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EFFECTS OF INTERGRANULAR GAS COMPOSITION AND FUMIGANTS ON MOULD GROWTH AND MYCOTOXIN PRODUCTION

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ABSTRACT

Fungi differ widely in their tolerances of high concentrations of CO_2 and of low concentrations of O_2 and some species may grow anaerobically. Generally, mycotoxin production is more sensitive to adverse environments than is growth and can be inhibited before any effect on growth is seen. Modified atmospheres recommended for insect control may inhibit fungal growth but must be maintained at inhibitory levels as long as other environmental factors favour fungal growth, and nitrogen should contain less than 1% O_2. Fumigants may also be used to inhibit fungal growth but the doses required may be much greater than those required to kill insects. Fumigants applied at commercial levels may inhibit mould growth or mycotoxin production for short periods but larger doses or supplementary treatments are necessary for long-term storage. The integration of physical and chemical treatments with modified atmosphere storage may prolong storage periods before moulding occurs.

INTRODUCTION

Moulding results in losses in dry matter and in quality for baking, malting, and seed, perhaps with the formation of mycotoxins. The numbers and species of fungi and their ability to form mycotoxins are determined chiefly by water activity (a_w), temperature, and intergranular gas composition. If grain cannot be dried to a safe a_w, other measures are necessary to prevent moulding. Storage in modified atmospheres, attained either naturally, through the respiration of damp grain and its microflora or by fermentation of waste materials (Paster *et al.*, 1990), or through the addition of carbon dioxide or nitrogen have been widely used to control insects and, to a lesser extent, to control moulding. However, the requirements are not necessarily the same. The effects of high CO_2 and N_2 atmospheres and low O_2 atmospheres on fungal growth have often been studied but there have been few systematic studies of the combined use of

high CO_2 and low O_2 atmospheres or of the effects of fumigants. These studies are reviewed in this paper and compared with the requirements for insect control.

FUNGAL GROWTH

Modified atmospheres

Changing the proportions of atmospheric gases in the atmosphere can affect fungal development in several interrelated ways. It can determine how many and how quickly spores germinate, hyphal growth rate, and how soon spores are produced. Fungi are usually considered to be aerobic but their tolerance of high CO_2 and low O_2 concentrations are often underestimated. Anaerobic growth has been demonstrated for some species of filamentous fungi and yeasts with *Geotrichum candidum, Mucor hiemalis, Fusarium oxysporum,* and *Fusarium solani* reported to grow extremely well in 100% N_2 and *Aspergillus niger, Aspergillus fumigatus, Penicillium aurantiogriseum, Penicillium brevi-compactum,* and *Aspergillus pullulans* to grow well if certain growth factors, such as vitamins, oxygen donors, and higher oxidation forms of certain elements are present (Gunner and Alexander, 1964; Tabak and Cook, 1968). However, most microorganisms are inhibited but not killed in 100% CO_2 (Fränkel, 1889). The concentrations of CO_2 and O_2 necessary to inhibit spore germination in different species of fungi can be modified by nutrients and by spore concentration (Brown, 1922) as in *Botrytis cinerea* where 1% O_2 without nutrient completely inhibited germination but the presence of some nutrient allowed 20% of spores in a dilute suspension to germinate and, depending on nutrient and spore concentration, 10 - 20% CO_2 was necessary to inhibit spore germination.

The time taken for spore germination (lag phase) is negatively correlated with O_2 concentration and positively correlated with CO_2 concentration (Magan and Lacey, 1984b). At 0.98 a_w and 23°C, decreasing O_2 from 21% to 0.14% increased the lag phases of *Alternaria alternata, Aspergillus candidus* and *Penicillium roquefortii* from ≤ 1 day, to 3, and 4 days, respectively. At 0.85 a_w/23°C in 1% O_2, the lag phase was increased to 13 and 19 days, respectively, for *A. candidus* and *P. roquefortii.* There were no consistent differences between field and storage fungi over the range 0.90 - 0.98 a_w in the effects of changing O_2 and CO_2 concentrations on the lag phases of field and storage fungi except that close to the limits for growth (<1% O_2), the lag phases of storage fungi showed much more marked increases than those of field fungi while the effect of increasing CO_2 to 15% became more marked as a_w decreased.

How much CO_2 affects fungal growth depends largely on how much O_2 is present in the atmosphere. Inhibition by increasing concentrations of CO_2 is much greater in the absence of O_2 then in its presence. With oxygen present, *A. alternata, B. cinerea, Rhizopus stolonifer,* and *Cladosporium*

herbarum grew in up to 45% CO_2 although growth rate decreased with increasing concentration of CO_2 and was halved at 20% CO_2 (Wells and Uota, 1970). However, *Alternaria tenuissima, Cladosporium cladosporioides, Aspergillus candidus, A. fumigatus, Eurotium repens (E. herbariorum), Penicillium aurantiogriseum,* and *Penicillium stoloniferum* failed to grow in even 10% CO_2 if oxygen was absent, although *Fusarium poae, Fusarium subglutinans, P. roquefortii,* and yeasts could all still grow (Pelhate, 1980) and *R. stolonifer, Mucor hiemalis,* and *Trichoderma* sp. grew slightly in 100% CO_2 and in 50% CO_2 + 45% N_2 + 5% O_2 (Stotzky and Goos, 1965). *P. roquefortii* can tolerate up to 80% CO_2 in the presence of oxygen (Golding, 1940). Mycelial weight may decrease linearly with increasing concentrations of CO_2 in air, e.g., *Byssochlamys nivea* (Yates *et al.*, 1967), or the logarithm of the percentage inhibition of growth may change linearly with the logarithm of CO_2 concentration (with 20% O_2), as shown for a range of fungi (Durbin, 1955). However, growth of *B. nivea* was stimulated in 50% CO_2 and decreased only slightly (85% of control) in 80% CO_2, when both atmospheres contained 20% O_2. Growth in 85% CO_2 + air was only 16% of that in air while in 100% CO_2 growth was only 4% of that in air (Yates *et al.*, 1967). *F. oxysporum* and *Mucor plumbeus* also grew in 97 - 99% CO_2 but only at 0.5 - 4% of their growth rate in air while seven other species failed to grow (Hocking, 1990). By contrast, 4-20% CO_2 can stimulate the growth of some fungi, even in low O_2 atmospheres (Wells and Uota, 1970; Gibb and Walsh, 1980; Magan and Lacey, 1984b). Growth of *Fusarium 'roseum'* was stimulated by 10% CO_2 but halved by 45% CO_2 (Stotzky and Goos, 1965). However, such stimulation could depend on the species concerned, incubation temperature, and a_w (Magan and Lacey, 1984b). Thus, growth of *A. alternata* growing at 23°C was stimulated most by 5% CO_2 (in air) at 0.95 a_w and by 10% at 0.90 a_w but not at all at 0.98 a_w. By contrast, *P. roquefortii* showed most stimulation at 0.98 and 0.95 a_w and 23°C but no stimulation at lower a_w. At 14°C, growth of *P. roquefortii* decreased sharply as CO_2 was increased from 0.03 to 5% but was then stimulated by 15% CO_2 at 0.98 a_w and by 10% CO_2 at 0.95 a_w. *E. repens* showed only slight stimulation by 5% CO_2 at 23°C and 0.95 a_w. Apart from this stimulation, growth rates declined with increasing CO_2 concentrations. Limiting concentrations (LCs) of CO_2 at which growth rates were halved (LC_{50} CO_2) are shown in Table 1. As found by Brown (1922), CO_2 affected growth more at lower temperatures than higher. *Penicillium brevicompactum, Aspergillus fumigatus, Aspergillus nidulans* , and *Aspergillus versicolor* were the most sensitive of 14 species to CO_2 concentration (Magan and Lacey, 1984b).

CO_2 concentration has been tested mostly for its effect on growth rate. However, Lopriore (1895) found sporangia to be inhibited more readily than spore germination while mycelial cells tended to be swollen. Germ tubes of *Rhizopus* were likewise short, swollen, and irregular in 50% CO_2,

resembling those formed in acid culture medium, but they could still grow normally when replaced in air (Brown, 1922). These effects are not confined to mucoraceous fungi as *Penicillium chrysogenum* forms mainly filamentous colonies in up to 8% CO_2 but in 15% CO_2 hyphae are swollen and stunted and many cells are spherical or yeast-like (Ho and Smith, 1986). Spores usually retain their viability in 50 - 80% CO_2, at least at low a_w, (Peterson *et al.*, 1956; Wells and Uota, 1970) but anaerobiosis decreased survival sharply, especially in *A. versicolor, A. fumigatus*, and *P. roquefortii* but also in *Aspergillus flavus* (Richard-Molard *et al.*, 1980) although spores of *Mucor mucedo* still germinated after 3 months in 100% CO_2 (Lopriore, 1895).

Fungi efficiently scavenge O_2, and field and storage fungi can both grow in atmospheres containing <1% O_2 (Magan and Lacey, 1984b). Brewer *et al.* (1972) detected respiration in *A. fumigatus, Mucor rouxii,* and *Sporormia minima* at partial pressures of oxygen (pO_2) <0.1 cm Hg while growth of *M. rouxii* was observed at 0.01 cm Hg. However, *Hyphopichia burtonii*, the only species of 75 yeasts tested that occurs commonly in grain, was unable to grow anaerobically (Visser *et al.,* 1990). Growth of field fungi declined linearly with O_2 concentration (Follstad, 1966; Wells and Uota, 1970) but growth of some *Aspergillus* and *Penicillium* spp. was affected only with <5% O_2 (Miller and Golding, 1949). *A. alternata* and *C. herbarum* sporulated in all atmospheres except 0% O_2 where there was no mycelial growth. Otherwise, growth declined with O_2 concentration. *B. cinerea* sporulated after 5 days in air but only with >1% O_2 whereas *R. stolonifer* produced no mature sporangia during 5 days in ≤0.5% O_2 and only a few hyphae appressed to the agar in ≤0.25% O_2. Many field fungi appear more tolerant of low O_2 atmospheres than storage fungi and the concentration of O_2 necessary to halve linear growth (LC_{50} O_2) is less than 0.14% (Table 2). For instance, the growth rates of most *Penicillium* spp., except for *P. roquefortii,* in <1% O_2 are <50% of those in air (Yanai *et al.,* 1980; Magan and Lacey, 1984b). As with CO_2, the effects of low O_2 concentrations on growth were enhanced by low temperatures.

N_2 is considered to affect fungi only through its low oxygen content. However, a range of fungi have been shown to grow in N_2 at rates close to those obtained in air (Hocking, 1990). Thus, *Fusarium equiseti* and *F. oxysporum* grew at 88 - 97% of the rate in air, *Cladosporium* at 95-100%, *E. repens* at 60 - 90% and *Penicillium corylophilum* and *P. glabrum* at 66 - 90%. *Mucor plumbeus* and *Absidia corymbifera* also grew strongly in N_2.

Fungi appear more sometimes tolerant of CO_2 in stored-products than in culture. Thus, *Eurotium* spp. survived and grew in 50% CO_2/21% O_2 and 79% CO_2/21% O_2 in grain (Petersen *et al.*, 1956; Wells and Uota, 1970) although not in 85% CO_2/3% O_2 (Hocking, 1990) even though growth in agar showed little evidence of such tolerance (Table 1). *Eurotium* spp. and *Penicillium* spp. were not affected directly in wheat bulks stored for up to 84 days in bins with 6 or 14-day half-lives, maintained at 15% CO_2 with surges

to 50% CO_2 every 7 days during the first month but with O_2 concentrations never below 11% (White and Jayas, 1991). By contrast, CO_2 treatment (2.4 kg CO_2/t) of maize stacks, enclosed in PVC sheet during 120 days storage, decreased *Eurotium chevalieri* significantly but not total fungi (Dharmaputra *et al.*, 1991).

Table 1: Concentrations of carbon dioxide (%) required to halve linear growth of field and storage fungi (LC_{50} CO_2) at 23 and 14°C.

	Temperature						
	23°C				14°C		
a_w	0.98	0.95	0.90	0.85	0.98	0.95	0.90
Field fungi							
Alt. alternata	>15.0	>15.0	>15.0	N.G.	>15.0	>15.0	4.5
C. cladosporioides	>15.0	>15.0	>15.0	N.G.	N.T.	-	-
C. herbarum	13.0	>15.0	>15.0	N.G.	N.T.	-	-
F. culmorum	14.0	13.5	>15.0	N.G.	8.0	11.5	14.0
Storage fungi							
P. brevicompactum	11.5	8.5	9.5	5.2	15.0	13.0	7.5
P.aurantiogriseum	4.5	4.0	>15.0	>15.0	11.0	12.0	8.0
P. hordei	>15.0	8.5	9.5	5.2	15.0	13.0	7.5
P. roquefortii	>15.0	>15.0	4.5	4.0	5.0	4.0	4.8
A. candidus	>15.0	>15.0	>15.0	10.0	4.5	13.0	4.0
A. fumigatus	>15.0	5.2	12.5	N.G.	N.T.	-	-
A. versicolor	12.0	>15.0	14.5	N.G.	N.T.	-	-
Eurotium repens	>15.0	>15.0	>15.0	13.0	>15.0	9.0	>15.0

A., Aspergillus; Alt., Alternaria; C., Cladosporium; E., Epicoccum; F., Fusarium; P., Penicillium; N.G., no growth; N.T., not tested.

Growth and sporulation of *A. flavus* on groundnut decreased with each 20% increment of CO_2 up to 80%, above which growth was totally inhibited (Landers *et al.*, 1967; Sanders *et al.*, 1968). Growth was much decreased with <5% O_2 and was almost completely inhibited with <1%. O_2 However, *A. flavus* populations in shelled groundnuts containing 5% water or unshelled containing 7.5% water in 3% O_2 or 82% CO_2 were unchanged from those stored in air over 12 months (Jackson and Press, 1967). Mould growth in maize stored at 0.75-1.00 a_w and 25-45°C in atmospheres containing 0.1% O_2 was decreased significantly over a 12 day period (Bottomley *et al.*, 1950). Also, moulding in wheat, containing 17.4% water, stored at 18 - 26°C was largely inhibited by <0.2% O_2, apart from *A. candidus* that developed late in storage (Shejbal and Di Maggio, 1976; Di Maggio *et al.*, 1976) and most

storage fungi in rice, stored with 0.87 a_w for 2 - 4 months, were inhibited by <1% O_2 (Richard-Molard *et al.*, 1986). Yeasts and *A. pullulans* developed, even with <0.5% O_2, especially at 0.94 a_w, but were inhibited completely by the absence of O_2, whether in CO_2 or N_2. Technical N_2, containing 0.3% O_2, decreased moulding from that in air and complete inhibition was possible only with <0.01% O_2 (Di Maggio, 1980).

Table 2: Concentrations of oxygen required to halve linear growth of field and storage fungi (LC_{50} O_2) at 23 and 14°C.

	Temperature						
	23°C				14°C		
a_w	0.98	0.95	0.90	0.85	0.98	0.95	0.90
Field fungi							
Alt. alternata	2.80	<0.14	0.14	N.G.	0.60	3.80	5.00
C. cladosporioides	1.30	5.10	0.14	N.G.	N.T.	-	-
C. herbarum	0.70	5.20	10.00	N.G.	N.T.	-	-
F. culmorum	<0.14	2.60	<0.14	N.G.	9.90	12.50	5.00
Storage fungi							
P. brevicompactum	1.10	0.60	0.40	1.00	<0.17	<0.17	<0.17
P. aurantiogriseum	0.60	5.30	2.40	13.00	0.50	<0.17	10.20
P. hordei	0.80	<0.14	1.30	12.50	<0.17	0.80	1.60
P. roquefortii	<0.14	<0.14	<0.14	<0.14	1.20	2.20	0.80
A. candidus	0.45	1.00	0.45	5.00	5.80	<0.17	9.40
A. fumigatus	3.40	5.40	6.20	N.G.	N.T.	-	-
A. versicolor	6.40	0.80	4.50	N.T.	N.T.	-	-
Eurotium repens	0.60	3.00	5.00	10.20	0.85	0.90	4.00

A., Aspergillus; Alt., Alternaria; C. Cladosporium; E., Epicoccum; F., Fusarium; P., Penicillium; N.G., no growth; N.T., not tested.

Ozone has also been used to extend the storage life of cereals, peas, beans, spices, and other seeds (Naito *et al.*, 1988).

Moist grain storage depends on the respiration of both the grain and its associated microflora to increase CO_2 and decrease O_2 concentrations sufficiently to inhibit fungal growth. Water contents of maize and barley in unsealed silos range from 20 - 40% (about 0.90 - 1.0 a_w). Peak CO_2 concentrations of 60 - >90% and minimum O_2 concentrations of <10% can be attained within three weeks of filling the silo but may decline, even in sealed silos, to only 15% CO_2 after 200 days as a consequence of imperfect sealing, daily temperature and pressure fluctuations and removal of grain. In spring,

towards the end of the storage period, respiration of the small amount of grain remaining is often insufficient to maintain an inhibitory atmosphere and microbial development may occur with spontaneous heating. A similar microflora develops in grain exposed at the surface of unsealed silos as air penetrates, allowing microbial activity and heating in the top 15 - 30 cm. When CO_2 concentrations are high and O_2 concentrations low, yeasts are the predominant fungi. Next to develop is *P. roquefortii* followed by *Penicillium rugulosum* as yeasts decline. Spontaneous heating commences when oxygen is sufficient, with the development of *Absidia corymbifera, Rhizomucor pusillus* and *A. nidulans,* and *A. fumigatus* (Burmeister and Hartman, 1966; Lacey, 1971). Similar patterns in maize were related to temperature and a_w (Bottomley *et al.* 1950). At 0.80 a_w, *Penicillium* spp. predominated at 25°C, *A. flavus* at 30°C, *Eurotium* spp. at 35°C, and "*Mucor*" (possibly *Rhizomucor*) at 40 and 45°C. Yeasts ("*Candida*" spp.) predominated at 0.90 a_w and 25°C but did not occur at higher temperatures. Mould growth was decreased significantly by 0.1% O_2 and 21% CO_2.

Fumigants

Fumigants have been chiefly used for the control of insects but some have also been shown to possess antimicrobial properties although much larger doses may be necessary to inhibit fungi than to kill insects. Also, fumigants may be less effective against spores than against mycelium. Thus, mycelium of *Penicillium islandicum* on autoclaved hulled rice was killed by 45 mg methyl bromide l^{-1} for 18 h at 25°C (concentration time product [ctp] 810 gh m^{-3}) but spores required 50 mg l^{-1} (ctp 900 gh m^{-3}). However, if the mycelium had penetrated to the centre of the grain, the larger dose was again required (Yanai *et al.*, 1964). By contrast, Paster *et al.* (1979) found that methyl bromide was only fungistatic to the internal microflora of wheat so that, if conditions are suitable, the spores may reinfect the grain after the fumigant has dispersed. This compares with a maximum dose of 100 gh methyl bromide m^{-3} for insecticidal use in France (Pierrot, 1988). Doses of 120 mg methyl bromide l^{-1} for 4 h (ctp 480 gh m^{-3}) at 25°C were required to kill all spores of A. ochraceus, A. flavus, Penicillium citrinum, *P. chrysogenum,* and *P. aurantiogriseum* but these failed to kill 40% of *A. niger* spores. However, 40 mg l^{-1} for 24 h (ctp 960 gh m^{-3}) killed all spores of all species. Mycelial growth was inhibited at both concentrations. However, wheat grain inoculated with *A. niger* fumigated with 100 mg l^{-1} (ctp 2,400 gh m^{-3}) yielded fungi from 16% of grains after 16 days and from 100% after 29 days. A given ctp was more effective if attained as a high dose over a short period than as a smaller dose over a longer period.

Treatment of maize with 0.1 - 0.3% sulphur dioxide controlled all moulds except *Penicillium* spp. Spore germination of a range of field fungi was affected little by 100 - 200 .µg SO_2 l^{-1} but 50 mg l^{-1} inhibited *A. flavus,*

A. ochraceus and *Aspergillus terreus* although not *A. niger* (Magan, unpublished data). The internal microflora of sorghum (8.4% water content) was totally eliminated by 16 mg ethylene oxide l^{-1} (ctp 768 gh m^{-3}), 96 mg methyl bromide l^{-1} (ctp 4,608 gh m^{-3}), 64 mg ethylene dibromide-methyl bromide mixture l^{-1} (ctp 3072 gh m^{-3}) while 95% were killed by 96 mg sulphur dioxide l^{-1} (4,608 gh m^{-3}). However, all fumigants caused loss of viability. Phosphine, even at 96 mg l^{-1}, had little effect on the internal microflora although seed germination was enhanced (Raghunathan *et al.*, 1969). In other tests, phosphine, as well as ethylene dibromide and ethylene dichloride, all applied at 100 mg l^{-1} for 15 days (ctp 36,000 gh m^{-3}) decreased the microflora of blackgram and field beans but ethylene dichloride was more effective against bacteria, ethylene dibromide against fungi, and phosphine against actinomycetes (Natarajan and Bagyaraj, 1984).

The most effective dose of phosphine against 106 fungal isolates was 3,000 - 3,500 ppm for 90 days (ctp 6,500,000 - 7,600,000 gh m^{-3}) killing 89% of 106 strains (Bailly *et al.*, 1987). Only five isolates (4.7%) were tolerant but these included four *Fusarium* spp. A smaller dose, 1200 ppm for 21 days (ctp 605,000 gh m^{-3}) was fungicidal to 41% of the isolates (Bailly *et al.*, 1985). However, these doses are greatly in excess of doses used insecticidally (72 gh m^{-3}) (Pierrot, 1988). In grain, phosphine has little effect on dormant microflora present as spores (Sinha *et al.*, 1967; Raghunathan *et al.*, 1969) but there is evidence of activity against actively growing mycelia. Non-fungicidal doses of phosphine (0.3 mg l^{-1} for 21 days, ctp 150 gh m^{-3}) decreased growth by *A. flavus* group isolates (Leitao *et al.*, 1987) and Hocking and Banks (1991) found that although *Penicillium* appeared particularly resistant to 0.1 mg phosphine m^{-3} for 14 and 28 days (ctp 33.6 and 67.2 gh m^{-3}), most storage fungi, especially *A. parasiticus* , developed less rapidly in inoculated freshly harvested paddy rice. Populations of *A. parasiticus* were two orders of magnitude smaller in phosphine treated samples after four weeks exposure. Larger doses of phosphine are obviously necessary for storage periods longer than a few days.

MYCOTOXINS

Modified atmospheres

Mycotoxin production is determined by water availability, temperature, and gas composition but is generally confined within narrower limits than growth that differ with species and toxin. Thus, aflatoxin was produced both in air and in a controlled atmosphere of 10% CO_2/1.8% O_2/88.2% N_2 at room temperature (25 - 35°C); no toxin was produced at 15°C although growth was not inhibited and weak growth still occurred in air at 12 °C but not in controlled atmospheres (Epstein *et al.*, 1970). Dharmaputra *et al.* (1991) also observed less aflatoxin production, but little effect on fungi, in

sheeted stacks of maize, whether these received 2.4 kg CO_2 ton^{-1} or were untreated, than in unenclosed stacks, in which CO_2 could not accumulate. Less than 1 μg aflatoxin kg^{-1} wheat was produced during storage in a nitrogen atmosphere (0.03% O_2) when over 1,000 μg kg^{-1} were produced during storage in air (Fabbri *et al.*, 1980). However, production in N_2 atmospheres could be enhanced 15-fold by the addition of B vitamins to the substrate and by traces of air (Clevström *et al.*, 1983). B vitamins failed to reverse CO_2 inhibition although formic acid allowed small amounts of aflatoxin to be produced. Diener, Davis and their colleagues have made the most extensive studies of how atmospheric gases affect mycotoxin production (Landers *et al.*, 1967; Sanders *et al.*, 1968; Diener and Davis, 1977) using groundnuts as substrate. Decreasing O_2 concentration from 21 to 15% did not affect aflatoxin production and, although production decreased with further decreases, the most marked inhibition occurred only when the O_2 concentration was <5% (Fig. 1a). Growth and sporulation were affected little above 5% O_2 but then declined markedly. Increasing CO_2 concentration from 0.03 - 20% had no visible effect on growth and sporulation but aflatoxin production was decreased to 25% of that in air (Fig. 1b). Increasing CO_2 concentration further decreased aflatoxin production with little production at ≥80% CO_2. Decreasing O_2 concentration while increasing CO_2 concentration increased greatly the effect on aflatoxin production (Table 3). A 20% CO_2/20% O_2 mixture decreased aflatoxin production to 11% of that in air while 20% CO_2/5% O_2 allowed only 0.6% to be produced with almost complete inhibition with higher concentrations of CO_2 and 5% O_2.

Water activity and temperature also affect interactions between the gaseous components of the environment. Atmospheres with 20% CO_2 in air allow aflatoxin production down to at least 0.86 a_w but little or no production at even 0.92 a_w with 40 or 60% CO_2 (Table 4). Decreasing temperatures from 25 - 17°C decreased aflatoxin production in all combinations of CO_2, O_2, and a_w tested. Wilson and Jay (1975, 1976) studied aflatoxin production in maize and groundnuts in different controlled atmospheres. Large amounts of aflatoxin accumulated in groundnuts stored in air or 13.6% CO_2/0.1% CO/1.5% O_2/83.9% N_2 but other atmospheres with <1% O_2 or >60% CO_2 restricted aflatoxin production to low levels although none prevented visible fungal growth. Buchanan *et al.* (1985) also found that CO suppressed fungal growth in pistachio nuts, cooked rice medium, and potato dextrose agar if O_2 concentrations were low but aflatoxin production was suppressed even if growth was normal. Atmospheres containing 2% O_2/10% CO inhibited aflatoxin production by 98% of that in 2% O_2 or air. Aflatoxin production was prevented in maize (18.8% water content) by 14 - 15% CO_2 with 0.5 - 1.0% O_2 for up to 109 days, although *A. flavus* could be found in 30 - 47% of kernels and 472 μg aflatoxin kg^{-1} was present in control grain (Wilson *et al.*, 1977).

Table 3: Effects of CO_2 and O_2 concentration on aflatoxin formation at 15°C and 99% r.h. after 42 days (Diener and Davis 1977).

Gas composition (%)		Kernel water content (%)	Aflatoxin content ($\mu g\ g^{-1}$)
CO_2	O_2		
0.03[1]	21	31.5	120.3
20	20	30.1	13.1
20	5	27.0	0.8
40	5	26.9	0
60	5	26.4	<0.1
80	5	25.9	0
Untreated		6.6	0

[1] air , all other treatments with CO_2 and balance made up of N_2.

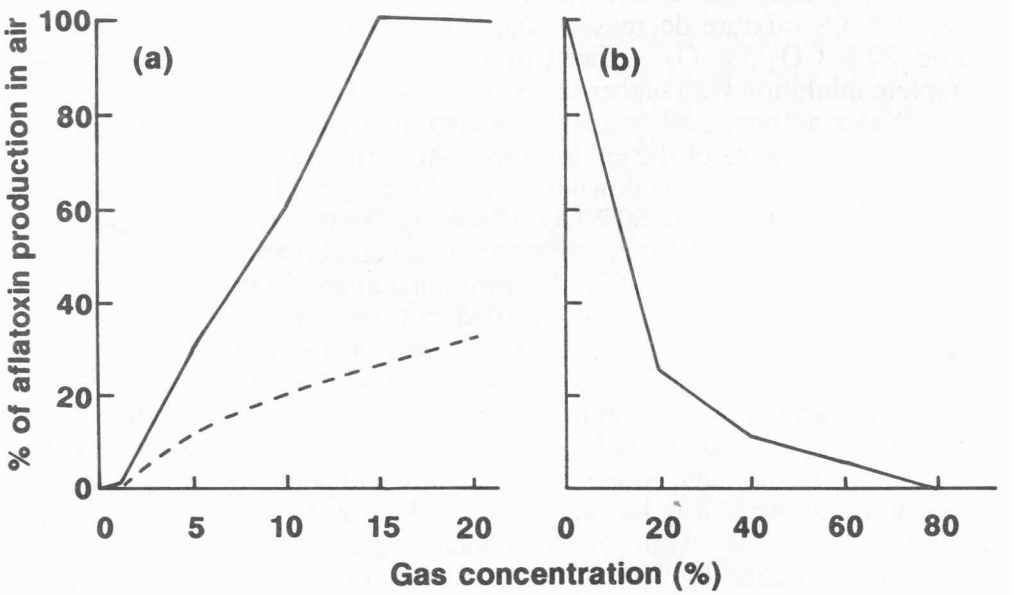

Fig. 1: Effects of (a) O_2 and (b) CO_2 concentration on aflatoxin formation after 14 days at 30°C and 99% r.h. (Diener and Davis 1977).
(a) ____, O_2 without CO_2; - -, O_2 with 20% CO_2; (b) CO_2 containing 20% O_2.

Table 4: Effects of CO_2 concentration and water activity and different water contents on aflatoxin formation at 25°C after 14 days (Diener and Davis 1977).

Gas composition (%)		Water activity (a_w)	Kernel moisture content (%)	Aflatoxin content ($\mu g\ g^{-1}$)
CO_2	O_2			
0.03[1]	21	0.99	22.4	206.3
0.03	21	0.92	26.5	185.2
0.03	21	0.86	15.0	72.1
40	20	0.99	29.2	3.8
40	20	0.92	24.8	0.3
40	20	0.86	17.4	0
60	20	0.99	24.5	0.2
60	20	0.92	20.0	<0.1
60	20	0.86	11.8	0
Untreated			6.0	0

[1]air, all other treatments with CO_2 and balance made up of N_2.

Ochratoxin is produced both by *A. ochraceus* and *P. verrucosum* but the effects of gas composition on production have been studied only in the former using semi-synthetic media at 16°C over 14 days (Paster *et al.*, 1983). Atmospheres containing 30% CO_2 or more completely inhibited ochratoxin formation, regardless of O_2 concentration, although growth was only inhibited when CO_2 concentrations exceeded 60% and was completely stopped by 80% CO_2. With atmospheres containing 10 or 20% CO_2, ochratoxin production was decreased if there was <20% O_2 but enhanced by 40 or 60% O_2. Without CO_2, ochratoxin production with 1 or 5% O_2 was as good as in air.

There are few studies of the effects of modified atmospheres on toxin production by *Penicillium* or *Fusarium* spp. Penicillic acid production by *P. aurantiogriseum* (*P. martensii*) decreased with increasing concentrations up to 60% CO_2 when, at both 5 and 10°C, toxin production fell below detectable levels although production was always small above 20% CO_2 at 5°C and 40% at 10°C. Penicillic acid was always detectable at 15 and 20°C (Lillehoj *et al.*, 1972). Patulin production by *Penicillium griseofulvum* (*P. patulum*) was decreased by >10% CO_2/20% O_2 and by 5% O_2 without CO_2 (Fig. 2; Paster and Lisker, 1985). T-2 toxin production in synthetic medium by *F. sporotrichioides* was decreased 80% by 50% CO_2/20% O_2 but growth was not affected with <60% CO_2. With 80%CO_2/20% O_2, T-2 toxin production was only 24 $\mu g\ l^{-1}$ medium. In maize, incubated at 26°C for 14 days after rewetting to 22% moisture content, T-2 production was totally inhibited by 60% CO_2/20% O_2 and only trace amounts were found with 40%

CO_2/5% O_2 even though there was no inhibition of fungal growth (Paster *et al.*, 1986; Paster and Menasherov, 1988). Zearalenone production by *F. equiseti* was almost completely inhibited by ≥20% CO_2 with 20 or 5% O_2. In all treatments, all grains were infected by *F. equiseti* but sporulation was inhibited in the modified atmospheres, especially when CO_2 was increased and O_2 decreased together (Paster *et al.*, 1991).

Fumigants

Fumigant activity against mycotoxin production may depend on fungal species, isolate, mycotoxin, substrate, fumigant, and storage conditions. Fumigation both enhanced and inhibited mycotoxin production by eight isolates (four *A. flavus*, two *A. parasiticus,* and one each of *A. ochraceus* and *P. verrucosum*). Six isolates were affected by methyl bromide, five by DDVP (O,O-dimethyl O-[2,2-dichlorvinyl] phosphate), four by ethylenedichloride/carbon tetrachloride and one by chloropicrin. Some of the effects were statistically significant but probably of no practical significance as any effect lasted only a short while (Vandegraft *et al.*, 1973). However, non-fungicidal doses of phosphine (0.3 mg l^{-1} for 21 days, ctp 150 gh m^{-3}) decreased both growth and aflatoxin production by *A. flavus* group isolates in synthetic medium (Leitao *et al.*, 1987). Similar results were obtained with freshly harvested paddy rice at 0.92 a_w (20.8% moisture content) inoculated with *A. parasiticus* and *E. chevalieri* and exposed to 0.1 mg m^{-3} phosphine for 14 and 28 days (ctp 33.6 and 67.2 gh m^{-3}) at 28°C (Hocking and Banks, 1991). *Penicillium* appeared particularly resistant to phosphine but there was less rapid development of most storage fungi and especially of *A. parasiticus* in phosphine treated samples. *E. chevalieri* was overgrown rapidly by *A. parasiticus*. Aflatoxin production was halved by phosphine but larger doses would be necessary for long-term storage.

INTEGRATED TREATMENTS

Although Bottomley *et al.* (1950) found the effects of atmosphere, humidity, and temperature on microbial development in maize to be highly significant, they found no interactions except between humidity and atmosphere. However, Lillehoj *et al.* (1972) found that interactions between temperature and atmosphere on penicillic acid production resulted in the inhibition of production by decreasing concentrations of CO_2 as temperature was decreased. By contrast, germination of *P. aurantio-griseum* spores was always optimum at 30°C but percentage germination was decreased from 37% to 3% by increasing concentrations of CO_2 from 0.03 - 60% and the temperature range allowing germination was also decreased. By contrast, germination of *P. aurantio-griseum* spores was always optimum at 30°C but percentage germination was decreased from 37% to 3% by increasing concentrations of CO_2 from 0.03 - 60% and the temperature range allowing

germination was also decreased. Tolerance of low water activity is greatest when temperatures are optimal (Northolt *et al.*, 1979; Magan and Lacey, 1984a) and Busta *et al.* (1980) concluded that three-way interactions between humidity, temperature, and atmosphere were also likely. The interactions between CO_2 and O_2 concentrations described above should also be considered. Interactions of physical and chemical treatments have been utilised to extend the period of storage possible in modified atmospheres without moulding (Lacey *et al.*, 1991; Paster *et al.*, 1992). Treatment with 0.2% propionic acid, 2 kGy γ-irradiation and modified atmospheres with 40 - 60% CO_2 and 20% O_2 prevented moulding better than any of the component treatments and allowed storage of maize with 18% moisture content for up to 45 days with little fungal development.

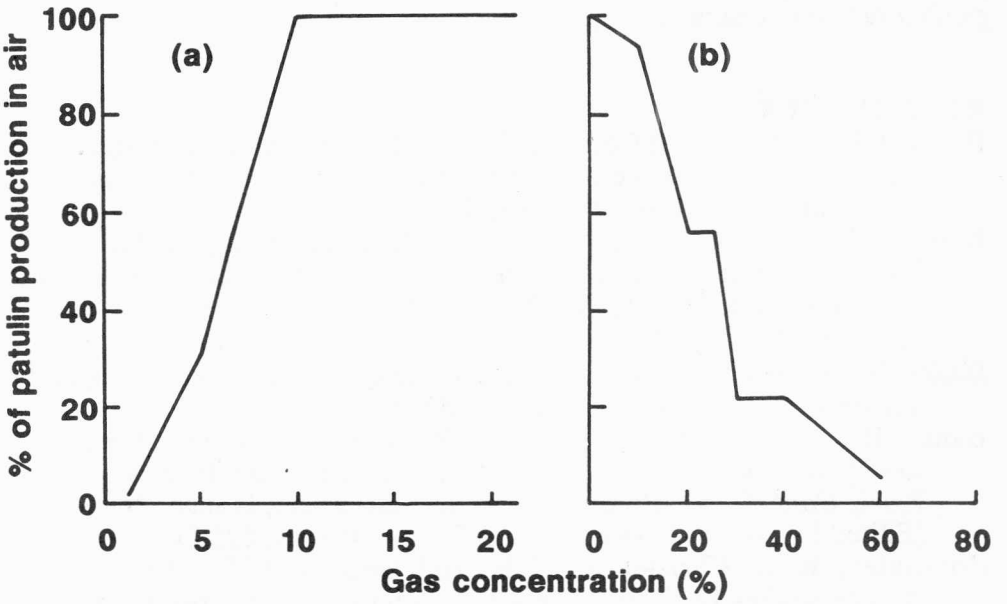

Fig 2: Patulin production in different modified atmospheres: (a) oxygen without carbon dioxide; (b) Carbon dioxide with 20% oxygen (Paster and Lisker, 1985).

CONCLUSIONS

Typical insecticidal atmospheres are 60% CO_2 in air or 1% O_2 in N_2 (Banks, 1981). Most fungi are inhibited by >50% CO_2, especially if O_2 is decreased simultaneously, but 1% O_2 will slow but not prevent growth. Moist grain storage should attain no more than 0.5 - 1% O_2 and, if possible, 0.2% O_2 with a simultaneous increase in CO_2 concentration to 50%. Thus nitrogen atmospheres suitable for insect inhibition are insufficient to inhibit fungi unless the O_2 content is decreased to <1% while CO_2 atmospheres are

adequate. These atmospheres kill most insects but fungal growth is only inhibited and mycotoxin production is blocked only temporarily. Thus, the atmosphere must be kept at inhibitory levels as long as the grain contains sufficient water for fungal growth and mycotoxin production. Structures for modified atmospheres to control insects allow for losses of, perhaps, half the CO_2 in 14 days or an increase of O_2 from 1 to 2% in only one day (Banks *et al.*, 1991). Requirements for fungal control are much more stringent unless the atmosphere is replenished repeatedly. In moist grain stores, O_2 concentrations are decreased to very low levels within hours at 0.95 a_w, 3 days at 0.90 a_w, 9 days at 0.86 a_w and 36 days at 0.76 a_w but there is only a slight decrease in concentration at 0.70 a_w (Diawara *et al.*, 1989). More than 0.90 a_w is necessary to obtain rapid anaerobiosis but *Lactobacillus* can still grow at this a_w. However, with both modified atmosphere and moist grain storage, preservation may be enhanced by low level chemical or gamma-radiation treatment.

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