The incidence of *Fusarium* infection and mycotoxin contamination in maize grain from the Manawatu

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Abstract

A survey of maize grain delivered to Hodder and Tolley Ltd., Gillespies Line, Palmerston North was carried out during the 1993 maize harvest season. Harvest date, hybrid, harvest moisture percentage, grain weight, grain yield, *Fusarium* infection and mycotoxin levels were recorded. The incidence of *Fusarium* infection was determined by plating out individual grains on potato dextrose agar and by counting resulting *Fusarium* colonies. Mycotoxin screening for zearalenone, α and β - zearalenols and the trichothecenes, nivalenol and deoxynivalenol was carried out at HortResearch, Ruakura using HPLC analysis techniques.

The 1992/93 season was cool which led to lower than average grain yields, delayed harvesting and high grain moisture percentage at harvest. All samples showed *Fusarium* infection. Average infection level was 62% but ranged from 51.7% to 78.1% of infected grains for different hybrids. P3902 and P3901 had significantly lower levels of infection than P3751, P3585 and Furio. All samples contained one or more mycotoxins. Nivalenol (0.70 mg/kg) and deoxynivalenol (0.41mg/kg) were present in 100% and 93% of samples respectively. Sixty three percent of samples contained nivalenol and 38% deoxynivalenol, at levels considered potentially toxic to animals. Zearalenone and alpha - zearalenol were also present in most samples but at relatively safe levels. There were significant hybrid differences in mycotoxin levels.

No correlation was found between *Fusarium* infection and mycotoxin concentration indicating that *Fusarium* infection alone cannot be used to predict mycotoxin levels. Harvest date was weakly correlated with *Fusarium* infection but was strongly correlated with total nivalenol and deoxynivalenol in P3751. No other factors had a significant effect on *Fusarium* infection or mycotoxin levels.

Additional key words: *Maize, hybrid, harvest date,* Fusarium, *mycotoxins, nivalenol, deoxynivalenol, zearalenone*

Introduction

The infection of grain by pathogenic fungi known to produce toxins is a global human and animal health problem (Hesseltine, 1977). In New Zealand, mycotoxins produced by *Fusarium* species are a major cause of animal health problems and could potentially affect human health. Levels of mycotoxin contamination in New Zealand grown cereal grains have been found to be high in maize (Lauren *et al.*, 1991), the major feed grain in New Zealand (Eagles and Wratt, 1985). Mycotoxins commonly found in New Zealand maize include the oestrogenic zearalenone (ZEA) (Hussein et al., 1989) and the trichothecenes nivalenol (NIV) and deoxynivalenol (DON) (Hussein *et al.*, 1989; Lauren *et al.*, 1991). In a few samples low levels of moniliformin (MON), diacetoxyscirpenol (DAS) and T-2 toxin have also been found (Hussein *et al.*, 1987).

Animals with a high proportion of maize based feeds in their diet are at risk from *Fusarium* mycotoxicoses. In New Zealand this is primarily poultry, pigs and to a lesser extent deer and horses. Current estimates of lost production on pig farms have been placed at up to $50,000 per farm per year (Dobson, 1993). Estimates of total costs to the poultry industry resulting from mycotoxicoses are in the order of one million dollars annually (Foulds, 1993).

The production of toxins in grain by field fungi of the genus *Fusarium* has been known overseas for at least two decades, but has been studied in New Zealand only recently (Agnew *et al.*, 1986; Hussein *et al.*,1987, 1989; Lauren *et al.*, 1991, 1992). These surveys and related studies (Sayer, 1991; Sayer and Lauren, 1991; Lauren, 1994) have revealed high levels of *Fusarium* infection and mycotoxin contamination of maize sampled after harvest, with all maize growing areas being affected. The problem can be more severe when seasonal climatic conditions are cool with high rainfall, resulting in a

delayed harvest. Agronomic factors influencing *Fusarium* infection and mycotoxin presence in feeds have been investigated on only a minor scale (Hussein *et al.*, 1989; Lauren *et al.*, 1992), with previous work concentrating on the distribution and magnitude of the mycotoxin problem.

This study describes the incidence of *Fusarium* produced mycotoxins (NIV, DON, ZEA), α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL) in post-harvest Manawatu maize grain collected during the 1993 harvest, and investigates the effects of various agronomic factors on mycotoxin contamination. The relationship between *Fusarium* infection and mycotoxin levels is also investigated. The ability to predict mycotoxin levels from *Fusarium* infection would improve the feasibility of testing maize for mycotoxins.

Materials and Methods

Sample collection

Grain harvested from 15 farms in the Manawatu and northern Wairarapa districts during 1993 was sampled within 12 hours of delivery to Rowe and Collis' (Hodder and Tolley) drying and storage facility, Gillespies Line, Palmerston North. A total of 52 samples representing the cultivars P3902 (n = 4), P3787 (n = 4), Furio (n = 10), P3751 (n = 18), P3901 and P3585 (n = 2), (in order of increasing thermal requirement to harvest maturity) were collected. Sites were mainly on recent soils associated with the Manawatu River and ranged from sands and sandy loams of wind-blown origin in the west to silt loams of higher fertility in the inland areas. Sampling occurred each day grain was delivered for drying over the harvest period (May 30 - July 29). Samples (approx. 1.5 kg) were taken by trier from truck and trailer units, sealed in plastic bags and processed daily. Each sample was representative of a particular hybrid and harvest date from a given farm.

All samples were analyzed for grain moisture, grain weight and *Fusarium* infection. Forty sub-samples (400g) were taken for determination of mycotoxin content, stored at -15°C, and sent to HortResearch, Ruakura, for analysis. Hybrids for which samples were submitted for analysis were P3902 (n = 4), Furio (n = 9), P3571 (n = 13) and P3901 (n = 14).

Assessment of *Fusarium* infection

Grain was surface sterilised by soaking for 3 minutes in 1% NaOCl, washed in distilled water and incubated in petri dishes on potato glucose agar at 20°C (12 hours light) for 4-7 days using the method outlined by Sayer and Lauren (1991). The number of *Fusarium* colonies which grew from the grain were recorded. Where more than one fungal colony grew from a grain, all were recorded. Identification of *Fusarium* was based on characteristic colony form and colour. Samples not clearly identifiable after four days were incubated for a further 3 days and reassessed. Doubtful positives (weak red in colour) were recorded as being negative. Individual species were not identified. The incidence of infection by *Fusarium* was calculated from the numbers of colonies present after a period of seven days.

Moisture percentage and grain weight

A sub-sample of approximately 120g of grain was weighed and oven dried for three days at 96°C, or until no further weight loss could be detected, after which it was reweighed and the moisture percentage calculated. Grain weight was calculated by counting four hundred grains per sample, drying at 96°C for three days and weighing. Grain weight is expressed as weight per 1000 grains at 14% moisture.

Mycotoxin levels

The multi-toxin screening method of Lauren and Agnew (1991) was used to detect trichothecenes. Briefly, a subsample of finely ground grain was extracted with acetonitrile-methanol-water (80:5:15). An aliquot of extract solution was "cleaned up" first through a cation-exchange/alumina-carbon column, hydrolysed to produce parent alcohols, then after neutralisation, passed through a carbon-Celite mini-column. This fraction was analyzed for NIV and DON using HPLC with UV detection at n (detection limit = 0.05 mg/kg). A second aliquot was analyzed directly without cleanup for ZEA and ZOLs using HPLC with fluorescence detection (detection limits = 0.05mg/kg for ZEA and α-ZOL; = 0.2mg/kg for β-ZOL).

Obtaining agronomic data

Weighbridge dockets were consulted daily to determine the site, date of harvest, and hybrid for each sample. Farmers were subsequently contacted and questioned in regard to the sowing dates and yields of their respective maize paddocks (sites). Yields were calculated on a 14% moisture basis.

Data analysis

Analysis was performed on the computerised Software Analysis System (SAS) using simple correlation and analysis of variance (generalised linear model) techniques. T-tests were used to test the significance of differences between hybrid means for *Fusarium* incidence and mycotoxin level.

Results

Climate and 1992/93 maize performance

The 1992/93 Manawatu growing season was cool, with an average monthly temperature of 14.0°C over the October-May period compared to the long term mean of 14.7°C (Table 1). The mean monthly temperature for December to April ranged between 0.8 - 2.2°C below average. One unseasonal ground frost occurred in December. The deficiency of heat units over the growing season (approx. 160 @ 10°C base temp.) resulted in delayed harvest and higher than usual grain moisture percentage. Poor grain drydown resulted in the first samples not being received until May 30. Peak harvest occurred in late June and the final sample was submitted on July 26, 1993.

At the beginning of harvest, grain moisture percentage was high (35.5%) and did not reach the recommended harvest moisture percentage of 22-23% (Sayer, 1991) until July 15. Grain bulk density was low (mean 62.2 kg/hl) and ranged from 59.4 - 65.2 kg/hl. Average grain yield of the crops surveyed was 7.25 t/ha. No significant differences in grain weight were observed among hybrids.

Table 1. Manawatu rainfall and temperature for 1992-93 and long-term means.

	Monthly rainfall total		Mean monthly temperature	
	1992/93[1] (mm)	Mean[2] (mm)	1992/93[1] (°C)	Long term[3] (°C)
Oct.	5.7	83	11.8	12.1
Nov.	60.8	63	14.7	13.7
Dec.	167.3	84	15.1	15.9
Jan.	5.4	65	15.7	17.4
Feb.	43.7	55	16.3	17.5
Mar	79.8	71	14.3	16.5
Apr.	5.4	74	12.6	13.7
May	66.9	83	11.4	10.6
Av.			14.0	14.7

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[2] 1943-1980: Palmerston North Airport, (Burgess, 1988).
[3] 1962-1980: Palmerston North Airport, (Burgess, 1988).

Fusarium infection

Fusarium was present in all samples with an average infection rate of 62.1% for all samples. In many cases individual grains produced more than one colony. There were significant differences (P = 0.05) among hybrids, with P3901 having significantly lower levels of *Fusarium* infection than P3751, Furio and P3585 (Table 2) and P3902 having significantly lower levels of infection than Furio and P3585. There were no other significant differences among any other hybrids. *Fusarium* infection was significantly, but weakly positively correlated with time of harvest (r =0.32) and was negatively correlated with grain moisture percentage (r = -0.38). This is probably due to the strong, negative (r = -0.69) correlation between grain moisture percentage and harvest date.

Mycotoxin levels

All mycotoxins screened for were detected (Table 3): NIV and α-ZOL were present in all 40 samples; DON was present in 37 samples while ZEA occurred in 26

Table 2. Mean incidence of *Fusarium* infection and mycotoxin levels for each hybrid. S.E.M. are in parenthesis.

	P3901	P3902	P3787	P3751	FURIO	P3585
Fusarium incidence (%)	56.5 (2.9)	51.7 (14.6)	63.5 (6.95)	65.3 (1.68)	69.4 (1.76)	78.1 (8.9)
Deoxynivalenol (mg/kg)	0.363 (0.085)	0.193 (0.10)	NA[1]	0.685 (0.12)	0.199 (0.06)	NA
Nivalenol (mg/kg)	0.55 (0.12)	0.555 (0.25)	NA	1.098 (0.18)	0.42 (0.10)	NA
Zearalenone (mg/kg)	0.270 (0.096)	0.025 (0.014)	NA	0.240 (0.06)	0.023 (0.013)	NA
α-Zearalenol (mg/kg)	0.147 (0.02)	0.098 (0.04)	NA	0.158 (0.03)	0.137 (0.23)	NA

[1] not submitted for analysis

Table 3. Incidence and concentration of Zearalenone, Nivalenol, Deoxynivalenol and Alpha-zearalenol.

	Number samples positive[1]	Mean level[2] (mg/kg)	Standard deviation (mg/kg)	Maximum level (mg/kg)
Deoxynivalenol	37	0.41	0.36	1.51
Nivalenol	40	0.71	0.51	2.57
Zearalenone	26	0.18	0.20	0.75
α-Zearalenol	40	0.14	0.06	0.31

[1] From a total of 40 samples
[2] Detection limit = 0.05mg/kg

samples. B-ZOL was found to be present in only one sample at a level of 0.2 mg/kg (Detection limit = 0.2 mg/kg). Twenty-five samples contained NIV and 11 samples DON above levels (0.4 mg/kg) at which subclinical animal health problems have been recorded (Lauren, pers. comm.). No samples had DON levels exceeding 4 mg/kg, the guideline tolerance level for feed ingredients (10% inclusion for pigs) in the USA (Van Egmond, 1989). (Note: maize is commonly included as 40% of pig diets in New Zealand, and the remainder may include processed maize by products which could also be contaminated).

Mycotoxin levels showed no association with any of the agronomic parameters collected in this study, with the exception of a weak correlation between NIV and *Fusarium* infection.

The effect of hybrid on mycotoxin content was dependant on the toxin (Table 2). Pioneer 3751 had significantly (P = 0.05) higher NIV and DON levels than Furio and P3901 and significantly higher levels of DON than P3902. Pioneer 3901 and P3751 had high levels of ZEA (0.270 and 0.240 mg/kg respectively), while P3902 and Furio had low (0.025 and 0.023 mg/kg respectively) levels of ZEA. However only the difference between P3901 and Furio was significant (P = 0.05). Correlation analysis was undertaken for each cultivar separately. Total trichothecenes (NIV and DON) and total ZEA and β-ZOL were significantly (P = 0.05) correlated with *Fusarium* infection, harvest date and grain moisture. There were no significant correlations between *Fusarium* infection and mycotoxin levels for any hybrid. Harvest date was correlated with total NIV and DON levels in P3751 (r = +0.76) and total ZEA and α-ZOL levels in P3901 (r = +60).

There were strong, significant (P = 0.01) correlations between ZEA and NIV (r = +0.98), ZEA and α-ZOL (r = +0.99), NIV and α-ZOL (r = +0.98) and a weak but significant (P = 0.05) correlation between NIV and DON (r = +0.37), indicating a high degree of co-existence among these mycotoxins.

Discussion

Manawatu maize yields from the 1993 harvest were significantly lower than the recent average of 9.0 t/ha (Anon. 1980-1992). Yields were similar to those of the 1982/83 season (6.7 t/ha) (Anon. 1984) which was characterised by strong *el nino* conditions and below average temperatures. Similarly, the 1992/93 season was unusually cool with yield losses being attributed to delayed silking and premature abscission resulting in reduced grain fill periods. There were no hybrid differences in grain yield, probably as a result of the confounding effects of different management practices at different sites. Under controlled conditions hybrid yield differences would be expected (Eagles, 1987). Under existing quality specifications (Hodder & Tolley New Zealand Ltd.) all grain samples were subject to discretional refusal and 15% received price penalties, primarily because of low bulk density. Most (78%) growers harvested grain with moisture above the recommended 22-23% (Sayer, 1991) resulting in increased drying costs and a high proportion of grain damaged during harvest (broken and cracked). High moisture and damaged grain can increase vulnerability to secondary storage fungi and pests.

The incidence of *Fusarium* infection was high, and all samples were infected. The average infection rate was 62%. *Fusarium* infection levels in the Manawatu (1987-1989) have previously been reported (Sayer and Lauren, 1991), with 67% of grain samples having an infection level greater than 20%. This is low compared to the samples in the current study where virtually all samples had infection rates above 20%. The high levels of infection in the current study are probably the result of the cool season favouring *Fusarium* infection (Hussein *et al.*, 1989). *Fusarium* infection levels increase during the extended period that maize is left standing in the field (Sayer, 1991). Hybrid differences in *Fusarium* infection have been reported (Miller, 1994); however little New Zealand information has been published. In the current survey hybrid differences in *Fusarium* infection were evident with P3901 and P3902 having significantly lower levels of infection than P3751, Furio and P3585. The limited published information available on the relative susceptibility of the hybrids used in this study to *Fusarium* infection may suggest that there are few differences between hybrids (Anon, 1993). However,

published rankings are based on American data and may not apply to the *Fusarium* species predominant in New Zealand or to New Zealand climatic conditions.

The prevalence of NIV is consistent with previous reports (Lauren *et al.*, 1991) but uncommon internationally. ZEA levels were below the levels (1-5mg/kg) reported by Chang *et al.*, (1979) to cause clinical oestrogenism in gilts, but effects at lower levels have been reported (Prelusky *et al.*, 1994). A significant number of samples contained NIV and DON at levels sufficient to produce sub-clinical animal health problems. Sub-clinical poisoning in animals is often observed with pigs when the combined level of NIV plus DON in the feed is at, or above, 0.4-0.5mg/kg (Lauren pers. comm., 1993). Although the effects of sub-clinical doses of mycotoxins are not well understood (Bottalico *et al.*, 1989), feed with these levels of NIV and DON is capable of depressing animal productivity. Since maize or maize by products can commonly represent 50% or more of pig diets, the presence of NIV plus DON combined in concentrations above about 0.4mg/kg is of concern. All hybrids screened for mycotoxin content (P3751, P3902, P3901 and Furio) had samples with NIV plus DON in excess of 0.4mg/kg. The presence of several mycotoxins in virtually all samples may increase the risk of animal mycotoxicoses. Synergistic and/or additive effects can occur with these mycotoxins (Hoerr *et al.*, 1982).

A number of *Fusarium* species are known to produce the mycotoxins found in this study (Lauren *et al.*, 1992). *F. culmorum,* (Sayer and Lauren, 1991), *F. graminearum,* (Hussein and Baxter, 1985) and *F. crookwellense* (Sayer and Lauren, 1991), are common in Manawatu maize crops.

A significant relationship between harvest date and NIV and DON levels in P3751 was established. The reason for this is unclear. P3751 has similar relative maturity and grain dry down to other hybrids in this study and there was little difference in average harvest moisture % between hybrids suggesting that hybrid maturity did not influence NIV and DON levels in this study. Other hybrid characteristics (husk tightness) may have had an effect (Enerson and Hunter, 1980), and there are certainly genetic effects amongst which trichothecene tolerance plays a part (Snijders, 1994). It is possible however that variation in site and management regime confounded the harvest date-NIV/DON level relationship in the other hybrids.

Hybrid differences in mycotoxin level have been noted in New Zealand (Lauren as cited by McCaw, 1993). This survey supports the view that hybrid selection may influence both *Fusarium* infection and mycotoxin production. Hybrid selection is a relatively simple management tool reinforcing the need for further investigation in this area.

The correlations between ZEA and its derivatives and the trichothecenes are in agreement with previously published material (Yoshizawa, 1983; Tanaka *et al.*, 1990; Lauren *et al.*, 1992) and indicates the strong potential for chronic animal health problems resulting from consuming feed contaminated with several different toxins.

One of the objectives of this study was to investigate the relationship between *Fusarium* infection and mycotoxin levels. If a direct relationship could be established, it may be possible to reduce the high costs associated with the determination of grain mycotoxin levels. However, in agreement with previous reports (di Menna *et al.*, 1987; Lauren *et al.*, 1992), a usable predictive relationship between *Fusarium* infection and mycotoxin levels was not found for total or individual mycotoxins. Despite the fact that *Fusarium* infection of maize results in the production of mycotoxins, it is apparent that the incidence of infection alone is not a good indicator of mycotoxin production.

Conclusion

The 1992/93 season was cool, resulting in lower than average maize yields, delayed harvesting and high grain moisture at harvesting. This resulted in high rates of *Fusarium* infection. Mycotoxins were present at levels potentially toxic to animals; however the relationship between *Fusarium* infection and mycotoxin levels was weak. There were significant hybrid differences in *Fusarium* infection and mycotoxin levels. High mycotoxin levels in P3751 appeared to be related to the build-up of mycotoxins in this hybrid when harvest was delayed.

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