EFFECT OF NITROGEN STORAGE ON THE FUNGAL CONTAMINATION OF CEREAL GRAINS

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ABSTRACT

The mycological contamination of soft and hard wheat, malting barley, rice, maize and sunflower seeds at various temperatures and moisture contents, experimentally stored in nitrogen, was studied.

Microbiological analysis showed that the stored grains are preserved as well in anoxia as in air at low moisture contents, not permitting fungal growth. In nitrogen the initial equilibrium of microorganisms is maintained more stable than in aerated grains.

At moisture contents permitting fungal but not yet bacterial growth, the proliferation of moulds in air was extremely high and within 3-4 weeks fungi could be observed macroscopically on the seeds. In nitrogen the development of fungi could be inhibited for several months, depending on the initial microbiological contamination of the grains and the oxygen impurities of the nitrogen used for the substitution of the interstitial atmosphere. The effect of nitrogen storage was shown to influence both mould counts and the selection of some genera of fungi.

INTRODUCTION

Food preservation research, in particular grain storage, demonstrates that fungi are a major cause of spoilage in stored grains. That fungi recently are recognized as a cause in the seeds of: discoloration, heating, biological decay (as decreasing germination percentage, increase of fatty acids, alteration of sugars, and diminution in weight), and production of mycotoxins (Christensen and Lopez, 1963; Christensen and Kaufmann, 1969; Roberts, 1972; Sinha and Muir, 1973).

From the standpoint of their ecology and the moisture content requirements these fungi can be divided into two groups: field fungi and storage fungi.

The field fungi invade seeds developing on the plants in the field, or after the seeds have matured and plants are still standing or are cut and swathed. All field fungi require a high moisture content in order to grow, a moisture content in equilibrium with relative humidities of 90-100%.

The storage fungi comprise several species of the genus Aspergillus and Penicillia, they are the major ones involved in the deterioration of stored grains. They have the ability to grow in materials the moisture contents of which are in equilibrium with relative humidities of 70-90%. All of these fungi invade the germ or embryo of the seeds preferentially.

In seed storage the basic requirements and factors influencing all fungal growth are a favourable humidity, temperature, a suitable atmosphere, the length of storage and the condition of the grain, amount of cracked and broken seeds, the nature and distribution of the debris, whether the embryos are alive or dead, the presence, numbers and activities of insects and mites (Christensen and Hodson, 1960; Christensen, 1964; Ayerst, 1969).

We have done intensive research on grain storage, especially under several moisture contents of seeds and different atmospheres. In this paper we report the mycological results of several experiments on grain storage, in air or under nitrogen. The species of seeds studied were soft and durum wheat, paddy, maize, malting barley and sunflower seeds at various moisture contents.

MATERIALS AND METHODS

The purpose of mycological examination on the seed is to give enough information about identification of common genera of moulds and the number count of the colonies. The fundamental methods for the mycological analysis of seeds are:

1) Samples taken by sterilized steel sampling devices.
2) External inspection of seeds under a binocular microscope.
3) Total mould counts, obtained by plating an inoculum from an extract of 10 g of grain in 100 ml physiological solution containing an antibiotic, in order to exclude bacterial growth. The values are reported

as number of germs/gramme dry matter (Di Maggio et al., 1976).
4) The internal fungal flora determined by surface - disinfecting 10 g aliquots of unbroken kernels by NaOCl and subsequent aseptical plating of the seeds; the values expressed as percent of the kernels from which fungi grew. For the two mycological examinations nutritive medium used: Mycological Agar (pH = 7 Difco).
5) Isolation and microscopic identification of the common genera of moulds and of some species.

RESULTS AND DISCUSSION

In this communication we report the mycological results of several typical storage studies on nitrogen atmosphere preservation of grain in laboratory and in pilot-scale storage bins at different moisture contents.

The first experiment was on 300 kg-aliquots of 12% moisture content soft wheat, variety "Conte Marzotto", preserved in silos for 4 ÷ 5 years in air and technical nitrogen ($<$ 0.3% oxygen). During this storage the fungi progressively decreased. These results were confirmed by one year preservation periods carried out with soft wheat at 14.5% and with durum wheat at 13% moisture contents both in laboratory silos and in 250 q-lots at ambient temperature (fig. 1).

Twenty three tons of malting barley, national production, 12% moisture content, were preserved for one year in technical nitrogen; total mould counts decreased although some storage fungi (Penicillia) appeared towards the end of the trial and internal moulds were frequent in the kernels even at the start of the experiment.

Other results on paddy, maize and sunflower seeds, at moisture contents up to 14.5%, confirmed that there is no difference between storage in air or in nitrogen (Table 1-2).

Storage problems are evident at high moisture contents, higher than 15%, because at the relative humidity in equilibrium with such moisture, moulding and heating of kernels increase.

At the moisture, between 15 and 18%, the beneficial effect of storage in nitrogen is evident also in short periods of time since mould attack is the main cause of fast deterioration.

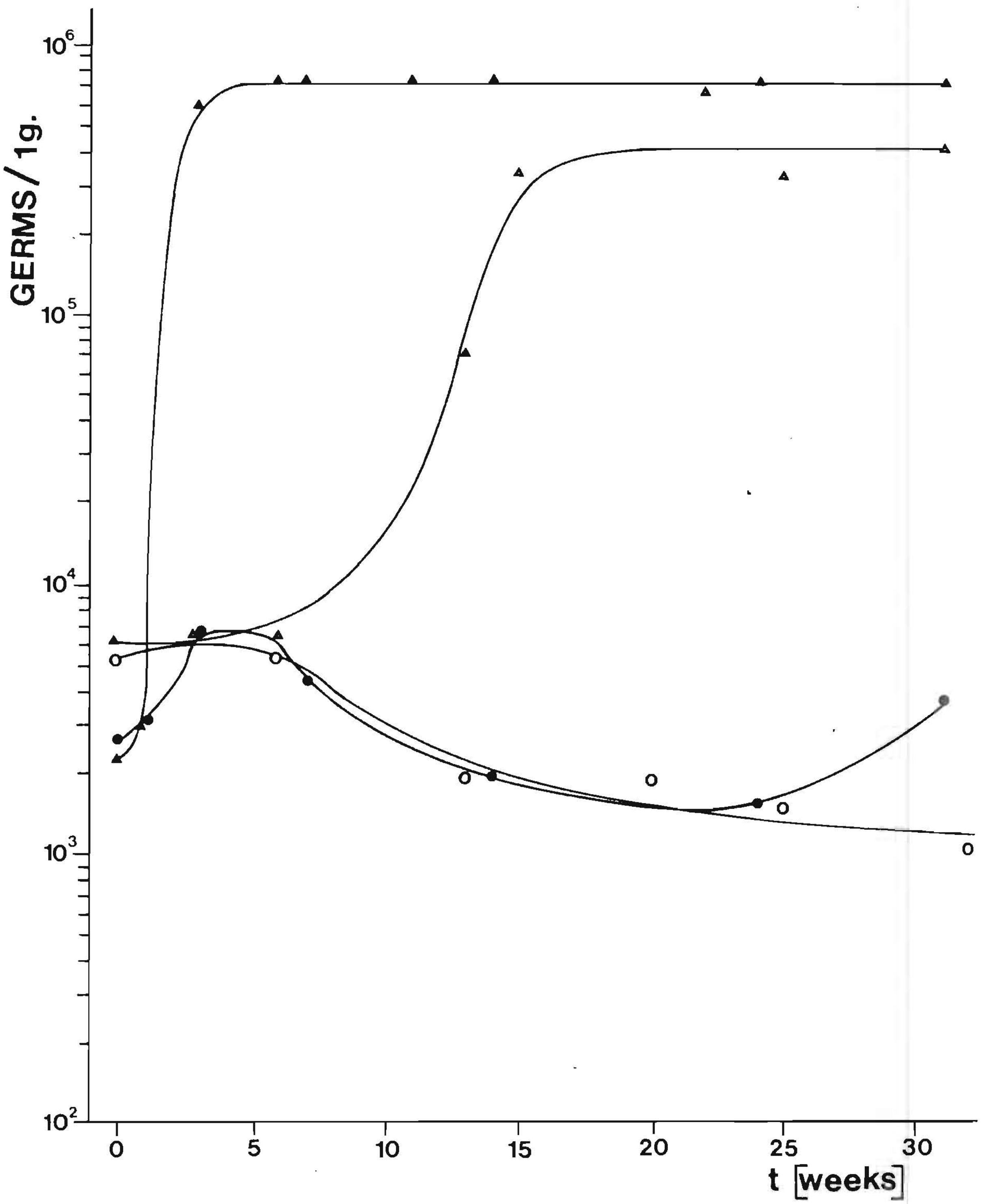

Fig. 1. Evolution of total mould counts in wheat at 14.5% (o-●) and 17% moisture contents (▵-▲), preserved in air (●-▲) and nitrogen (o-▵).

TABLE 1

Mould counts, expressed as germs per g dry matter, of low moisture grains (11%-13%) preserved in air and technical nitrogen.

Grain	Gas	Storage period (weeks)					
		0	5	16	28	40	50
Wheat	N_2	10^3	10^2				10^2
	Air	6.10^3	3.10^3				3.10^3
Paddy	N_2	3.10^4		5.10^3	10^3	2.10^3	
	Air	2.10^4		4.10^3	8.10^3	10^4	
	N_2	4.10^3	9.10^2	5.10^2	2.10^2	5.10^2	
	Air	4.10^3	10^3	$8\ 10^2$	10^3	10^3	
Maize	N_2	6.10^3	9.10^2	3.10^2	5.10^2	2.10^2	
	Air	6.10^3	1.10^3	1.10^3	2.10^3	1.10^3	

TABLE 2

Mould counts, expressed as germs per g dry matter, of low moisture content (6%) sunflower seeds stored in air and N_2 at 30°C.

	Storage period (weeks)							
	0	9	11	17	19	21	36	45
Air	1.10^5		7.10^4		3.10^4	2.10^4	2.10^4	3.10^3
N_2	1.10^5	8.10^4		2.10^4		4.10^4	3.10^4	2.10^3

In technical nitrogen, containing up to 0.3% oxygen, moulds develop slower than in air but are not inhibited (fig. 1). The better storability of the grains in technical nitrogen is evident especially over long periods of time, but after the lag-phase of fungal development, deterioration cannot be avoided at these residual oxygen concentrations.

In pure nitrogen (less than 0.01% oxygen) fungal proliferation was found to be completely inhibited and total germ counts tended to diminish in time (fig. 2), while internal moulds were very scarce (2-3%).

The same results were obtained for sunflower seeds with 9.5% moisture content, and for maize at 18.2% (Tables 3 and 4).

TABLE 3

Mould counts, expressed as germs per g dry matter, of high moisture (9.5%) sunflower seeds stored in air and N_2 at 25°C.

	Storage period (weeks)			
	0	4	9	13
Air	2.10^5	5.10^4	7.10^5	5.10^5
N_2	2.10^5	5.10^3	4.10^3	1.10^3

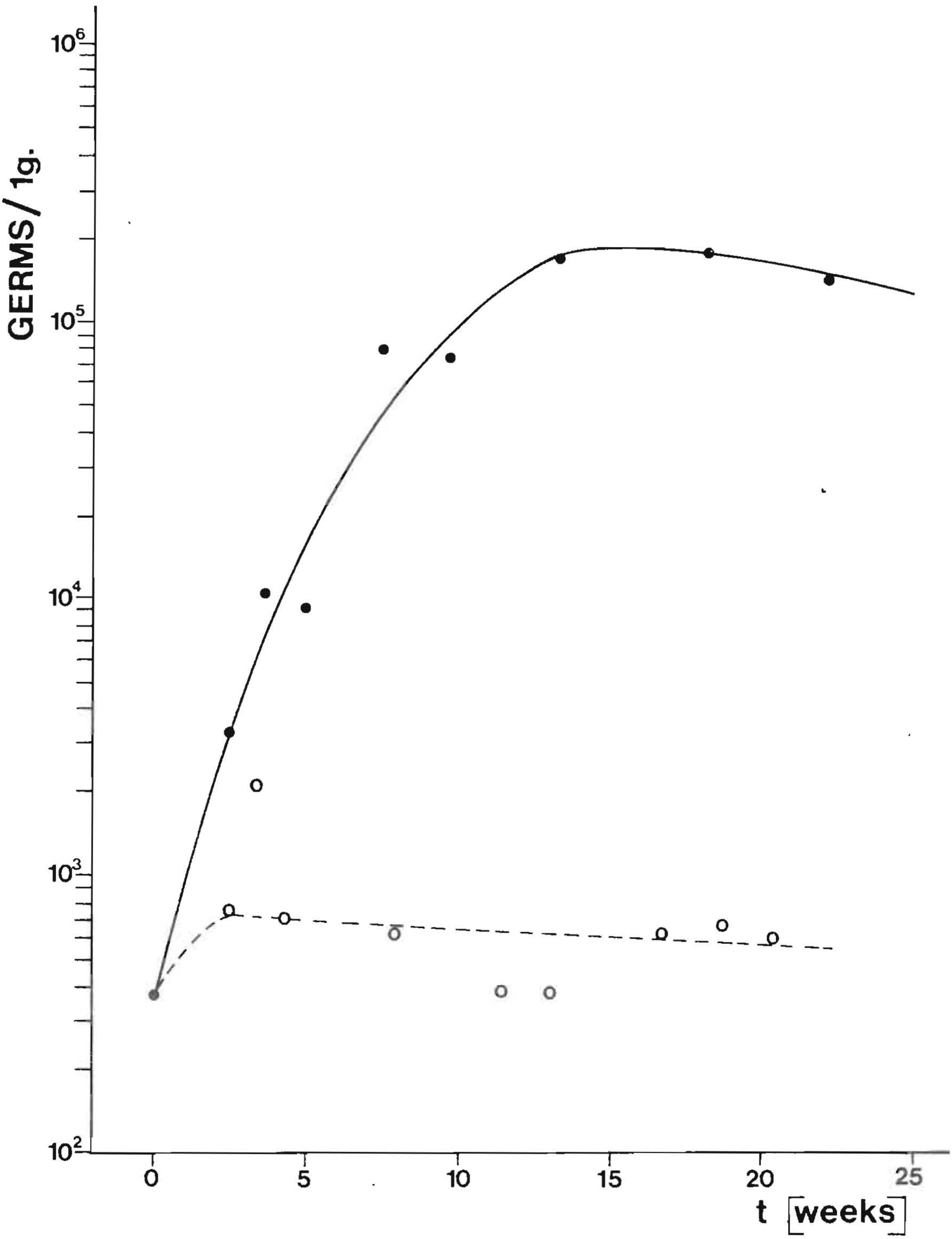

Fig. 2. Evolution of moulds in 18% moisture content wheat, stored in air (●) and pure nitrogen (O).

TABLE 4

Mould counts, expressed as germs per g dry matter, of high moisture (18.2%) maize stored in air and N_2.

	Storage period (weeks)			
	0	4	11	18
Air	4.10^2	1.10^4	1.10^5	8.10^5
N_2	4.10^2	1.10^2	8.10^3	1.10^3

These mycological results are in accordance with the loss of viability of seeds and technological quality, and are strictly correlated to the quality of the seeds at the start of storage.

As to the type of moulds present, the situation was as follows: field fungi (such as: Cladosporium and Alternaria) decreased during storage, while storage moulds (such as: Penicillium and Aspergillus) increased in all the batches. In air Aspergillus and Penicillium growth is faster.

The results of the laboratory and pilot-scale trials were confirmed for wheat and malting barley in two large scale storage trials: 500 t-silos for preservation of wheat and 1500 t for malting barley storage.

In conclusion there can be no doubt on the advantages presented by the storage of grains in nitrogen especially for high moisture content. It permits longer storability of grains with exclusion of temperature and moisture increase, exclusion of off-odours, retarded loss of viability and retarded loss of technological and nutritional quality.

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