J. Sci. Fd Agric. 1974, **25**, 1089–1093

Production of the Mycotoxin, Zearalenone, by *Fusarium graminearum* Growing on Stored Grain

II. Treatment of wheat grain with organic acids

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(Manuscript received 31 October 1973 and accepted 17 April 1974)

The concentrations of formic, acetic, propionic and butyric acids required to prevent growth of *Fusarium* in wheat grain cultures were determined. The cultures were maintained at 31% moisture content and stored for 4 weeks at 25 °C followed by 6 weeks at 12 °C, which were optimum conditions for growth of *Fusarium* and synthesis of the mycotoxin, zearalenone. Under these conditions the critical concentration of all the acids, whether used singly or in combination, was between 1000 and 10 000 parts/million (equivalent to 0.1% and 1% w/w). At 1000 parts/million and below the fungus was able to develop and zearalenone synthesis was not affected except where acetic acid was used alone, when higher toxin yields were recorded.

1. Introduction

High moisture content grain may be protected from deterioration by micro-organisms by chemical treatment. Ideally the antimicrobial agents must be effective against a wide range of organisms, be non-toxic to man or animals and not affect the quality of grain in other ways. These may include reducing grain viability, which would make it unsuitable for seeding or malting purposes or chemically modifying the grain to make it unsuitable for food manufacturing processes. A chemical which satisfies all these criteria has yet to be found but a number of compounds have been considered in the past and subjected to experimental trials. Methyl bromide has been used as a soil fumigant to control growth of fungi and other soil micro-organisms.[1] Lee and Riemann,[2] working with rice grain cultures, showed that spores of *Aspergillus parasiticus* and *Penicillium rubrum* were killed by prolonged exposure to low concentrations of methyl bromide. At high grain moisture levels, however, bacterial spoilage still occurred. Muir and Wallace[3] used formaldehyde to treat damp wheat grain (17.9% on a wet-weight basis), but found that although *Altenaria* was reduced to almost zero levels, *Aspergillus* and *Penicillium* spp. were able to re-infect treated grain after 13 weeks' storage. Formaldehyde had a second disadvantage in that it destroyed the baking quality of the gluten in flour, resulting in abnormal dough.

Recently, increasing commercial use has been made of propionic acid to control growth of fungi, yeasts and bacteria in high moisture grain.[4] Because it inhibits grain germination, affects the bread-making characteristics of the resulting flour and leaves

a residual taint in grain, which makes it unacceptable for human consumption, it has been used mainly in the preservation of cereals for animal feedstuffs, being considered non-toxic to animals at the concentrations used (approximately 1% w/w). After receiving reports that strains of *Fusarium culmorum* had been isolated from grain treated with propionic acid and seemed less susceptible than other fungi, experiments were carried out to investigate the effects of propionic and other organic acids on the growth of *Fusarium* and the production of the oestrogenic mycotoxin, zearalenone.[5]

2. Experimental

2.1. Grain cultures

Grain cultures were set up using wheat grain (cultivar Capelle). Duplicate flasks each containing 20 g of grains were autoclaved and adjusted to 31% moisture content, on a wet-weight basis, with sterile distilled and deionised water. The flasks were inoculated with 0.1 g of soil culture of *Fusarium graminearum* IMI 155426 and incubated for 4 weeks at 25 °C followed by 6 weeks at 12 °C. These were optimum conditions of moisture content and temperature for growth of the fungus and zearalenone synthesis.[5] Growth of the fungus was assessed as visible mycelium and by the loss in the weight of grain dry matter.

2.2. Acid treatment

Formic, acetic, propionic and isobutyric acids were used singly and in combination for treatment of the grain culture. Acid concentrations ranged from 10 to 10 000 parts/million and the desired concentration was achieved by adding the required weight of each acid in the volume of water needed for moisture content adjustment. Where mixtures of acids were used, total acid concentration was the sum of equal concentrations of each of the acids in the mixture.

2.3. Estimation of zearalenone

Methods of extraction, separation and estimation of zearalenone have been described previously.[6]

3. Results

Fusarium graminearum grew in wheat grain cultures where the concentration of organic acids, added singly or in combination, was 1000 parts/million (equivalent to 0.1% w/w) or below (Figures 1, 2 and 3). All points plotted represent the average of the results from duplicate grain cultures. Growth of the fungus did not occur at 10 000 parts/million. Where the fungus did grow there was little effect on zearalenone synthesis using the different acid treatments compared to control culture levels. Where acetic acid was added singly, higher toxin yields were recorded, but when used in combination with other acids this effect was not seen.

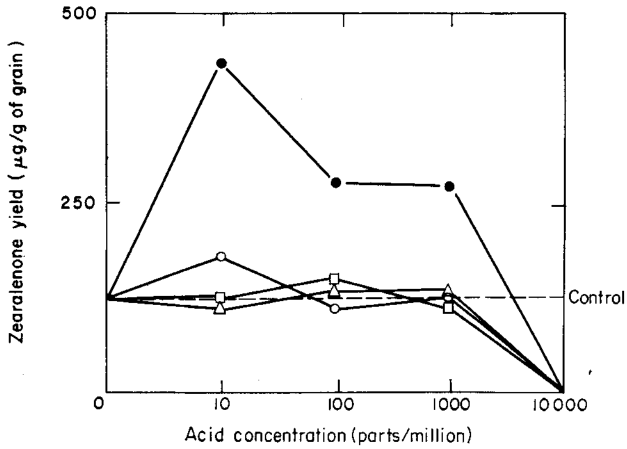

Figure 1. The effects of increasing concentrations of organic acids on zearalenone yield from *Fusarium graminearum*. Single acids: -○—○-, formic acid (F); -●—●-, acetic acid (A); -□—□-, propionic acid (P); -△—△-, isobutyric acid (B).

4. Discussion

The action of propionic and other organic acids in preventing mould development is unclear. It has been suggested that it is the undissociated propionic acid that has antifungal action. Previous experiments[7] involving the treatment of wheat grain with Propcorn (B.P. Chemicals, Ltd), a commercial preparation of propionic acid, showed that strains of *F. graminearum*, *F. culmorum* and *F. moniliforme* were inhibited at 2000 parts/million of the antifungal agent. At 1000 parts/million and below, these

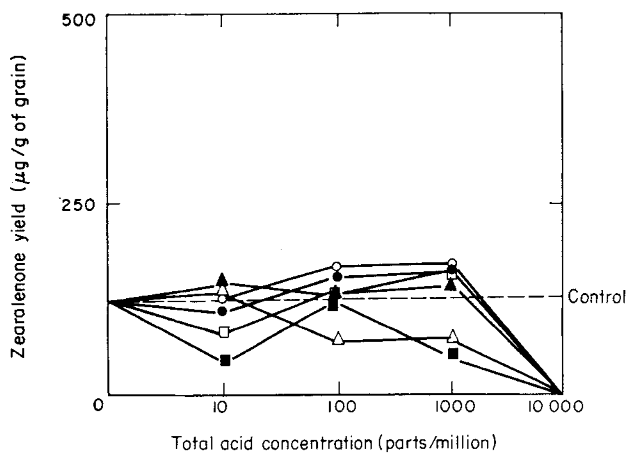

Figure 2. The effects of increasing concentrations of organic acids on zearalenone yield from *Fusarium graminearum* growing on wheat grain. Binary mixtures: -●—●-, FA; -□—□-, FP; -■—■-, FB; -▲—▲-, AP; -○—○-, AB; -△—△-, PB.

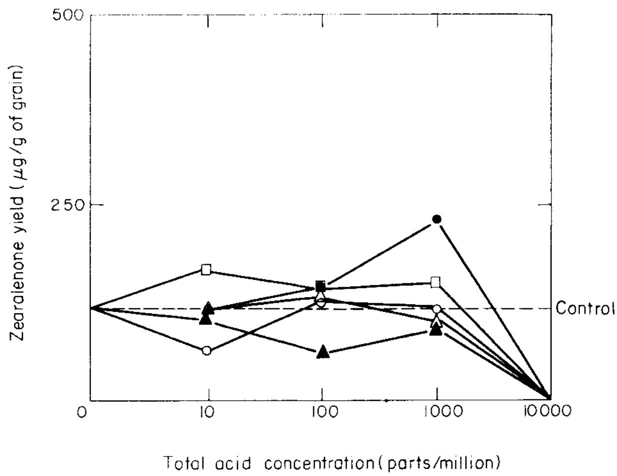

Figure 3. The effects of increasing concentrations of organic acids on zearalenone yield from *Fusarium graminearum* growing on wheat grain. Ternary and Quaternary mixtures: -○—○-, FAP; -●—●-, FAB; -□—□-, FPB; -△—△-, APB; -▲—▲-, FAPB.

fungi were able to develop and zearalenone synthesis was unaffected. This latter result compared with those in this investigation using the various acids singly and in combinations at 1000 parts/million, which also had no inhibitory effect on the fungus and toxin production. Grain moisture content and storage temperature were at optimum values for *Fusarium* growth and zearalenone synthesis, but if these were more limiting fungal growth might be precluded at lower concentrations of these organic acids.

Kozakiewicz and Clarke,[8] using *in vitro* tests, have recently shown that growth of *F. culmorum* on agar media and in shake cultures was inhibited at 1000 parts/million propionic acid. These tests were carried out at a pH range 4.0 to 4.5 compared to pH 6.05 in the wheat grain cultures described in this paper, where *F. culmorum* grew at 1000 parts/million propionic acid. This variation in pH might explain the differences in these results as there would be a higher concentration of the undissociated acid at the lower pH value.[9] Bandelin[10] showed that a lower concentration of propionic acid was needed to inhibit growth of *Aspergillus niger* under more acid pH conditions. Further evidence that it is the undissociated acid that is effective in inhibiting *Fusarium* is the ability of this fungus to grow on defined media at pH 2.0, whereas the pH of grain cultures did not fall below 4.7 when treated with 10 000 parts/million propionic acid.[11] In contrast the increased yield of zearalenone recorded in grain cultures when acetic acid was added alone, might be related to some dissociation of the acid and the resulting uptake of the acetate ion by the fungus. Wolf, Liebermann and Mirocha[12] have shown the incorporation of [1-^{14}C]acetate into the zearalenone molecule when *F. graminearum* was grown on rice.

In practice the amounts of propionic acid recommended for use in the field are approximately five times the concentration necessary to prevent growth of *Fusarium*. This is due to the technical difficulties of uniform distribution of the acid on all kernels. It is usually added in the range 0.8 to 1.5% w/w to grain (equivalent to 8000 to 15 000

parts/million) for storage of grain at 17 to 30% moisture content, respectively. Grain may be stored in this manner for periods in excess of six months.

Acknowledgement

The authors wish to thank the Home Grown Cereals Authority for a grant to support this work.

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