Session 3. Biological responses of microflora to treatment with CA/fumigation#

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Rapporteur's Report

This session dealt with the effects of controlled atmosphere and fumigation treatments on the development, growth and survival of microorganisms in stored grains and related products. Each paper stressed the importance of interactions within the stored grain mass between the treatment (CA or fumigation) and factors such as water activity, temperature, pH and nutritional status. Of these, water activity is the most important. Interactions between the various microorganisms present is also critical in establishment of microbial succession, and in some cases, the production of mycotoxins. The first two papers of this session dealt with microbial ecosystems in stored grains. The last three papers related to antimicrobial effects of grain fumigants or preservatives in stored-products. Two papers on the effects of phosphine were withdrawn.

Dr. John Lacey delivered the first paper, which reviewed the effects of modified atmospheres on microbial development in grain. Fungi are more tolerant of marginal conditions of atmosphere if all other conditions are favorable, thus their tolerance to elevated carbon dioxide (CO_2) and/or reduced oxygen (O_2) is affected by a_w, nutrient, temperature, etc. Tolerance to high CO_2 or low O_2 atmospheres varies between fungal species, with some *Fusarium* species being quite resistant. There is interaction between concentrations of CO_2 and O_2, so that CO_2 is more inhibitory to fungi if O_2 is reduced, particularly below 1%. Slightly elevated levels of CO_2 between 5-10% can be stimulatory to some fungi. Mycotoxin production can be inhibited by CA treatment of stored grains. Toxin production is more sensitive than growth to elevated CO_2 and reduced O_2. Toxin production, like growth, is also affected by other physical and chemical parameters in the grain mass.

Dr. Lacey also briefly reviewed the effects of fumigants in grains: in general, high doses are required to kill fungi, with spores being more resistant than mycelium. Fumigants in the dosages used in normal fumigation practice are fungistatic rather than fungicidal.

Editors' comment: The proceedings includes the paper of Dr. Dharmaputra on "The effects of phosphine on storage fungi of soybean meal". Dr. Dharmaputra who was planning to attend the conference could not finalize his travel plans. Because of the importance of his report, the Organizing Committee made an exception and agreed to include this paper in the proceedings.

Dr. Daniel Richard-Molard (with B. Cahagnier) gave a very interesting account of microbial succession in high (0.95 a_w) and intermediate moisture (0.90 a_w, 18-21% m.c.) grain. At 0.95 a_w, lactic acid bacteria develop, i.e. the stored grain undergoes a lactic fermentation. At slightly lower a_w, 0.90, storage yeasts become important, particularly *Hyphopichia burtonii*. These yeasts are capable of growth at extremely low O_2 tensions, and establishment of populations of *H. burtonii* and other storage yeasts may protect high moisture grain and silage against mould deterioration.

The use of ergosterol for measurement of fungal growth is not a good indicator of growth of storage yeasts in sealed storages, as there is significantly less ergosterol formed under low oxygen conditions.

Dr. Naresh Magan's paper on the effects of sulphur dioxide (SO_2) on fungi in grains stressed the importance of pH, as this preservative is active only in the pH range 1 - 3.5. Water activity and contact time are also important. A number of the fungi tested were quite tolerant of SO_2: *Botrytis cinerea* was the most tolerant of the field fungi, while *Aspergillus niger, A. versicolor* and *Penicillium aurantiogriseum* were the most resistant of the storage fungi. High concentrations of SO_2 were required for inhibition of fungi in grains, possibly due to absorption of the preservative by the grain. Treatment of grain with SO_2 will change the microbial ecology of the grain, and if tolerant species are present, this may encourage the growth of mycotoxigenic fungi, with consequent potential for increased mycotoxin production.

Dr. Ailsa Hocking (with H.J. Banks) presented results of a study on the effects of low level, long term application of phosphine to wheat stored at marginal a_w values. Over a period of six months, storage fungi, particularly *Aspergillus* Series *restrici* began to establish in grains at a_w levels as low as 0.67. The application of 0.1 $g.m^{-3}$ phosphine prevented the growth of these fungi and *Eurotium* species at this a_w but not at 0.70 and above. At 0.72-0.73 a_w, 1.0 $g.m^{-3}$ phosphine was needed to prevent establishment of storage fungi. This paper demonstrated the practicality of preventing fungal deterioration of stored grains by the SIROFLO technique, but only if the a_w of the grain is below 0.70.

Ms. Valerie Ducom (with P. Drouin, P. Ducom, J. Protais and J.Y. Toux) presented the last paper in this session, on the efficacy of methyl bromide as a fumigant for control of *Salmonella* in poultry feed and poultry litter. In general, a CT product of 2,000 $g.h.m^{-3}$ was required to control *S. typhimurium* in samples contaminated with $>10^6$ cfu/g. However, even this level was insufficient to kill $5x10^6$ cfu/g *S. typhimurium* in wet chicken faeces.

All the papers drew attention to the importance of interactions within the stored grain mass, both physical factors such as a_w (moisture content), O_2 and CO_2 tensions, temperature, pH etc., and biological factors, particularly interactions between the various components of the microflora. All these factors will impact on the efficacy of controlled atmosphere storage and/or fumigation, in preventing mould growth in stored commodities.

Discussion following this session centered on the future of CA technologies for control of fungi in stored commodities, and the relationship between laboratory studies and the field situation. It was felt that CA technologies show great promise in extending the mould-free storage life of grains, particularly in the humid tropics, where moisture control is difficult. Low O_2 high CO_2 atmospheres in closed systems can slow the growth of fungi, and inhibit mycotoxin production. The application of fumigants, particularly phosphine, for insect control, can have the added benefit of inhibiting fungal growth, but is probably less effective than high CO_2 atmospheres. Laboratory studies are needed to define the levels of O_2 and CO_2 necessary to prevent growth of important fungal species such as the primary invaders of stored grains (*Eurotium, A. restrictus* and related species) and mycotoxigenic fungi.