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THE AIR SPORA OF A PORTUGUESE CORK FACTORY

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Abstract—Cork particles and fungus spores were commonly airborne in a cork factory in Portugal where some workers suffered from suberosis. Where cork was being handled and shaped, fungus spores were usually more common than cork particles. In a warehouse (*fundão*) where cork had moulded, up to 54×10^6 spores/m^3 were general in the air, but workers handling the mouldy cork were exposed to as many as 128×10^6 spores/m^3. Cork particles were most common close to cutting and sanding machines, but seldom exceeded 2×10^6 particles/m^3 air. The most abundant species were *Penicillium frequentans* Westling, *P. granulatum* Bain., *Aphanocladium album* (Preuss) W. Gams, *Monilia sitophila* (Mont.) Sacc. and *Mucor plumbeus* Bon. *P. frequentans* was common everywhere in the factory and mostly in units small enough to penetrate into alveoli.

INTRODUCTION

CORK is the bark of an oak tree, *Quercus suber* L., grown widely in Portugal and other Mediterranean countries. Bark stripped from the trees is stacked outside for about a year, then cut to size, baled, boiled in water and restacked outdoors until required. At the factory at Seixal near Lisbon, where these observations were made, some cork was re-boiled and stacked while still hot in a poorly ventilated, damp warehouse (*fundão* 1) for several days before use. This cork was mostly used to make discs but sometimes also corkskin for wall coverings and stoppers. Other cork was wetted with cold water and stacked in a larger, drier warehouse (*fundão* 2). This was steamed before it was used to make stoppers ("corks"). Tiles were made from unprocessed cork, while balls, fishing rod handles and other items were made from resin-bonded granulated cork, shaped by sanding machines. The process is summarised in Fig. 1.

The Portuguese cork industry employs 20,000 workers. Many suffer from a respiratory disease known as suberosis, a form of extrinsic allergic alveolitis, caused by inhaling dust from mouldy cork (CANCELLA, 1955, 1963; ÁVILA and VILLAR, 1968). Recently, PIMENTEL and ÁVILA (in press) reported precipitins to *Penicillium frequentans* Westling in sera from suberosis patients. This fungus was isolated from mouldy cork, but nothing was known of its incidence in the air of cork factories nor of its frequency relative to other fungi and airborne cork particles. The study of the air spora described in this paper was combined with a study of suberosis by the Department of Chest Diseases, Lisbon University Faculty of Medicine, who will describe epidemiological and immunological aspects later.

METHODS

The air spora in different parts of the factory were assessed using a cascade impactor (MAY, 1945) and a modified Andersen sampler (MAY, 1964). The exposure of individual workers was determined with a small suction trap, the "Casella"

personal sampler, worn on a harness, with the sampling orifice on the shoulder so that the air sampled was similar to that inhaled. Slides from the cascade impactor and membrane filters from the personal sampler were examined microscopically. Spores were classified as described by LACEY (1971b). Some slides were stained with cochineal red (PIMENTEL, 1971) to identify and count cork particles.

Isolations were made with an Andersen sampler on 2% malt extract agar and half-strength "Oxoid" nutrient agar (GREGORY and LACEY, 1963). Malt plates were incubated at 25 and 37°C, nutrient plates at 25, 37 and 55°C, and counted respectively after 7, 6 or 3 days' incubation.

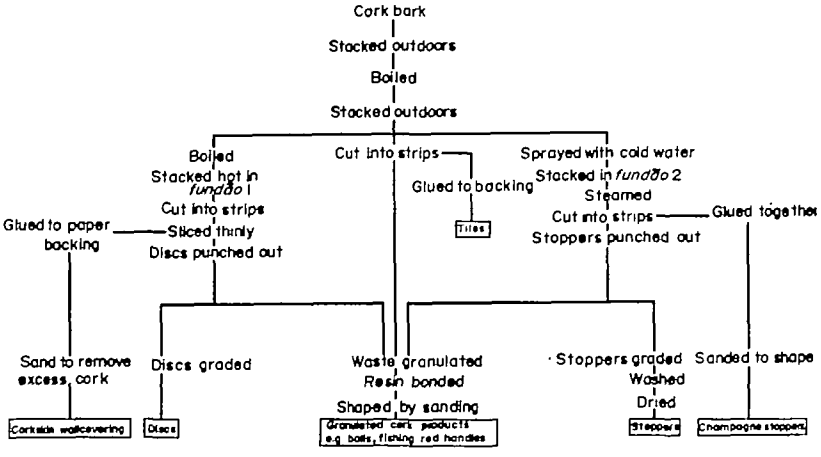

FIG. 1. Work flow diagram of the Seixal cork factory.
[ ] = end products.

RESULTS

The general air spora

Within buildings, airborne spores, mostly of fungi, numbered from 35×10^3 to $54\times10^6/m^3$ when there were $36–76\times10^3$ spores/m^3 outdoors. Departments differed greatly, depending on the mouldiness of the cork and the handling it received (Table 1). Fewest spores were found in the department where bales of cork were boiled before placing in a *fundão*. Most were in *fundão* 1 where the boiled cork was stacked while still hot. Mould was visible macroscopically after 4 days and was extensive after 7 when the cork was usually removed. During removal, concentrations exceeded 50×10^6 spores/m^3, although at other times fewer than 2×10^6 spores/m^3 were found.

Most cork in *fundão* 1 was used in the disc department where many spores were dispersed as the mouldy cork was cut into strips, sliced and cut into discs. Successive operations were separated by about 2–3 m along one side of the department. Along the middle of the department another conveyor carried discs as they were graded, and stoppers were similarly graded on other conveyors on the far side. Up to 22×10^6 spores/m^3 were found where the cork was cut into strips, but concentrations declined rapidly along the first conveyor, so that where discs were punched there were few more than the average for the department (Table 2).

Stoppers were also made from strips of cork which were cut at one end of the room and punched out on machines spaced throughout the rest of the department.

TABLE 1. MEAN CONCENTRATIONS OF AIRBORNE SPORES AND CORK PARTICLES IN DIFFERENT PARTS OF A CORK FACTORY

Department	No. samples	No. occasions	Concentration (10^6/m^3) Spores	Cork particles
Outdoors	3	2	<0·1	<0·1
Boiling shed	3	2	<0·1	<0·1
Fundão 1	9	4	26·4	<0·1
Fundão 2	1	1	1·4	<0·1
Disc	19	4	9·7	0·3
Stopper	11	3	2·5	0·7
Stopper grading	5	2	1·1	0·3
Stopper drying	2	1	0·4	5·7
Waste collection (disc)	1	1	2·1	0·9
Waste collection (stopper)	3	2	1·3	1·1
Stopper collection	2	1	2·3	0·9
Paper cutting	4	1	1·7	1·4
Gluing	2	1	0·1	0·6
Sanding	3	2	0·3	1·1
Champagne stopper	2	1	3·2	1·3
Ball	5	2	1·1	1·7
Tile	2	1	3·8	1·0

TABLE 2. SPORE CONCENTRATIONS ACROSS THE DISC DEPARTMENT

Work station				
Cork cut into strips	Strips sliced thinly	Discs punched from slices	Grading discs	Grading corks
Spore concentrations (10^6 spores/m^3 air)				
16·7	8·5	2·3	1·7	1.9

There were many fewer airborne spores ($1–3 \times 10^6$/m^3) when cork from *fundão* 2 was being cut, compared with almost 10^7 spores/m^3 with the mouldier cork from *fundão* 1. Spores were fewer among the machines making stoppers. Numbers varied little along the length of the room when the windows were shut, but opening them decreased numbers at the far end by more than half, relative to those close to the slicing machines.

Discs or stoppers and waste cork were collected in rooms below each department, where numbers of spores were similar to the average in the source departments, from 1 to $2·5 \times 10^6$ spores/m^3. The spore content of air progressively decreased in departments where the stoppers were successively graded, washed and dried.

The preparation of corkskin wall-covering was accompanied by few airborne spores. Up to 2×10^6 spores/m^3 were found close to the microtome cutting cork paper from blocks of cork. There were fewer than $0·5 \times 10^6$ spores/m^3 where the cork paper was glued on supporting paper and the excess was sanded-off. Machines sanding blocks of cork or granulated cork to shape were fitted with dust extractors, but up to 6×10^6 spores/m^3 were still found nearby. Surprisingly large numbers of spores were also found where decorative tiles were being made.

Spores of *Penicillium*, *Aphanocladium*, *Mucor* and *Monilia* predominated on cascade impactor slides, and there were few actinomycetes and bacteria. Table 3 shows the species isolated with the Andersen sampler and their relative abundance.

TABLE 3. SPECIES ISOLATED FROM AIR OF A CORK FACTORY

Species	Frequency
Aphanocladium album (Preuss) W. Gams	3
Monilia sitophila (Mont.) Sacc.	3
Mucor plumbeus Bon.	3
M. racemosus Fres. var *sphaerosporus* (Hagem) Schipper	1
Penicillium frequentans Westling	3
P. granulatum Bain.	3
P. funiculosum Thom.	1
P. brevicompactum Dierckx	1
P. corylophilum Dierckx	1
Trichoderma spp. (*T. harzianum* Rifai, *T. koningii* Oudem.)	2
Oidiodendron spp. (*O. rhodogenum* Robak, *O. tenuissimum* (Peck) Hughes)	2
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind	1
Chrysosporium pannorum (Link) Hughes	1
Cladosporium spp.	2
Sporobolomyces spp.	1
Papularia sp.	1
Aureobasidium pullulans (de Bary) Arnaud	1
Beauveria bassiana (Bals.) Vuill.	1
Humicola nigrescens Omvik	1

Key to frequency:
1: in fewer than 25% of samples;
2: in 25–75% of samples;
3: in more than 75% of samples.

The predominant *Penicillium* species was usually *P. frequentans*, but *P. granulatum* Bain. was widespread, abundant in *fundão* 2 and predominant in the ball department. In *fundão* 1 and the disc department up to 6×10^6 *P. frequentans* were isolated/m^3 air, while numbers exceeded $10^5/m^3$ air in most other departments (Table 4). Most *P. granulatum* ($4\times10^5/m^3$ air) occurred in the ball department. *Aphanocladium album* (Preuss) W. Gams, *Monilia sitophila* (Mont.) Sacc. and *Mucor plumbeus* Bon., although widespread, were abundant only in *fundão* 1 and the disc department. *A. album* numbered up to $2\times10^6/m^3$ air, but *Monilia* and *Mucor* were less abundant.

Cork particles were also airborne in all departments (Table 1). Most, up to $46\times10^6/m^3$ air, were found close to the sanding machines in the ball department, but many of these were large particles that soon settled so that the general concentration was about 2×10^6 particles/m^3 air. Concentrations exceeded $10^6/m^3$ air in other departments where cork was sanded or microtomed, where waste collected beneath the stopper department, where tiles were made and, unexpectedly, where washed corks were dried by circulating in hot air through large enclosed drums. In contrast to the fungus spores, few cork particles were found in the *fundões*, disc and stopper departments.

Variation with time

Air in some departments was sampled on several occasions (Table 5). Usually concentrations of both spores and cork particles varied little. The largest differences were in *fundão* 1 where spore concentrations were $2\times10^6/m^3$ when cork was not being

handled or when freshly boiled cork was being stacked, but about 20 times greater when mouldy cork was being removed from stacks. The number of airborne cork particles differed less.

TABLE 4. ABUNDANCE OF *Penicillium frequentans* COLONIES ON ANDERSEN SAMPLER PLATES AND *Penicillium* TYPE SPORES ON CASCADE IMPACTOR SLIDES EXPOSED IN DIFFERENT PARTS OF THE FACTORY

Department	*P. frequentans* colonies (10^6/m^3 air)	Total "*Penicillium*" spores (10^6/m^3 air)
Outdoors	<0·01	<0·1
Boiling	<0·01	<0·1
Fundão 1 (17 March)	2·40	13·9
Fundão 1 (28 March)	0·55	1·4
Fundão 2	0·08	1·1
Stopper, slicing end	0·22	0·6
Stopper, far end	0·05	0·1
Disc, near slicing	5·59	10·3
Disc, far side	1·10	3·1
Grading stoppers	0·12	1·1
Waste from stoppers	0·14	1·3
Ball	0·25	0·6
Champagne stopper	0·59	2·4
Corkskin	0·04	<0·1
Tiles	0·80	4·9

TABLE 5. CONCENTRATIONS OF AIRBORNE SPORES AND CORK PARTICLES IN DIFFERENT PARTS OF THE FACTORY ON DIFFERENT DATES

Department	17 March	21 March	23 March	28 March	30 March	11 April
	Spore concentrations (10^6 spores/m^3 air)					
Fundão 1	35·2	2·2	43·4	1·5	—	—
Disc: cutting into strips	20·9	—	16·7	11·2	—	17·0
: far side	—	—	1·9	3·6	—	—
Stopper: cutting into strips	—	3·1	—	1·0	—	6·3
: far end	—	1·4	—	0·2	—	—
: waste collection	—	1·3	—	—	1·4	—
: grading	—	—	1·2	—	1·3	—
Ball	—	—	—	—	1·5	0·2
	Concentration of cork particles (10^6/m^3 air)					
Fundão 1	<0·1	0	<0·1	0·1	—	—
Disc: cutting into strips	0·3	—	0·1	0·5	—	<0·1
: far side	—	—	0·4	0·2	—	—
Stopper: cutting into strips	—	1·6	—	0·5	—	0·5
: far end	—	0·4	—	0·1	—	—
: waste collection	—	0·9	—	—	1·6	—
: grading	—	—	0·4	—	0·4	—
Ball	—	—	—	—	0·5	4·2

— indicates that no sample was taken.

Exposure of workers to dust

The personal sampler was worn by representative workers in most departments where air was sampled by cascade impactor (Table 6).

TABLE 6. COMPARISON OF CASCADE IMPACTOR AND PERSONAL SAMPLER CATCHES AT DIFFERENT WORK STATIONS

	Concentration (10^6/m^3 air) estimated by			
	Cascade impactor		Personal sampler	
	Fungus spores	Cork particles	Fungus spores	Cork particles
Fundão 1: 17 March	35·0	<0·1	89·6	0·6
: 23 March	43·3	<0·1	55·2	0·6
Disc: cutting into strips	16·6	0·1	23·8	0·7
: slicing	8·3	0·1	44·9	0·7
: cutting discs	1·3	0·2	4·7	0·4
: Supervisor	—	—	25·6	1·3
Stopper: cutting into strips	3·9	<0·1	4·7	0·9
: cutting stoppers	1·2	1·0	3·4	0·8
: Supervisor	—	—	2·8	0·3
Grading stoppers	1·2	0·4	1·2	1·1
Champagne stoppers	2·4	1·3	2·7	1·6
Paper cutting	2·7	1·8	1·7	<0·1
Corkskin sanding	0·4	1·2	0·3	1·2
Ball	1·3	0·5	0·6	2·5

Usually workers in the first *fundão*, disc and stopper departments were exposed to more spores than the general air samples indicated, even when the latter were taken near their work stations. Workers in the first *fundão* were exposed to the highest mean daily concentrations—up to 128×10^6 spores/m^3. However, as they spent only part of each day loading mouldy cork on trolleys, and the remainder on less dusty work, this was probably an underestimate of the highest concentrations they breathed. The workers cutting and slicing cork in the disc department were also heavily exposed, as also was the local supervisor. By contrast, the supervisor in the stopper department was exposed to fewer spores than his workers and to many fewer than the disc supervisor.

Usually estimates of particle concentration by the personal sampler and cascade impactor were similar, but ball makers were exposed to more cork dust than the general atmosphere of the department suggested.

Particle size and lung deposition

The cascade impactor and Andersen sampler separate particles according to their aerodynamic size. About 50 per cent of unit density spheres of 4·5 μm diameter penetrate the first two stages of the cascade impactor when sampling at 20 l./min. and so can be considered capable of reaching the alveoli (GREGORY and LACEY, 1963). Table 7 shows the proportions of the most common spore types penetrating stage 2.

TABLE 7. PERCENTAGE OF PARTICLES PENETRATING STAGE 2 OF THE CASCADE IMPACTOR

Cork	22·4
Penicillium	68·3
Aphanocladium	95·3
Mucor	16·1
Monilia	10·1

Most *Monilia* and *Mucor* spores occurred on stages 1 and 2, while two-thirds of *Penicillium* and nearly all *Aphanocladium* spores penetrated further and so should reach the alveoli when inhaled. By contrast, only a fifth of the cork particles penetrated stage 2. Some of the larger particles soon settled out of the air so that the proportion of non-penetrating particles decreased with distance from the cutting and sanding machines. For instance, close to slicing machines 85 per cent of cork particles and 38 per cent of *Penicillium* spores were classified as non-penetrating while further away the figures were, respectively, 74 per cent and 11 per cent.

The only available pump allowed the Andersen sampler to be operated only at slightly less than the recommended sampling rate, so that the separation of particles between stages differed from that described by ANDERSEN (1958). Nevertheless, the proportion of colonies occurring on stages 3–6, which Andersen described as "penetrating", agreed closely with cascade impactor results for *Penicillium* and *Aphanocladium*. More *P. granulatum* colonies (73 per cent) occurred on stages 3–6 than *P. frequentans* colonies (64 per cent), probably resulting from the known tendency of *P. frequentans* spores to remain aggregated.

DISCUSSION

Sera from cork workers with suberosis yield multiple precipitin arcs with extracts of mouldy cork (ÁVILA and VILLAR, 1968). Of the fungi isolated from the air, only *Penicillium frequentans* extracts reacted similarly (PIMENTEL and ÁVILA, in press), but it is still not clear whether sensitization results from inhalation of the fungus spores or of cork particles containing fungal metabolites and hyphae. *Penicillium* spores were generally more numerous and smaller than cork particles in the factory air, and so more likely to penetrate deeply in the lungs, but lung lesions consistently contain cork dust (PIMENTEL, 1971; PIMENTEL and ÁVILA, in press). A precedent for a *Penicillium* sp. causing allergic alveolitis may be found in cheesewasher's lung caused by *P. casei* (DE WECK *et al.*, 1969). Actinomycetes were uncommon in the air and so are unlikely to be implicated as in farmer's lung and bagassosis, two other forms of allergic alveolitis (PEPYS *et al.*, 1963; LACEY, 1971a).

Factory hygiene could be greatly improved if moulding could be decreased. The contrast between the two *fundões* shows that improvement is possible, provided that cork prepared by wetting with cold water and steaming was acceptable for making discs. Other precautions might include adding fungicide to the boiling water or to a dip after boiling, as suggested by SARDICA (1971), providing these did not interfere with subsequent use; the *fundões* might be fumigated to kill fungus spores and decrease reinoculation of the cork; and ventilation and cleanliness might be improved. In other parts of the factory, dust levels could be decreased by improved ventilation and dust extraction close to the sources of dust, especially where the cork was first sliced. Respirators would decrease the number of spores inhaled but would be unpleasant to wear in the warm, humid *fundão*.

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