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Characterization of Some Mushroom and Earthy Off-Odors Microbially Induced by the Development of Rot on Grapes

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Grape rot is one of the major causes of degradation of many grape components and, thus, of deterioration in wine quality. In particular, the association of *Botrytis cinerea* with other, less visible, fungi frequently leads to the development of organoleptic defects in grapes and sometimes in wines. This study examines the nature of the volatile compounds responsible for mushroom, mossy, or earthy odors detected by gas chromatography−olfactometry in organic extracts of rotten grapes and musts. 2-Methylisoborneol, (−)-geosmin, 1-octen-3-one, 1-octen-3-ol, 2-octen-1-ol, and 2-heptanol were identified or tentatively identified. Their concentrations in musts were determined, and the impact of alcoholic fermentation by the yeast *Saccharomyces cerevisiae* was studied. The ability of fungi isolated from rotten grapes (*Botrytis cinerea*; *Penicillium* species including *P. brevicompactum*, *P. expansum*, *P. miczynskii*, *P. pinophilum*, *P. purpurogenum*, and *P. thomii*; *Aspergillus* section *nigri*; *Rhizopus nigricans*; and *Coniothyrium* sp.) to produce some of the identified compounds was evidenced.

KEYWORDS: ***Vitis vinifera*; grape; gray rot; earthy; 2-methylisoborneol; geosmin; 1-octen-3-one; 1-octen-3-ol; *Saccharomyces cerevisiae***

INTRODUCTION

Grape rot, mainly due to the *Botrytis cinerea* fungus, is one of the major causes of damage of a number of grape components and, thus, of deterioration in wine quality. In particular, phenolic compounds (anthocyanins, hydroxycinnamic acids, and flavanols) are oxidized by the polyphenol oxidase activity of *B. cinerea* (laccase activity) (*1*), and quinones resulting from the enzymatic oxidation of phenolic compounds are highly reactive with grape glutathion (*2*). Some aromatic components, like the monoterpenes that play a major role in the aromas of Muscat grapes and wines, are transformed into less odorous compounds (*3*, *4*). Ethyl esters of fatty acids, which contribute to the fermentation aromas of wine, are hydrolyzed by the esterase activity of *B. cinerea* (*5*). Moreover, several studies have shown that grape rot, due to the association of *B. cinerea* with other, less visible, fungi frequently leads to the development of organoleptic defects in grapes and wines (*6*−*8*).

Over the past 20 years, wine growers have observed organoleptic defects with mushroom, moldy, camphoric, or earthy odors in must made with rotten grapes and sometimes in wine (*6*). These effects are exacerbated by high rainfall during the vintage and hail damage. The compounds responsible for some of these defects have been identified. 1-Octen-3-ol is a well-known compound associated with fresh mushroom odors in grapes and wines (*9*, *10*). In previous studies, we identified (−)-geosmin [octahydro-4,8a-dimethyl-4a(2*H*)-naphthalenol], a powerful aromatic compound with an earthy smell, at levels above its olfactory perception threshold in some must and wine made with at least partially rotten grapes (*11*, *12*). La Guerche et al. (*13*) interpreted the origin of geosmin in the metabolism of *Penicillium expansum* fungus, in contaminated grapes modified by *B. cinerea*.

In this project, we identified several compounds with mushroom or earthy odors in rotten grapes. Concentrations were measured in grapes and wine and the impact of *Saccharomyces cerevisiae* yeast metabolism during alcoholic fermentation was estimated. The ability of various fungi isolated on rotten grapes to produce the identified compounds was determined.

MATERIALS AND METHODS

Chemicals and Biological Compounds. Sodium sulfate, 3-octanol, and pentane were from Sigma-Aldrich (St. Louis, MO). Pentane was distilled in order to improve its purity. Anhydrous ethyl alcohol was from Carlo Erba (Milan, Italy). The degree of purity of odorous compounds was determined by GC−MS analysis of an alcoholic

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solution of each volatile. 1-Octen-3-ol (93% purity), 1-octen-3-one (89% purity), fenchone (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one) (95% purity), 2-heptanol (94% purity), geosmin (93% purity), and 2-methylisoborneol (95% purity) were provided by Sigma-Aldrich (St. Louis, MO). Fenchol [(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol] was from two different suppliers: Sigma-Aldrich (St. Louis, MO) at 92% purity and Acros (Geel, Belgium) at 95% purity. 2-Octen-1-ol (91% purity) was from Lancaster (Morecambe, UK). Agar, malt agar medium, and chloramphenicol were from Sigma-Aldrich (St. Louis, MO).

Grape Samples and Volatile Extraction. Cabernet Sauvignon, Merlot, Gamay, Pinot Noir, Sauvignon, and Sémillon grapes visibly affected by gray rot were collected during the harvest and postharvest period in various French vineyards (Bordeaux, Beaujolais, Champagne, and Burgundy). To extract the volatile compounds, a whole grape bunch was put directly into 100 mL of distilled pentane for 1 h at room temperature, without agitation, with 1 mL of 3-octanol (10 mg/L) as internal standard. The pentane extract was then dried with anhydrous sodium sulfate, vacuum-concentrated to 10 mL using a rotary evaporating apparatus at 4 °C to prevent the volatilization of aroma compounds, and concentrated to 500 μL under nitrogen flow (approximately 100 mL/min).

Volatile Extraction from Must and Wine. A 500-mL sample of grape juice or wine was supplemented with 1 mL of 3-octanol (10 mg/L) as internal standard and then extracted in a 1-L flask by three successive extractions with distilled pentane (20, 20, and 10 mL, respectively) with magnetic stirring for 10 min each time. The combined organic phases were then dried using anhydrous sodium sulfate and concentrated to 100 μL under nitrogen flow (100 mL/min).

Fungus Isolation and Identification. First, visible rot was taken directly from grapes with a Pasteur pipet under a binocular microscope (×20) and spread on Petri dishes containing malt agar medium supplemented with 250 mg/L chloramphenicol. After 7−10 days' incubation at 20 °C, colonies were counted under a binocular microscope (×20). The microorganisms were identified, according to macroscopic and microscopic criteria (*14*, *15*). Some *Penicillium* species were also identified by ITS sequence analysis (*P. expansum*, *P. thomii*, and *P. purpurogenum*) (*16*), or by β-tubuline gene sequence analysis (*P. brevicompactum*, *P. miczynskii*, and *P. pinophilum*) (MUCL; Belgium).

Fungus Culture and Volatile Extraction on Solid Media. Spore suspension (20 μL) containing 1×10^7 conidia/mL was inoculated in polystyrene Petri dishes containing 20 mL of malt agar (MA) or grape juice medium (GJ; 15 mL of commercial grape juice and 5 mL of agar solution at 60 g/L) and stored at 20 °C for 4−6 days for *Penicillium* spp. and 6−10 days for other fungi prior to volatile extraction (*16*).

For each extraction, the solid medium was put directly into 100 mL of alcohol solution (20%; v/v) with magnetic stirring at 20 °C for 1 h. Then, 100 μL of 3-octanol (10 mg/L in ethanol) was added as internal standard and 5 and 2 mL of pentane were used in turn to extract the solution for 5 min each time. The combined organic phases were dried with anhydrous sodium sulfate and concentrated to 50 μL under nitrogen flow before analysis.

Gas Chromatography−Olfactometry (GC−O). The analysis was carried out on a Hewlett-Packard HP5890 gas chromatograph (Agilent, Palo Alto, CA) coupled with an olfactory detector using the ODO1 installation [Scientific Glass Engineering (SGE), Ringwood, Australia]. Two microliters of the extract were injected at 175 °C in splitless mode (valves opening at 60 s.) into a BP20 capillary column (SGE, 50 m, internal diameter (i.d.) 0.22 mm, μm film thickness 0.25). The GC temperature program started at 45 °C for 1 min, increasing to 230 °C at a rate of 3 °C/min, and maintained at 230 °C for 15 min. The carrier gas was hydrogen (U, Air Liquide, Floirac, France) with a column head pressure of 140 kPa (total flow 1 mL/min; purge flow 50 mL/min). The GC−O analysis of each extract was performed by two experimenters.

GC−Mass Spectrometry (GC−MS). An HP6890 gas chromatograph coupled to an HP5973 mass spectrometer in SCAN mode was used for analysis [electronic impact (EI) 70 eV; MSD interface temperature at 250 °C]. The carrier gas was helium (N60, Air Liquide, Floirac, France) with a column head pressure of 140 kPa (total flow 1 mL/min). The GC temperature program was as previously described.

Multidimensional GC Coupled with Mass Spectrometry (MDGC−MS). This analysis was carried out using an HP5890 connected to an HP6890 coupled with an HP5973 mass spectrometer. The two chromatographs were connected with a temperature-controlled transfer line following the chromatograph temperature program (45 °C for 1 min, increasing to 230 °C at 3 °C/min, and constant at 230 °C for 15 min). A Gerstel multicolumn switching system (Gerstel, Mülheim, Germany) was used for multidimensional analysis inside the HP5890 oven. The precolumn (BP20, 30 m, 0.32 mm i.d., 0.25 μm) was connected to the column switching device, where the compounds eluted from the first column could be eliminated or transferred directly into the analysis column (BPX5, 50 m, 0.22 mm i.d., 0.25 μm). The column head pressure was programmed to maintain the same helium flow (1.4 mL/min) during capillary analysis [i.e., 230 kPa at 45 °C for the (30 + 50) meter column, then 1.8 kPa/min increase]. At 27.5 min, there was a 4-min cut and the fraction was trapped on a BPX5 column at −50 °C under liquid nitrogen until the end of the temperature program. The trap was then warmed to 150 °C (temperature increase 50 °C/min) and then subjected to another program from 45 to 230 °C at 3 °C/min. The carrier gas was helium (N60, Air Liquide, France).

GC−MS Quantification. The compounds were quantified using the ratio of the areas of their main characteristic ions (*m*/*z* 112 and 111 for geosmin, *m*/*z* 95 and 108 for 2-methylisoborneol, *m*/*z* 81 and 154 for fenchol, *m*/*z* 81 and 152 for fenchone, *m*/*z* 55 and 70 for 1-octen-3-one, *m*/*z* 57 and 72 for 1-octen-3-ol, *m*/*z* 57 and 68 for 2-octen-1-ol, and *m*/*z* 45 and 55 for 2-heptanol), and of 3-octanol, the internal standard (*m*/*z* 59 and 83). The levels of each compound were quantified by comparison with standard graphs obtained by extracting grape bunches, musts, wines, and Petri dishes supplemented with several well-defined concentrations. For each compound, the regression coefficient (R^2) was calculated with five concentrations and the relative standard deviation of the method (RSD) was determined with three similar assays (*11*, *12*, *17*).

Olfactory Perception Threshold Determination. The perception thresholds of 2-methylisoborneol, (−)-geosmin, 1-octen-3-one, 1-octen-3-ol, 2-octen-1-ol, and 2-heptanol were determined by a directional triangular test at five increasing concentrations in distilled water, model solution similar to wine [12% ethanol, 5 g/L tartaric acid, pH 3.5 (*11*)], and neutral red wine. The solutions, presented in glasses corresponding to AFNOR (Association Française des Normes) standards, were tasted by a 60-person jury, except 2-heptanol and 2-octen-1-ol, when the jury consisted of 12 tasters. The odor perception threshold corresponded to the minimum concentration recognized by 50% of the tasters. The odor perception threshold of the 10% higher persons was also specified.

Alcoholic Fermentation of Rotten Grape Juices in the Presence or Absence of (−)-Geosmin, 2-Methylisoborneol, 1-Octen-3-one, or 1-Octen-3-ol; Storage in Model Solution, Similar to Wine. Grape juices were inoculated with 100 mg/L active dry yeast (strain VL3c, *Saccharomyces cerevisiae*; Laffort Oenologie, France) and stored at 24 °C for about 2 weeks of alcoholic fermentation.

1-Octen-3-one (5 μg/L) and 1-octen-3-ol (5 μg/L) were added to 1.5 L of botrytized Sémillon must. The must was put into five 375-mL bottles, inoculated with 100 mg/L active dry yeast (strain VL3c, *S. cerevisiae*; Laffort Oenologie, France), and stored at 24 °C. Must samples (200 mL) were taken during alcoholic fermentation. Volatiles were extracted from the 200-mL samples and then subjected to GC analysis. The same protocol was applied for (−)-geosmin (100 ng/ L), added to 1.5 L of botrytized Sémillon must, and 2-methylisoborneol (3.5 μg/L), added to 1.5 L of botrytized Sémillon must and 1.5 L of Cabernet Sauvignon must. All experiments were done separately for 1-octen-3-one and 1-octen-3-ol, (−)-geosmin, and 2-methylisoborneol.

(−)-Geosmin (100 ng/L) and 2-methylisoborneol (35 μg/L) were separately added to 1 L of model solution [12% ethanol, 5 g/L tartaric acid, pH 3.5 (*11*)]. The (−)-geosmin solution was stored at 10 or 20 °C for several months and the 2-methylisoborneol solution at 20 °C for up to 3 weeks. Volatiles were extracted from 200-mL samples of these solutions taken during storage and subjected to GC analysis.

Table 1. Main Volatiles with Mushroom or Earthy Odors in Rotten Grapes and Musts Made with Rotten Grapes, Detected by Gas Chromatography Coupled with Various Detection Modes

	Kovats indices				olfactory perception threshold[b] (μg/L)					
					water		model solution		red wine	
compd	BP20	BPX5	odor	identification mode[a]		10% higher		10% higher		10% higher
1, 1-octen-3-one	1290	973	mushroom	GC–O, KI, MS	0.003	0.001	0.03	0.015	0.07	0.03
2, 2-heptanol	1320	–	mushroom	GC–O, KI, MS	100	50	–	–	–	–
3, fenchone	1388	1014	earthy–camphor	MS, KI	500	250	–	–	–	–
4, 1-octen-3-ol	1438	978	mushroom	GC–O, KI, MS	2	0.5	20	10	40	25
5, unknown	1471	1003	mushroom	GC–O	–	–	–	–	–	–
6, unknown	1528	1344	moss	GC–O	–	–	–	–	–	–
7, 2-methylisoborneol	1537	1210	earthy–camphor	GC–O, KI, MS	0.012	0.006	0.04	0.02	0.055	0.04
8, fenchol	1549	1182	earthy–camphor	MS, KI	50[c]	30	–	–	–	–
9, 2-octen-1-ol	1598	1211	mushroom	GC–O, KI	50	40	–	–	–	–
10, unknown	1667	–	earthy	GC–O	–	–	–	–	–	–
11, geosmin	1869	1497	earthy	GC–O, KI, MS	0.01	0.005	0.04	0.015	0.05	0.02
12, unknown	1891	1459	mushroom	GC–O	–	–	–	–	–	–

[a] GC–O, gas chromatography coupled with olfactometry; KI, Kovats indices; MS, mass spectrometry. [b] Thresholds determined in a tasting room specifically used for sensorial analysis. [c] Fenchol was determined for the (1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol.

Table 2. Levels of Some Volatiles with Mushroom or Earthy Odors in Rotten Grapes and Musts Made with Rotten Grapes, Determined by Gas Chromatography–Mass Spectrometry

	in must			in grapes		
compd	samples[a]	concn ranges (μg/L)	RSD[b] (%)	samples[a]	concn ranges (μg/bunch)[c]	RSD[b] (%)
1, 1-octen-3-one	1 CS^1, 2 M^2, 5 $S^{2,3}$, 3 PN^5	0.01–0.13	6.4	–	–	–
2, 2-heptanol	2 $S^{2,3}$	0.8–4	10	–	–	–
3, fenchone	2 S^2	0.06–0.25	7.9	1 S^2	0.06	5.2
4, 1-octen-3-ol	1 CS^1, 2 M^2, 2 G^4, 4 $S^{0,2,3}$	6–21	4.3	1 M^1, 2 G^4	10–50	3.9
7, 2-methylisoborneol	1 CS^2, 3 $PN^{3,4}$	0.045–0.15	3.9	1 CS^1, 2 M^2, 1 PN^5	0.17–0.5	9.1
8, fenchol	1 S^2	0.01	5.1	–	–	–
9, 2-octen-1-ol	2 S^2	0.35–0.5	7.9	–	–	–
11, geosmin	5 G^4, 1 PN^4	0.08–0.23	5.1	1 CS^4, 3 G^4, 1 PN^5	0.5–2	8.2

[a] CS, Cabernet Sauvignon; M, Merlot; G, Gamay; PN, Pinot Noir; S, Sémillon; ^{0}vintage 2000, ^{1}vintage 2001, ^{2}vintage 2002, ^{3}vintage 2003, ^{4}vintage 2004, ^{5}vintage 2005. [b] RSD: relative standard deviation of the method of quantification. [c] Average weight of a grape bunch 200 g.

RESULTS AND DISCUSSION

Characterization of Volatiles with Mushroom or Earthy Odors in Grapes and Musts Made with Rotten Grapes. Cabernet Sauvignon, Merlot, Gamay, Pinot Noir, and Sémillon grapes and musts with mushroom or earthy odors were collected from various French vineyards (Bordeaux, Beaujolais, Champagne, and Burgundy) between 2000 and 2005 and extracted before analysis of volatile compounds by gas chromatography−olfactometry (GC−O) and gas chromatography−mass spectrometry (GC−MS). The olfactory perception thresholds of the compounds identified were determined in a tasting room specifically used for sensorial analysis.

Six odorous zones with mushroom odors were detected in grapes and musts extracts by GC−O analysis and characterized by their Kovats Indices (KI) on a BP20 capillary column: (**1**) KI 1290, (**2**) KI 1320, (**4**) KI 1438, (**5**) KI 1471, (**9**) KI 1598, and (**12**) KI 1891 (**Table 1**). Three of them (**1**, **2**, and **4**), corresponding to 1-octen-3-one, 2-heptanol, and 1-octen-3-ol, respectively, were identified by GC−MS. Another was tentatively identified as 2-octen-1-ol (**9**). 1-Octen-3-one (**1**) was frequently detected by GC−O in extracts from musts made with rotten Cabernet Sauvignon, Merlot, Sémillon, and Pinot Noir grapes, and it could be assayed at levels up to 0.13 μg/L (Pinot Noir musts, 2005) (**Table 2**). Considering its very low perception threshold in water (in the vicinity of 0.003 μg/L), smaller that what was described in the literature [0.05 μg/L (*18*)], this compound is presumed to be present at concentrations above its perception threshold in musts. 1-Octen-3-ol (**4**) was present at concentrations between 6 and 21 μg/L in several musts made with different grape varieties (Cabernet Sauvignon, Merlot, Gamay, and Sémillon) and up to 50 μg/grape bunch in Merlot and Gamay grapes, i.e., 270 μg/kg (**Table 2**). The olfactory perception threshold of 1-octen-3-ol determined in the laboratory (3 μg/L in water) is a 1000-fold higher than that of 1-octen-3-one. These results corroborate a recent study relating this threshold at 11 μg/L in water (*19*). 1-Octen-3-one and 1-octen-3-ol are well-known metabolites of various ascomycetes and basidiomycetes as well as unsaturated lipid degradation products (*20*). 1-Octen-3-one has been identified in grapes (*21*) and in some wines potentially contaminated by tainted cork (*22*, *23*), whereas 1-octen-3-ol, which constitutes one of the main characteristic fungal odor (*24*−*26*), has frequently been cited as a marker for rotten grapes and wine (*9*, *10*).

2-Octen-1-ol (**9**) has already been reported to be produced by molds like *Aspergillus* and *Penicillium* (*24*, *25*, *27*). It was quantified in different Sémillon musts at levels up to 0.5 μg/L (**Table 2**), but was not detected by GC−O in red musts.

Considering its olfactory threshold, close to 50 μg/L in water, its impact on wine aroma is likely to be limited.

2-Heptanol (**2**) was quantified in two Sémillon musts obtained from rotten grapes at levels up to 4 μg/L (**Table 2**), a concentration lower than its perception threshold (100 μg/L in water). This compound has surprisingly been detected in Monastrell and Cabernet Sauvignon grape juices with a positive impact (*28*). Its evidence as an off-flavor in virgin olive oil, with an earthy odor (*29*), is more in accordance with our observations. The recent identification of this compound as a metabolite of *Penicillium camemberti* and *P. roqueforti* (*30*) makes it possible to postulate for its fungal origin in must.

The five odorous zones detected by GC−O corresponding to earthy odors could be divided into two groups (**Table 1**). One group of three odorous zones had earthy and camphor odors (**3**, KI 1388; **7**, KI 1537; and **8**, KI 1549 on a BP20 capillary column), while the group of two odorous zones corresponding to an unidentified compound (**10**) (KI 1667) and geosmin (**11**) had specific earthy odors. In the first group, fenchone (**3**) (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one) was identified in Sémillon grapes and musts by GC−MS and matching the Kovats indices with those of a pure compound and was quantified at levels close to 0.25 μg/L and 0.06 μg/bunch (**Table 2**). We determined an olfactory perception threshold of this compound in water at 500 μg/L, a value in close accordance with published data (*18*). So, it is clear this compound will be only perceived in must or wine at high concentrations. Fenchol (**8**) was also identified by GC−MS and matching the Kovats indices with those of a pure compound. The olfactory perception threshold in water was only determined for (1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]-heptan-2-ol at concentrations of 50 μg/L for 50% of the tasters. Fenchol was rarely detected by GC−O and assayed at low concentration in a Sémillon must (0.01 μg/L), a concentration well below its olfactory perception threshold.

An extract of rotten Merlot grapes was analyzed by multidimensional GC coupled with mass spectrometry (MDGC−MS) and zone **7** on the chromatogram revealed a mass spectrum corresponding to 2-methylisoborneol (1,2,7,7-tetramethyl-*exo*-bicycloheptan-2-ol or MIB) (**Figure 1**). Unlike fenchone and fenchol, MIB was only found in black grapes (Cabernet Sauvignon, Merlot, and Pinot Noir). In Merlot grapes from the Graves region (Bordeaux) in 2002, MIB levels reached to 0.5 μg/grape bunch, i.e., 2.5 μg/kg. Concentrations in musts made with black grape varieties (Cabernet Sauvignon and Pinot Noir) were between 0.045 and 0.15 μg/L, but this compound has never been detected in botrytized Sémillon musts, irrespective of the vintage (**Table 2**). Moreover, MIB has never been detected in wine. MIB has frequently been found in rotten grapes and musts made with rotten grapes, as well as in wine marked by cork taint (*22*, *23*). We determined for this compound a low perception threshold of 0.012 μg/L in water, similar with what has already been described, 0.017 μg/L (*19*). MIB is a metabolite of *B. cinerea* (*31*), and some *Penicillium* species as well as bacteria in the *Streptomyces* genus have also been found to produce this compound (*32*, *33*).

In the second group, earthy odorous zone **10** (KI 1667) was only detected in a Sémillon must, whereas geosmin (**11**) was not only assayed in grapes and must from Cabernet Sauvignon, Merlot, and Sémillon varieties (*11*) but also in Gamay, Pinot Noir, and Chenin Blanc (**Table 1**). (−)-Geosmin concentrations up to 0.3 μg/L have already been measured in must and wine made with several black and white grape varieties (*11*, *12*). Then, in 2004, geosmin levels were measured in three Gamay bunches and one Cabernet Sauvignon bunch. They varied from

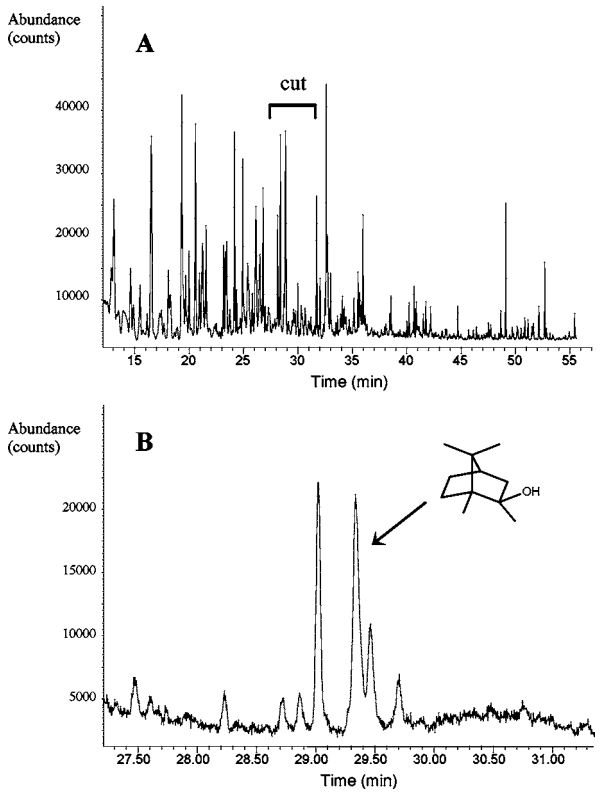

Figure 1. Multidimensional gas chromatography analysis of rotten Merlot grapes extract: (**A**) precolumn chromatogram of the extract (10% effluent detected by flame ionization) and (**B**) main column chromatogram of the extract.

0.7 to 2 μg/bunch in the Gamay bunches, and the Cabernet Sauvignon bunch contained 0.5 μg of this compound (**Table 2**). Considering the low perception threshold of this earthy compound, close to 0.010−0.023 μg/L (*19*), the average weight of a grape bunch (200 g), and the approximate volume of 0.75 L obtained from 1 kg grapes, only 1−3% contaminated grape bunches will result in at least 0.1 μg/L geosmin in the must, a concentration which is above the perception threshold of this compound. Geosmin is a very well-known metabolite of several microorganisms such as bacteria (*Streptomyces* sp.) or fungi (*Penicillium* sp.) (*34*, *35*), and the study of its origin in grapes has already demonstrated that the action of two fungi, *B. cinerea* and *P. expansum*, is necessary to generate this compound on grapes (*13*).

Odorous zone **6** (KI 1528), which has a mossy aroma, was also found in a Sémillon must in 2002 (**Table 1**).

Chemical Transformation Kinetics of the Main Mushroom and Earthy Compounds in Must and Model Solution. Among the odorous zones characterized, some corresponding to **2**, **5**, **6**, **9**, **10**, and **12** were not detected on both capillary columns by GC−O in wines done with rotten Sémillon musts, which may suppose of chemical transformation of the volatiles or a masking effect by GC−O due to other volatiles produced during alcoholic fermentation.

First, the behavior of mushroom compounds, 1-octen-3-one (**1**) and 1-octen-3-ol (**4**), was tested. After 2 days of alcoholic fermentation by *S. cerevisiae* of a Sémillon must supplemented with the volatiles, the totality of 1-octen-3-one had disappeared, whereas only 20% of 1-octen-3-ol did (**Figure 2**). Previous studies had already described the relative stability of 1-octen-3-ol and the disappearance of 1-octen-3-one during alcoholic fermentation of a Cabernet Sauvignon must, interpreted by the enone reductase activity of *S. cerevisiae* yeast (*21*, *36*).

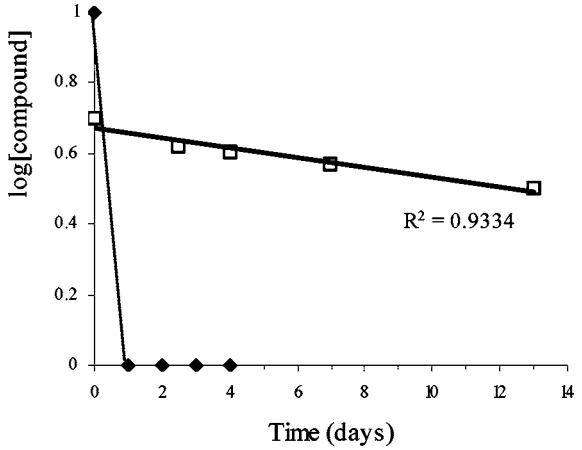

Figure 2. Degradation kinetics of 1-octen-3-one (□) and 1-octen-3-ol (◆) in a botrytized Sémillon must during alcoholic fermentation.

However, a mushroom odorous zone at the retention time of 1-octen-3-one was sometimes detected by GC−O in wines made with rotten Sauvignon Blanc and Pinot Noir grapes (results not shown). Furthermore, another paper has cited 1-octen-3-one concentrations in wines from 0.007 to 0.061 μg/L (*37*). These results suggest either that 1-octen-3-one concentration was very high in musts at the beginning of fermentation and therefore was not completely metabolized, that some yeast strains had a limited enone reductase activity during alcoholic fermentation, or that this compound has another origin. Moreover, fenchone (**3**) and fenchol (**8**) added to a red must and a botrytized Sémillon must have been detected at concentrations 30% lower after alcoholic fermentation (*38*).

Next to these compounds, (−)-geosmin (**11**) and 2-methylisoborneol (**7**) are two main compounds considered to be responsible for earthy defects. Their behavior during the alcoholic fermentation of must and storage in model solution was then investigated. Only 20% of the geosmin in a botrytized Sémillon must disappeared after 2 weeks of alcoholic fermentation (**Figure 3A**). In a model solution similar to wine, 50% of the initial geosmin content had disappeared after 2 months at 20 °C and 8 months at 10 °C (**Figure 3B**). (−)-Geosmin was relatively stable during alcoholic fermentation and storage, especially at the optimum temperature for aging wine (15 °C), which corroborated previous studies (*23*). Other results demonstrated the disappearance of 90% of the geosmin in a model solution after 24 h at 90 °C (*17*). Geosmin degradation is apparently highly dependent on temperature. Geosmin chemical transformation leads to argosmin, a much less odorous compound (*39*).

In a model solution, the MIB concentration decreased by 80% after only 7 days at 20 °C and no MIB was quantified after 20 days (**Figure 4**). After 2 days of alcoholic fermentation, the MIB concentration of a botrytized Sémillon must decreased by 40% and was only 10% of the initial value after 2 weeks. Moreover, MIB degraded faster during the fermentation of Cabernet Sauvignon must, as it was no longer detectable after only 6 days. 2-Methylenebornane and 2-methyl-2-bornene have previously been identified as dehydration or degradation byproducts of 2-methylisoborneol (*40*). These compounds are not supposed to have any earthy odor (*41*). We did not detect them by GC−MS and no earthy odorous zone was evidenced by GC−O in model solution or wine obtained at the end of these experiments.

Fungi Responsible for Mushroom or Earthy Odors on Solid Media. In order to identify the origin of the compounds

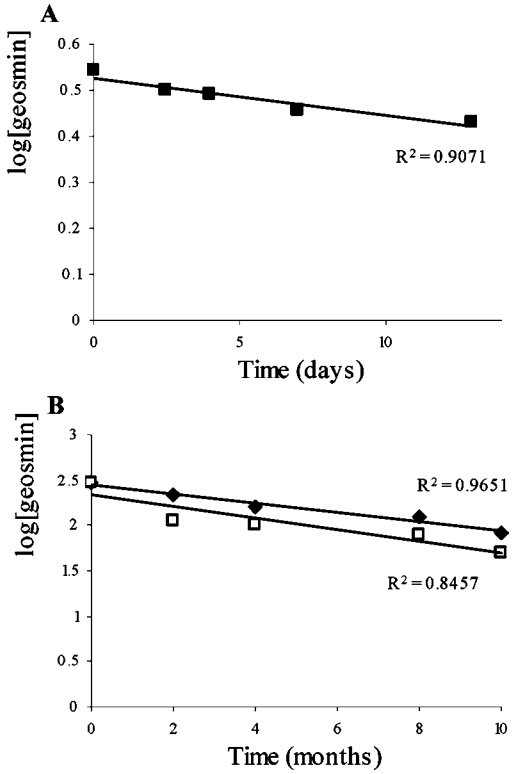

Figure 3. Degradation kinetics of (–)-geosmin: (**A**) in a botrytized Sémillon must during alcoholic fermentation and (**B**) over 10 months of storage in model solution at 10 °C (◆) and 20 °C (□).

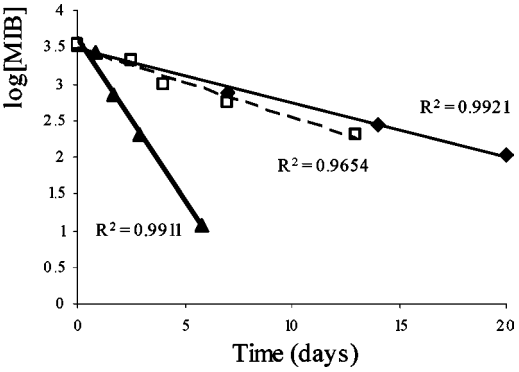

Figure 4. Degradation kinetics of 2-methylisoborneol (MIB) in model solution (◆), Cabernet Sauvignon must during alcoholic fermentation (▲), and botrytized Sémillon must during alcoholic fermentation (□).

responsible for mushroom or earthy odors in grapes, must, or wine, the microbiota on grapes from areas affected by this problem were analyzed and their potential to produce any of the compounds identified was tested on two media: malt agar (MA) and grape juice (GJ).

Among the fungi isolated from grapes, no representative was found to produce fenchol, fenchone, 2-heptanol, and 2-octen-1-ol. Only five genera were able to produce mushroom or earthy odors on MA and GJ media (**Table 3**). *Aspergillus* section *nigri* synthesized 1-octen-3-ol and 1-octen-3-one on MA, resulting in mushroom odors. These results corroborate previous studies on 1-octen-3-ol production by *Aspergillus* sp. (*42*, *43*).

Several *Penicillium* species isolated from grapes were able to produce compounds with mushroom or earthy odors: *P. expansum* synthesized geosmin on MA, and *P. carneum* synthesized this compound on MA and GJ; among the 49 *P. expansum* isolates tested, 37 from Bordeaux region had already been described to produce geosmin on MA (*16*), and the 12

Table 3. Fungi Isolated from Rotten Grapes Responsible for Mushroom or Earthy Odors in Solid Media

fungi	no. of isolates tested	odor[a] on MA	odor[a] on GJ	identified compds[b]	concentration ranges (ng/Petri dish)[c] on MA	concentration ranges (ng/Petri dish)[c] on GJ
A. nigri	1	mushroom	–	1-octen-3-ol	<5–50	<5
				1-octen-3-one	nq	<5
B. cinerea	7	–	–	–	nq	nq
	34	mushroom	mushroom	1-octen-3-ol	<5–30	<5
				1-octen-3-one	<5–5	<5
	32	earthy–camphor	earthy–camphor	MIB	<5–14	<5
	1	mushroom	–	unknown	nq	nq
Coniothyrium sp.	1	mushroom	–	unknown	nq	nq
	1	earthy–camphor	–	MIB	<5–9	<5
P. brevicompactum	1	mushroom	–	1-octen-3-ol	nq	nq
				1-octen-3-one	nq	nq
P. carneum	1	earthy	earthy	geosmin	650–800	700–1000
P. citreonigrum	1	cellar	–	unknown	nq	<5
P. decumbens	1	–	earthy	geosmin	<5	66
P. expansum	49	earthy	–	geosmin	30–140	<5
P. miczynskii	2	earthy	earthy	geosmin	210–250	130–190
P. pinophilum	1	earthy	earthy	geosmin	150	<5–180
P. purpurogenum	3	–	camphor	unknown KI 1479	nq	nq
	1	earthy	–	geosmin	15–37	<5
	1	cellar	–	unknown	nq	nq
P. thomii	8	–	–	–	nq	nq
	3	mushroom	mushroom	1-octen-3-ol	<5–42	<5
	3	earthy–camphor	–	MIB	<5–9	<5
R. nigricans	1	earthy–camphor	–	MIB	<5–11	<5

[a] No odor detected; MA, malt agar medium, GJ, grape juice medium. [b] Kovats index (KI) on a BP20 capillary column; MIB, 2-methylisoborneol. [c] Each result corresponds to the average value of three repetitions of Petri dish cultures; <5 means less than 5 ng/Petri dish, the limit of quantification; nq, not quantified.

last tested, coming from three other French vineyard, corroborate these results. In contrast to *P. carneum*, which synthesized geosmin on both media, any *P. expansum* isolate produced this compound on GJ. Thus, geosmin genesis by *P. expansum* seems to be independent of the isolate but dependent on the culture conditions. Both *P. miczynskii* isolates produced geosmin on MA and GJ, as well as *P. pinophilum*; to our knowledge, these two species (*P. miczynskii* and *P. pinophilum*) had never been cited to produce this compound, whereas *P. expansum* and *P. carneum* had (*16*, *32*, *44*). Among the 11 *P. thomii* isolates tested, three synthesized 1-octen-3-ol on both media and MIB on MA, whereas other isolates did not produce any odor. *P. thomii* had never previously been found to produce MIB, unlike *P. camemberti*, *P. discolor*, and *P. caseicolum* (*32*, *45*, *46*). Therefore, the ability of *P. thomii* to synthesize 1-octen-3-ol or MIB seems to be dependent on the isolate and culture conditions. As *P. glabrum*, *P. roqueforti* (*47*), *P. chrysogenum* (*24*, *32*), and *P. verrucosum* (*48*), or *P. brevicompactum* produced 1-octen-3-ol and also 1-octen-3-one, smelling of mushrooms, on MA, *P. citreonigrum* and some *P. purpurogenum* isolates synthesized cellar odors on MA, whereas another isolate of *P. purpurogenum* produced a camphor odor on GJ. The compound responsible for this odorous zone has a Kovats index of 1479 on a BP20 column, but it has not yet been identified.

Two other fungi, *Rhizopus nigricans* and *Coniothyrium* sp., produced MIB on MA. They had never been described to produce this compound before.

Isolates of *B. cinerea* may be divided into several types. Some did not produce any mushroom odor, irrespective of the medium, while a number of isolates synthesized 1-octen-3-ol and 1-octen-3-one. This was also true for the production of earthy-smelling 2-methylisoborneol.

These different aromatic profiles illustrate the potential intraspecific variability among isolates of some species in terms of aroma compounds produced. This is especially true for *B. cinerea*, *P. purpurogenum*, and *P. thomii*. These results constitute a preliminary study on the potential of some fungi to produce mushroom- and earthy-smelling compounds. However, the influence of some biotic and abiotic factors on the metabolism of production of these compounds by these fungi should be specified, in particular in the context of bunch rot complexes (*49*).

Conclusions. Some volatiles with mushroom or earthy odors have been identified or tentatively identified and assayed in rotten grapes of various grape varieties, as well as in musts made with rotten grapes. Then, the incidence of alcoholic fermentation by *S. cerevisiae* on the evolution of the most odorous compounds was measured in order to quantify their impact on wine aroma.

Fenchone and fenchol, as well as 2-heptanol and 2-octen-1-ol, have high perception thresholds, so only high concentrations will be perceived in must or wine. 2-Methylisoborneol and 1-octen-3-one have low perception thresholds and disappear during alcoholic fermentation, so they may not have a negative impact on wine aroma. However, 1-octen-3-one is sometimes detected in wines made with Sauvignon and Pinot Noir grapes, which suggests that the laboratory conditions are not similar to those of alcoholic fermentation in wineries, or that this compound has another origin during winemaking. 1-Octen-3-ol and geosmin have relatively low perception thresholds, persist after alcoholic fermentation, and may be responsible for defects in wine.

Microflora analysis has made it possible to assess the potential of some fungi to produce mushroom- and earthy-smelling compounds. Some of them have been evidenced to contribute to the development of off-flavors on grape juice and their study is important for developing targeted strategies in order to prevent problems in the vineyard.

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