



Survey of viable airborne fungal propagules in French wine cellars

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Abstract

A study was carried out of the viable airborne mycological flora of 12 wine cellars in the Arbois region in France. Results show that there were considerable variations in the quantities of propagules: from 57 to 2547 CFU m⁻³. There was a relatively large number of species (48), which are more frequently recovered from certain cellar types. The most common were *Cladosporium sphaerospermum*, *Aspergillus versicolor*, *A. restrictus*, *Penicillium glabrum*, *P. dierckxii*, *P. implicatum* and *P. brevicompactum*. So the risk of allergies subsequent to inhalation of large quantities of spores is far from minimal.

1. Introduction

Aeromycology, a field undergoing constant development, covers the study of a number of indoor atmospheres, in hospital as much as in work environment. Agricultural facilities seem to be particularly prone to indigenous or fungal invasion. Possible consequences include the risk of allergies for workers and the deterioration of foodstuffs, of their packaging and of the equipment in general. Such problems can occur in the storage cellars of wineries.

Some research has been done on the subject: Schanderl (1962) pointed to the presence of *Zasmidium cellare* and *Verticillium*, Dittrich (1977) mentioned *Cladosporium* and *Aureobasidium* and Moreau (1978) *Penicillium* and *Aspergillus*. A recent study in France and Germany (Goto et al., 1989) has produced qualitative results only, without the slightest allusion to spore invasion and the genera mentioned, *Penicillium*, *Cladosporium*, *Aspergillus* and *Alternaria*, are usually encountered in aeromycology.

Other more detailed works deal with specific ecological niches: Marais and Kruger (1975), Moreau et al. (1976), Moreau (1978) and Danesh et al. (1997) studied the mycoflora in bottle stoppers which, apart from destroying the cork, can, through the produc-

tion of certain metabolites, contribute to the development of the much feared 'corky taste' affecting wines. And the powdery blackish film peculiar to old bottles derives from the proliferation of *Zasmidium cellare*. A rather surprising phenomenon described by Bertrand and Barrios (1994) and Chatonnet et al. (1994) is the fungal degradation of the chlorophenols used in disinfecting the worksites, paddles and other equipment. The chloroanisols formed are themselves also responsible for the mouldy odour and taste of certain wines stocked close by (in their vicinity).

With the exception of these precise, though fragmented, findings, no exhaustive or detailed study has been undertaken in this field.

2. Materials and methods

Samples were collected from 12 cellars situated in 3 districts belonging to the Jura vineyards in eastern France, namely Arbois, Montigny-les-Arsures and Pupillin.

2.1. Description of the facilities

These sites, all of them very different, can nevertheless be organised into three categories:

- Old, partially subterranean type storage cellars with packed sod or gravel floors, limestone walls and vaulted stone ceilings; the temperature and relative humidity, measured at the same place of the sampling, with a thermohygrometer (Hanna HI 93640), range respectively from 9 to 14 °C, and from 40 to 60%.
- Modern storage cellars, non-subterranean, entirely built in concrete with or without insulating material. The temperature range is 8 to 16 °C, with a relative humidity between 43 and 50%.
- ‘Vin jaune’ surface cellars situated in the upper part of the buildings, are subjected to significant differences in temperature variation. The conditions described are ideally suited to the specific vinification of ‘vin jaune’.

2.2. Samples and culture

Samples were collected between 10 and 11 a.m., exposed sequentially over a period of 15 min, during 1997 winter, from the centre of the cellar at a height of 1.2 m above ground level, using a one-stage volumetric sieve sampler (SAS Compact, Pool Bioanalysis Italiana, Milan, Italy), at a flow of 90 l/min. For each cellar, 8 plates (RODAC type, special for SAS) with a diameter of 84 mm were used, each plate receiving the equivalent of 60 litres of air, on the basis of two plates for each type of medium and each incubation temperature.

The culture media chosen were: malt extract (1%) agar = MEA, widely used in aeromycology and recommended by the American Conference of Governmental Industrial Hygienists' Committee for indoor Environment Studies (1986), malt extract (1%) agar supplemented with 20% sucrose = MESA for osmophilic species. Incubation took place at 22 °C or at 37 °C (for the possible detection of thermotolerant species). Qualitative and quantitative evaluation of CFU (Colony Forming Unity) was carried out following 8 days of culture; 15 days for slow growth species. The CFU were identified by their microscopic and macroscopic morphology using standard mycologic literature (Raper and Thom, 1949; De Vries, 1967; Ellis, 1971; Barnett and Hunter, 1972; Raper and Fennell, 1977; Von Arx, 1981; Klich and Pitt, 1988; Pitt, 1991). These methods have been described with precision in previous publications (Simeray et al., 1995, 1997). Daily viable propagules counts were converted to the number of CFU m⁻³ air.

2.3. Statistical analysis

Description of the data was expressed using means and their confidence interval (95%). Multifactor analysis of variance (ANOVA) was performed to compare simultaneous culture medium (MESA versus MEA) and type of cellar (old versus modern). Statistical package SAS V-6.12 for Windows was used to analyse.

3. Results and discussion

3.1. Number of colony forming unity

The number of CFU m⁻³ in cellars is generally elevated, with a mean density of 783 and a median density of 614. Variations can, however, be considerable, with a maximum of 2547 and a minimum of 57.

The culture medium MESA is not statistically best ($P = 0.48$) to the development of colonies (Table 1) with a mean of 825.8 [IC (95%): 506.8–1144.9] CFU m⁻³ compared to 665.3 [IC (95%): 346.3–984.4] for MEA (Table 2) take into account old cellars and modern cellars. We have the same result for all cellars (793 versus 721).

Significant variations in the number of CFU were observed in the cellars studied, which can be explained according to the following characteristics:

- The type of cellar: old traditional semi-subterranean cellars with hard packed sod floors offer a higher concentration of spores than more recent constructions with concrete walls and floors ($P = 0.046$). Indeed, whatever the type of medium might be (MEA or MESA), the mean values of CFU are always higher in old cellars: 975.2 [IC (95%): 690.3–1261.0] than in recent ones: 515.5 [IC (95%): 166–865]. Colonies of *Penicillium*, *Aspergillus* and *Zasmidium cellare* are commonly observed on the walls, floors, and for the last mentioned species, on the bottles.
- The cleaning techniques: the atmosphere of cellars where the walls are ‘brushed’ occasionally, presents fewer spores (cellar no. 6).
- Cellar activity prior to sample collection: in cellar no. 9, we observed an elevated number of *Botrytis cinerea* CFU, which can be attributed to the pressing, a few days before, of the grapes used in the making of the ‘vin de paille’.
- Viticulture and vinification methods: in the cellars where winegrowers proceed using natural methods (little or no pesticide, vinification without SO₂,

Table 1. Occurrence of mould species (CFU m⁻³) isolated on MESA (incubated at 22 °C)

	Old cellars						Modern cellars				“Vin jaune” cellars	
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Acremonium</i> sp.	–	–	16	–	–	–	–	–	–	–	–	–
<i>Arthrographis</i> sp.	–	–	–	–	–	16	–	–	–	–	–	–
<i>Aspergillus restrictus</i>	542	–	83	92	8	67	–	1008	–	–	42	8
<i>A. versicolor</i>	25	33	58	683	8	75	25	8	–	–	150	–
<i>Beauveria bassiana</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>Botrytis cinerea</i>	8	–	–	–	8	–	–	16	125	8	16	–
<i>Cladosporium cladosporioides</i>	–	8	–	–	–	–	–	–	–	–	–	–
<i>C. herbarum</i>	8	–	8	8	–	–	–	–	–	–	8	–
<i>C. sphaerospermum</i>	16	–	208	1275	–	–	–	–	–	–	858	16
<i>Dicyna ampullifera</i>	–	–	33	–	–	–	–	–	–	–	–	–
<i>Emericella nidulans</i>	8	–	100	–	–	50	–	–	–	–	–	–
<i>Eurotium</i> sp.	8	–	16	–	–	8	–	–	–	–	–	–
<i>Geotrichum</i> sp.	–	–	–	–	–	33	–	–	–	–	–	–
<i>Oidiodendron cerealis</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>O. griseum</i>	–	–	125	–	–	–	–	–	–	–	–	–
<i>Penicillium aurantiogriseum</i>	–	–	–	–	–	–	8	–	–	–	–	–
<i>P. brevicompactum</i>	8	67	16	142	92	8	–	42	33	16	33	8
<i>P. canescens</i>	–	–	–	–	–	–	–	–	–	–	8	–
<i>P. chrysogenum</i>	–	–	8	–	8	–	8	–	–	–	8	–
<i>P. citrinum</i>	–	–	–	–	16	–	–	–	–	–	–	–
<i>P. commune</i>	–	–	8	58	16	–	–	–	–	8	16	8
<i>P. decumbens</i>	–	–	–	–	–	–	–	8	–	–	–	–
<i>P. dierckxii</i>	217	–	–	16	16	8	8	8	–	317	–	–
<i>P. echinulatum</i>	–	8	–	–	–	–	–	–	–	–	–	–
<i>P. expansum</i>	–	–	25	–	–	–	25	8	16	8	–	–
<i>P. glabrum</i>	8	433	42	108	16	–	16	8	–	25	33	–
<i>P. implicatum</i>	8	533	25	25	33	8	183	25	8	16	8	–
<i>P. montanense</i>	–	–	–	–	16	–	–	–	–	–	–	–
<i>P. restrictum</i>	–	–	–	–	–	–	–	16	–	–	–	–
<i>P. roqueforti</i>	–	–	–	33	–	–	–	–	–	108	8	8
<i>P. rugulosum</i>	–	–	–	–	–	–	–	–	–	8	–	–
<i>P. sclerotiorum</i>	–	16	–	–	–	–	–	–	–	–	–	–
<i>P. solitum</i>	–	8	–	16	8	–	–	–	8	–	25	42
<i>P. spinulosum</i>	–	50	–	8	–	–	–	–	–	–	–	–
<i>P. variable</i>	–	16	92	–	–	–	8	–	–	–	–	–
<i>P. verrucosum</i>	–	16	–	–	–	–	–	–	–	–	–	–
<i>P. waksmanii</i>	–	–	–	42	25	–	50	–	8	108	25	–
<i>Penicillium</i> sp.	–	–	–	–	8	–	–	–	–	–	–	–
<i>Phoma</i> sp.	–	–	–	–	–	–	–	–	–	–	42	–
<i>Rhinochlaia atrovirens</i>	–	–	–	8	–	–	–	–	–	–	–	–
<i>Thysanophora penicillioides</i>	–	–	–	–	–	–	–	–	8	–	–	–
<i>Wallemia sebi</i>	–	–	16	–	–	–	–	–	–	–	–	–
<i>Zasmidium cellare</i>	–	–	250	–	–	–	–	–	–	–	–	–
Non sporulating isolates	108	–	25	8	–	8	50	–	8	–	–	8
Total	964	1188	1145	2547	278	281	356	1164	206	630	1288	98

Table 2. Occurrence of mould species (CFU m⁻³) isolated on MEA (incubated at 22 °C)

	Old cellars						Modern cellars				'Vin jaune' cellars	
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Arthrimum arundinis</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>A. phaeospermum</i>	–	–	–	–	–	–	8	–	–	–	–	–
<i>Aspergillus versicolor</i>	16	16	200	750	8	25	33	16	–	–	58	–
<i>Botrytis cinerea</i>	–	–	–	–	–	–	–	8	125	–	8	–
<i>Cladosporium cladosporioides</i>	–	–	16	25	–	–	–	–	–	–	8	–
<i>C. herbarum</i>	8	–	33	–	–	–	–	–	–	16	8	–
<i>C. macrocarpum</i>	–	–	–	–	–	–	–	–	–	–	16	–
<i>C. sphaerospermum</i>	8	42	333	1167	16	–	25	8	–	8	883	–
<i>Dicyma ampullifera</i>	–	–	25	–	–	–	–	–	–	–	–	–
<i>Echinobotryum</i> sp.	–	–	8	–	–	–	–	–	–	–	–	–
<i>Emericella nidulans</i>	–	–	50	8	–	42	–	–	–	–	–	–
<i>Eurotium</i> sp.	–	8	–	25	–	8	8	–	–	–	–	–
<i>Geotrichum</i> sp.	–	–	–	–	–	33	–	–	–	–	–	–
<i>Mucor racemosus</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>Mycosphearella</i> sp.	–	–	8	–	–	–	–	–	–	–	–	–
<i>Oidiodendron cerealis</i>	–	–	16	–	–	–	–	–	–	–	–	–
<i>O. griseum</i>	–	–	16	–	–	–	–	–	–	–	–	–
<i>Penicillium brevicompactum</i>	8	517	75	125	83	–	8	25	66	16	42	33
<i>P. camemberti</i>	–	8	–	–	–	–	–	–	–	–	–	–
<i>P. chrysogenum</i>	–	33	–	–	–	8	–	–	–	–	–	–
<i>P. citreonigrum</i>	–	8	–	–	–	–	–	–	–	–	–	–
<i>P. citrinum</i>	–	–	–	–	16	–	–	–	–	–	–	–
<i>P. commune</i>	–	–	42	33	8	8	–	–	8	–	8	–
<i>P. expansum</i>	–	–	–	25	–	–	–	–	–	–	–	8
<i>P. dierckxii</i>	25	83	–	50	–	–	58	–	–	617	25	–
<i>P. glabrum</i>	525	142	–	75	8	–	8	8	–	16	8	–
<i>P. implicatum</i>	–	125	66	41	–	16	183	–	–	16	16	–
<i>P. italicum</i>	–	–	16	–	–	–	–	16	–	–	–	–
<i>P. montanense</i>	–	8	–	–	–	–	–	–	–	8	–	–
<i>P. restrictum</i>	–	8	–	–	–	–	–	–	–	–	–	–
<i>P. roqueforti</i>	–	–	–	33	25	–	–	–	–	217	–	8
<i>P. sclerotiorum</i>	–	16	–	–	–	–	–	–	–	–	–	–
<i>P. solitum</i>	–	–	–	–	–	–	–	–	–	–	8	–
<i>P. spinulosum</i>	–	–	–	16	–	–	–	–	–	–	–	–
<i>P. thomii</i>	–	–	–	8	–	–	–	–	–	–	–	–
<i>P. variable</i>	–	25	33	16	–	–	25	–	–	–	8	–
<i>P. verrucosum</i>	–	16	–	–	–	–	–	–	–	–	–	–
<i>P. waksmanii</i>	–	–	25	25	33	–	8	–	16	108	33	8
<i>Penicillium</i> sp.	–	16	–	–	–	–	25	–	–	–	–	–
<i>Phoma</i> sp.	–	–	25	–	–	–	–	–	–	–	192	–
<i>Spiniger meinelkellus</i>	–	–	–	–	–	8	–	–	–	–	–	–
<i>Wardomyces inflatus</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>Zasmidium cellare</i>	–	–	50	–	–	–	–	–	–	–	–	–
Non sporulating isolates	8	–	8	–	25	–	33	–	–	–	–	–
Total	598	1071	1075	2422	222	156	414	81	215	1022	1321	57

Table 3. Occurrence of mould species (CFU m⁻³) isolated on MESA and MEA (incubated at 37 °C)

	Old cellars						Modern cellars				'Vin jaune' cellars	
	1	2	3	4	5	6	7	8	9	10	11	12
<i>MESA</i>												
<i>Aspergillus fumigatus</i>	–	–	–	–	–	8	–	–	–	–	–	–
<i>A. niger</i>	8	8	–	–	–	8	–	–	–	–	–	–
<i>Eurotium amstelodami</i>	–	–	–	–	8	–	–	–	–	–	–	–
<i>E. intermedium</i>	–	–	–	8	–	–	–	–	–	–	–	–
<i>Eurotium</i> sp.	8	–	–	–	–	–	–	–	–	–	–	–
<i>Penicillium decumbens</i>	–	–	–	16	–	–	–	–	–	–	–	–
<i>P. pinophilum</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>Trichoderma koningii</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>MEA</i>												
<i>Penicillium pinophilum</i>	–	–	8	–	–	–	–	–	–	–	–	–
<i>P. variable</i>	–	25	–	–	–	–	–	–	–	–	–	–
<i>Scopulariopsis brevicaulis</i>	–	25	–	–	–	–	–	–	–	–	–	–
Non sporulating isolates	–	–	–	8	–	–	–	–	–	–	–	–

no wine filtration), a high number of CFU was observed (cellar no. 4).

3.2. Number of species and composition of the aeromycological flora

Forty-eight different species were identified. The average number per cellar was between 10 and 11. Old cellars harboured a greater variety than those of recent construction. Goto et al. (1989) had isolated 24 species only.

Penicillium, *Cladosporium* and *Aspergillus* were the three dominant genera. According to their relative frequencies, three variants could be distinguished:

- Cellars where *Penicillium* dominated (cellars no. 2, 5, 7, 10), the most frequently encountered case: the species present in the largest quantities were, for the monoverticillates: *P. glabrum*, *P. implicatum*, for the biverticillates: *P. dierckxii*, *P. waksmanii* and for the triverticillates: *P. brevicompactum*, *P. roqueforti* and to a lesser degree, *P. expansum*. *P. glabrum* and *P. expansum* are well known contaminants of cork bottle stoppers (Moreau, 1978).
- Cellars where *Cladosporium* was dominant (less common, cellars no. 3, 4, 11), especially in old facilities: large quantities of *C. sphaerospermum* were sometimes found.

- Cellars where *Aspergillus* dominated (less common, cellars no. 6, 8): the main species were *A. versicolor* and *A. restrictus*. This last species, well known as xerophilic, thrives only in sucrose-rich culture medium and is often overlooked when standard isolation media are used (Flannigan et al., 1994). *A. fumigatus* was encountered just once, in small quantities.

Other species can abound periodically, such as *Botrytis cinerea* (cellar no. 9), *Phoma* sp. (cellar no. 11), *Oidiodendron griseum* (cellar no. 3). *Zasmidium cellare* whose mycelium commonly covers the walls and sometimes the bottles in old cellars, was seldom isolated in the samples; this was perhaps due to poor sporulation, or to a culture medium poorly adapted to the development of this type of mould.

No rational explanation could be offered to account for the presence of thermotolerant species found exclusively in old cellars (Table 3).

4. Conclusions

The aeromycological flora of wine cellars are varied, and of high spore density. Reponen et al. (1992) and Gravesen (1979) considered as unacceptable a spore level of 500 CFU m⁻³ which indicates an abnormal indoor atmosphere. Several potentially

allergy-provoking species are present in considerably large quantities (Van Bronswijk et al., 1986). This is the case for *Cladosporium sphaerospermum*, *Botrytis cinerea*, diverse *Aspergillus* (*A. versicolor*, *A. restrictus*) and *Penicillium* (*P. brevicompactum*). A more detailed study of the seasonal variations of these species should be undertaken to complete our findings. France, a country where wine producing traditions differ significantly, would be the ideal choice for further research into the mycoflora of wineries, with the aim of enhancing the quality of the wines and improving workers' protection against the likelihood of allergies to fungal spores.

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