Responses of Fungi to Modified Atmospheres

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Abstract

The use of controlled atmospheres (CA) for insect control in the bulk storage of grains can have the added benefit of controlling mould growth and mycotoxin production. Mould deterioration accounts for significant losses in stored grains, particularly in tropical countries where the temperature and relative humidity are high. The problem is exacerbated where grains are inadequately dried before entering storage.

Atmospheres high in CO_2 are more effective in controlling fungal growth than those which exclude O_2 by replacement with nitrogen. Although most fungi require some oxygen for growth, many spoilage species are efficient scavengers and are capable of near normal growth in O_2 concentrations of <1%. Atmospheres containing about 20% CO_2 generally inhibit mould growth, but >80% CO_2 may be required to prevent fungal deterioration of high moisture commodities. Some *Fusarium, Aspergillus,* and *Mucor* species are particularly tolerant of high levels of CO_2 Mycotoxin production is more sensitive than fungal growth to low O_2 and high CO_2. Concentrations of CO_2 between 20 and 60% have been demonstrated to prevent or significantly reduce mycotoxin production by some *Fusarium, Aspergillus,* and *Penicillium* species. Reduction of O_2 content is less effective in preventing mycotoxin formation.

MEDIUM to long term storage of grains in tropical regions presents many problems, as grain is frequently stored at a higher moisture content than is desirable, and invasion pressure from insects is often high. Both these factors will also encourage mould growth, and postharvest losses in stored grains due to insects and other pests, and fungal spoilage are considerable.

The introduction of controlled atmosphere storage of commodities for insect control also offers considerable scope for control of fungal deterioration. However, many storage fungi are capable of growth in low partial pressures of oxygen, and reduction of available oxygen is often not sufficient to prevent moulding, particularly of high moisture grains. Elevated levels of CO_2 are more inhibitory to mould growth, but other factors, such as temperature and moisture content, will affect the degree of inhibition exerted by controlled atmospheres.

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Effect of Controlled Atmospheres on Mycoflora of Stored Commodities

Investigations into the effects of reduced O_2 and increased CO_2 on moulds in stored commodities date back at least to the early 1950s, when hermetic storage of grains was proposed as a new technology (Vayssiére 1948). The earliest studies were undertaken with maize (Bottomley et al. 1950) and wheat (Peterson et al. 1956) and dealt mainly with spoilage by storage fungi. Studies undertaken after the mid 1960s were more concerned with the proliferation of mycotoxigenic fungi, and the effects of controlled atmospheres on mycotoxin production.

Maize

Storage of high moisture content maize presents a significant problem in many parts of the world, including the USA. Bottomley et al. (1950) investigated the effects of reduced

oxygen on maize stored at relative humidities between 75 and 100%, and temperatures from 25 to 45°C, but their storage period was only 12 days. They found that mould growth was significantly reduced but not prevented by storage in an atmosphere of 0.1% O_2 and 21% CO_2. Different moulds predominated depending on the storage conditions. At 80% relative humidity, *Penicillium* species were dominant at 25°C, *Aspergillus flavus* at 30°C, and *Eurotium* species at 35°C (Table 1). Mould growth was less at 40 and 45°C, but *Mucor* was predominant at 45°C, especially when the oxygen concentration was 5% or less. In maize at 90% ERH or higher, *Candida* species proliferated in the 0.1% O_2 and 21% CO_2 atmosphere at 25°C, but not at higher temperatures.

Wilson et al. (1975) investigated the effects of modified atmospheres on the survival of the toxigenic moulds *A. flavus* and *Fusarium moniliforme* in freshly harvested high moisture maize (moisture content 29.4%) and maize rewetted to 19.6% moisture. The maize was inoculated with *A. flavus* and exposed to atmospheres of air, N_2 (99.7%, balance O_2), CO_2 (61.7%) and low O_2 (8.7%), and a CA mixture of 13.5% CO_2, 0.5% O_2 and 84.8% N_2. In the freshly harvested maize, *A. flavus* levels increased in the air control to 90% kernel infection after 2 weeks, but with the other treatments kernel infection rate was only 5-18% after 4 weeks (Fig. 1). *F. moniliforme* was recovered from 21% of the kernels initially, but in subsamples exposed to modified atmospheres for four weeks, then held for 1 week in air, was present in 100% of kernels from all three treatments. The CO_2 + low O_2 sample developed an unpleasant odour, and was visibly overgrown with an unidentified yeast. In the rewetted maize, *A. flavus* did not decrease in any of the treatments, and increased in the N_2 and CA treatments. The incidence of *F. moniliforme* increased from 67% to near 90% in all treatments. The incidence of other fungi (*Penicillium, Eurotium,* other *Aspergillus* species, *Rhizopus* and *Mucor*) was low, and did not increase during modified atmosphere storage.

In a longer term experiment, Wilson et al. (1977) used maize with a moisture content of 18.8% for a storage trial in an atmosphere of 14–15% CO_2 and 0.5–1.0% O_2. Maize stored for 35 and 109 days in this atmosphere was tested for aflatoxins and the presence of *A. flavus* and *F. moniliforme*. No aflatoxin was detected after 35 or 109 days, whereas a control sample stored in air contained 472 µg/kg total aflatoxins. A significant proportion of the kernels contained *A. flavus* (30–47%) and *F. moniliforme* (35–47%), after both storage periods, and 27% of kernels contained a *Penicillium* species after 109 days storage. The

Table 1. Predominant mycoflora in maize stored for 12 days at 80% ERH in 20% CO_2 and 0.1% O_2. Data of Bottomley et al. (1950).

Temperature (°C)	Species (%)	
25	*Penicillium*	(55)
	A. flavus	(45)
30	*A. flavus*	(90)
35	*Eurotium*	(50)
	Penicillium	(50)
40	*Eurotium*	(70)
	A. flavus	(25)
45	*Mucor*	(25)
	Penicillium	(50)
	A. flavus	(15)

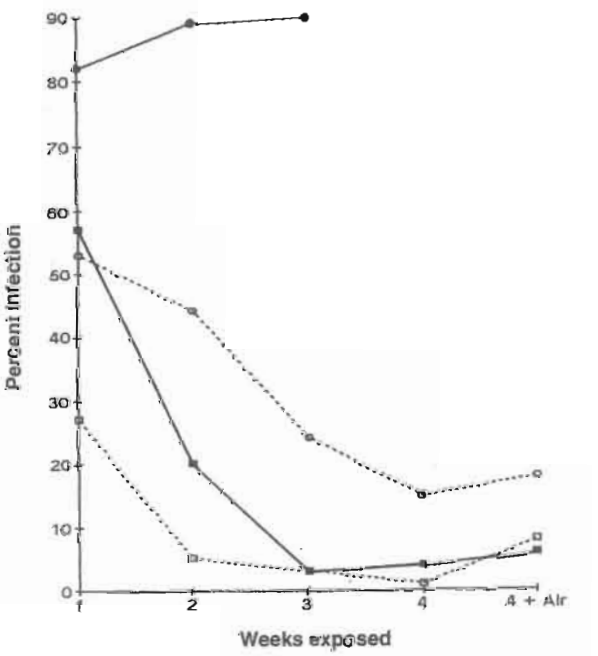

Fig. 1. Effects of modified atmospheres on *Aspergillus flavus* infection of inoculated high-moisture maize. Data of Wilson et al. (1975).
(●) Air (0.03% CO_2, 21% O_2, 78% N_2)
(○) CO_2 + low O_2 (61.7% CO_2, 8.7% O_2, 29.6% N_2)
(■) N_2 (99.7% N_2, 0.3% O_2)
(□) CA (13.5% CO_2, 0.5% O_2, 84.8% N_2)

maize was not assayed for *Fusarium* or *Penicillium* toxins.

Controlled atmosphere storage of high moisture maize in atmospheres containing <1% O_2 could be used for temporary holding before drying, or, at lower temperatures, for longer-term storage, the main advantages being residue-free insect control and retardation of fungal growth. However, because most fungi are not killed by low O_2 atmospheres, the safe storage period for high moisture maize is limited and the maize will deteriorate rapidly upon exposure to the normal atmosphere.

Peanuts

Much attention has been paid to control of aflatoxin production in stored peanuts by use of controlled atmospheres, and this aspect will be addressed later in this paper. However, relatively little has been published on the mycoflora changes that occur in peanuts stored under controlled atmospheres over long periods.

The effects of CO_2 on growth and sporulation of *A. flavus* on high moisture peanuts were reported by Landers et al. (1967) and Sanders et al. (1968). Growth and sporulation were reduced with each 20% increase in CO_2 from 20% to 80%, with no growth occurring in 100% CO_2. Growth was much reduced in atmospheres of < 5% O_2, and almost completely inhibited at < 1% O_2. Concentrations of CO_2 in excess of 20% were required before there was any inhibition of growth of *A. flavus* in high moisture peanuts. However, Jackson and Press (1967) reported that incidence of *A. flavus* at 27°C on shelled peanuts of 5.0% moisture content (m.c.) or unshelled peanuts at 7.5% m.c. was not reduced by storage in atmospheres containing 3% O_2 or 82% CO_2 in air compared with air storage over 12 months.

Wilson et al. (1985) used pilot scale experiments to determine if long-term storage of peanuts was practical in modified atmospheres with minimal deterioration due to mould spoilage, aflatoxin contamination and insect infestation, without use of refrigeration or pesticides. Two large bins of peanuts (1996 kg and 6451 kg) were stored in an atmosphere of approximately 60% CO_2 (balance air), at a moisture content of 6–7% for one year.

The smaller (metal) bin experienced moisture migration due to condensation of water on or near the surface at night, the moisture content of the peanuts at the top rose to 11.1% and they were visibly mouldy after 16 weeks. After this time, the atmosphere was recirculated, and moisture contents rapidly equilibrated throughout the bin. The most common species at the top of the bin were *A. flavus*, *Eurotium* species, and an unidentified white yeast, possibly a *Candida* species (Table 2). Other *Aspergillus* species (*A. candidus*, *A. ochraceus* and *A. niger*) were also recorded on 18% of kernels, while *Rhizopus* and *Penicillium* were less frequently isolated. The same species of fungi were isolated from kernels at the bottom of the bin, but in much lower numbers (Table 2). Despite the high incidence of *A. flavus*, no aflatoxins were detected.

In the second trial with the larger (fibre-glass) bin of peanuts, the atmosphere of 55–60% CO_2 was recirculated, and there was no moisture migration. The only major change observed in the mycoflora was a decrease in superficial *Penicillium* contamination for 64 to 16%. Aflatoxins were not detected during the 54 week trial.

Wheat

The mycoflora of wheat differs from that of maize and oilseeds. Wheat is usually drier when harvested, and in general *A. flavus* and *F. moniliforme* cause fewer problems in this commodity.

Petersen et al. (1956) stored wheat of 18% m.c. for 16 days at 30°C under atmospheres with varying concentrations of oxygen and carbon dioxide. In 4.3% O_2, the mycoflora was dominated by *Eurotium* species (80%), with *Penicillium* species and *A. flavus* also present (10% each). When O_2 was reduced to 2.3%,

Table 2. Fungal colonisation of peanut kernels stored at 7% m.c. in 50–60% CO_2 in an outside bin at ambient temperatures for 12 months. Data of Wilson et al. (1985).

Species	Percent kernel invasion	
	Top of bin	Bottom of bin
A. flavus	95	18
A. candidus	5	1
A. niger	9	20
A. ochraceus	4	1
Eurotium	87	21
Penicillium	3	4
Rhizopus	12	38
Candida	100	11

only *Eurotium* (67%) and *Penicillium* (33%) were present (Table 3). In 0.2% O_2 only *Eurotium* species were detected, and their numbers were much reduced (Table 3). With gas mixtures containing 21% O_2 and varying concentrations of CO_2, there was no significant change in numbers of fungi present in up to 18.6% CO_2. However, growth was almost completely inhibited by 50% and 79% CO_2 (Fig. 2). *Eurotium* species were the most tolerant of elevated levels of CO_2

Shejbal and Di Maggio (1976) and Di Maggio et al. (1976) stored wheat of 18% m.c. in pure nitrogen, and found that mould growth was inhibited, and fungi gradually decreased with time. After 30 weeks, there was an increase in *Aspergillus candidus*. After 54 weeks, the total mould count was 6 x 104/gram, a quite acceptable level for wheat. Under 0.2% O_2, mould growth at 18-26°C on wheat of 17.4% m.c. was substantially inhibited in comparison with the air control. However, with both treatments, *A. candidus* eventually proliferated, reaching counts of 6×10^5/gram after 3 and about 20 weeks, respectively.

Rice

In a study on naturally contaminated rice, Richard-Molard et al. (1986) investigated the effects of oxygen deficiency on microflora of grain rewetted to 0.87 and 0.94 a_w and stored for 2-4 months. They found that in the samples where the moisture content was low enough to prevent bacterial growth (0.87 a_w), most storage fungi, including *Penicillium* and *Aspergillus* were inhibited by atmospheres of less than 1% O_2. However, yeasts (*Candida* spp.) and the yeast-like fungus *Aureobasidium pullulans* were able to develop, even with less than 0.5% O_2, and the higher the a_w, the more rapid the growth. In the complete absence of O_2 (under 100% CO_2 or N_2), there was no fungal growth. At a_w, values higher than 0.90, lactic acid bacteria proliferated, and were not inhibited by any of the atmospheres studied.

Table 3. Effect of oxygen concentration on mould population and distribution in wheat stored for 16 days at 18% moisture and 30°C. Data of Peterson et al. (1956).

Oxygen (%)	Moulds/g	Species
0.2	7.0×10^3	*Eurotium*
2.3	1.9×10^5	*Penicillium*
	2.9×10^5	*Eurotium*
4.3	1.0×10^5	*Penicillium*
	8.0×10^5	*Eurotium*
	1.0×10^5	*A. flavus*
8.0	1.0×10^5	*Penicillium*
	5.6×10^5	*Eurotium*
20.6	6.8×10^5	*Penicillium*
	3.9×10^5	*Eurotium*
	5.6×10^4	*A. flavus*

Effect of Gas Mixtures on Growth of Fungi

The two factors that need to be considered in preventing fungal growth in controlled atmospheres are (1) the minimum amount of oxygen required for fungal growth and (2) the inhibitory effects of high levels of CO_2. Atmospheres high in nitrogen are only effective because of their low O_2 content, as nitrogen itself has no inhibitory effects.

Oxygen Requirements

Many fungi are able to grow in the presence of very small amounts of oxygen (Miller and Golding 1949; Follstad 1966; Wells and Uota 1970; Walsh 1972; Yanai et al. 1980; Gibb and Walsh 1980; Magan and Lacey 1984). Anaerobic growth has also been reported for several fungi, for example, *Fusarium oxysporum* (Gunner and Alexander 1964) and some species of *Mucorales* that are used as starter cultures for

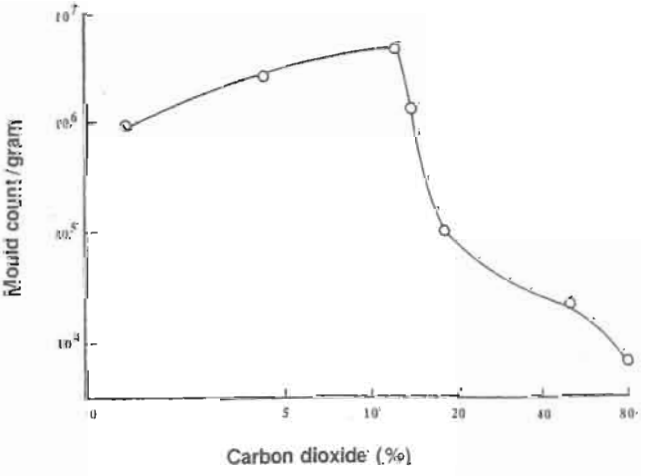

Fig. 2. Effect of CO_2 tension on mould count in wheat incubated 20 days at 30°C and 18% moisture. All gas mixtures contained 21% O_2. Data of Peterson et al. (1956).

food fermentations in Asia (Hesseltine et al. 1985). Tabak and Cook (1968) reported 'good to very good' growth of a range of species under 100% nitrogen. The strongest growth was exhibited by *Geotrichum candidum*, a yeast-like fungus, *Mucor heimalis, Fusarium oxysporum*, and *F. solani*. However, 'good' growth was observed in *Aspergillus niger, A. fumigatus, Penicillium aurantiogriseum*, and *P. brevicompactum*, and the black yeast-like fungus *Aureobasidium pullulans*. Such anaerobic growth can take place only if a number of growth factors (vitamins, oxygen donors in the form of higher oxidation states of certain elements) are supplied.

What is perhaps more relevant to CA storage of commodities, is the ability of many common field and storage fungi to grow in atmospheres containing <1% O_2 (Fig. 3). Of the field fungi present on grains at harvest, e.g. *Fusarium* species, *Alternaria*, other dematiaceous hyphomycetes, *Rhizopus*, yeasts, etc., some grow very well in low levels of oxygen. *Fusarium moniliforme, F. oxysporum, F. culmorum*, and *F. solani* all grow strongly in atmospheres containing 1.0% to 0.1% O_2 or even less (Gunner and Alexander 1964; Tabak and Cook 1968; Walsh 1972; Gibb and Walsh 1980; Magan and Lacey 1984), provided that other growth conditions such as temperature and water activity are favourable. Some *Rhizopus* and *Mucor* species can also grow at low oxygen tensions (Wells and Uota 1970; Gibb and Walsh 1980; Yanai et al. 1980) or even anaerobically (Hesseltine et al. 1985), and can proliferate in high moisture commodities stored under low oxygen atmospheres (Bottomley et al. 1950; Wilson et al. 1975). Other field fungi such as *Alternaria* and *Cladosporium herbarum* are more sensitive to reduced oxygen tensions (Magan and Lacey, 1984) and gradually die out during storage.

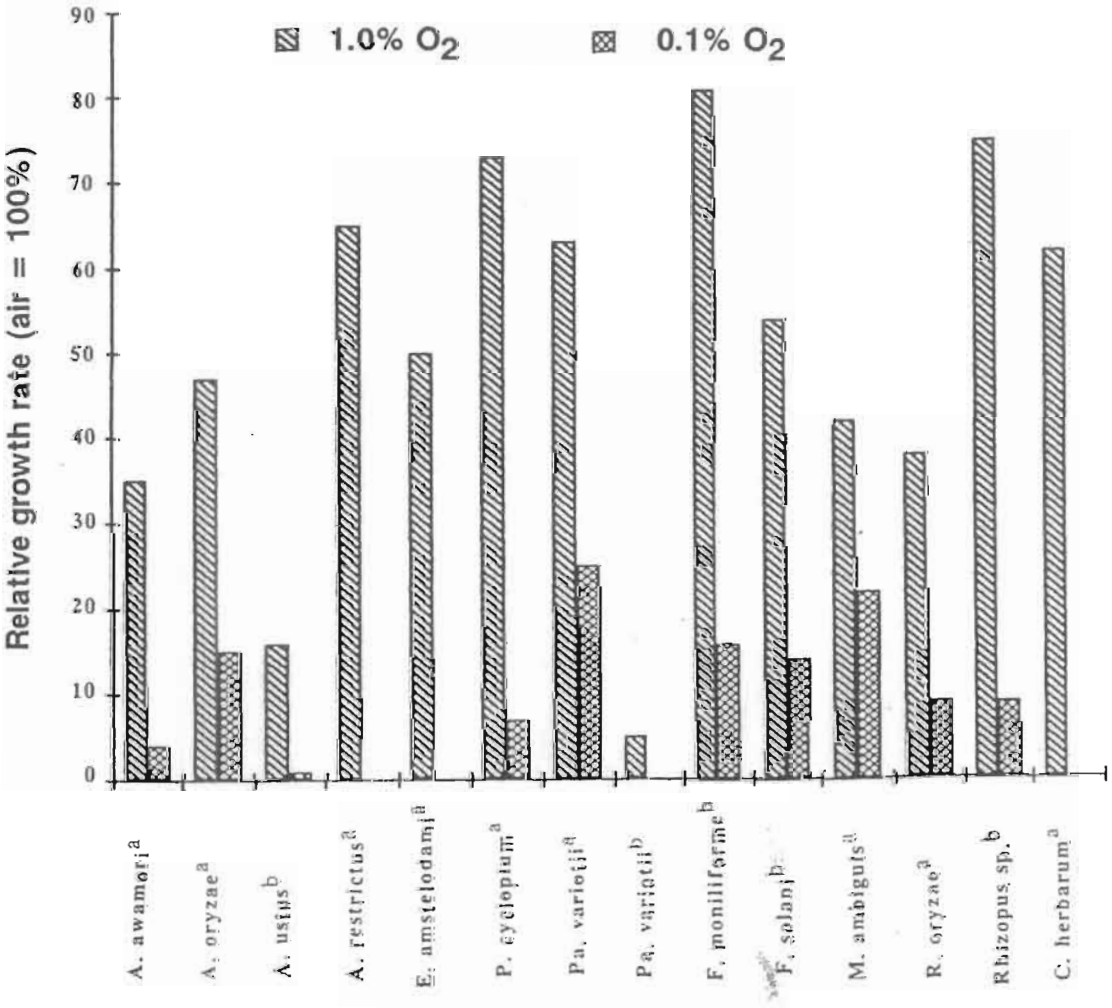

Fig. 3. Effects of reduced O_2 tensions on growth of some field and storage fungi. Data of a. Yanai et al. (1980); and b. Gibb and Walsh (1980).

Storage fungi such as *Penicillium* and *Aspergillus* species are generally more sensitive to low levels of O_2 than the more tolerant field fungi. With the exception of *P. roquefortii*, the growth rates of most *Penicillium* species are reduced by more than 50% in atmospheres of 1% O_2 or less (Yanai et al. 1980; Magan and Lacey 1984). Of the *Aspergillus* species, *A. candidus* is the most tolerant of reduced O_2 conditions (Magan and Lacey 1984) and thus can proliferate in CA stored wheat (Shebjal and Di Maggio 1976; Di Maggio et al. 1976). Some *Eurotium* species are also reasonably tolerant of low O_2 levels (Petersen et al. 1956; Yanai et al. 1980).

In our laboratory, studies on a number of spoilage fungi isolated from low O_2 environments have shown that most are inhibited only slightly when grown in nitrogen atmospheres, with 0–1.0% O_2 (Fig. 4). Isolates of *Penicillium corylophilum* and *P. glabrum* from vacuum-packed jams were able to grow at 66–90% of their control rate (air) when sealed in barrier film with an atmosphere of nitrogen. *Fusarium equiseti* and *F. oxysporum* which caused fermentative spoilage of UHT fruit juices grew at 88–97% of their normal rate. A *Cladosporium* species isolated from the inside of a UHT pack of apple juice was little affected by lack of oxygen, growing at 95–100% when sealed in an atmosphere of nitrogen. *Mucor plumbeus* and *Absidia corymbifera* also grew strongly in nitrogen. The xerophilic fungus *Eurotium repens* grew at 60–90% of the control rate, depending on the growth medium, and the extreme xerophile *Xeromyces bisporus* grew at the same rate in air and in nitrogen (Fig. 4).

Effects of Increased Carbon Dioxide Levels

Levels of CO_2 from 4% to 20% can be stimulatory to growth of many fungi in atmospheres containing low levels of O_2 (Wells and Uota 1970; Gibb and Walsh 1980), conditions that may well arise during sealed storage of commodities. However, elevated CO_2

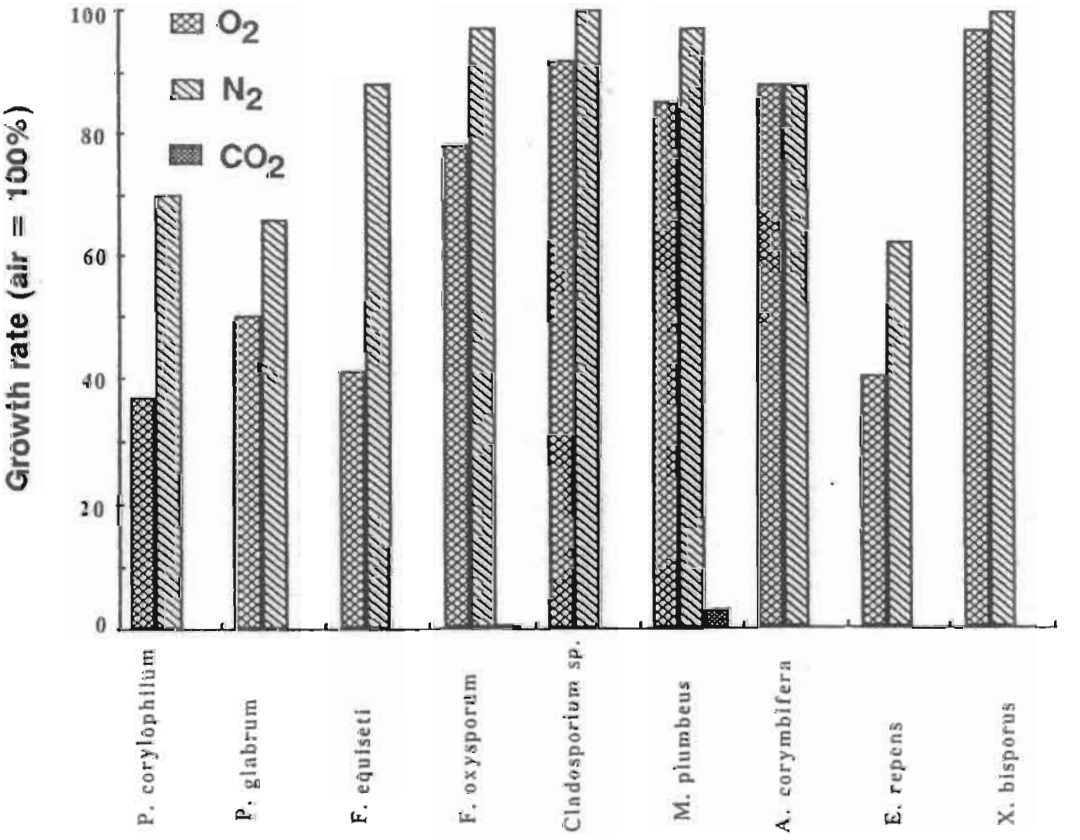

Fig. 4. Growth rates (relative to air) of nine fungi in O_2, N_2, and CO_2. Fungi were grown in pure culture on Petrislides (Millipore Corp.) sealed inside barrier film containing the appropriate atmosphere, and incubated at 25°C. Growth was measured by radial growth rate of colonies.

concentrations are generally much more effective in controlling fungal growth than oxygen depletion. Thus, atmospheres rich in CO_2 are more likely to prevent mould deterioration of CA stored high moisture commodities than atmospheres of nitrogen with traces of O_2. Typical insecticidal atmospheres used for grain storage are 1% O_2 in nitrogen and 60% CO_2 40% air (Banks 1981), and while both may be equally effective in controlling insect populations in stored grain, the CO_2–enriched atmosphere would be more effective in controlling fungal growth in high-moisture commodities.

Atmospheres containing > 50% CO_2 will substantially inhibit growth of most spoilage fungi (Petersen et al. 1956; Wells and Uota 1970) but there is little information in the literature on their actual CO_2 tolerances. Stotzky and Goos (1965) recorded slight growth of *Rhizopus stolonifer, Mucor hiemalis,* and a *Trichoderma* species in 100% CO_2. The same three species grew well in an atmosphere of 50% CO_2, 45% N_2, and 5% O_2. *Fusarium oxysporum* grew in 95% CO_2, 5%N_2 but not in 95% CO_2, 5% O_2. *Paecilomyces lilacinus* did not grow in either of these atmospheres, but grew reasonably well in 50% CO_2, 45% N_2, and 5% O_2.

Magan and Lacey (1984) reported that >15% CO_2 was required to halve the linear growth rate of most of the 14 species of field and storage fungi tested at 0.98–0.90 a_w and 23°C. The species most sensitive to elevated CO_2 concentrations were *Penicillium brevicompactum, Aspergillus fumigatus, A. nidulans,* and *A. versicolor* (Table 4). However, no upper limits of CO_2 tolerance were determined, as the maximum concentration of CO_2 tested was 15%.

Nine species were tested in our laboratory for their ability to grow in an atmosphere of 97–99% CO_2 with trace amounts of O_2 and N_2. Only *Fusarium oxysporum* and *Mucor plumbeus* grew, and their growth rates were only 0.5–4% of those in air (Fig. 4).

Wells and Uota (1970) showed that growth of *Alternaria alternata, Botrytis cinerea, Rhizopus stolonifer,* and *Cladosporium herbarum* in atmospheres of 10, 20, 30, and 45% CO_2 plus 21% O_2 decreased linearly with increasing CO_2 concentrations and was inhibited about 50% in an atmosphere of 20% CO_2 (Fig. 5). Growth of a *Fusarium* species, cited as *F. roseum* was stimulated at 10% CO_2, and inhibited 50% at 45% CO_2

Table 4. Concentrations of CO_2 required to halve the linear growth rate of field and storage fungi at 23°C. Data of Magan and Lacey (1984).

	Water activity		
	0.98	0.95	0.90
Field fungi			
A. alternata	>15.0	>15.0	>15.0
C. cladosporioides	>15.0	>15.0	>15.0
C. herbarum	13.0	>15.0	>15.0
E. nigrum	>15.0	>15.0	>15.0
F. culmorum	14.0	13.5	>15.0
Storage fungi			
P. brevicompactum	11.5	8.5	15.0
P. aurantiogriseum	4.5*	4.0*	>15.0
P. hordei	>15.0	8.5	9.5
P. piceum	>15.0	>15.0	14.5
P. roquefortii	>15.0	>15.0	4.5
A. candidus	>15.0	>15.0	>15.0
A. fumigatus	>15.0	5.2	2.5
A. nidulans	>15.0	6.5	13.5
A. versicolor	12.0	>15.0	14.5
E. repens	>15.0	>15.0	>15.0

* Stimulation of growth occurred at higher CO_2 concentrations.

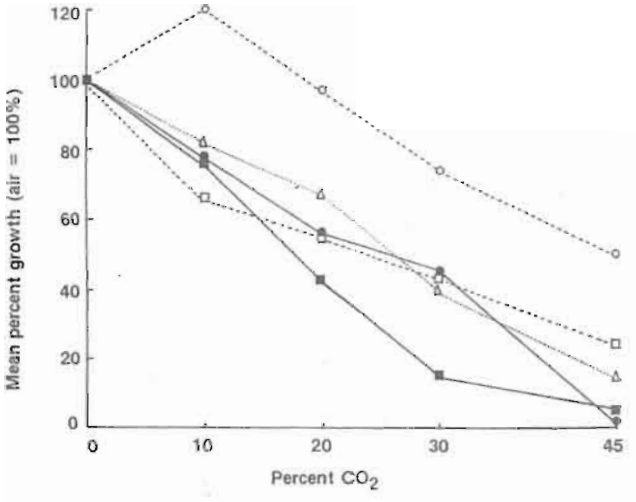

Fig. 5. Growth of five fungi in 21% O_2 with different levels of CO_2, cultured on liquid media at 19°C. Growth was measured by dry weight of mycelia. Data of Wells and Uota (1970). (○) *Fusarium 'roseum'*; (△) *Rhizopus stolonifer;* (□) *Alternaria alternata;* (●) *Botrytis cinerea;* (■) *Cladosporium herbarum.*

The effects of CO_2 concentrations on fungi growing in stored commodities, rather than in pure culture, seem to vary. Landers et al. (1967) and Sanders et al. (1968) reported that growth of *A. flavus* on high moisture peanuts was inhibited by 80% CO_2/20% O_2, but Jackson and Press (1967) found no reduction in *A. flavus* on peanuts stored at 5% m.c. (approximately 0.7 a_w) in 82% CO_2 in air for 12 months. This perhaps indicates that although 80% CO_2 will inhibit growth of *A. flavus*, conidia of this species are not killed by exposure to high levels of CO_2 at low a_w. Peterson et al. (1956) reported that *Eurotium* species survived and grew in wheat stored in 50% CO_2/21% O_2 and 79% CO_2/21% O_2. However, there is little evidence that *Eurotium* species are particularly tolerant of high concentrations of CO_2 in pure culture. Magan and Lacey (1984) found that >15% CO_2 was required to halve the linear growth rate of *E. repens*, but this species will not germinate or grow in an atmosphere of 85% CO_2, 12% N_2, and 3% O_2 (Hocking, unpublished).

The exact mechanisms of CO_2 inhibition of microbial growth are unknown. It is obvious that it is not simply an oxygen displacement effect. Most studies have been carried out on bacteria, and little is known of the effects on fungi. Research on mechanisms of inhibition of bacterial growth have been summarised by Daniels et al. (1985) as follows: (a) the exclusion of oxygen by replacement with CO_2 may contribute slightly to the overall effect; (b) the ease with which CO_2 penetrates cells may facilitate its chemical effects on the internal metabolism; (c) carbon dioxide is able to produce a rapid acidification of the internal pH of cells with possible ramifications relating to metabolic processes; and (d) carbon dioxide appears to exert an effect on certain enzyme systems, though these effects differ for different species and with differing growth conditions.

Effects of Gas Mixtures on Mycotoxin Production

Aflatoxins

A number of studies have investigated the effects of various atmospheres and other environmental conditions on aflatoxin production, both in stored commodities and in pure culture. Landers et al. (1967), investigating aflatoxin production in stored peanuts, reported that aflatoxin production decreased with increasing concentrations of CO_2 from 0.03% (air) to 100%, and that, in general, reducing the O_2 concentration also reduced aflatoxin production, particularly from 5% to 1% O_2 (Fig. 6). The inhibitory effect of CO_2 was greater at 15°C than at 30°C. At 15°C, aflatoxin production in 20% CO_2, 5% O_2, and 75% N_2 was less than 1% of that in air, and was barely detectable in an at-mosphere of 40% CO_2, 5% O_2, and 55% N_2. Sanders et al. (1968) reported similar results in storage experiments with peanuts at reduced a_w and temperature. They found that aflatoxin levels decreased as a_w decreased from 0.99 to 0.86. At a constant temperature, an increase in CO_2 concentration caused a decrease in aflatoxin formation, and lowering the temperature also decreased the amount of toxin formed.

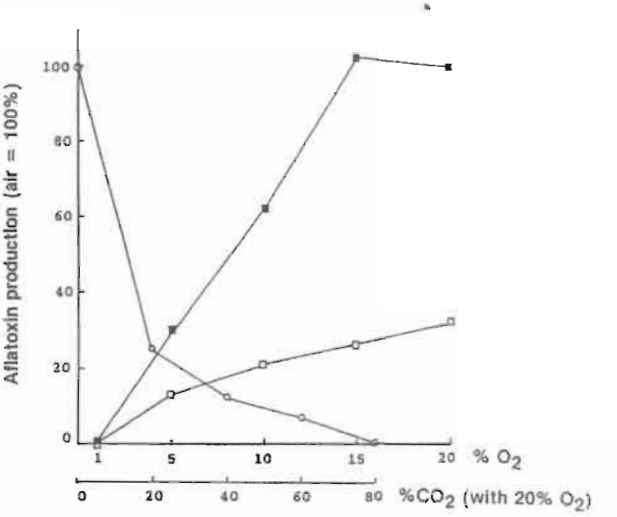

Fig. 6. Influence of various concentrations of O_2 and CO_2 on aflatoxin production in peanuts with kernel moisture content of 27-30% held at 30°C for 2 weeks. Data of Landers et al. (1967). (○) CO_2 with 20% O_2; (■) O_2 with no CO_2; (□) O_2 with 20% CO_2

Epstein et al. (1970) studied the effects of controlled atmosphere (10% CO_2, 1.8% O_2, and 88.2% N_2) on aflatoxin production in liquid medium and in inoculated maize at room temperature (which varied from 25 to 35°C) and at temperatures from 29°C to 1°C. At room temperature, *A. flavus* grew well and produced toxin in both air and CA. At 15°C, aflatoxin production, but not growth, was inhibited in CA. Aflatoxin was not produced at 12°C, and there was little growth at this temperature in air and none in CA. The minimum temperature for

aflatoxin production varies with strains, but is generally 10–12°C (Northolt et al. 1977).

Wilson and Jay (1975) found that maize inoculated with *A. flavus* and stored at 27°C for four weeks in three different modified atmospheres accumulated less than 20 µg/kg total aflatoxin compared with up to >1021 µg/kg for the air control. Remoistened maize was more susceptible to aflatoxin production than freshly harvested high moisture maize. Aflatoxin production in moistened (18.5% m.c.) wheat incubated at 32°C for up to 21 days was minimal (<1 µg/kg) in an atmosphere of N_2 compared with 123 µg/kg in air (Fabbri et al. 1980). Clevström et al. (1983) also found that small quantities of aflatoxins were produced when *A. flavus* was cultured under an atmosphere of nitrogen, and that production increased approximately 15-fold with the addition of B vitamins and a supply of traces of air. Carbon dioxide enrichment hindered aflatoxin formation on a defined medium even in the presence of B vitamins, but small quantities (5 to 15 µg/litre) were formed when formic acid was added.

Carbon monoxide can also suppress growth of *A. flavus* and aflatoxin formation. Buchanan et al. (1985) reported that after growth of *A. flavus* for 32 days in cooked rice medium or raw pistachio nuts in an atmosphere containing 2% O_2 and 10% CO, aflatoxin production was <2% of the production in an atmosphere containing 2% O_2 or air without CO.

Other *Aspergillus* Toxins

Ochratoxin is the only other *Aspergillus* toxin that has been studied under modified atmospheres. Paster et al. (1983) grew *A. ochraceus* on solid synthetic medium at 16°C±1°C for 14 days in atmospheres containing various concentrations of O_2 and CO_2 (Fig. 7). In atmospheres of 1% and 5% O_2without CO_2, ochratoxin production was similar to the air control. Increasing the O_2 level up to 40% reduced ochratoxin production by 75%, whereas at 60% O_2, ochratoxin production was enhanced. In atmospheres of 10% and 20% CO_2, ochratoxin production decreased when O_2 concentrations were below 20%, and was enhanced when they were 40% or 60%. Ochratoxin production was completely inhibited by 30% or more CO_2, regardless of the oxygen concentration. Colony growth was partially inhibited at 60% CO_2, and there was no growth in 80% CO_2.

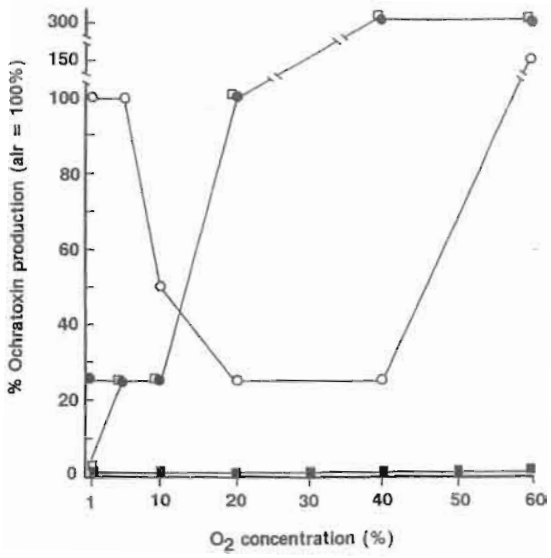

Fig. 7. Ochratoxin production by *Aspergillus ochraceus* grown under modified atmospheres on solid synthetic medium at 16°C±1°C for 14 days. Data of Paster et al. (1983). (○) 0% CO_2; (●) 10% CO_2; (❑) 20% CO_2; (■) 30% CO_2.

Penicillium Toxins

The effect of modified atmospheres on growth and toxin production by *Penicillium* species has not been thoroughly investigated, and there are few reports in the literature. However, in general, it can be assumed that elevated levels of CO_2 will inhibit toxin production to some degree. The effect of limiting O_2 supplies is less predictable. The effect of modified atmospheres on patulin production by *Penicillium patulum* (now *P. griseofulvum)* has been investigated by Paster and Lisker (1985) (Fig. 8). Cultures grown for 7 days in 1% or 5% O_2 but no CO_2 produced less toxin than the control (1 and 14 mg/40 mL compared with 45 mg/40 mL for the control). In 10% O_2 without CO_2 patulin production and mycelial dry weight were similar to the controls. Increasing the O_2 content to 60% or 70% decreased patulin production to 20 and 1.3 mg/40 mL respectively. Toxin production was also inhibited when CO_2 concentration was raised to 20% or more in the presence of 20% O_2. Spores incubated in 100% CO_2 or N_2 did not germinate, but grew normally and produced patulin in amounts comparable to the controls when subsequently exposed to air.

Penicillic acid production by *Penicillium martensii* (now *P. aurantiogriseum)* was studied in mould inoculated maize over a

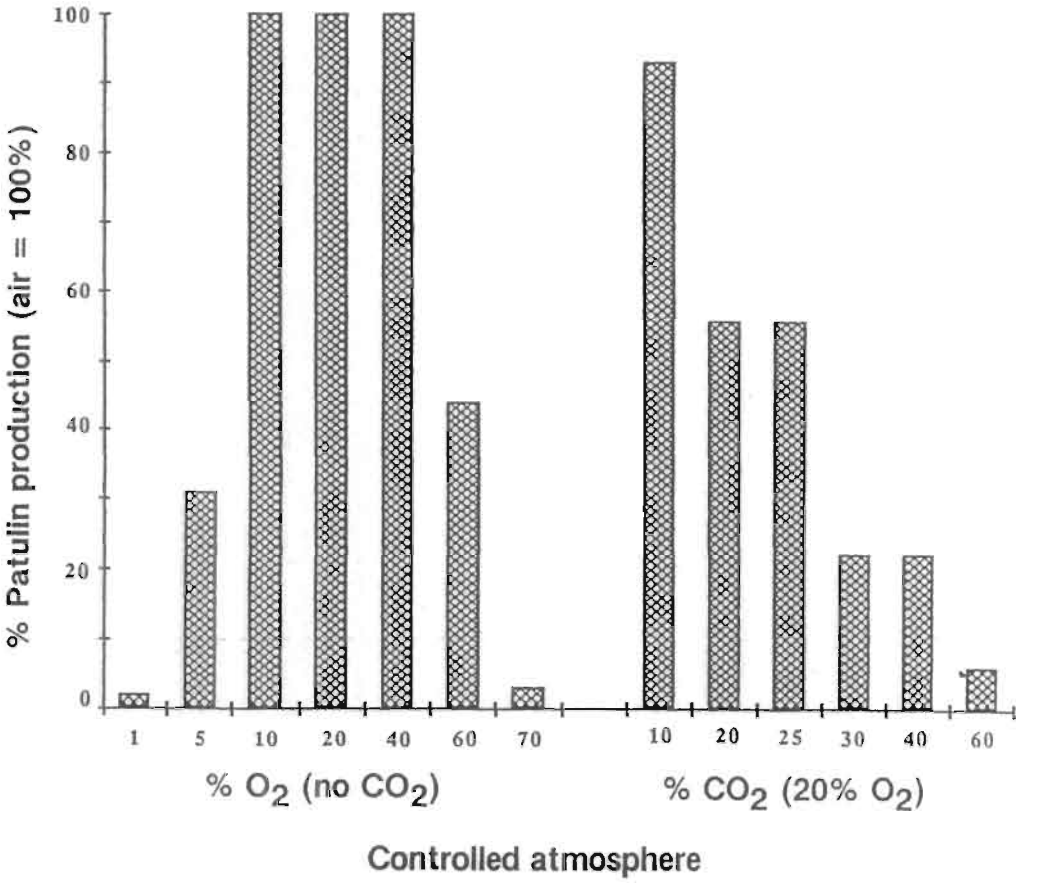

Fig. 8. Effects of controlled atmospheres on patulin production by *Penicillium aurantiogriseum* grown for 7 days in Czapek agar at 26°C. Data of Paster and Lisker (1985)

temperature range of 5° to 20°C in air and in atmospheres containing 20%, 40% or 60% CO_2, with 20% O_2 (Lillehoj et al. 1972). Penicillic acid production decreased with increasing CO_2 concentration. Toxin production was greatest in air at 5°C, but was completely blocked at this temperature by 20% CO_2, and by 40% CO_2 at 10°C over a four week incubation period.

Fusarium Toxins

As with *Penicillium* species, little work has been done on the effects of modified atmospheres on toxin production by *Fusarium* species, although it is known that many *Fusarium* species are tolerant of low O_2 tensions and high CO_2 concentrations.

The effects of MA on production of T–2 toxin by *F. sporotrichioides* has been investigated both in synthetic media (Paster et al. 1986) and in remoistened irradiated maize (Paster and Menasherov, 1988). In the synthetic medium, T–2 production after 7 days at 27°C in an atmosphere of 50% CO_2/20% O_2 was reduced to about 20% of the air control (Fig. 9). At 60% and 80% CO_2 with 20% O_2, there was a significant reduction in fungal growth. Toxin production in 80% CO_2 was only 1.1 µg/45 mL. When the same strain of *F. sporotrichioides* was grown for 14 days at 26°C±1°C on irradiated maize remoistened to 22% m.c., the production of T–2 toxin was totally inhibited under 60% CO_2/20% O_2, and only trace amounts were detected when the gas combination was 40% CO_2/5% O_2 (Fig. 10). Fungal growth was not inhibited by any of the gas mixtures examined, and the growth rate was identical to that for grains kept under air.

Implications for CA Storage of Commodities

Storage of commodities in controlled atmospheres containing high (>60%) levels of CO_2 to prevent insect infestation can also inhibit mould growth and mycotoxin production, while atmospheres of nitrogen need to contain <1% O_2 to retard fungal growth. Mycotoxin produc-

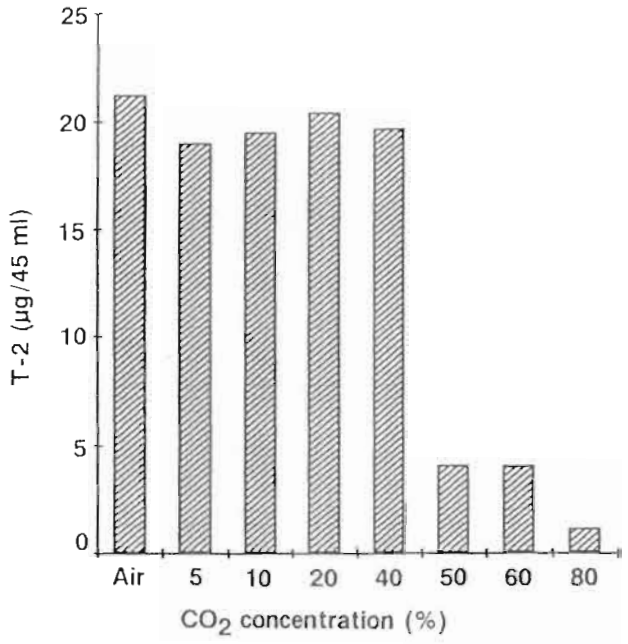

Fig. 9. T–2 toxin production by *Fusarium sporotrichioides* under controlled atmospheres containing various concentrations of CO_2 in 20% O_2. Cultures were grown on potato dextrose agar for 7 days at 27°C. Data of Paster et al. (1986)

tion is more sensitive than fungal growth to CA conditions, but may still occur if other conditions (temperature and a_w) are favourable.

Fungal deterioration cannot be completely prevented in high moisture commodities (a_w between about 0.90 and 0.80) by CA storage, as some fungi, particularly some *Fusarium*, *Mucor* and *Aspergillus* species, are tolerant of levels of 60–80% CO_2. Yeasts and yeast-like fungi can also develop in CA stored high moisture commodities, causing rancidity and off odours. At very high moisture levels, above 0.90 a_w, lactic acid bacteria may develop, irrespective of the concentrations of CO_2 or O_2 used in the storage atmosphere.

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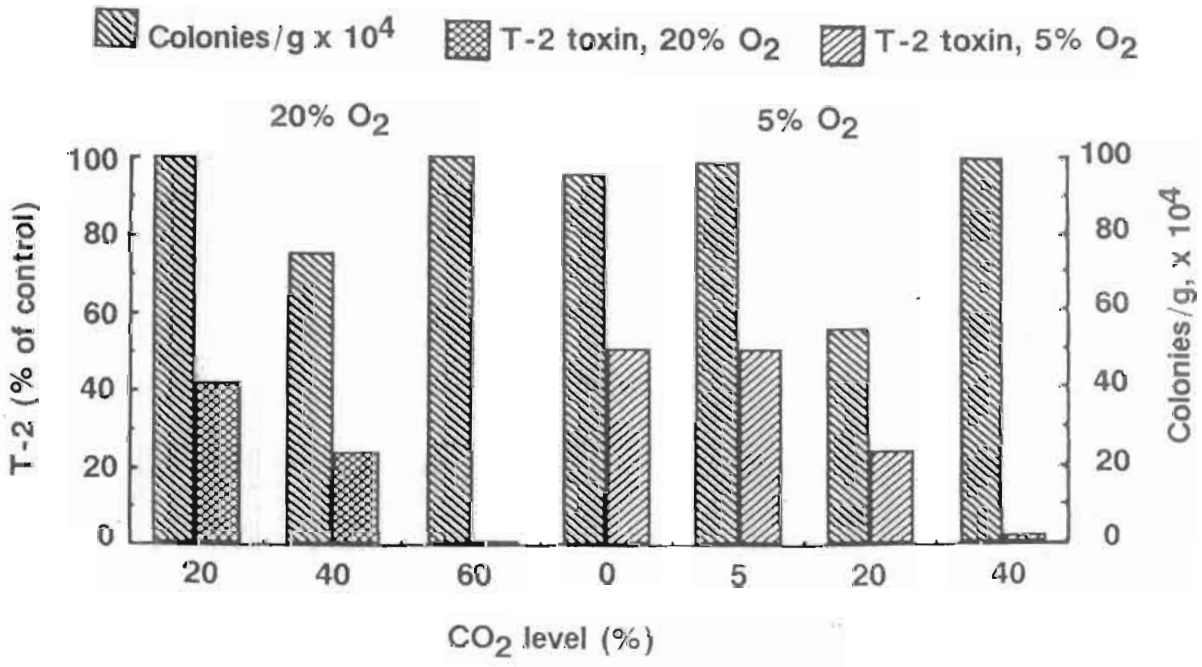

Fig. 10. Effects of various levels of CO_2 and O_2 on colony counts of *Fusarium sporotrichioides* and T–2 toxin production in maize stored under modified atmospheres at 26°C±1°C for 14 days. Data of Paster and Menasherov (1988)

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