STUDIES OF THE MYCOFLORA OF BARLEY DURING STORAGE

AND ITS RELATION TO

MYCOTOXIN CONTAMINATION

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ABSTRACT

Much of the economic loss occurring during storage of barley grain is that due to fungal deterioration. In addition, the development of mycotoxins during storage may result in contamination of animal feedstuffs.

In this study barley from samples obtained at harvest, from commercial silos after 5 and 9 months' storage, from farm silos after 5 months' storage and from laboratory-stored samples held at 4^{o}C and at ambient temperature were subjected to various fungal isolation techniques. These techniques were designed to provide information on the total viable counts of the whole grain and of the outside and inside surfaces of the husks and caryopses. Isolates of *Aspergillus flavus* were screened for toxigenicity and the barley itself was subjected to multimycotoxin analysis.

To investigate levels of contamination, dilution plating of supernatants from the inner and outer parts of grains were examined. Differential viable counts revealed wide variations between samples at harvest and after storage with most contamination on the outer surface. "Clean" grain showed higher outer than inner counts, but for mouldy grains inside counts were greatly increased. It was concluded that inside counts give a better indication of grain condition.

Commercially-stored grain showed a marked decrease in viable counts over time, as did the laboratory-stored grain although those held at 4^{o}C showed a smaller decrease. In contrast, the farm-stored grain continued to yield high viable counts. These counts could be related to storage conditions.

Amongst the genera isolated from dilution plates, *Alternaria* was the most frequent and persistent, whilst others, including *Cladosporium* and *Fusarium*, showed falling levels over the period of investigation. The genera *Penicillium* and *Aspergillus* showed rising frequencies with storage and were predominant in mouldy samples.

Microscopic examination and culturing of caryopsis sections and husk surfaces revealed the significance and distribution of various genera. Alternaria was found in all fractions whereas Penicillium was completely absent from grain at harvest, later appearing on the outer husk and finally on the inner husk after prolonged storage at ambient temperature.

Microscopy of stained caryopsis sections showed hyphae only in those from mouldy grain. Microscopy of stained husks revealed hyphae on and in both husk surfaces of all grains, with a greater abundance on the inner surface and in mouldy husks. S.E.M. observations confirmed these findings and established the adherence of spores and hyphae to grain structures and their rough surfaces.

Some hyphal fragments associated with the grain can cause mycotoxin contamination. Loosely-attached hyphae were examined using membrane filtration and micro-manipulation techniques. It was found that whilst total levels of hyphal fragments showed little decrease during storage, their viability dropped considerably. For silo- and laboratory-stored grain, the viability dropped from over 20% to 5%. In contrast, over 45% were viable in a mouldy sample.

Of the fungal species isolated, species of the Aspergillus flavus group were screened for aflatoxin production using coconut agar fluorescence. Positive isolates ranged from 3% for farm-stored grain to 25% of those from grain at harvest. Selected A. flavus isolates were cultured on moist barley which when analysed for aflatoxin gave identical results to those from the coconut agar.

A multimycotoxin technique was used to screen 14 barley samples for aflatoxins, citrinin, ochratoxin, T-2 toxin and zearalenone. Only one obviously-mouldy sample proved positive for aflatoxin, citrinin and ochratoxin. It appears that although A. flavus and other potentially toxigenic fungi can be regularly isolated from barley grains, only in exceptional circumstances are they of significance in relation to mycotoxin contamination of stored grain.

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PREFACE

In spite of advances in technology, millions of dollars worth of cereals are lost each year through spoilage of various sorts. In technologically-advanced countries, (such as New Zealand), where insect and rodent infestations of stored grains can usually be kept under reasonable control, fungi are considered to be the major cause of deterioration and spoilage of newly-harvested and stored grains (Ciegler, 1978). Incipient deterioration in grain quality begins as soon as grain is harvested and under suitable conditions, fungi readily proliferate resulting in gross spoilage.

For many years, there was a tendency to regard fungal growth on food commodities as harmless; a nuisance affecting commodity appearance and perhaps a cause of loss and spoilage. When contamination was severe, commodities deemed unfit for human consumption might perhaps still be used as animal feed. However, ever since the discovery of the aflatoxins and toxigenic *Aspergillus flavus* species two decades ago, fungal growth in cereal grains was viewed as a potential health hazard.

The genera of fungi most implicated belonged to the "storage fungi" (Ciegler, 1978), but an increasing number of studies have shown the elaboration of toxins by other fungal genera. The range of fungi which can be implicated in toxin formation is now known to be almost limitless. The widespread and frequent occurrence of toxigenic fungi in agricultural commodities indicate a constant potential hazard (Davis, 1981).

The development of specific methods of analysis has led to the discovery of a wide spectrum of mycotoxic compounds at low but significant levels in many samples of food contaminated with fungi. Cereal grains represent the most important commodity contaminated by mycotoxins (Hesseltine, 1974). Contamination is usually the result of faulty harvesting and improper storage, although mycotoxins can be produced before harvest (Scott, 1978; Stoloff, 1979). The presence of fungal toxins is potentially a serious problem as most mycotoxins are stable and even persist in food commodities after the fungi that produced them are killed (Moreau, 1979).

Ingestion of contaminated food can result in acute or chronic symptoms of toxicoses as well as insidious effects in both man and animals (Wyllie & Morehouse, 1978; National Academy of Sciences, 1979; W.H.O., 1979; Pier *et al*, 1980; Pier, 1981). Thus, the economic consequences of crop losses due to fungal attack and concomitant mycotoxin production are of concern when ensuring the production of wholesome food products and livestock feeds for the markets of today.

Barley is widely grown in New Zealand as a feed for livestock, for export and for human consumption and brewing. The studies on grain deterioration and mycotoxin contamination overseas have concentrated mainly on cereal grains other than barley, whilst similar studies are very limited in New Zealand. These factors, together with the increasing production and use of barley particularly in animal feed-stuffs in this country, meant that more intensive studies would be useful in understanding the local situation.

The investigations reported here concern the changing fungal flora of barley grains during storage, the topographic origins of the fungi in relation to grain deterioration and also their potential to produce toxins. Particular attention is paid to *Aspergillus flavus*, as this species is considered to be of considerable importance in causing gross spoilage and many isolates are important mycotoxin producers. Aflatoxins, the formidable mycotoxins produced by many isolates of *A. flavus*, are potent carcinogens, mutagens and hepatoxins (Goldblatt, 1969; Heathcote & Hibbert, 1978). *A. flavus* isolates have been screened for aflatoxin production and selected barley samples analysed for the presence of aflatoxins and other mycotoxins.