MOULDY-EARTHY AROMAS IN WINES: FIRST PRACTICAL RESULTS OBTAINED BY A MUTLIDISCIPLINARY PARTNERSHIP

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Introduction

The organoleptic faults called mouldy-earthy aromas (MEA) have been identified in some vineyards for several years. The financial impact of these faults has been significant in numerous vineyards in 2002, and again in 2004. These defects have been detected in the Bordeaux, Beaujolais and Bourgogne regions, the Loire valley, and are suspected in Alsace, Jura, Savoie, the South-Western vineyards, Cognac,…The list of potentially affected varieties, both reds and whites, is getting longer: Gamay, Chenin, Pinot Noir, Cabernet Franc, Cabernet Sauvignon, Sauvignon, Semillon, …

1. The molecules responsible and their chemical analysis

1.1. Geosmin

Geosmin is a naturally occurring molecule, which can be produced by various microorganisms. It is characterized by an earthy odour. Its odour threshold in water is 1 – 10 ng/l. Two enantiomers exist, with the (-) form being the only one biosynthesized by microorganisms such as *Streptomyces sp.* and *Penicillium sp*., and the only one to be found in wines.

Geosmin analyses have been performed by solid phase microextraction (SPME) followed by GC-MS with a sensitivity of 5 ng/l and a repeatability between 5 and 12%.

1.2. 2-methylisoborneol (MIB)

2-methylisoborneol is part of the alkyl fenchol family. It is also produced by different microorganisms. Its odour threshold in water is about 3 ng/l. During alcoholic fermentation it is degraded into two metabolites.

2-methylisoborneol can be extracted using SPME based techniques. In musts and wines, its detection limit is approximately 10 ng/l with a 2% repeatability.

1.3. 3-isopropyl-2-methoxypyrazine (IPMP)

Pyrazines are very active aromatic substances that can be of biological origin, as well. 3-isopropyl-2-methoxypyrazine is described as "earthy", "potato". Its odour threshold in water is 2 ng/l.

From an analytical perspective, SPME combined with GC/MS is suited best. The method sensitivity is about 0.5 ng/l.

2. Organoleptic characterization

These three compounds only affect wine aroma, and do not influence the other flavour elements (taste, astringency, pseudo-thermal sensations). The sensory descriptors used for other foods (water, fish, seafood,…) are also suitable to represent the "olfactory image" of wines contaminated by these malodorous molecules.

When perceived in wines, geosmin is described by the terms "wet earth", "cooked beetroot", "humus" and "moss (under-woods)". If red wines are artificially spiked with geosmin, the odour threshold is about 50 ng/l. In “naturally” contaminated wines, the odour threshold is about 20 ng/l, and even 10 ng/l in certain wines.

MIB is responsible for musty taints, but an effect of its metabolites in wines is suspected. Spiking wines with 2-methylisoborneol produces “dry mould”, “wet cardboard” and “rotten apple” notes, which may be perceived at 30 ng/l and are obvious at 60 ng/l.

Finally, IPMP confers aromas to wines that tend towards vegetal notes: “bud”, “asparagus” and “celery”.

Geosmin is the principal cause for contamination of wines with mouldy-earthy aromas, and thus, appears to be the best marker for this taint, to date.

3. Origin and geographical distribution

3.1. Methods:

Grape and earth samples were taken in 2002 and 2003. Microbiological analyses were performed by ENSAT or ITV-France. The objective was to isolate moulds, study their geosmin, MIB and IPMP production potential, and to identify them.

3.2. Results – discussion:

Several hundred strains were isolated from the collected samples. Nearly all strains producing geosmin and/or MIB and/or IPMP belonged to the *Penicillium* genus. The main results are presented in Table 1.

Fungi	Vineyards[1]	Grape varieties
P. expansum	B- C - E	Chenin - Gamay
P. spinulosum	E	Chenin - Gamay
P. canescens	D - E	Tokay - Gamay
P. restrictum	A - E	Semillon - Gamay
P. minioluteum	B - E	Gamay - Chenin
P. digitatum or crustosum	E	Pinot Noir - Chenin
P. brevicompactum	A	Semillon
P. thomii or glabrum	E - F	Chenin - Folle blanche - Gamay
P. geastrivorus	A	Sauvignon
P. ochrochoron	A	Semillon
P. paraherquei	C	Gamay
P. digitatum or hirsutum	E - F	Chenin - Folle blanche - Gamay

[1] Vineyards: Bordeaux: A, Beaujolais: B, Maconnais: C, Alsace: D, Touraine: E, Pays Nantais: F

Table 1: Geosmin producing Penicillium species according to vineyards and grape varieties

This table clearly shows the diversity among geosmin producing *Penicillium* species. With few exceptions, these strains also produced MIB and, some of them IPMP, as well.

Of the 200 *Penicillium* strains tested, 21% produced geosmin and 40% MIB.

3.3. Production capacity:

In addition to studying production capacity in synthetic medium, the Syngenta laboratories developed a method for the artificial contamination of grape clusters with *P. expansum*. After sterilization of the grape clusters, berries are artificially injured, and then contaminated with *P. expansum*. High levels of geosmin were measured after 5 days of incubation in a dark and humid room at 24°C (184 and 265 ng/l).

These results show that, independent from the presence of any other microorganism, *Penicillium* strains can produce geosmin on grape berries.

4. Finding solutions in the vineyard:

The absence of an authorized oenological remedy to remove these molecules from wines without affecting their quality led us to look for solutions in the vineyard in order to deliver grapes with a minimum of contamination to the winery.

4.1. Favouring factors

Observations made in the vineyard allowed the listing of a certain number of factors that seem to favour the development of *Penicillium sp*. responsible for the production of geosmin:

- Significant rain at maturity and during harvest.
- Temperatures of 15 to 25°C
- Very ripe berries
- Excessive vigour and tight clusters
- Skin damage caused by parasites (specifically grey rot, but probably also sour rot, grape berry moths, or even Oidium); physiological factors (berry burst or micro-lesions); climatic factors (hail); or mechanical (trimming, leaf-thinning).

4.2. *In vitro* fungicide tests (Petri dish)

The first evaluation of the potential efficiency of fungicides was done *in vitro*, which allowed the calculation of the concentrations that inhibited spore germination and mycelial growth to 50% (IC50).

Active anti-botrytis compounds of the main chemical families were tested on 13 strains of 5 geosmin and MIB producing *Penicillium* species isolated from our samples.

	cyprodinil	fludioxonil	fluazinam	ref 1	ref 3
Spore germination	0.14	0.57	0.83	14.23	6.46
Mycelial growth	0.25	0.12	0.60	15.00	3.91

Table 2: Average IC50 in mg/l of anti-botrytis fungicides

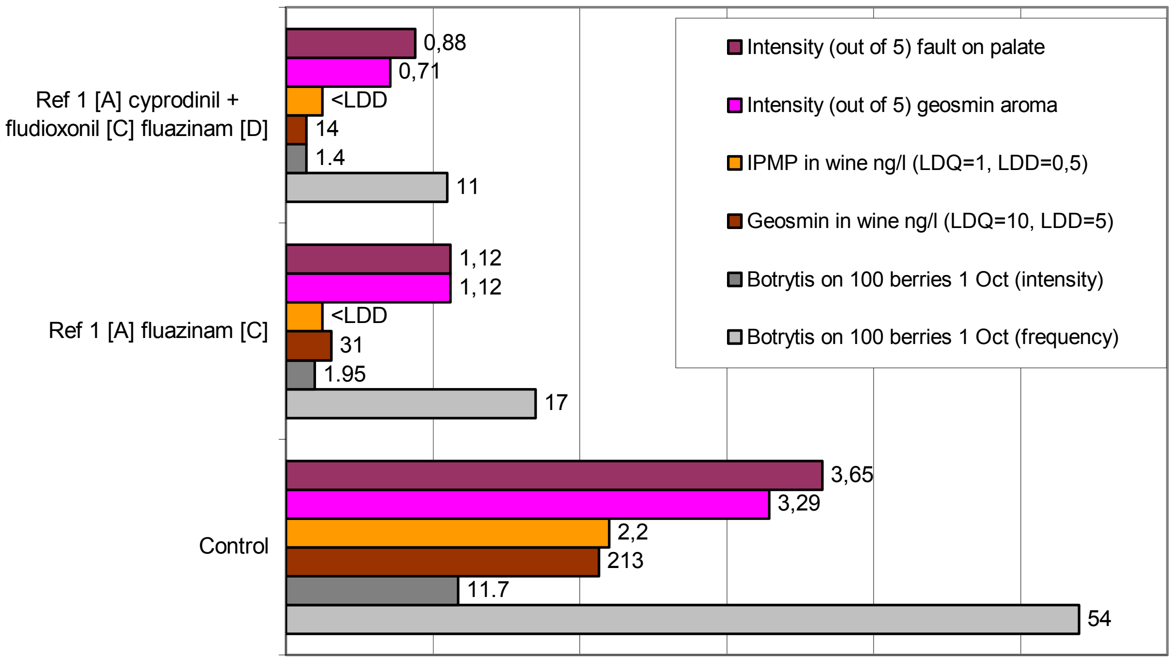
4.3. Fungicide tests with laboratory contaminated grapes:

Fungicide efficiency against growth of *P. expansum* was tested using the artificial contamination method described previously. The clusters were immersed into solutions containing three concentrations of one anti-botrytis agent (n, n/10 and n/100, n being based on the active substance dose by hectare, approved for grey rot). They were contaminated 24 hours later. The tests confirmed the *in vitro* results. The mixture cyprodinil + fludioxonil provides a satisfactory control of the fungus as shown in the picture below taken 5 days after incubation. Similar results were obtained with fludioxonil alone and fluazinam, while the other products were not effective.

---------------------- cyprodinil + fludioxonil ------------------------- control
625 mg a.c*./l 62.5 mg a.c./l 6.25 mg a.c./l
* a.c., active compounds

4.4. Results of trials in the vineyard:

Trials were carried out in the vineyard to compare different anti-botrytis programmes with a non-protected control. The programmes were tested and assessed on small plots containing 12 vines with three replicates each. In each plot, 40 to 50 kg of grapes were harvested and micro-vinified by ITV or SICAREX depending on the trials. One example is shown in Figure 1.



Harvest 1 October (40kg per trial), vinification and tasting by SICAREX in Beaujolais.

Figure 1: 2004 trials, Beaujolais Gamay variety:
Botrytis assessment, levels of geosmin and IPMP, and tasting notes

In this trial, a low percentage of grapes had been naturally contaminated by green moulds of the *Penicillium* type in the different treatments. It could be observed that this was sufficient to cause a high level of geosmin in the wine produced from the plot without botrytis protection.

In a similar trial carried out with Chenin in the Touraine region, the must produced from the control plot had 42 ng/l of geosmin compared with 16-17 ng/l in the musts produced from the treated plots. This trial was performed in a plot where significant MEA problems had been identified in preceding years. Despite this potential and a significant development of grey rot, geosmin levels stayed relatively low.

5. Summary and suggestions:

- Numerous moulds of the *Penicillium* genus are able to produce geosmin and/or MIB and/or IPMP.
- *Penicillium* capable of producing these molecules and wines contaminated by them could be found in several of the French vineyards studied.
- A low percentage of contaminated grapes were sufficient to taint a wine.
- Traditional assessment of *Botrytis* contamination of plots did not correlate well with the levels of geosmin found in the wines obtained from these plots.
- The control of grey rot by anti-botrytis products in a plot contaminated by *Botrytis* and responsible for wine taints reduced the level of geosmin significantly.

Based on our actual knowledge, we propose two types of MEA management:

<u>Indirect control measures</u>: Prevention is based on limiting grape skin damage as much as possible. To this end, excellent sanitary condition of grapes should be maintained. This is all the more essential if advanced maturity is preferred. Grey rot should be controlled as a priority as it is appears that this is the main causative agent. In addition, other parasites such as sour rot and grape berry moth should not be neglected. Mechanical damage should also be considered. A good overall vineyard management program, specifically including the control of vine vigour and watering, will help to limit the risk of berry micro-lesions.

<u>Direct control measures</u>: The choice of cyprodinil, fluazinam or fludioxonil based products to develop anti-botrytis programmes, which are tailored to each vineyard, will provide good management of *Botrytis cinerea* and, at the same time, limit the development of the *Penicillium* species able to produce geosmin and/or MIB.