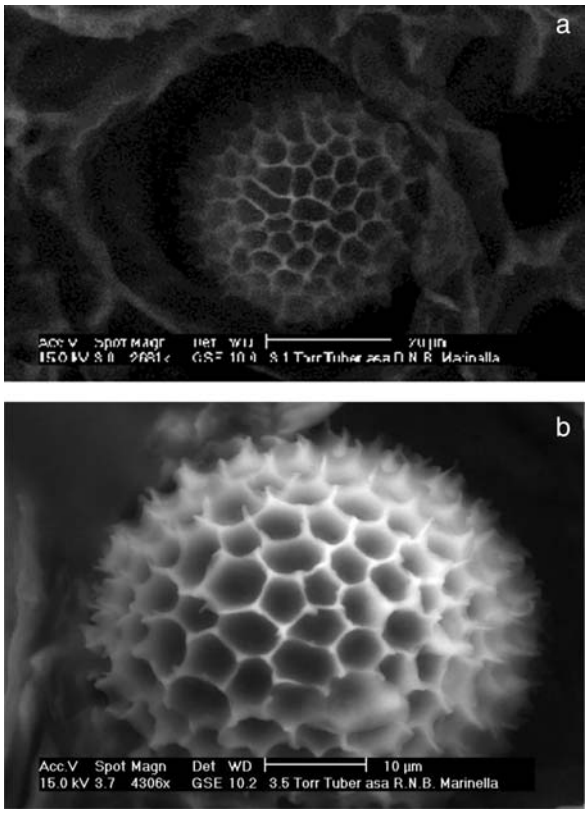

Figure 2. ESEM of *Tuber asa-foetida*.

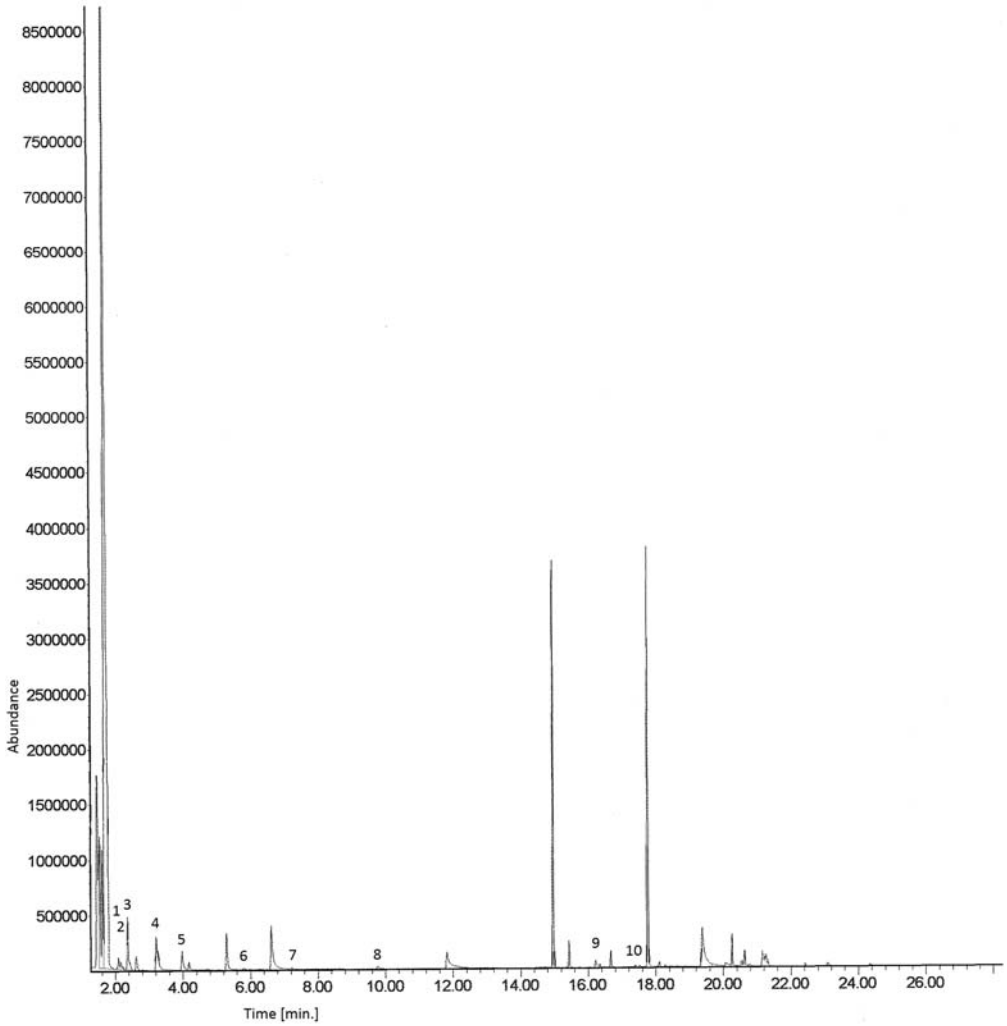

Figure 3. SPME–GC–MS chromatogram of a sample of *T. borchii*. Peak 1: 2-methylfuran, 2: 2-methyl-1-propanol, 3: 3-methylpropanal, 4: 3-methyl-1-butanol, 5: 3-methylthiophene, 6: xylene, 7: α-pinene, 8: 3,7-dimethyl-1,3,6-octatriene, 9: tetradecane, 10: 3-acetyl-1-propyl-5,6-dihydro-2-naphthol.

Another species of *Tuber* that naturally grows in Basilicata and can be macroscopically confused with *T. borchii* is *T. asa-foetida* Tul. and C. Tul., although it primarily grows in symbiosis with various species of *Cistus, Helianthemum, Ephedra* and other *Cistaceae* in sandy soils close to sea, has a strong, mouldy and nauseating odour and generally matures after *T. borchii*.

This study had the primary goal of differentiating the last two species of *Tuber*, either with an ultra-microscopic point of view, at spore level, or on the basis of their respective volatile organic compounds (VOCs), with the goal of eventually discovering those that render the ascomata of *T. asa-foetida* so repulsive and fetid.

Material and Methods

Truffle specimens were collected, with well-trained Epaniel Breton and Lagotto dogs in different maturation phases, in the following months of 2011 and areas of Basilicata: *T. borchii*, March, Foresta Mantenera (Tricarico, Matera province); *T. asa-foetida*, April, Riserva Naturale Biogenetica "Marinella-Stornara" (Bernalda, Matera).

Identification of the two truffle species was achieved on the basis of ascospore morphology using an Axioscop optical microscope (Karl Zeiss, Germany) furnished with a DS-U1 digital photocamera (Nikon, Japan). Ultra-microscopic features of ascospores of *T. borchii* and *T. asa-foetida* were observed with a scansion electron microscope for environmental analysis [Philips XL 30 Environmental Scanning Electron Microscope (ESEM)].

The collected specimens (ascomata at the beginning, middle and complete maturation) were carefully cleaned and then stored at 2–4°C in plastic bags for 48–72 h until the analysis was performed. The technique used to determine VOCs produced by the two truffle species was solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS), as described in detail by Mauriello *et al.* (5, 6).

In particular, a 100-μm PDMS-SPME module (57300-U, Supelco; Milan, Italy) was employed to determine VOCs. The

Table I
VOCs Identified in *T. borchii* and *T. asa-foetida*

Compound	Retention time (min)	Area %						
		T. borchii			*T. asa-foetida*			
		1	2	3	1	2	3	4
2-Methyl-1,3-butadiene	1.63	18.1	50.3	59.9	17.3	17.2	23.8	44.9
2-Butanone	2.07							3.9
2-Methylfuran	2.09		0.3	0.3		0.6		0.8
2-Methyl-1-propanol	2.16		0.3			8.1		5.2
Tetrahydrofuran	2.26				3.5		1.2	
3-Methylbutanal	2.30	0.4	1.5			0.3		
Benzene	2.49				0.6		0.8	
1-Methylpropyl formate	2.64							0.2
3-Methyl-1-butanol	3.30	0.9	1.8			16.9		0.4
2-Methyl-1-butanol	3.32							6.1
Toluene	3.81				2.1	1.1	1.2	0.2
3-Methylthiophene	4.06	0.9	0.8	0.5				
Xylene	5.80		0.1	0.3				
α-Pinene	7.21		0.1					
3,7-Dimethyl-1,3,6-octatriene	9.78		0.2					
Tetradecane	16.27	0.4	0.2	0.2		1.5		
3-Acetyl-1-propyl-5,6-dihydro-2-naphthol	17.39		0.1					
9-(Diphenylmethylene)-9H-fluorene	29.88	1.9						

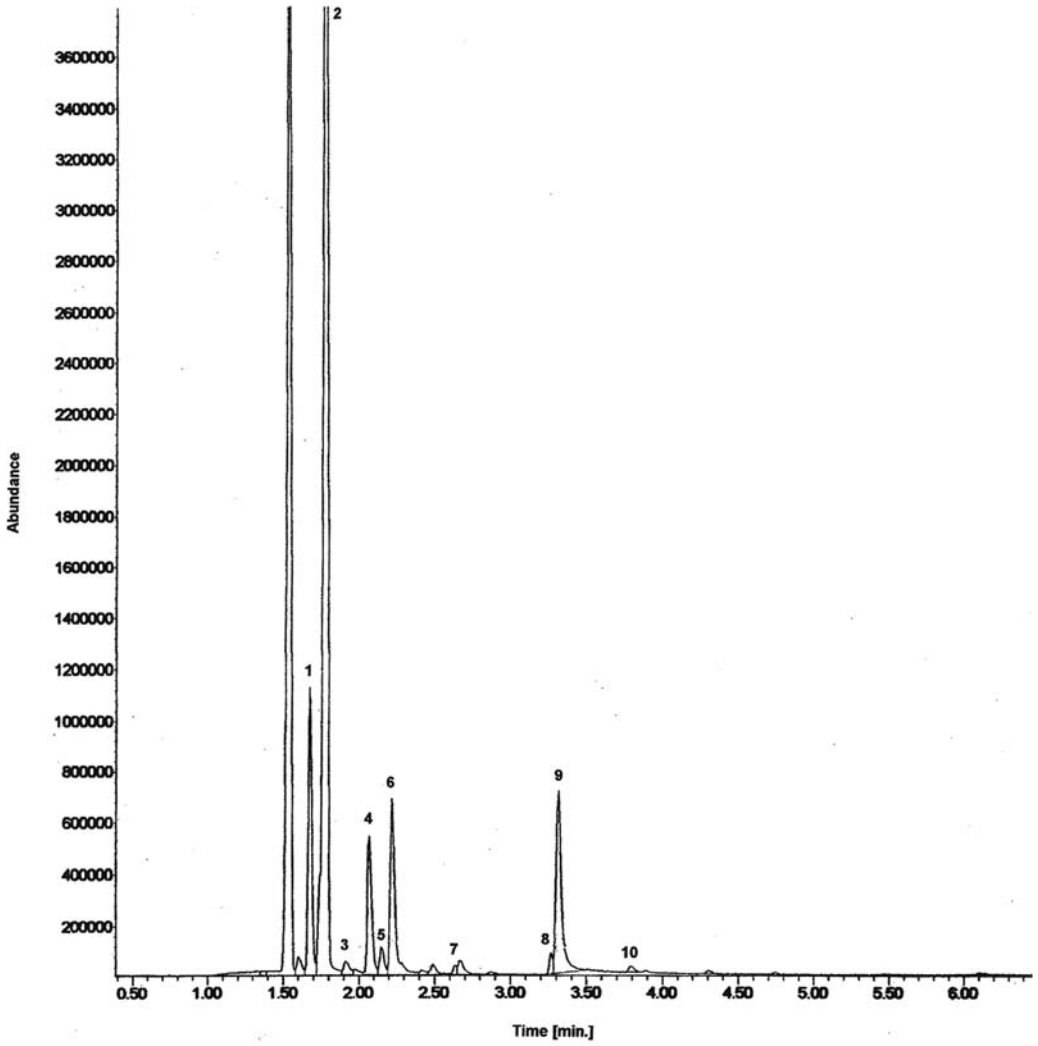

Figure 4. SPME–GC–MS chromatogram of a sample of *T. asa-foetida*. Peak 1: ethanol, 2: 2-methyl-1,3-butadiene, 3: propanol, 4: 2-butanone, 5: 2-methylfuran, 6: 2-methyl-1-propanol, 7: 1-methylpropyl formate, 8: 3-methyl-1-butanol, 9: 2-methyl-1-butabol, 10: toluene.

Table II
Concentration of Selected VOCs Identified in *T. borchii* and *T. asa-foetida*

Compound	Concentration (mg/g)						
	Tuber borchii			*Tuber asa-foetida*			
	1	2	3	1	2	3	4
2-Methyl-1-propanol		0.029			0.072		0.263
3-Methyl-1-butanol	0.170	0.322			1.139		0.046
2-Methyl-1-butanol							0.837

SPME fiber was maintained over a 1.0-g sample in a 20-mL vial at 36°C for 20 min. Analyses were accomplished with an HP 6890 Plus gas chromatograph equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm FT) (Agilent; Milan, Italy). An HP 5973 mass selective detector (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. A splitless injector was maintained at 250°C and the detector at 230°C. The oven was held at 40°C for 2 min, then gradually warmed, 8°C/min, up to 250°C and held for 10 min. Tentative identification of aroma components was based on mass spectra and Wiley 6 and NITS 98 library comparison. A single VOC peak was considered as identified when its experimental spectrum matched a score over 90% that present in the library.

Results

Ascospores of *T. borchii* and *T. asa-foetida* were easily distinguished from each other. In fact, in the first case, they primarily appeared to be ellipsoid, reticulate-alveolate with more than 7–8 poligonal meshes along the longer axis, but also subglobose, and variable in dimension (25–40 μm on average), but rarely reaching 25.6 × 48 μm (Figures 1A and 1B).

Ascospores of the second fungus species were, ellipsoid-subglobose, but also spherical, reticulate-alveolate with 6–8 meshes along their diameter and had dimensions averaging from 25–30 μm up to 35–45 μm (Figures 2A and 2B).

The SPME–GC–MS chromatogram of a sample of *T. borchii* is shown in Figure 3. We analyzed three samples and the results are reported in Table I. In a previous work in this field, we found that all samples lacked dimethylsulfide and that 2-methyl-1,3-butadiene and 1,2-pentadiene were the primary components of the volatile fractions (6). Furthermore, lower percentages of 1-methylpropyl formate, tetradecanal, tetradecane and 3-octanone were found (6). In other studies on this *Tuber*, both 2-methyl-1,3-butadiene and 1,2-pentadiene were not found, whereas 1-octen-3-ol was found to be the primary component of the VOCs. Furthermore, some sulfur compounds were found (7–9). The results we present here confirm our previous reported identification of the components of volatile fraction of *T. borchii* (6). In this study, we found that the primary component of the VOCs in *T. borchii* is 2-methyl-1, 3-butadiene, while the other components are present in very low amounts. Minor components were 3-methylbutanal, 3-methyl-1-butanol and tetradecane.

The SPME–GC–MS chromatogram of a sample of *T. asa-foetida* is shown in Figure 4. We analyzed four samples at different maturation levels (from immature, Sample 1, to very mature, Sample 4) and the results are reported in Table I. In this species of *Tuber*, the amount of 2-methyl-1,3-butadiene is lower than in *T. borchii*, although we observed the presence of other volatile compounds such as 2-butanone, 2-methyl-1 -propanol, 2-methyl-1-butanol and toluene in relevant amounts. Most likely, the latter compound is responsible for the "solvent" smell of this *Tuber*. In the case of 2-methyl-1-propanol, 3- methyl-1-butanol and 2-methyl-1-butanol, we were able to give a quantitative evaluation of the concentration of these compounds in the two considered *Tuber* species. The results are reported in Table II.

Conclusion

We have shown that both electron microscopy and gas chromatographic analysis of volatile compounds can be useful methods to characterize *T. borchii* and *T. asa-foetida*.

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