WET GRAINS STORAGES UNDER MODIFIED ATMOSPHERES. MICROBIOLOGICAL ASPECTS

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INTRODUCTION

Grain molds have long been considered as strict aerobic microorganisms but several microbiologists have recently said that under particular conditions fungi may show some metabolic activities and even mycelial growth in oxygen-free atmospheres (GUNNER and ALEXANDER, 1964 ; TABAK and COOKE, 1968 ; BULL and BUSHELL, 1976).

Those recent data could question the feasibility of controlled atmospheres storages from a microbiological point of view.

To investigate further what happens with stored grains, experiments have been carried on with maize at different moisture content levels, in airtight conditions and under nitrogen.

Under anaerobic conditions, molds that are said to be able to grow or to develop metabolic activities, cannot sporulate (TABAK and COOKE, 1968). So, enumeration of conidia by the classical suspension-dilution method cannot be used to demonstrate a possible mold activity. This method has nontheless been used as a control for anaerobiosis. To some extent, the "Ulster" method (MUSKET and MALONE, 1940) allows to account for mycelial growth but is not really quantitative. That is why the mycological analysis has been complemented with a gas-chromatographic determination of specific volatile compounds which indicate a fungal activity and besides with the high pressure liquid chromatography analysis of fungal ergosterol for mycelial growth examination.

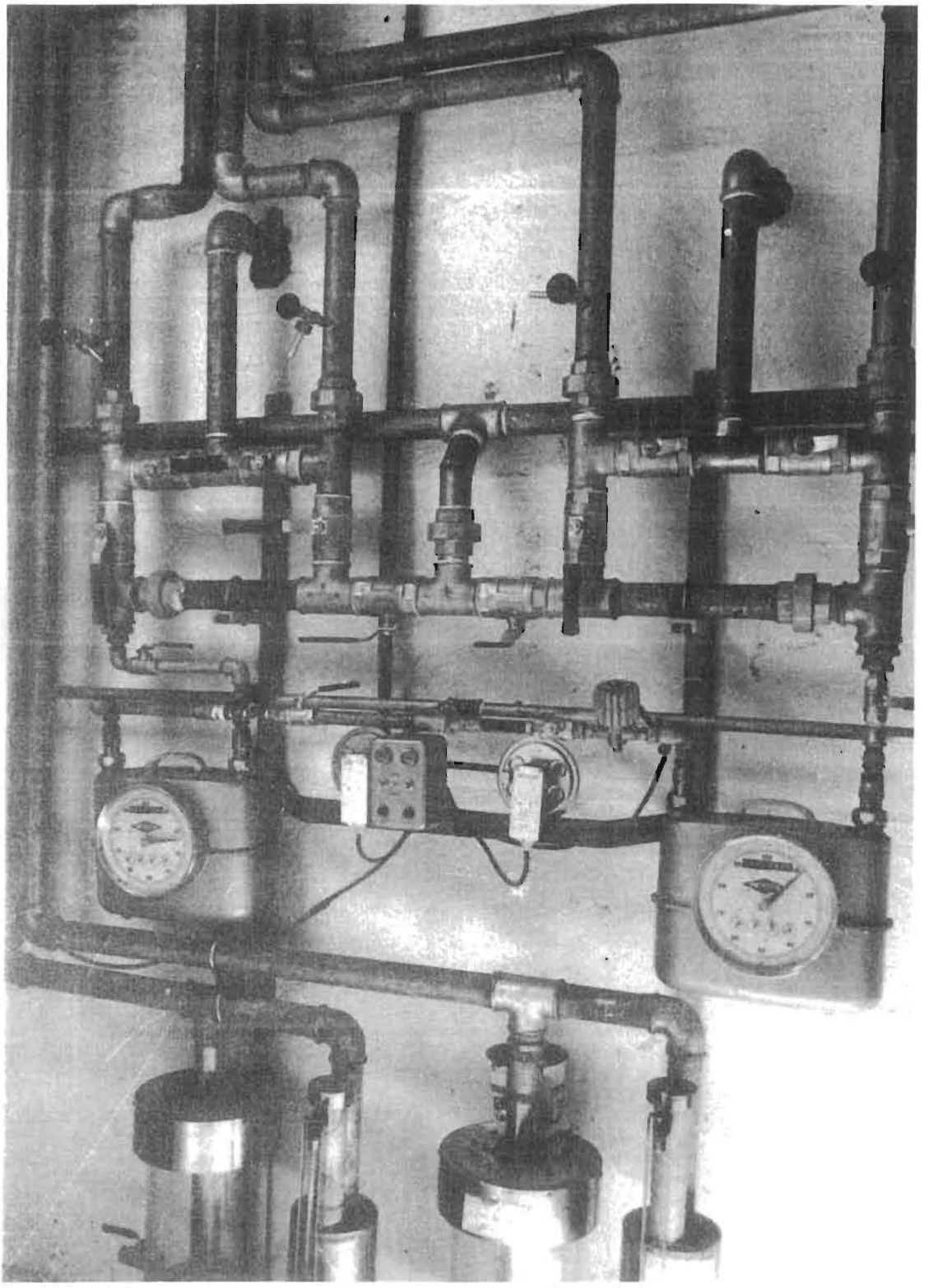
1. MATERIALS AND METHODS

1.1 Operating conditions

High moisture grains harvested at 36 p.100 moisture content (M.C.), are used without treatment. Intermediate moisture grains (17 and 23 p.100 M.C.) are heat-dried grains.

In airtight storage experiments, grains are placed into one liter glass containers hermetically closed. For experimental storages under nitrogen, anaerobic jars are used after modification allowing gas introduction. After filling up with grains, air is evacuated and replaced by very high purity nitrogen, three times running and introduced endly with a slight over-pressure.

In each case, anaerobiosis is checked by means of a methylene blue indicator.



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1.2 Methods

1.2.1. Enumeration of bacteria

The classical microbiological methods have been employed for enumeration of the following bacteria groups : General mesophilic bacteria (P.C.A.), Enterobacteriaceae (VRBG), Lactobacilli (Rogosa agar), Anaerobes (TGY agar) and especially sporulated Clostridia.

1.2.2. Yeasts and molds

As previously indicated, the suspension-dilution method has been used, especially for yeasts counts (CAHAGNIER, 1973). Concerning the Ulster method (malt extract agar) 400 grains are analysed each time, 200 grains being superficially disinfected, 200 grains being not. Disinfected grains lead to aberrant results probably due to an increasing permeability of stored kernels (PELHATE and THERIAULT, 1979). For this reason those results are not considered there.

1.2.3. Collection and analysis of volatile organic compounds

The volatiles are desorbed under vacuum, collected in a dry-ice cooled trap and recovered with methylene chloride. The extract is then concentrated by solvent evaporation and analysed by gas-chromatography, using a glass capillary column (50 m length, 0,5 mm i.d.) coated with carbowax 20M. Oven temperature is programmed from 70° to 150°C at a rate of 2°C/min. with helium as carrier-gas (RICHARD-MOLARD et al., 1976).

1.2.4. Fungal ergosterol assay

Fungal ergosterol is extracted with methanol from grounded kernels, saponified with KOH, extracted from methanol with petroleum ether, purified and analysed by HPLC using a 5µ -Spherisorb Column (SEITZ and al., 1977).

2 AIRTIGHT STORAGES

2.1. Microflora evolution

2.1.1. Bacteria

In high-moisture grains (36 p.100 M.C.) the evolution of bacterial microflora is mainly characterised by an active growth of Lactobacilli (fig. 1) during the first 15 days, at 22°C and 30°C. As a consequence, the pH is decreasing from 6 to about 4 in the same time. Enterobacteriaceae are rapidly decreasing and cannot be detected anymore after 2 months. Anaerobic bacteria, others than Lactobacilli, do not grow and sporulated Clostridia practically disappear within three months, probably because of the low pH value.

In grains stored at 23 p.100 M.C., at 22°C and 30°C, all bacterial populations are decreasing slowly (fig. 2) and the pH remains constant. Same results are observed at 17 p.100 moisture content.

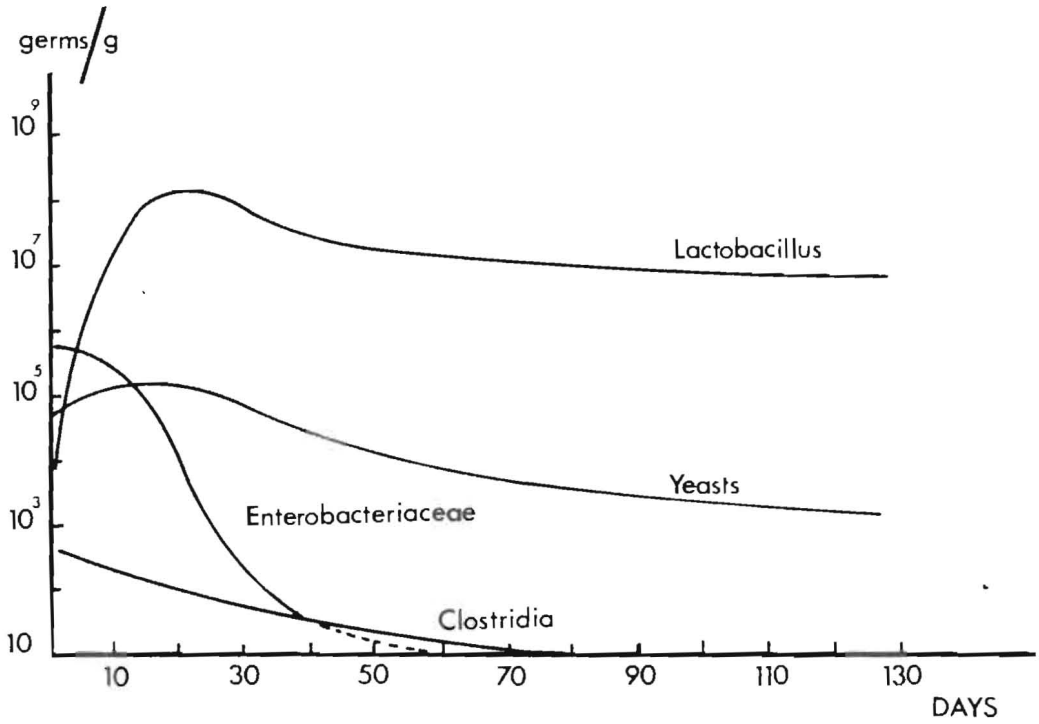

Fig. 1 Airtight stored grains (36 p.100 M.C., 22°C). Evolution of bacteria and yeasts.

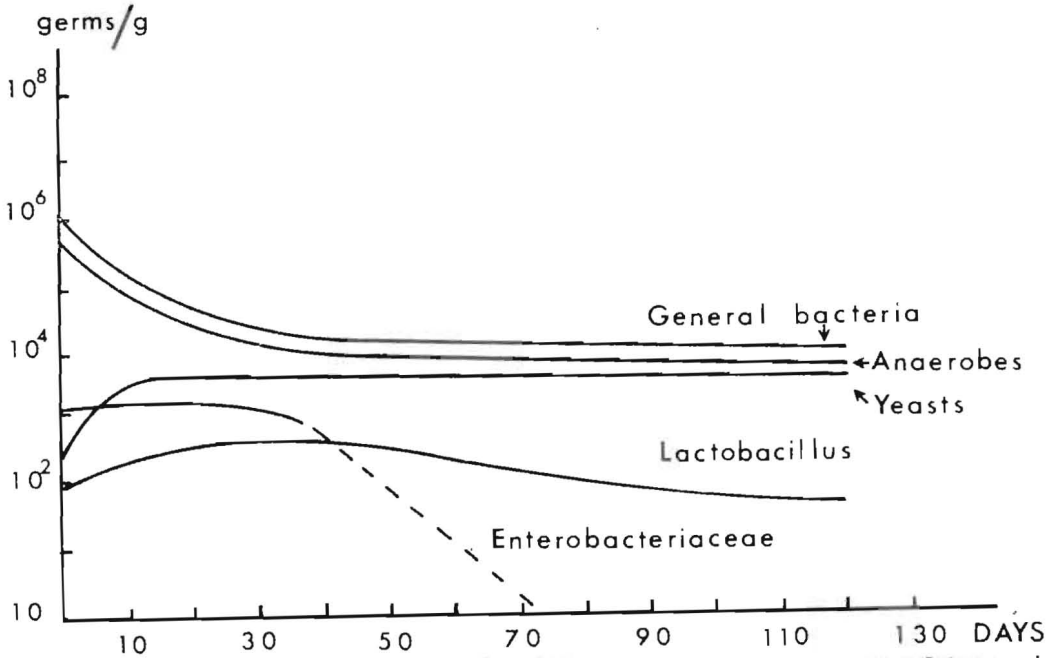

Fig. 2 Airthight stored grains (23 p.100 M.C., 22°C). Evolution of bacteria and yeasts.

2.1.2. Yeasts

In wet grains, yeast counts do not change during about 1 month and then are significantly decreasing. At 23 p.100 M.C., the repartition of yeasts is very heterogeneous and results are more dispersed. Nevertheless the Ulster method results show the population being increasing very slowly during all the experiment, but such an increase does not occur at 17 p.100 M.C.

2.1.3. Molds

On grains stored at 36 p.100 M.C., the initial contamination is about $2{,}5.10^4$ germs/g. Two weeks later, it becomes impossible to detect molds by dilution method. Results obtained by Ulster method are resumed in table I.

TABLE I. : Grains airtight stored (36 p.100 M.C., 22°C). Percentage of contaminated grains.

FUNGI	TIME IN DAYS					
	0	21	36	65	96	122
FUSARIUM	38	38	0,5	-	-	-
EPICOCCUM	10	2	-	-	-	-
MUCORALES	10	17	9	-	-	-
PENICILLIUM	20	22	11	-	-	-
TRICHODERMA	3	5	-	-	-	-
YEASTS	60	72	70	67	63	71

Field species like Fusarium sp., Alternaria sp., Epicoccum sp., are rapidly decreasing in such conditions of humidity and acidity. Fusarium species, which are present on 38 p.100 of the grains at the begining disappear within one month. The same evolution is observed with storage species (Aspergillus and Penicillium) : within two months, the grains seem practically sterile.

The evolution observed at 23 p.100 M.C. is quite different (Table II).

The percentage of grains contaminated by Fusarium sp. remain almost constant. Other species are decreasing slowly, except Penicillia which seem to be able to grow very slightly.

For the grains stored at 17 p.100 M.C. the decrease of molds population is slower but the growing of Penicillia does not occur. No significative difference has been observed between 22°C and 30°C.

TABLE II. : Grains airtight stored (23 p.100 M.C., 22°C) Percentage of contaminated grains.

FUNGI	TIME IN DAYS					
	0	7	35	69	96	118
FUSARIUM	38	34	36	36	28	25
EPICOCCUM	10	2	-	-	-	-
CLADOSPORIUM	5	-	-	-	-	-
MUCORALES	10	13	10	10	7	8
PENICILLIUM	-	9	22	46	25	21
TRICHODERMA	3	15	6	-	1	-
YEASTS	60	70	73	78	80	78

2.2. Fungal ergosterol

Preliminary assays have shown that a very good correlation does exist between mold growth and ergosterol content of open-stored grains (24 p.100 M.C.). Ergosterol is not detectable in non-moldy grains. Results obtained with grains stored at 36 and 23 p.100 M.C. show that no fungal growth occurs in airtight conditions and confirm the results of mycological analysis (fig. 3). It is interesting to notice that ergosterol determination does not allow to take into account the fungal population decrease, ergosterol remaining present on grains while the percentage of contaminated grains is decreasing.

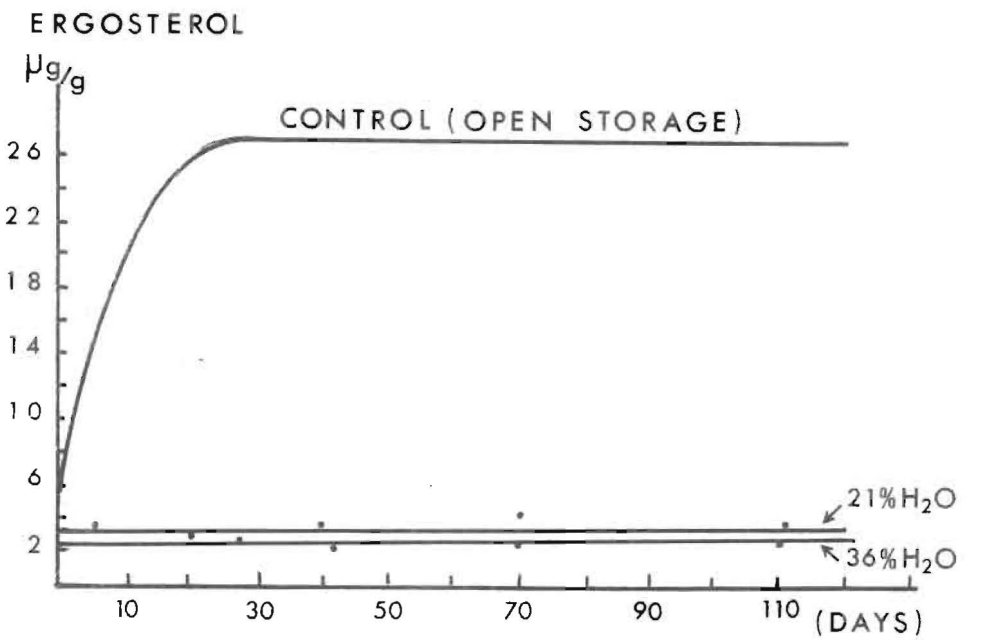

Fig. 3 Fungal ergosterol evolution in grains airtight stored at 21 p.100 and 36 p.100 moisture content.

2.3. Volatile organic compounds

Chromatograms shown in the figure 4 are those obtained with grains being stored during 17 days at 22°C.

On wet grains (36 p.100 M.C.), the metabolic activity of yeasts and heterofermentative Lactobacilli is clearly shown and active lactic and alcoholic fermentations occur. Characteristic compounds like ethanol (peak 1), isobutyl and isoamyl alcohols (peaks 2 and 3) and 3-hydroxy-butanone (peak 4) are produced.

But in grains at 23 and 17 p.100 M.C., the metabolic activity remains undetectable and fungal specific compounds such as 1-octene-3-ol or 3-octanone (RICHARD-MOLARD and al., 1976 ; STAWICKI and al., 1973) are not produced.

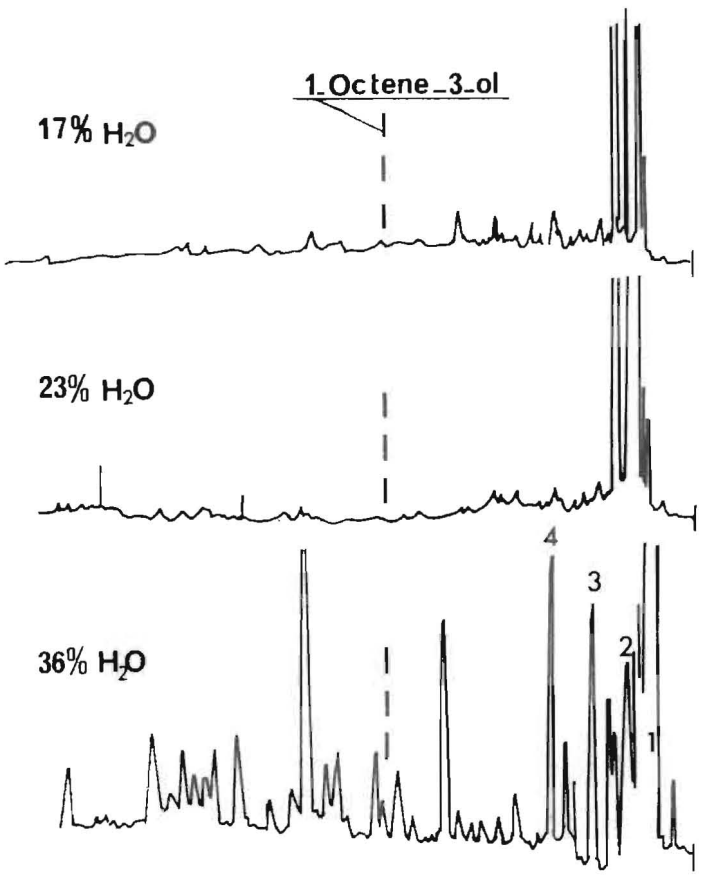

Fig. 4 Volatiles produced in airtight stored grains.

3 STORAGE UNDER NITROGEN

In this part of the study, experiments have been carried on at 30°C with grains at 17 and 21 p.100 moisture content.

The results obtained are quite similar to those previously described for airtight stored grain. Therefore it seems not necessary to detail them.

Table III show the molds evolution on grains stored under pure nitrogen.

TABLE III : Grains stored under nitrogen. Percentage of contaminated grains

FUNGI	21 p.100 M.C. T I M E (days) 0	38	164	17 p.100 M.C. T I M E (days) 0	38	164
FUSARIUM	78	60	37	49	57	10
A. FLAVUS	78	-	-	74	66	27
A. NIGER	10	-	-	10	3	1
PENICILLIUM	10	0,5	-	5	-	-
RHIZOPUS	36	-	-	43	18	-

As in airtight stored grain, the initial mycoflora is progressively decreasing, the regression of different species being more rapid at 23 p.100 M.C. than at 17 p.100, Fusarium spp. appearing the most resistant to the anaerobiqsis. At 23 p.100 M.C. a slight alcoholic fermentation occurs (shown by gas-chromatography) possibly due to yeasts activity.

4 SURVIVAL OF CONIDIA IN AIRTIGHT CONDITIONS

4.1. Experimentals

The pure cultures of storage fungi used in this part of the study were isolated from ensilated damp grains. In a preliminary step, autoclaved maize grains 30 p.100 M.C., are inoculated with conidia and incubated 8 days at 24°C in air. The moldy grains obtained are then divided in three parts : the first one, used as control, is simply stored in air ; the second part is placed in Gas-Pack anaerobic jars and so stored in strictly anaerobic conditions. A small nylon net-bag is filled with the third part of moldy grains and suspended in a glass container which is then completely filled with freshly harvested damp grain (30 p.100 M.C.) and hermetically closed in order to obtain airtight conditions.

Conidia germinative capacity is checked four month later in the following manner : the total number of conidia produced on grains is determined with the Coulter counter, while conidia that are still able to germinate are numbered on malt agar plates. The germinative capacity is expressed as surviving conidia to total conidia count (Coulter counter) ratio, in percentage.

4.2. Results

As can be seen on fig. 5, conidia survival is drastically reduced for Aspergillus versicolor in anaerobiosis or airtight conditions.

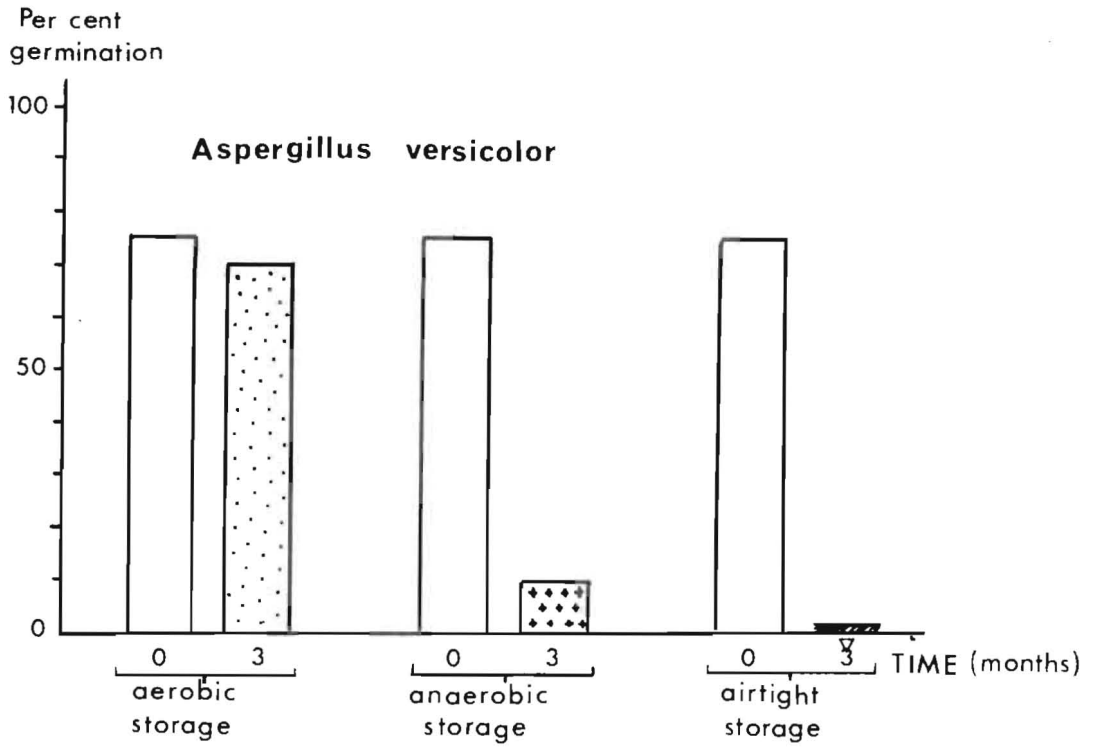

Fig. 5 Germinative capacity of conidia of Aspergillus versicolor

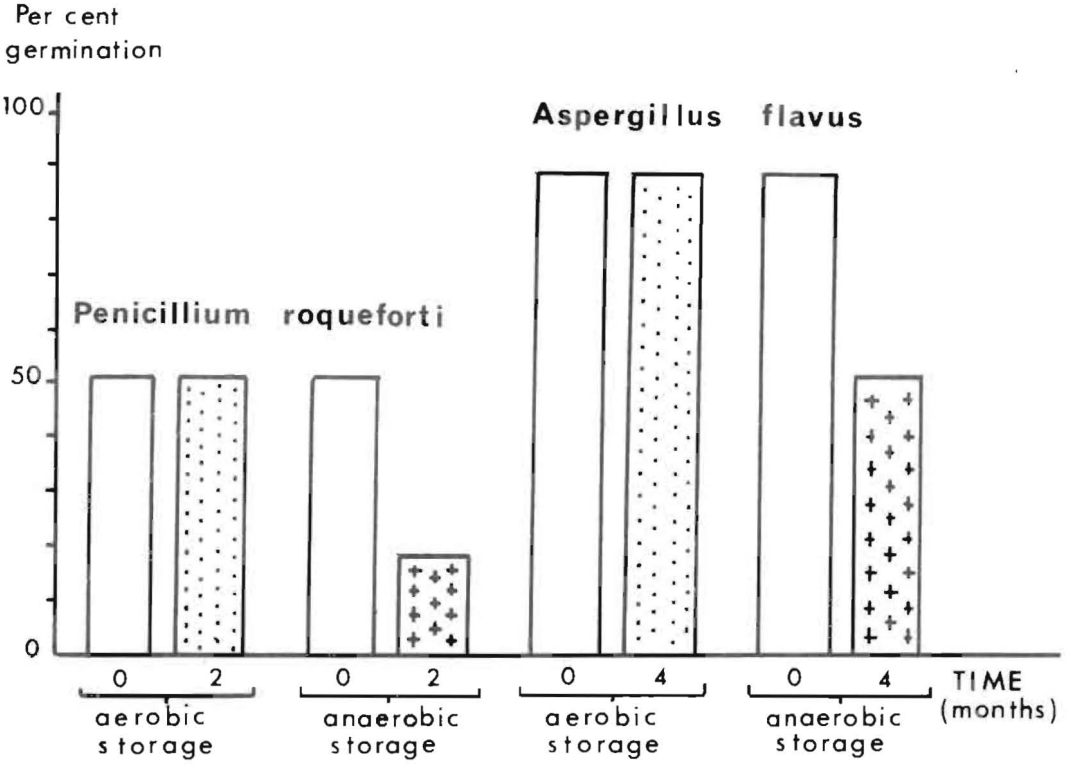

Fig. 6 Germinative capacity of A. flavus and P. roqueforti.

As a general rule, conidia survival is rather better under strict anaerobiosis than under airtight atmospheres. The behaviour of A. flavus and P. roqueforti is shown on fig. 6. After four months in anaerobiosis, 50 p.100 of A. flavus conidia are still able to germinate but with P. roqueforti which is considered as micro-aerophilic, less than 20 p.100 of produced conidia are still able to germinate after only two months.

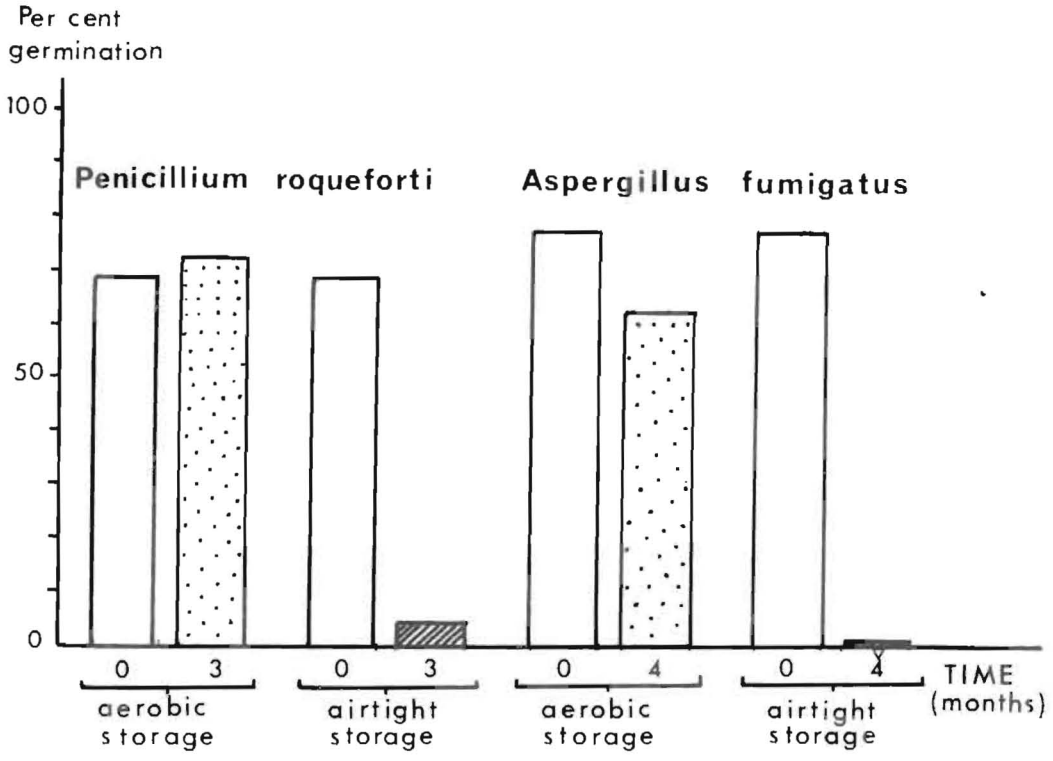

Fig. 7 Germinative capacity of A. fumigatus and P. roqueforti.

In airtight conditions (fig.7), the decrease of conidia survival is greater for P. roqueforti and A. fumigatus is shown to be very affected by the lack of oxygen, conidia destruction being nearly complete within four months.

CONCLUSION

Results obtained in this study show that no fungal growth or activity can be observed in grains stored under very low oxygen tensions. In all experimented conditions, fungal populations are decreasing, the regression being more rapid when grain moisture content is higher.

Technics of grains storage under modified atmospheres, which are as yet not very wide-spread in France, will certainly undergo an important development in the coming years.

The rapidly increasing cost of energy leads to considering other storing technics than the usual grain drying. The question is of particular interest in France for maize which is very often harvested at very high moisture content such as 30 p.100 to 40 p.100, or more. Considering the results obtained in this study, it is possible to say that maize which is largely used for animal feeding can be stored under nitrogen or more simply in airtight conditions without microbiological objections. With high-moisture grains, such storage technics lead to lactic and alcoholic fermentations which modify mainly the organoleptic characteristics of the product, but this seems to be no real problem for use in animal feeding.

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