SESSION 2: BIOLOGICAL RESPONSES OF MICROFLORA TO TREATMENT WITH CA AND/OR FUMIGANTS

J. LACEY
IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK

Rapporteur's Report

Fungi remain important causes of deterioration of stored grain that has been insufficiently dried, especially in humid, tropical climates. They cause grain to discolor, heat and lose dry matter, and they attract insects and mites. Some species may also produce toxic secondary metabolites (mycotoxins) that can cause diseases in man and animals through toxic, immunotoxic and carcinogenic action on specific organs. Fungal growth is completely prevented only if grain is dried to water activities (a_w) under 0.65–0.70 a_w, equivalent to water contents of 12–13% in cereal grains or 6–7% in oilseeds. Mycotoxin production may be prevented within narrower limits. The six papers presented in this session illustrated many of the problems of doing, and interpreting the results of, laboratory and field experiments.

Bagged paddy with 14% water content generally stored well at the center of Volcani cubes, constructed of gastight, heavy-duty plastic liners. However, *Eurotium chevalieri* increased in number, even though an atmosphere containing up to 15% CO_2 and down to 3–4% O_2 was generated. Numbers of fungi generally increased at the periphery of the bulk. With 16% water content in a plastic silo, *Aspergillus flavus* increased as other species declined, even though CO_2 increased to 18% and O_2 became undetectable (Caliboso *et al.*). It remains questionable whether the modified atmosphere or water activity had the greater effect on colonisation.

E. chevalieri also increased in bagged maize treated with PH_3 but declined in similar maize enclosed in plastic sheeting and treated with CO_2. However, *A. flavus* continued to grow at up to 80% CO_2, and aflatoxin was produced in significant amounts at up to 60% CO_2. *Aspergillus permicilloides* increased in similarly treated rice. Although CO_2, PH_3 and MB decreased fungal growth, no treatment eliminated it (Dharmaputra *et al.*).

In general, attempts to prevent moulding and mycotoxin contamination of stored grain have utilised only one strategy. The utilisation of synergistic and additive effects from applying different methods of control in an integrated strategy has seldom been considered. The use of biological, chemical and physical control methods in a single control strategy could inhibit moulding without having to use extreme levels of any one factor. Thus storage periods for damp grain have been extended by the combined use of 0.2% propionic acid, 2–4 kGy gamma irradiation and a 60% CO_2 modified atmosphere although no single treatment would alone have prevented moulding. Natural products inhibiting mould growth or mycotoxin production could be substituted for propionic acid, while partial drying could increase the effects of other treatments (Paster).

The tolerance of fungi for low O_2 atmospheres has been largely underestimated. Although generally considered aerobic, fungi can be efficient scavengers of O_2 and grow with <5% O_2. A combination of high CO_2 and low O_2 concentrations is most effective in preventing fungal growth, but results of experiments in culture sometimes contrast with those using a grain substrate. Increasing O_2 concentration from 0.5% to 20% O_2 with 80% CO_2 could sometimes negate the inhibitory effect of the larger CO_2 concentration. Mycotoxin production could be found with up to 60% CO_2 + 0.5% O_2. Although 20% CO_2 + 0.5% O_2 allowed only slight growth and no aflatoxin production by *A. flavus* on agar, aflatoxin concentrations in cheese were sufficient to be of concern with 20 or 40% CO_2 and 1 or 5% O_2 (Hocking and Taniwaki).

Elevated CO_2 concentrations in stored grain often result from respiration of the grain and its associated microflora. Continuous measurement of respiration has previously been difficult, but a new electrolytic respirometer has made possible the measurement of O_2 production in response to CO_2 absorption into alkali. This has allowed input of data into models of ambient-air drying and could be used in modelling CO_2 production during damp-grain storage. The contribution of fungi to total grain respiration remains controversial, and different species differ widely in their respiration rates. It seems likely that respiration at low a_w (where germinability remains high) is primarily attributable to the grain, while that at high a_w (where germinability has been lost) is mainly due to microorganisms (Lacey *et al.*).

To draw a single conclusion from the data presented is not possible, given the diverse nature of the papers. Considerably more work is required before the tolerances of fungi for controlled atmospheres and fumigants are fully understood. This will require fully replicated experiments with adequate statistical control and an attention to detail that is not always evident in published reports. Methods must be described in such a way that work can be interpreted and repeated. Water activity must be determined in different parts of a bulk of stored grain, both near the centre and at the periphery, especially if this is covered by an impermeable membrane. Actual concentrations of CO_2, O_2 and fumigants attained during treatment, and their persistence, also need to be measured, while the experimental design needs to be adequate to distinguish between the effects of controlled atmosphere or fumigant and those of water activity. The measurement of fungal growth in grain continues to exercise microbiologists. Neither plate counts of colony-forming units nor direct plating of grain (to determine the percentage infected), estimates fungal biomass. Mycelial growth and sporulation may respond differently to treatment. Ergosterol allows a measure of total biomass, but it is subject to limitations. Another method that responds equally to all fungi is urgently required.