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ASPERGILLUS FLAVUS AND THE DETERIORATION OF FARM-STORED BARLEY GRAIN

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SUMMARY

Inadequate farm storage of barley can result in moulding of the grain mass with a corresponding danger of mycotoxin production in that grain and subsequent risks to animal health. Dilution plating techniques utilising progressive washing and surface sterilization have been used in this study to investigate the mycoflora of the husks of 12 samples of farm-stored barley grains, with particular reference to the presence of *Aspergillus flavus*, the producer of the potent mycotoxin, aflatoxin. These techniques allowed differentiation of fungi and fungal numbers on the inner and outer surfaces of the husks to be made and related to the extent of deterioration of the grain.

The dilution plating method used for examining the husks revealed that total viable counts of the outer surface were not a reliable index of the condition of the samples, whereas inner surface counts were consistently related to the degree of mouldiness. A "condition line" could be established at 2.0×10^2 CFU/g grain for such inner surface counts.

The most common *Aspergillus* species isolated by dilution plating were *A. flavus*, *A. glaucus* and *A. fumigatus*. The most common of the other genera were *Alternaria*, *Cladosporium* and *Aureobasidium*. *A. flavus* was the most widely distributed species in both clean and mouldy samples, but was present mainly on the outer surface. The distribution of the various genera on the outer and inner surfaces of the husks was also found to be related to the degree of mouldiness of the sample. In clean samples the field fungi (*Alternaria*, *Cladosporium* and *Aureobasidium*) were dominant, but they were replaced by storage fungi (*Aspergillus* and *Penicillium*) in mouldy samples.

A further technique allowing direct examination of the fungal mycelium within husk tissue using a vital stain was developed. This allowed an assessment to be made, by means of three comparative scales (relative mycelial score, comparative mycelial score and relative viability score), not only of the abundance of such mycelium but also of its viability. Most samples of husk tissue showed abundant mycelium

but estimation of viability obtained by this direct plating technique showed that whilst hyphae in husks from mouldy samples were active, much of the mycelium in clean samples was dead. The most common species of *Aspergillus* in the husk tissue of mouldy samples were the spoilage fungi *A. glaucus*, *A. restrictus* and *A. fumigatus*. Only 2 samples yielded *A. flavus*. Fungal genera isolated mainly from clean samples were *Alternaria*, *Monilia* and *Papulospora*. This technique thus reinforces the findings obtained by dilution plating and emphasises the location of spoilage fungi within the husk tissue of mouldy samples.

Barley isolates of *A. flavus* have been compared to soil isolates for their ability to produce aflatoxin on different media. *A. flavus* isolates from barley were first screened for aflatoxin production on coconut agar. All were negative. Several isolates from soil, however, were found to be toxigenic. Selected barley and soil isolates were examined for their ability to form aflatoxin on various media (semi-synthetic, Weet-bix, pearled barley and barley husk), culture filtrates being analysed by the minicolumn technique and by TLC. Aflatoxin B_1 and traces of B_2 were detected by the TLC method in culture extracts from 7 out of 9 soil isolates of *A. flavus*. No aflatoxin was detected in cultures of barley isolates.

The studies reported suggest that although *A. flavus* is common in stored barley, it is mainly a surface contaminant and present largely as spores. It seemed to play little part in the actual spoilage of the grain, as indicated by its infrequent occurrence as mycelium within the husk tissue. Furthermore, elaboration of aflatoxin does not appear to be a problem in the barley samples examined, as judged by the absence of toxigenic *A. flavus* strains in those samples. However, soil isolates were toxigenic, and it is possible that other samples of stored grain may on occasions become contaminated with these strains, with the concommitant danger of aflatoxin production if the grain is not adequately stored.

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