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Influence of some nutrional factors on the development of *Penicillium sp.* and *Botrytis sp.* responsible of the "musty-earthy" odor in wine

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Abstract

After 1999, the white and red wines from France presented an earth-mold taste "musty-earthy" associated with the development of moulds more or less visible on grapes.

The aim of this study was the influence of different factors (copper, the nature of the nitrogen source, linoleic acid and ergosterol) on the development of Penicillium sp. and Botrytis sp. (isolated or in association) and on the production of the "musty-earthy" odor. If this odor were perceived, the extraction of the substances responsible for musty-earthy taste (geosmin, 2-methylisoborneol) was carried out and the compounds identified by gas chromatography coupled with the mass spectrometry.

Keywords: wine quality, geosmin, *Botrytis sp.*, *Penicillium sp.*

Introduction

For several years, a certain number of French vineyards have been marked by aromatic deviations evoking the wet ground, the humus, the fresh mushroom [3, 4, 5, 8]. The development of micro-organisms (yeasts, bacteria, mushrooms) on grape bay can have, inter alias consequences, of the effects prejudicial on organoleptic quality of musts and the wines (Semillon, Cabernet Sauvignon and Gamay) producing a fungic, musty or earthy character [1, 2, 6, 11]. These defects are associated to the presence of specific odorous molecules. Analyses by olfactometry coupled with the mass spectrometry made it possible to highlight, in the majority of these wines, the presence of trans-1, 10 dimethyl-trans-9-decalol, more known under the name of geosmin [7, 8, 12, 16]. Geosmin has been identified from pure cultures *Penicillium sp*. (fungi) and *Streptomyces sp* (filamentous bacteria) isolated on rotten grapes. The analysis of microflora grape was maked by gas-chromatography coupled with mass spectrometry method [10, 14, 15, 16]. This method demonstrated that only two species of *Penicillium sp* produced geosmin in the presence of *Botrytis cinerea: Penicillium expansum* and *Penicillium roqueforti* [8, 9, 12, 13, 16]. Further, it has been considered that *Penicillium expansum* on grape produces geosmin only in presence of *Botrytis cinerea* leading to the "musty-earthy" odor of wine [1, 2, 10, 13].

The aim of our research was to study the influence of various factors (copper) or the composition of the grape (natural source of nitrogen, linoleic acids and ergosterol) on the

production of musty-earthy odor by of *Penicillium sp.* and *Botrytis sp*. isolated or in association.

Materials and Methods

Strains

- *Botrytis cinerea 1(B1)* isolated from Givry region
- *Botrytis cinerea 2 (B2)* isolated from Givry region
- *Penicillium expansum 1619 (P1619)* isolated from ITV de Tours
- *Penicillium expansum 1627 (P1627)* isolated from ITV de Tours

Botrytis sp. and *Penicillium sp.* were cultivated on proposed culture media as explants mycelium form. These fungal cultures were incubated at 28°C.

Culture medium

For the study of the influence of different factors (copper, the nature of the nitrogen source, linoleic acid and ergosterol) on the development of *Penicillium sp.* and *Botrytis sp*. and on the production of the odorant molecules by molds, we added these compounds in various concentrations to the basal Czapek medium (table 1):

Table 1 Modified Czapek medium

N°	Basal medium	Cu(mg/l)	Nitrogen (g/l)	linoleic acid 2.5g/l	Ergosterol 2.5g/l
1.	Czapek medium	12,725	$SO_4(NH_4)_2$ **2,26**	-	-
2.	Czapek medium	12,725	NO_3Na **3**	-	-
3.	Czapek medium	-	$SO_4(NH_4)_2$ **2,26**	-	-
4.	Czapek medium	-	NO_3Na **3**	-	-
5.	Medium Czapek + NO_3^- + Cu^{2+}	12,725	NO_3Na **3**	+	+
6.	Medium Czapek + NO_3^- + Cu^{2+}	12,725	NO_3Na **3**	+	-
7.	Medium Czapek + NO_3^- + Cu^{2+}	12,725	NO_3Na **3**	-	+
8.	Medium Czapek + NO_3^- + Cu^{2+}	12,725	NO_3Na **3**	-	-

Gas chromatography-mass spectrometry analysis and quantification of odorant's molecules

Extraction volatiles compounds (geosmin and 2-methylisoborneol) were achieved by magnetic stirring (250 rpm) at room temperature for 1 hour. After filtration, the supernatant was diluted in ultra pure water for analysis. The analysis volatiles compounds were carried out by the method of Dumoulin and Riboulet [6]. Gas chromatography-mass spectrometry method is based on the adsorption and the desorption of the volatile compounds on an adapted fiber (one noted that the mixed fibers e.g. PDMS-Divinyl-benzen-Carboxen, PDMS-DVB are often adapted better than simple fibers e.g. polydimethylsiloxan (PDMS), polyacrylat) on samples heated between 40 and 65°C, for a duration from 30 to 60 minutes and under agitation.. Solutions of 10µg/litre of geosmin and geosmin d5 were prepared by dilution in absolute ethanol. The geosmin d5 was used as internal standard.

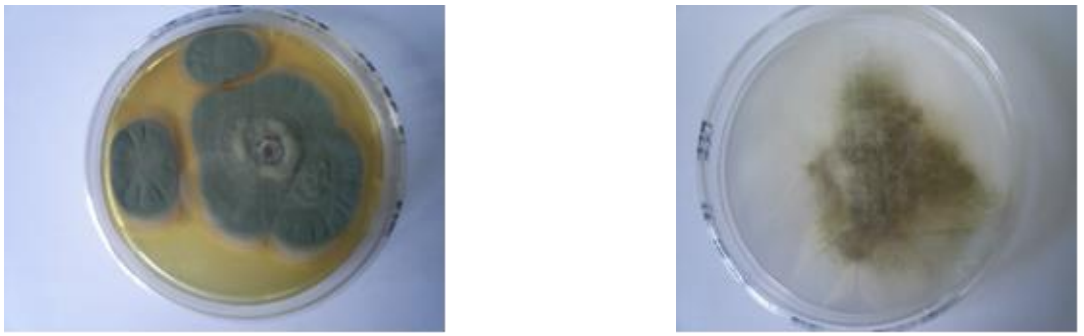
Results evaluation

A jury of 4 people appreciated the growth and the sporulation, to which it allotted notes ranging between 0 and 3 according to their importance. The produced odor was described; it was allotted a note of 1 to qualitative “musty” and a note of 0.5 to qualitative fungal and yeasts. The results were also presented in percent %.

Results and Discussions

For a better understanding of the production metabolism of the geosmin and the 2-methylisoborneol, two isolates (P1619 and P1627) of *P. expansum* and two isolates (B1 and B2) of *Botrytis cinerea* were cultivated single or associated on various modified Czapek media, presented in the Materials and Method chapter. The influence (activities or inhibiting) of various factors (copper, the source of nitrogen, linoleic acids, and ergosterol) was studied.

The macroscopic morphological analysis carried out after 7 to 10 days of incubation made it possible to put in evidence the following characters: the aspect of the colony (colour, form), the presence of hyphes, the presence of sexual reproduction forms, the spores’ aspect. The production of odorous molecules follows the formation of the mycelium and the spores (5 to 7 days of incubation to 28°C). The production of these molecules was evaluated by an olfactory way.



Photographic N ° 1: Culture aspects of Penicillium sp. and Botrytis sp. on Czapek medium.

At 28°C, *Botrytis cinerea* presents more colonies of 5 cm diameter after 10 days, hyaline at the beginning, becoming later grey to brown grey, presenting conidiophores frequently brown coloured, having a diameter of 16-30µm.

The colony of *Penicillium expansum* is green grey to dark green and produces an exudate as well as a yellow pigment which diffuses in the medium.

Influence copper and source of nitrogen on the development of Botrytis cinerea and Penicillium expansum in monoculture, their sporulation and the production of "musty-earthy" odor

For the strains of *Penicillium sp.* the growth is more considerable in the presence of nitrate (medium 4) than in the presence of ammonium (medium 3) and decreases copper the growth (media 1 and 2). The sporulation is more important on the medium with ammonium (medium 3) than on the medium with nitrate (medium 4). As for the sporulation, it is supported by the presence of copper in the medium with nitrate (medium 2).

Table 2 (a and b). Growth, sporulation and production of the "musty-earthy" odor by *Botrytis sp.* and *Penicillium expansum* in monoculture on modified Czapek medium

a. Penicillium expansum 1627 and Penicillium expansum 1619

Incubation 168 h	**Medium 1**		**Medium 2**		**Medium 3**		**Medium 4**	
	P. e. 1619	*P. e. 1627*	*P. e.1619*	*P. e.1627*	*P. e.1619*	*P. e. 1627*	*P. e 1619*	*P. e. 1627*
The growth	12	15	17	22,5	15	23	15	30
The frequency (%)	*51*		***55***		***53***		***63***	
The sporulation	2	0	12	8	3	8	0	6
The frequency (%)	*6*		***56***		*31*		*17*	
The aroma	3,5	5,5	3	1,5	0	1	0	0
The frequency (%)	***75***		*38*		*8*		*0*	

b. Botrytis cinerea 1 and Botrytis cinerea 2

Incubation 168 h	**Medium 1**		**Medium 2**		**Medium 3**		**Medium 4**	
	B. c. 1	*B. c. 2*	*B. c. 1*	*B. c. 2*	*B. c. 1*	*B. c. 2*	*B. c. 1*	*B. c. 2*
The growth	9	24	16	34	30	34	16	24
The frequency (%)	46		**69**		**89**		**56**	
The sporulation	0	6	1	6	6	9	0	6
The frequency (%)	17		19		**42**		17	
The aroma	5	5	1	2	4	0	0	2,5
The frequency (%)	**83**		25		33		21	

Concerning the production of "musty-earthy" odor, copper supports its production and the most favourable medium is the medium containing of copper and ammonium (medium 1). This result is in agreement with that of Dionigi [5] which showed the influence of copper on the production of geosmin by *Penicillium sp.*

For the strain of *Botrytis sp*. the growth is more considerable in the presence of ammonium (medium 3) than in the presence of nitrate (medium 4), and by adding the copper the growth decreases in the presence of ammonium (medium 1) and increases it in the presence of nitrate (medium 2). The sporulation is important on the medium with ammonium (medium 3). The production of "musty-earthy" odor seems more important on medium with ammonium (medium 3). It is increased by the presence of copper as in the case of *Penicillium sp*. (medium 1).

During the time it was noted that the frequency of the "musty-earthy" odor evolves differently according to associations *Botrytis-Penicillium*. In the presence of *B. cinerea 1* only the medium containing of ammonium and copper (medium 1) allows an increase in the production of the "musty-earthy" odor.

On the other hand, the first medium has always increased in the production of "musty-earthy" odor per *P. expansum 1627* during the cultivation.

Table 3 (a, b, c and d). Perception of the "musty-earthy" odor produced by *B. cinerea* and *P. expansum* in co-culture on medium of Czapek modified according to the duration of culture

a. Botrytis cinerea 1 + Penicillium expansum 1619

	Medium 1		**Medium 2**		**Medium 3**		**Medium 4**	
The incubation	168 h	240 h	168 h	240 h	168 h	240 h	168 h	240 h
The frequency (%)	11	38	20	0	25	0	45	6
The kinetics		↗		↘		↘		↘

b. Botrytis cinerea 1 + Penicillium expansum 1627

	Medium 1		**Medium 2**		**Medium 3**		**Medium 4**	
The incubation	168 h	240 h	168 h	240 h	168 h	240 h	168 h	240 h
The frequency (%)	25	**50**	35	38	**50**	**69**	45	**88**
The kinetics		↗		↗		↗		↗

c. Botrytis cinerea 2 + Penicillium expansum 1619

	Medium 1		**Medium 2**		**Medium 3**		**Medium 4**	
The incubation	168 h	240 h	168 h	240 h	168 h	240 h	168 h	240 h
The frequency (%)	**55**	**65**	**50**	35	35	20	**75**	45
The kinetics		↗		↘		↘		↘

d. Botrytis cinerea 2 + Penicillium expansum 1627

	Medium 1		**Medium 2**		**Medium 3**		**Medium 4**	
The incubation	168 h	240 h	168 h	240 h	168 h	240 h	168 h	240 h
The frequency (%)	**65**	**75**	**60**	**90**	**65**	30	**70**	40
The kinetics		↗		↗		↘		↘

In the presence of *B. cinerea 2*, *P. expansum 1619* has behaved identically as in the case with *B. cinerea 1*. The production of "musty-earthy" odor by *P. expansum 1627* in the presence of *B. cinerea 2* increases during time only for the media containing of copper (media 1 and 2). This *Penicillium expansum* variable reaction according to the stock of *Botrytis cinerea* is to be brought closer the observations made by La Guerche [8], which distinguished if the stocks of *Botrytis* Bot+ and Bot- supports or not the production of geosmin by *Penicillium sp*

Influence of linoleic acid and ergosterol on the development, the sporulation and the production of "musty-earthy" odor of Botrytis cinerea and Penicillium expansum

Cultures were carried out on Czapek medium added with linoleic acid and /or ergosterol. In order to introduce these two substances into the medium we diluted them in alcohol 95% (v/v). Taking into account the low solubility of the products, the quantity of alcohol presents in the media ready to be used, was close to 10% (v/v). The obtained results are presented in table 2.

It was observed that, after 192 hours of cultivation in the presence of linoleic acid inhibits partially the growth of *P. expansum 1619* and *1627*. The ergosterol (medium 7) seems more strongly to inhibit the growth of *P. expansum 1619* than *P. expansum 1627*. The simultaneous addition of linoleic acid and ergosterol (medium 5) does not modify the growth of *Penicillium sp*. The sporulation of *Penicillium* sp. is important only on the control medium (medium 8) w/o alcohol and it is weaker but similar on the medium with linoleic acid (medium 6) and the linoleic acid medium + ergosterol (medium 5).

After 332 hours, the growth is the same for all the media and the two strains of *Penicillium sp*. The sporulation is very weak for the control medium (medium 8) and important for the media added with lipids. The production of "musty-earthy" odor is maximal for the medium added with linoleic acid and ergosterol (medium 5). After 332 hours of culture the "musty-earthy" odor decreases especially for the control (medium 8).

Table 4 (a and b). The effect of the ergosterol and linoleic acid on growth, sporulation and production of the "musty-earthy" odor of *B. cinerea 2* and *P. expansum 1619 and 1627* in monoculture according to the culture length

a. ***P. expansum 1619 and 1627***

Incubation 192 h	**Medium 5**		**Medium 6**		**Medium 7**		**Medium 8**	
	P. e. 1619	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*
The growth	24	24	16	16	8	16	24	24
The frequency	***100***		***75***		***50***		***100***	
The sporulation	24	8	8	0	0	0	8	4
The frequency	*75*		***20***		*0*		*25*	
The aroma	0	8	3	0	3	0	3	8
The frequency (%)	***50***		*19*		*19*		***69***	

Incubation 332 h	**Medium 5**		**Medium 6**		**Medium 7**		**Medium 8**	
	P. e. 1619	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*	*P. e. 1619*	*P. e. 1627*
The growth	*24*	*24*	*24*	*24*	*24*	*24*	*24*	*24*
The frequency	***100***		***100***		***100***		***100***	
The sporulation	*8*	*4*	*24*	*24*	*24*	*24*	*24*	*24*
The frequency	***25***		***100***		***100***		***100***	
The aroma	*1*	*0*	*0,5*	*0*	*0*	*0,5*	*3*	*0*
The frequency (%)	***6.5***		*3*		*3*		***19***	

b. ***Botrytis cinerea 2***

Incubation 192 h	**Medium 5**	**Medium 6**	**Medium 7**	**Medium 8**
	B. c. 2	*B. c. 2*	*B. c. 2*	*B. c. 2*
The growth	24	16	0	12
The frequency (%)	**100**	**67**	**0**	50
The sporulation	0	0	0	0
The frequency (%)	0	**0**	0	0
The aroma	8	0	0	0
The frequency (%)	**100**	0	0	0

Incubation 332 h	**Medium 5**	**Medium 6**	**Medium 7**	**Medium 8**
	B. c. 2	*B. c. 2*	*B. c. 2*	*B. c. 2*
The growth	*24*	*24*	*0*	*20*
The frequency (%)	***100***	***100***	***0***	*83*
The sporulation	*24*	*24*	*0*	*24*
The frequency (%)	*100*	***100***	*0*	*100*
The aroma	*7*	*5*	*0*	*7,5*
The frequency (%)	***88***	***63***	*0*	***94***

After 192 hours of culture *B. cinerea 2* grows on the control medium (medium 8), on medium added with linoleic acid (medium 6) and on medium added with linoleic acid + ergosterol (medium 5). The last two media are however less favourable to the growth. There is no spore formation on these media.

After 332 hours the growth of *B. cinerea 2* is identical on the control medium (medium 8) and on the medium added with linoleic acid (medium 6). On the other hand, there is no growth on the medium supplemented with the ergosterol (medium 7) and a weaker

growth on the medium added with linoleic acid + ergosterol (medium 5). The sporulation is the same on the control medium (medium 8) and on the media added with lipids (medium 5). The production of "musty-earthy" odor takes place only on the control medium after 192 hours. After 332 hours this production decreases on the control medium (medium 8), but is important on the medium added with linoleic acid + ergosterol (medium 5), a little weaker on the medium only added with linoleic acid (medium 6).

B. cinerea 2 and *P. expansum* results are shown simultaneously on different the media used previously. The obtained results are presented in table 5.

Table 5 (a and b). The effect of ergosterol and the linoleic acid on the production of musty-earthy odor by *B. cinerea 2* and *P. expansum 1619* and *1627* in mixed culture according to the culture lenght

a. ***Botrytis cinerea 2 + Penicillium expansum 1619***

	Medium 5		**Medium 6**		**Medium 7**		**Medium 8**	
The incubation	192 h	332 h	192 h	332 h	192 h	332 h	192 h	332 h
The frequency (%)	75	7	50	7	25	13	25	0
The kinetics	↘		↘		↘		↘	

b. ***Botrytis cinerea 2 + Penicillium expansum 1627***

	Medium 5		**Medium 6**		**Medium 7**		**Medium 8**	
The incubation	192 h	332 h	192 h	332 h	192 h	332 h	192 h	332 h
The frequency (%)	100	0	0	31	0	13	100	38
The kinetics	↘		↗		↗		↘	

It was observed that during the time the production of "musty-earthy" odor decreases for the mixed cultures of *B. cinerea 2* and *P. expansum 1619* on all media.

On the other hand, for the association *B. cinerea 2* and *P. expansum 1627* the production of "musty-earthy" odor decreases in time for the control medium (medium 8) and the medium added with lipids (medium 5). For the medium with linoleic acid and the medium with ergosterol (media 6 and 7) this production increases.

Quantification of the compounds responsible for the "musty-earthy" odor

Culture media analyzed contain neither 2-isopropyl-3-methoxypyrazin (IPMP), nor geosmin, as both of them were under the limit of detection. Only *B. cinerea 1* product (the 2-methylisoborneol) whatever the culture medium was used and *B. cinerea 2* does not produce this compound on Czapek medium supplemented with alone nitrate. The presence of different interferences makes difficult the quantification of the 2-methylisoborneol. A modification of the method will be able to carry out this quantification.

Table 6. Proportioning of the "musty-earthy" compounds produced by *Botrytis cinerea 1* and *2* in the presence or absence of *Penicillium sp.* on modified Czapek media by gas chromatography-mass spectrometry

Reference	**IPMP (ng/l)**	**Geosmin (ng/l)**	**2-MIB (ng/l)**

CONTROL	<LDD	<LDD	<LDD
B. cinerea 1 on Cu + ammonium medium	<LDD	<LDD	+
B. cinerea 1 on Cu +nitrate medium	<LDD	<LDD	+
B. cinerea 1 on nitrate medium	<LDD	<LDD	+
B. cinerea 2 on Cu + ammonium medium	<LDD	<LDD	+
B. cinerea 2 on Cu +nitrate medium	<LDD	<LDD	+
Botrytis 2 on nitrate medium	<LDD	<LDD	<LDD
B. cinerea 2 + Penicillium sp. on Cu + ammonium medium	<LDD	<LDD	<LDD
B. cinerea 2 + Penicillium sp. on Cu +nitrate medium	<LDD	<LDD	<LDD
B. cinerea 2 + Penicillium sp. on nitrate medium	<LDD	<LDD	<LDD
B. cinerea 1 + Penicillium sp. on Cu + ammonium medium	<LDD	<LDD	<LDD
B. cinerea 1 + Penicillium sp. on Cu +nitrate medium	<LDD	<LDD	<LDD
B. cinerea 1 + Penicillium sp. on nitrate medium	<LDD	<LDD	<LDD

IPMP - 2-isopropyl-3-methoxypirazin
2-MIB - 2-methyilisoborneol
LDD – limit of the detection
+ - presence

Conclusions

The results obtained in this study were in accordance with de La Guerche [8]. In his PhD paper, the *Botrytis* strains Bot + and Bot – were divided, depending on the influence of the production of geosmin by *Penicillium sp*. Olfactive analysis at different various times of culture made possible to check the appearance of the musty-earthy odor. The extraction of the substances responsible for musty-earthy odor (geosmin, 2-methylisoborneol, and 2-isopropyl-3-methoxypyrazin) was carried out and the compound identified by gas chromatography coupled with the mass spectrometry. Chromatographic analysis didn't put in evidence the presence of geosmin or 2-isopropyl-3-methoxipirazin, the compounds responsible for "musty-earthy" odor. Only 2-methylisoborneol, other compound responsible of "musty-earthy" odor was presented in culture media tested, but the presence of the interfering substances makes difficult its quantification.

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References

1.Begue G., *Recherche des micro-organismes responsables des goût moisi-terreux dans les vins*, Diplôme National d'œnologue, Institut Jules Guyot, DIJON, 2002-2004.

2. Beldame G ., Borselli C., Deriencourt A., *Le goût moisi terreux dans le vin*, Projet de 2^{éme}, ENSBANA, DIJON, 2004-2005.

3. Darriet P., Pons M., Lamy S., Dubourdieu D., *Identification and quantification of geosmin, an earthy odorant contaminating wines*, J. Agric. Food Chem., 48, 2000, pp. 4835-4838.

4. Delanoë D., Maillard C., Maisondieu D., *Le vin. De l'analyse à l'élaboration*, 5^{e}édition, Editions TEC&DOC, 2002.

5. Diongi C. P., *The effects of copper sulphate on geosmin biosinthesys by Streptomyces tendae, Streptomyces albidoflavus, and Penicillium expansum*, Wat. Sci. Tech., vol. 31, no. 11, 1995, pp. 135-138.

6. Dumoulin M., Riboulet J. M., Analyse *de géosmine dans les vins : apport de la microextraction en phase solide, (SPME), couplée à l'analyse par GC-MS*, Revue Française d'œnologie, septembre/octobre, n° 208, 2004, pp. 28-30.

7. La Guerche S., Blancard D., Chamont S., Dubourdieu D., Darriet P., *Origine de la géosmine, composé responsable d'un arôme terreux dans les vins - étude des micro-oranismes impliqués et leur métabolisme,* Œnologie, 7e Symposium International d'œnologie, Ed . TEC&DOC, 2003, p. 22-25.

8. La Guerche S., *Recherches sur les déviations organoleptiques des moûts et des vins associées au développement de pourritures sur les raisins. Etude particulière de la géosmine,* Thèse pour le doctorat de l'Université Bordeaux 2, 2004.

9. La Guerche S., Garcia C., Darriet P., Dubourdieu D., Labarère J., *Characterization of Penicillium species isolated from grape berries by their internal transcribed spacer (ITS1) sequences and by gas chromatography-mass spectrometry analysis of geosmin production,* Curr. Microb., vol. 48, 2004, pp. 405-411.

10. Leclerc P., 2004, *Recherché des molécules responsables des déviations de type « moisi-terreux » dans les vins de Pinot Noir de Bourgogne*, Mémoire de fin d'études, Centre Technique Interprofessionnel de la Vigne – Unité de Beaune.

11. Mattheis J. P., Roberts R. G., *Identification of geosmin as a volatile metabolite of Penicillium expansum*, Appl. and Environ. Microb., vol. 58, no. 9, 1992, pp. 3170-3172.

12. Sautour M., Rouget A., Dantigny P., Divies C., Bensoussan M., *Prediction of conidial germinationof Penicillium crysogenum as influenced by temperature, water activity, and pH*, Lett. in Appl. Microb., 32, , 2001, pp. 131-134.

13. *Applications courantes de la micro-extraction en phase solide (SPME) à l'analyse des vins*, Revue des Œnologues, n°114 HS, 2005, pp.19-20.

WEB SITES:

14. Blancard D., S. Chamont, S. La Guerche, P. Darriet, D. Dubourdieu, *Arôme terreux des vins : premiers éléments d'explication du phénomène*, www.bordeaux.inra.fr [17.07. 2005]

15. www.labo-exact.com [17.07. 2005]

16. www.syngenta-agro.fr [17.07.2005]