F U S A R I A A N D *F U S A R I U M* T O X I N S

I N M A I Z E

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HASSAN M. HUSSEIN

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ABSTRACT

Many species of Fusarium are commonly associated with cereals, particularly maize, but in New Zealand, little is known of their significance as mycotoxin producers. These studies have examined the prevalence of fusaria and other fungi in maize and maize fields and have investigated the presence and sources of some major Fusarium toxins in maize.

Fungi in maize, husk, litter and soil from maize fields and in grain at harvest and in storage were assayed. The distribution of fungi was found to be uneven within maize, husk and litter substrates within a field but in soil was more homogeneous. Sampling techniques were therefore developed to ensure representative subsamples were obtained from each source.

Similarly isolation procedures were chosen to ensure adequate recovery of fungi. Dilution and direct platings were used to provide information on total populations and on fungi actually invading kernels, with two media, PDA-D and PCNB. The medium used showed no significant influence on either viable counts or kernel contamination rates nor on the number of different Fusarium spp recovered on the two media, but PDA-D supported a greater overall variety of fungi. The numbers of genera and of Fusarium spp recorded by direct plating were significantly higher than with dilution plating.

The total population and the number of different genera and of Fusarium spp were compared for the four "field" substrates. A total of 25 genera was isolated, most being recovered from soil and litter. Fusarium was present in all samples. Acremonium, Cladosporium, Penicillium and Mucor occurred regularly. The four substrates gave up to ten different Fusarium spp, F. graminearum, F. culmorum and F. acuminatum being the most frequent. Husk and litter samples gave the highest viable counts for both total fungi and Fusarium spp.

Field samples of maize kernels showed 13 genera and ten Fusarium spp. At harvest time total genera increased to 17 but Fusarium spp remained constant. While the total genera remained constant at 17 in stored samples, the number of Fusarium spp dropped to three, only F.

subglutinans, F. graminearum and F. poae being detected. The contamination rate of kernels by fusaria also changed significantly from field samples (75.8%) to harvest samples (58.3%) to only 1.5% in stored maize.

As with Fusarium, Acremonium and Mucor populations decreased from harvest to storage but other genera (e.g. Aspergillus, Beauvaria) were only found in stored maize. The frequency of occurrence of Penicillium remained stable over the whole period.

Three analytical methods, TLC, GC and GC-MS were used for screening maize, poultry ration samples and cultures of Fusarium isolates for five Fusarium toxins. The GC-MS method was the most reliable and sensitive for detection and quantitation of DON, DAS and T-2 toxin, but not for quantitation of ZEA, due to derivatisation problems. TLC and TLC-densitometry were sensitive and reliable enough for detection and quantitation of ZEA and MON respectively. Although the GC results were closer to the GC-MS results, a high percentage of false positives, particularly for T-2 toxin, was noticed.

Of the examined maize samples, 85% were contaminated with fungal toxins. The majority contained ZEA and three samples were each contaminated with four toxins. No MON was detected.

Many isolates, particularly of F. graminearum, were found to be ZEA-producers. Some 63% produced ZEA at >2 ppm. T-2 toxin was produced by 46% of the isolates but at low levels (<1.7 ppm). Low levels of DON and DAS were produced by a few isolates. MON was produced by 30% of isolates, particularly F. subglutinans, and in large amounts (up to 64 ppm).

This thesis is the first report on the natural occurrence of Fusarium toxins in New Zealand maize. T-2 toxin and DAS have not been reported as natural contaminants in this country. MON production has also not been reported in New Zealand.

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ABBREVIATIONS USED IN THIS THESIS

3-ADON	3-acetyl-deoxynivalenol
15-ADON	15-acetyl-deoxynivalenol
BSA	N,O-Bis (trimethylsilyl) acetamide
BSTFA	N,O-Bis (trimethylsilyl) trifluoroacetamide
CFU/g	Colony-forming units per gram
CDA	Czapek-dox solution agar
CLA	Carnation leaf agar
DAN	Diacetylnivalenol
DAS	Diacetoxyscirpenol
2,4-DNPH	2,4-dinitrophenylhydrazine
DON	Deoxynivalenol
ECD	Electron capture detector
EI	Electron impact
FID	Flame ionization detector
FUS-X	Fusarenon-X
GC	Gas liquid chromatography
GC-ECD	Gas liquid chromatography with electron capture detector
GC-FID	Gas liquid chromatography with flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GYEP	Glucose-yeast-extract-peptone
HFB	Heptofluorobutyryl
HFBI	Heptofluorobutyryl-imidazole
IMC	Initial moisture content
LEM	Leukoencephalomalacia
MAS	Monoacetoxyscirpenol
MC	Moisture content
MID	Multiple ion detection
MON	Moniliformin
MTM	Mycotoxin standards mixture
MS	Mass spectrometry
MS-MS	Mass spectrometry-mass spectrometry
M/Z	Mass/charge ratio of ion fragments in mass spectrometry
NEO	Neosolaniol
NIV	Nivalenol
PCNB	Pentachloronitrobenzene
PDA	Potato dextrose agar
PDA-D	Potato dextrose agar-dichloran
ppb	Part per billion (ng/g)
ppm	Part per million (mg/kg)
PSA	Potato sucrose agar
RIA	Radioimmunoassays
sdw	Sterile distilled water
SIM	Selective ion monitoring
TAS	Triactoxyscirpenol
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSI	N-trimethylsilyl-imidazole
TCMS	Trimethylchlorosilane
TIM	Total ion monitoring
ZEA	Zearalenone