

Cultivar and crop management influences on fusarium head blight and mycotoxins in spring wheat (*Triticum aestivum*) in New Zealand

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Abstract Cultivar and crop management influences on fusarium head blight (FHB) of wheat (*Triticum aestivum* L.) were investigated in cultivar field trials and commercial wheat crops in the North Island of New Zealand over two growing seasons. There were consistent differences between cultivars in their susceptibility to FHB. The Chinese wheat ‘Nanjing’ had the lowest level of FHB, mycotoxins, and *Fusarium* infection in grain. Although no New Zealand cultivars approached an equivalent level of resistance, FHB in some cultivars was low in most situations, and these cultivars had a useful level of resistance. FHB and mycotoxin levels varied widely between crops surveyed. Two *Fusarium* mycotoxins, deoxynivalenol (DON) and nivalenol (NIV), were detected in grain samples from crops and trials. Overall, DON levels were higher than NIV in crops in both years. FHB incidence and levels of *Fusarium* infection and mycotoxins in grain were closely

related in samples from a particular crop, but the relationships were much less apparent between crops. *F. graminearum* predominated in grain samples, although *F. avenaceum*, *F. culmorum*, and *F. poae* were also common. Highest levels of *F. graminearum* were recorded in grain samples from crops that followed maize, whereas *F. avenaceum* and *F. poae* were more common in samples from crops that did not follow maize.

Keywords *Fusarium graminearum*; head scab; mycotoxins; fungicides; host resistance

INTRODUCTION

In recent years, diseases caused by *Fusarium* spp. (foot rots, snow mould, and fusarium head blight (FHB)) have become an increasing concern in New Zealand wheat (*Triticum aestivum* L.) crops, and to a lesser extent in barley (*Hordeum vulgare* L.) (Braithwaite et al. 1998). These diseases are caused by complexes of *Fusarium* spp. The species mixture has been shown to vary according to region, with *F. graminearum* often predominating in samples from the North Island (Sayer & Lauren 1991). The diseases can affect yield by reducing grain number and weight. The quality of seed for sowing is also adversely affected through reduced rates of germination and emergence.

Fusarium head blight, also known as head scab, is most easily recognised on immature ears where one or more spikelets in each ear become prematurely bleached. Sometimes large areas of ears may be affected and, where infection is severe, pink or orange spore masses occur on diseased spikelets. FHB can cause yield losses of 30–70% where conditions favour the disease, but, more importantly, grain from affected crops may be less palatable to stock than healthy grain and may contain mycotoxins such as the trichothecenes deoxynivalenol (DON) and nivalenol (NIV) (Bai & Shaner 1994).

Lauren et al. (1991) reported a high incidence of trichothecene contamination in grain from wheat

crops from the North Island of New Zealand (levels as high as 1.27 and 11.95 mg/kg for NIV- and DON-type trichothecenes respectively), whereas levels never exceeded 0.1 mg/kg in South Island crops. A maximum trichothecene level of 1 mg/kg is the United States Food and Drug Administration guideline for human food components, whereas some recommendations for animal food are below that level (Cromey et al. 2001a). Also, levels below 1 mg/g for uncleaned wheat can result in levels well above this for some milled fractions such as bran and in screenings (Young et al. 1984).

There are two stages of infection of wheat ears by *Fusarium*. Host plants are most susceptible to primary infection during anthesis when florets can be infected, especially during wet conditions (Bai & Shaner 1994). Secondary spread between florets and spikelets occurs at later stages of crop development.

All of the *Fusarium* species that infect cereals are capable of surviving saprophytically on crop debris (Parry et al. 1995). This debris is considered to be an important reservoir of *Fusarium* inoculum for the infection of wheat ears (Sutton 1982), although alternative hosts, such as grasses and broadleaf weeds, may also provide important sources of inoculum (Parry et al. 1995).

Some fungicides have been shown to provide a degree of control of FHB when applied to wheat crops during anthesis (Cromey et al. 2001a, 2002). Levels of control can be inconsistent, probably because of such factors as the complex of causal species, timing of application, coverage, and inherent fungicidal activity (Parry et al. 1995).

Wheat cultivars differ in resistance to FHB. It is generally considered that no cultivar is immune, a few are moderately resistant and most are susceptible (Parry et al. 1995). Published information on the relative resistance of New Zealand wheat cultivars to FHB is not available, although differences between cultivars in levels of *Fusarium* on harvested grain have been recorded (Cromey et al. 2001b).

Reported correlations between FHB severity, *Fusarium* frequency in grain, and mycotoxin concentrations vary. Cromey et al. (2001a) reported a positive correlation ($r = 0.84$) between *Fusarium* frequency and mycotoxin concentration amongst samples from a fungicide trial. Salas et al. (1999) reported a range between years in correlations (between $r = 0.28$ and 0.83) in surveys of grain samples. Many factors influence mycotoxin production (Cooney et al. 2001). For instance it has been suggested that resistance to FHB and

mycotoxin accumulation may be controlled by different genes (Bai & Shaner 1994).

Given the moderate levels of resistance and lack of highly effective fungicides, management of FHB will probably rely on the integration of a number of control options (Dill-Macky & Jones 2000). However, although it is known that FHB and mycotoxins occur in New Zealand wheat, the crop management factors associated with the disease, and the relationships between FHB parameters are not. In this paper we report a series of cultivar trials aimed at determining the levels of resistance available in New Zealand wheat cultivars. A 2-year survey of commercial North Island wheat crops was also conducted to examine factors, such as cultivar, cropping history and fungicide usage, associated with FHB and mycotoxin contamination. The relationships between FHB parameters was also examined between and within sites to determine the extent to which one parameter can be predicted from the measurement of another parameter.

METHODS

1998/99 field trials

Three cultivar field trials (randomised complete block design with eight cultivars and four replicates) were sown on growers' properties at Greytown (Wairarapa), Marton (Rangatikei), and Kairanga (Manawatu). Plot dimensions were 1.5 m by 10 m. Soil types were Ruamahanga silt loam (Greytown), Kiwitea silt loam (Marton), and Kairanga silt loam (Kairanga). Incidence of FHB was estimated by examining c. 500 ears in each plot. Grain samples combined over the four replicates were collected for each trial at harvest from eight genotypes (FHB37, 'Karamu', 'Kohika', 'Monad', 'Nanjing', 'Norseman', 'Otane', 96WFHB5568) and assessed for visibly infected grains, mycotoxins, and levels of *Fusarium* in grain using the methods described below. Samples of these lines were also collected and analysed from each of four harvests at another Kairanga site (Aorangi Research Farm, Kairanga silt loam).

1999/2000 field trial

A field trial was located at Kairanga, Manawatu (Kairanga silt loam), where FHB is common. The previous crop was maize. The trial was of randomised complete block design (seven cultivars, four replicates), with the blocks arranged in a single

row, and plot dimensions 1.5 m by 10 m. The trial was sown in late August and harvested in mid February. No fungicides were applied to the trial. Incidence of FHB, visibly infected grains, mycotoxins, and levels of *Fusarium* were assessed as before.

Crop surveys

Twenty-two and 32 commercial wheat crops in 1999 and 2000 respectively were selected to cover a range of management regimes in the southern North Island cropping regions. In each season three cultivars were represented, with 10, 7, and 5 crops in 1999 and 11, 11, and 10 crops in 2000 of the cultivars 'Monad', 'Otane', and 'Kohika', respectively. Crops were inspected c. 3 weeks after flowering, and the proportions of ears with FHB symptoms were estimated by examining c. 500 ears in each of five places within each crop. Samples (2 kg) of unscreened grain harvested from each crop were received from growers. From each grain sample, a 50 g subsample was taken and the visibly infected (tombstone) grains were separated from the healthy grains, counted, and weighed. Numbers of visibly infected grains/100 g and proportion of sample weight in visibly infected grains were determined. Subsamples were also used to determine mycotoxin and *Fusarium* levels (see below). Information was collected from growers on previous crop, location, cultivar, sowing and harvest dates, and timing, active ingredients, and rate of fungicide applications for each crop, as well as weather conditions during flowering and grain fill.

Mycotoxin analysis

Grain samples were analysed for the presence of DON and NIV, the trichothecene mycotoxins commonly found in infected New Zealand wheat crops (Lauren et al. 1991). Analysis was by a method adapted from that described by Lauren & Ringrose (1997). The samples were extracted with acetonitrile-methanol-water (85:5:15), and aliquots subjected to clean-up through an alumina-carbon/cation exchange column, followed by hydrolysis to convert all trichothecenes to parent alcohols. After neutralisation, the extracts were passed through a 216 Trichothecene Charcoal-Column (Romer Labs, MO, United States). These refined extracts were then used for analysis by High Performance Liquid Chromatography with UV detection at 245 nm and with confirmation at 254 nm. Analysis for NIV and DON used a Zorbax SB-C8 column held at 35°C with a mobile phase of methanol-water (12:88).

Under these conditions NIV in some samples was subject to interference, and in these instances NIV was analysed using a Zorbax SB-Phenyl column held at 35°C with a mobile phase of methanol-water (1:99). Concentrations of mycotoxins were expressed on a grain fresh weight (c. 5–10% moisture) basis.

Assessment of *Fusarium*

To measure infection by *Fusarium* spp., 100 seeds/plot were surface sterilised in 1% sodium hypochlorite for 10 min, and plated onto Potato Dextrose Agar (PDA) at 10 seeds/plate. Plates were incubated for 4–8 days on a laboratory bench and numbers of *Fusarium* colonies were counted. Species identification was carried out on PDA and on Carnation Leaf Agar (CLA) using the methods of Burgess et al. (1994). Plates for species identification were incubated for 7 days in an alternating temperature regime, 25°C light/20°C dark, with a 12 h photoperiod.

Statistical analyses

Data from field trials were statistically analysed with ANOVA. Crop survey data were not examined statistically. Instead, factors commonly associated with high mycotoxin levels were identified, and these factors were then examined with reference to mean and range for each variable, and the proportions of samples with high mycotoxin levels, removing as far as possible influences of complicating factors. Relationships between variables were examined graphically, and by calculated correlation co-efficients.

RESULTS

Field trials

Statistically significant differences between cultivars in the incidence of FHB and levels of grain *Fusarium* and mycotoxins were observed in the 1998/99 trials (Table 1). Of the four trial sites, FHB levels were very low in the Greytown and Marton trials. In the Marton trial, FHB in ears and visibly infected grains were observed only in 'Otane' and 'Kohika' and mycotoxins were detected only in 'Kohika'. Levels of FHB were considerably higher in the Kairanga and Aorangi trials, where greater differentiation of cultivars was observed. Weather conditions during flowering and grain fill were mostly warm and dry, especially in the Greytown and Marton trials.

Cultivar had a significant effect on the percentage of ears with FHB, visibly infected grains, grain infection with *Fusarium*, and mycotoxin contamination. The cultivars 'Otane' and 'Kohika' had the highest levels of FHB, visibly infected grains, mycotoxins, and grain infection with *Fusarium*. Two cultivars ('FHB37' and 'Nanjing'), which had resistance derived from known Chinese resistant sources, had the lowest levels of all FHB measurements, including negligible mycotoxin levels. The New Zealand cultivars 'Karamu', 'Monad', and 'Norseman' were intermediate in levels of FHB, *Fusarium*, and mycotoxins.

Statistically significant differences in FHB, frequency of visibly infected grains and grain *Fusarium* and mycotoxin levels between cultivars were again observed in the trial in the 1999/2000 growing season (Table 1). There was occasional rain during flowering, with cool damp weather during grain fill. Overall, FHB levels were relatively high

compared with the drier 1998/99 season. Only 'Torlesse' and 'Monad' had mean mycotoxin levels of <1 mg/kg. Levels of each were highest in 'Otane' and 'Kohika', with mean mycotoxin levels of 3.8 and 2.2 mg/kg respectively, and grain *Fusarium* levels of more than twice those of other cultivars. Low levels of FHB, grain *Fusarium*, and mycotoxins were recorded in 'Torlesse' and 'Monad', and intermediate levels in 'Impact', 'Karamu', and 'Sapphire'. DON levels were at least 3 times as high as NIV in each cultivar. 'Otane' had proportionally higher levels of DON than other cultivars, but also had the highest level of NIV of any cultivar.

Crop surveys

Crops in the North Island wheat survey showed a wide variation in FHB, visibly infected grains, levels of *Fusarium* in grains, and mycotoxin levels. Twelve crops were recorded with over 0.5 mg/kg combined NIV and DON, four (of 22) from 1998/99 and eight

Table 1 Mean fusarium head blight (FHB) incidence and visibly infected grains, *Fusarium* and mycotoxin levels in harvested grain in wheat (*Triticum aestivum*) cultivars in field trials in 1998/99 (mean of four trials, except FHB, which is the mean of the Kairanga, Greytown, and Marton, New Zealand, trials only) and 1999/2000 (one trial). (NIV, nivalenol; DON, deoxynivalenol.)

Cultivar	% ears with FHB	% sample weight in visibly infected grains	No. of visibly infected grains in 100 g sample	% grains with <i>Fusarium</i>	Mycotoxin (mg/kg)		
					NIV	DON	NIV + DON
1998/99							
FHB37	0.1	0.1	2.1	8	0.00	0.00	0.00
Karamu	1.4	0.7	10.6	14	0.05	0.04	0.09
Kohika	1.7	1.3	22.3	13	0.13	0.04	0.17
Monad	0.3	0.5	7.3	10	0.03	0.02	0.05
Nanjing	0.0	0.0	0.6	6	0.00	0.01	0.01
Norseman	0.1	0.5	9.0	11	0.07	0.02	0.09
Otane	2.4	1.4	24.7	13	0.26	0.14	0.40
96WFHB5568	0.0	0.5	4.3	12	0.04	0.03	0.07
LSD _{0.05}	2.3	0.5	8.7	7	0.17	0.09	0.16
d.f.	14	42	42	42	42	42	42
1999/2000							
Impact	1.8	3.4	66.0	10.5	0.41	1.34	1.75
Karamu	0.8	2.3	41.0	17.0	0.23	1.00	1.23
Kohika	2.8	4.8	74.5	36.0	0.36	1.80	2.16
Otane	3.5	5.3	78.0	45.0	0.57	3.25	3.82
Sapphire	0.5	2.4	35.7	13.0	0.33	0.93	1.26
Torlesse	0.0	1.0	19.3	5.0	0.06	0.30	0.36
(Monad*)	–	(0.5)	(9.0)	(4.5)	(0.0)	(0.1)	(0.1)
LSD (0.05)							
d.f. = 15	2.2	1.8	22.3	7.5	0.09	0.7	0.69

*'Monad' was not included in the trial, but was the surrounding crop and was in a neighbouring trial. Results presented are the mean of two samples, one from the surrounding crop, and one from the neighbouring trial. Results were not used in the statistical analyses.

(of 32) in 1999/2000 (Table 2). Of the 12 crops, five (of 18 in the survey) were 'Otane', five (of 15) were 'Kohika', and two (of 21) were 'Monad'. Six of the 12 crops had maize as the previous crop (there were 11 ex-maize crops), and all crops were located in southern Manawatu. There was no obvious association of these crops with sowing or harvest dates, fungicide usage or weather data provided by growers.

In the 1998/99 growing season, mean FHB, visibly infected grains, levels of *Fusarium* in grains, and mycotoxin levels were much higher in 'Otane' and 'Kohika' than in 'Monad', with total mycotoxin levels in 'Otane' and 'Kohika' 3–6 times that of 'Monad' (Table 3). Maximum NIV + DON levels were 2.0 and 0.7 mg/kg in 'Otane' and 'Kohika' respectively compared with 0.2 mg/kg in 'Monad'. Incidence of FHB and numbers of visibly infected grains were lower in 'Monad' than in 'Otane' and 'Kohika'.

Frequency of visibly infected grains, FHB, grain *Fusarium*, and mycotoxin levels also differed markedly between cultivars in the 1999/2000 survey (Table 3). Mean levels of each were considerably lower in 'Monad' than in 'Otane' or 'Kohika'. The mean mycotoxin level of 0.2 mg/kg in 'Monad' compares with 0.5–0.6 for the other two cultivars, and *Fusarium* was isolated from 5% of 'Monad' grains compared with 8–9% for the other two cultivars. Maximum mycotoxin levels were 1.0 mg/kg in 'Monad', compared with 2.3 and 2.2 for 'Otane' and 'Kohika' respectively.

The proportions of visibly infected grains, grain *Fusarium*, and mycotoxins were higher in crops that followed maize immediately than any other previous crop, although there were no obvious differences in the proportions of ears with FHB (Table 4). Mean mycotoxin levels in crops out of maize were twice those of crops out of grass and c. 5 times those of crops out of other cereals or non-cereals. Forty-five percent of crops out of maize had over 1 mg/kg NIV + DON, compared with 13% of crops out of grass, and no crops out of other cereal or non-cereals.

Because of the strong association with cultivar, and because all but one of the crops that followed maize were grown in the southern Manawatu region, previous crop associations were further examined amongst southern Manawatu crops of 'Otane'/'Kohika' and 'Monad' separately (Table 4). Again, crops that followed maize had higher levels of visibly infected grains, *Fusarium* incidence and mycotoxins than crops following other previous crops. In 'Otane'/'Kohika', mycotoxin levels where maize was the previous crops were about twice those following another Graminae (other cereal or grass) and 3 times those which followed a non-Graminae crop. In 'Monad', mycotoxin levels were negligible except where crops followed maize.

Mean combined NIV + DON levels were over 4 times greater in southern Manawatu than in the northern region but more importantly, all of the crops with >0.5 mg/kg NIV + DON were located in southern Manawatu (Table 4). Mycotoxin levels were negligible in East Coast crops, although it

Table 2 Cultivar, previous crop, location, and growing season of the 12 of 54 wheat (*Triticum aestivum*) crops surveyed which had over 0.5 mg/kg combined nivalenol (NIV) + deoxynivalenol (DON).

Sample	Cultivar	Previous crop	Location	Year	Mycotoxin (mg/kg)		
					NIV	DON	NIV + DON
8	Monad	Maize	S Manawatu	1999/2000	0.22	0.65	0.87
10	Monad	Maize	S Manawatu	1999/2000	0.26	0.75	1.01
18	Otane	Non-Graminae	S Manawatu	1999/2000	0.44	0.36	0.80
19	Otane	Maize	S Manawatu	1999/2000	0.51	1.82	2.33
25	Kohika	Maize	S Manawatu	1999/2000	0.19	0.86	1.05
27	Kohika	Maize	S Manawatu	1999/2000	0.93	1.24	2.17
28	Kohika	Wheat	S Manawatu	1999/2000	0.32	0.30	0.62
30	Kohika	Wheat	S Manawatu	1999/2000	0.29	0.22	0.51
40	Otane	Grass	S Manawatu	1998/99	0.36	1.60	1.96
41	Otane	Maize	S Manawatu	1998/99	0.33	0.69	1.02
44	Otane	Grass	S Manawatu	1998/99	0.46	0.56	1.02
45	Kohika	Grass	S Manawatu	1998/99	0.24	0.42	0.66

Table 3 Mean and range (in parentheses) of fusarium head blight (FHB) incidence in crops and visibly infected grains, *Fusarium* and mycotoxin levels in harvested grain from spring crops of three wheat (*Triticum aestivum*) cultivars in the North Island of New Zealand in 1998/99 and 1999/2000. (NIV, nivalenol; DON, deoxynivalenol.)

Cultivar	No. crops	% ears with FHB	% sample weight in visibly infected grains	% grains with <i>Fusarium</i>	Mycotoxins (mg/kg)			% crops over 0.5 mg/kg NIV + DON	% crops over 1.0 mg/kg NIV + DON
					NIV	DON	NIV + DON		
1998/99									
Otane	7	5 (2-6)	1.2 (0-2.4)	17 (2-54)	0.16 (0-0.46)	0.41 (0-1.60)	0.6 (0-2.0)	43	43
Kohika	5	5 (4-5)	0.5 (0.2-1.2)	9 (0-18)	0.09 (0-0.24)	0.16 (0.02-0.42)	0.3 (0-0.7)	20	0
Monad	10	1 (0-1)	0.4 (0-0.8)	12 (4-19)	0.01 (0-0.07)	0.03 (0-0.12)	0.1 (0-0.2)	0	0
All crops	22	3 (0-6)	0.7 (0-2.4)	13 (0-54)	0.08 (0-0.46)	0.18 (0-1.60)	0.26 (0-1.96)	18	14
1999/2000									
Otane	11	29 (0-50)	0.9 (0-3.2)	11 (2-21)	0.20 (0.0-0.51)	0.29 (0-1.82)	0.5 (0-2.3)	18	9
Kohika	10	23 (5-40)	1.5 (0.3-5.8)	10 (2-26)	0.26 (0.05-0.93)	0.35 (0.09-1.24)	0.6 (0.1-2.2)	40	20
Monad	11	3 (0-10)	0.3 (0-1.0)	6 (0-18)	0.06 (0-0.26)	0.17 (0-0.75)	0.2 (0-1.0)	9	9
All crops	32	18 (0-50)	0.9 (0-5.8)	9 (0-26)	0.17 (0-0.93)	0.27 (0-1.82)	0.44 (0-2.33)	25	13

should be noted that only three East Coast crops were sampled. Because of the possible influence of previous crop (all crops out of maize were in southern Manawatu) and cultivar, regional associations were further examined in crops which were not out of maize (Table 4). Amongst 'Otane'/'Kohika' crops which did not follow maize, mean mycotoxin levels in southern Manawatu crops were 4 times those of northern Manawatu crops, and all crops with over 0.5 mg/kg were in southern Manawatu.

Weather information supplied by growers indicates that conditions during flowering and grain fill were generally drier in the 1998/99 than 1999/2000 growing season, in line with the 40% lower mean mycotoxin level in the former season. There were differences in weather conditions between crops in both years, but no relationship was detected between rainfall information and FHB, *Fusarium* levels in grain, or mycotoxin levels. No clear relationship was apparent between FHB variables and time of sowing or harvest.

The variation in fungicide usage and timing between crops, along with complicating factors (in particular cultivar and previous crop) make it difficult to extract firm associations between fungicide and FHB. Although there was a trend across years for mycotoxin levels to be lower in crops treated with triazole fungicides than in untreated crops, several crops treated with triazoles had mycotoxin levels over 0.5 mg/kg. However, in each case the cultivar was highly susceptible to FHB ('Otane' or 'Kohika') and the previous crops was in the Graminae. The strobilurin fungicide azoxystrobin was first registered in the 1999/2000 season. Eight of nine crops treated with azoxystrobin alone recorded over 10% isolation of *Fusarium* compared with two of 13 crops treated with other fungicides and two of 10 untreated.

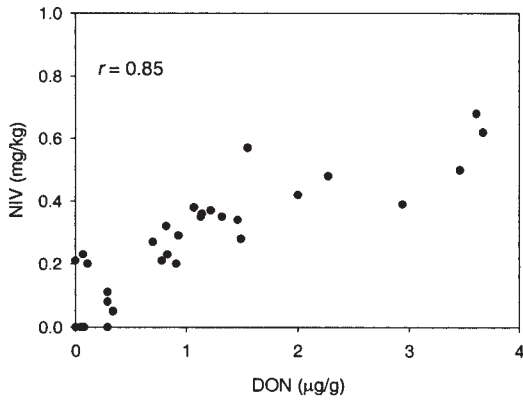
Fusarium graminearum was the most commonly isolated *Fusarium* species from grain harvested from North Island wheat crops in the 1999/2000 growing season (Table 5). Other species isolated were *F. avenaceum*, *F. poae*, and *F. culmorum*, with lower levels of several other species also isolated. Whereas infection levels were lower in 'Monad' than in 'Otane' and 'Kohika', 67% of 'Monad' isolates were *F. graminearum*, compared with 50-56% of isolates from 'Otane' and 'Kohika'. *F. avenaceum* also formed a greater proportion of isolates from 'Monad' (16%) than from 'Otane' (8%) or 'Kohika' (9%). Crops following maize, as well as having higher overall levels of *Fusarium*, tended to have higher

Table 4 Mean and range (in parentheses) of fusarium head blight (FHB) incidence in crops and visibly infected grains, *Fusarium* and mycotoxin levels in harvested grain from 1998/99 and 1999/2000 spring crops of three wheat (*Triticum aestivum*) cultivars following different previous crops. (Ot/Ko, 'Otane' and 'Kohika'; N Man./Ran., northern Manawatu and Rangitikei; S Man., southern Manawatu; NIV, nivalenol; DON, deoxynivalenol.)

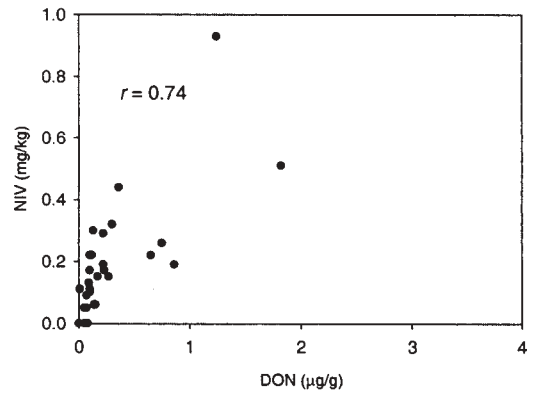
Cultivar	Region	Previous crop	No. crops	% ears with FHB	% sample weight in visibly infected grains	% grains with <i>Fusarium</i>	Mycotoxins (mg/kg)		% crops over 0.5 mg/kg		% crops over 1.0 mg/kg	
							NIV	DON	NIV + DON	DON	NIV + DON	DON
All	All	Maize*	11	11 (0-50)	1.5 (0-5.8)	15 (1-54)	0.24 (0-0.93)	0.60 (0-1.82)	0.84 (0-2.33)	55	45	
		Other cereal	14	11 (0-40)	0.5 (0.2-1.7)	11 (0-19)	0.08 (0-0.32)	0.09 (0-0.29)	0.17 (0-0.62)	14	0	
		Grass	15	13 (0-40)	0.8 (0-2.4)	9 (0-28)	0.17 (0-0.46)	0.25 (0-1.60)	0.42 (0-1.96)	20	13	
		Non-Graminae	13	11 (1-40)	0.5 (0-1.3)	9 (0-21)	0.06 (0-0.44)	0.07 (0-0.36)	0.14 (0-0.80)	8	0	
Ot/Ko	S Man.†	Maize	5	19 (6-50)	2.7 (0.7-5.8)	22 (3-54)	0.42 (0.15-0.93)	0.98 (0.27-1.82)	1.40 (0.42-2.33)	80	80	
		Other Graminae	9	19 (5-40)	1.1 (0.3-1.2)	11 (2-28)	0.28 (0.14-0.46)	0.43 (0.13-1.60)	0.71 (0.34-1.96)	56	22	
		Non-Graminae	3	17 (5-40)	0.7 (0.5-0.9)	13 (5-21)	0.21 (0.06-0.44)	0.20 (0.09-0.36)	0.41 (0.21-0.80)	33	0	
Monad	S Man.†	Maize	5	5 (1-10)	0.5 (0.1-1.0)	10 (1-18)	0.11 (0-0.26)	0.33 (0.05-0.75)	0.44 (0.05-1.01)	40	20	
		Other Graminae	6	1 (0-1)	0.3 (0-0.6)	6 (0-15)	0.04 (0-0.11)	0.05 (0-0.12)	0.09 (0-0.19)	0	0	
		Non-Graminae	5	1 (1-2)	0.3 (0-0.5)	8 (0-14)	0.04 (0-0.07)	0.04 (0-0.07)	0.04 (0-0.07)	0	0	
All	E Coast	All	3	1 (0-2)	0.4 (0-1.2)	9 (2-22)	0	0.02 (0-0.03)	0.02 (0-0.03)	0	0	
	N Man./Ran.	All	18	12 (0-40)	0.6 (0-1.7)	10 (0-19)	0.06 (0-0.22)	0.06 (0-0.22)	0.12 (0-0.41)	0	0	
	S Man.	All	33	11 (0-50)	0.5 (0-5.8)	11 (0-54)	0.18 (0-0.93)	0.35 (0-1.82)	0.53 (0-2.33)	33	21	
Ot/Ko	N Man./Ran.	Not maize†	14	19 (3-40)	0.7 (0-1.7)	9 (2-15)	0.08 (0-0.22)	0.07 (0-0.22)	0.15 (0-0.41)	0	0	
Ot/Ko	S Man.	Not maize	12	19 (5-40)	1.0 (0.5-2.4)	12 (2-34)	0.26 (0.06-0.46)	0.37 (0.12-1.60)	0.63 (0.22-1.96)	50	17	
Monad	N Man./Ran.	Not maize	4	0	0.5 (0.3-0.8)	14 (10-19)	0	0	0	0	0	
Monad	S Man.	Not maize	11	1 (0-2)	0.3 (0-0.6)	7 (1-15)	0.02 (0-0.11)	0.04 (0-0.12)	0.07 (0-0.19)	0	0	

*One crop was omitted from this section because of the absence of information on previous crop history.

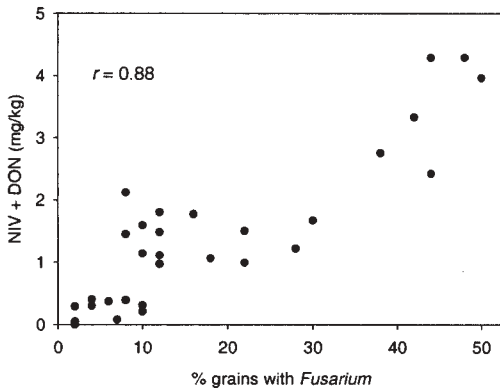
†No crops in N Manawatu/Rangitikei had maize as the previous crop.



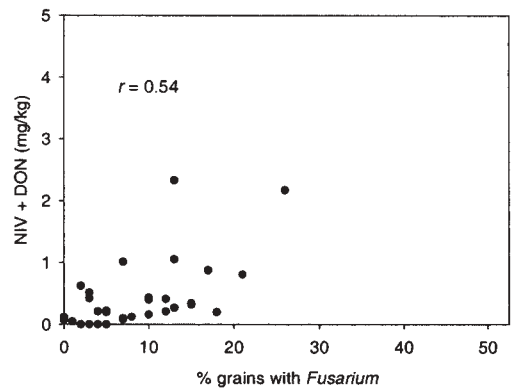
A, Relationship between levels of the mycotoxins NIV and DON



E, Relationship between levels of the mycotoxins NIV and DON



B, Relationship between *Fusarium* levels in grain and mycotoxin



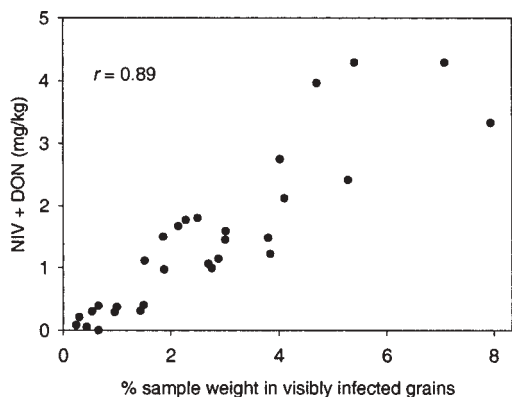
F, Relationship between *Fusarium* levels in grain and mycotoxin

Fig. 1 (and opposite) Relationships between fusarium head blight measurements in 30 grain samples from trial plots within one crop (A–D) and in samples from 32 crops (E–H) of North Island, New Zealand, spring wheat (*Triticum aestivum*) harvested in 2000. (NIV, nivalenol; DON, deoxynivalenol.)

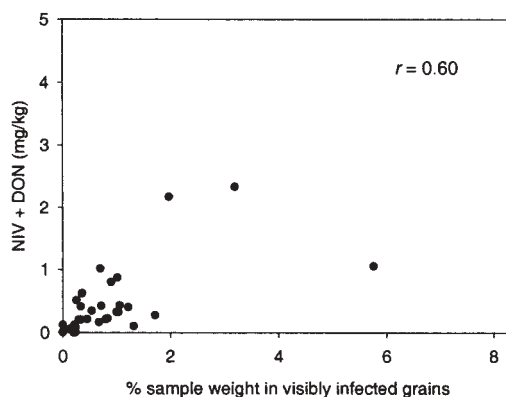
proportions of *F. graminearum* isolates and lower proportions of *F. avenaceum*, *F. poae*, and *F. culmorum* isolates than crops that did not follow maize. *F. poae* was rarely isolated from crops that followed maize, but represented 15% of isolations from crops that did not follow maize. Crops from the northern area tended to have lower proportions of *F. graminearum* and higher proportions of *F. poae* and *F. culmorum* than crops in the southern area, although it should be noted that all of the crops that followed maize crops were in the southern area. In the southern area, proportions of *Fusarium* spp. were similar in the three cultivars.

Relationships between FHB parameters were examined (Fig. 1) using 1999/2000 data from the field trial site (30 samples) and crops (32 samples) separately. The 30 samples from the trial site comprised 29 trial plots (six cultivars replicated 4 times, and one replicate of each of five further cultivars) and the surrounding crop. Trial plots were managed as the surrounding crop, which did not receive fungicides. All correlation coefficients in Fig. 1 were statistically significant ($P < 0.05$).

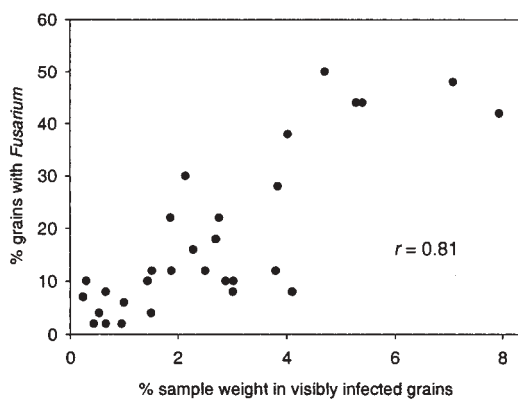
In virtually all samples from the trial site, DON levels were substantially higher than NIV levels and increased DON levels were highly



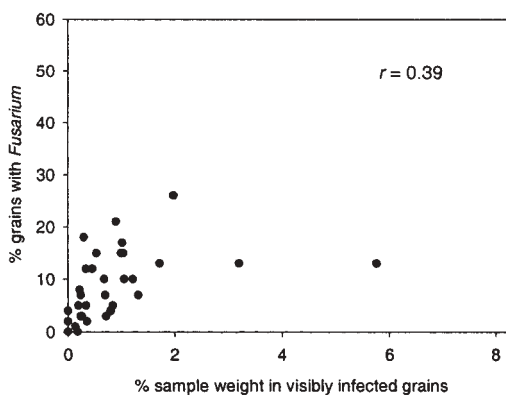
C, Relationship between proportion of visibly infected grains and mycotoxins



G, Relationship between proportion of visibly infected grains and mycotoxins



D, Relationship between proportion of visibly infected grains and levels of *Fusarium* in grain



H, Relationship between proportion of visibly infected grains and levels of *Fusarium* in grain

correlated ($r = 0.85$) with increased NIV levels (Fig. 1A). The correlation between NIV and DON was not quite as strong ($r = 0.74$) between crops in the survey (Fig. 1E) as in the trial site samples. DON levels were not consistently higher than NIV amongst the crops. Crops where NIV levels were higher than DON levels occurred most often where *F. poae* was isolated.

An association ($r = 0.88$) between levels of grain *Fusarium* and mycotoxins occurred at the Kairanga site (Fig. 1B). Samples could be grouped into three clusters based on their relationship between grain *Fusarium* and mycotoxin levels. One group of nine samples had maximum mycotoxin levels of 0.4 mg/kg, and less than 10% of grains infected. Another

group of 14 samples ranged in mycotoxin level between 1.0 and 2.1 mg/kg and grain *Fusarium* levels between 8 and 30%. The third group, of six samples, had a range in mycotoxin between 2.8 and 4.3 mg/kg and in grain *Fusarium* between 38 and 50%. The relationship between grain *Fusarium* and mycotoxin levels was relatively poor for the crops sampled ($r = 0.54$), although there was a trend towards higher mycotoxin levels with higher *Fusarium* levels (Fig. 1F).

A close association ($r = 0.89$) occurred between the percentage of the weight of the sample made up of visibly infected grains and mycotoxin levels in the Kairanga plot samples (Fig. 1C). However, the association was poor ($r = 0.60$) amongst survey crops

Table 5 Percentage of grains infected with different *Fusarium* species from crops of three spring wheat (*Triticum aestivum*) cultivars grown in the North Island, New Zealand, in 1999/2000 (N Man./Ran., northern Manawatu and Rangaitikei; S Man., southern Manawatu). Figures in parentheses are percentage of isolates from a crop or region.

	% grains infected									
	<i>F. graminearum</i>	<i>F. avenaceum</i>	<i>F. poae</i>	<i>F. culmorum</i>	<i>F. crookwellense</i>	<i>F. sambucinum</i>	<i>F. equiseti</i>	<i>F. heterosporium</i>	Other <i>F. spp.</i>	
Otane	5.4	0.9	1.5	1.8	0.5	0.1	0.0	0.5	0.1	
Kohika	5.4	0.9	1.0	0.2	0.3	0.3	0.8	0.4	0.3	
Monad	4.0	1.1	0.5	0.5	0.4	0.2	0.1	0.0	0.0	
Ex maize	8.9 (76)	0.6 (5)	0.1 (1)	0.7 (6)	0.6 (5)	0.2 (2)	0.1 (1)	0.1 (1)	0.4 (3)	
Ex other	3.3 (42)	1.1 (14)	1.2 (15)	0.9 (12)	0.3 (4)	0.2 (3)	0.3 (4)	0.4 (5)	0.1 (1)	
N Man./Ran.	3.4 (37)	0.9 (10)	1.8 (20)	1.8 (20)	0.2 (2)	0.1 (1)	0.4 (4)	0.4 (4)	0.1 (1)	
S Man.	5.5 (62)	1.0 (11)	0.6 (7)	0.5 (6)	0.4 (4)	0.2 (2)	0.2 (2)	0.3 (4)	0.2 (2)	
All crops	5.0 (53)	1.0 (11)	1.0 (11)	0.8 (8)	0.4 (4)	0.2 (2)	0.3 (3)	0.3 (3)	0.2 (2)	

in general, although the crops with the three highest levels of visibly infected grains also had the highest mycotoxin levels (Fig. 1G).

An association ($r = 0.81$) between the percentage weight of visibly infected grains and the proportion of grains with *Fusarium* occurred in samples from the Kairanga site (Fig. 1D), and all samples with over 35% of grains infected with *Fusarium* also had over 4% of the sample weight taken up with visibly infected grains. The association was relatively poor ($r = 0.39$) for crops in general. There were two outlier crops; they had the highest proportion of visibly infected grains, but only average *Fusarium* levels. Both crops had maize as the previous crop, and in both cases all or almost all isolations were of *F. graminearum*. Removal of these two outlier crops resulted in a modest improvement in the correlation ($r = 0.62$).

DISCUSSION

Results of field trials and crop surveys reported here show consistent differences between cultivars in susceptibility to FHB. The New Zealand cultivars ‘Otane’ and ‘Kohika’ consistently had the highest levels of FHB in ears, visibly infected grains, *Fusarium*, and mycotoxins in grain samples. The Chinese cultivar ‘Nanjing’, reported to have a high level of resistance to FHB (Snijders 1990), had the lowest level of FHB components in the 1998/99 field trial. This cultivar, and the similarly resistant line FHB37 serve as resistant standards against which to compare New Zealand cultivars. Although no New Zealand cultivars approach this level of resistance, FHB levels in ‘Monad’ were low in almost all situations, and ‘Torlesse’ appeared also to have a useful level of resistance, supported by results from other trials not reported here (Cromey unpubl. data).

Infection levels in the trials and crops relied on natural inoculum and environmental conditions. Flowering is the critical time for infection to occur (Parry et al. 1995), and rain during flowering increases the likelihood of infection. Rain during grain fill will enhance the amount of spread of FHB along each spike (Parry et al. 1995). Because ‘Otane’ and ‘Kohika’ have consistently shown relatively high FHB levels across environments, they can be confirmed to be highly susceptible. There were occasional instances of higher than usual levels of FHB and mycotoxins in ‘Monad’, which indicates that its resistance is partial, and may be insufficient

under conditions particularly favourable to FHB. Wheat genotypes resistant to FHB caused by *F. graminearum* have been shown to also be resistant to *F. culmorum* (Snijders 1990). The nature of resistance to FHB is complex, and involves a wide variety of factors that range from flowering habit to physiological defensive reactions (Schroeder & Christensen 1963). Resistance can also be to initial infection or to hyphal spread along spikes (Schroeder & Christensen 1963).

Two *Fusarium* mycotoxins, DON and NIV, were detected in grain samples from crops and trials in the present study. Overall, DON levels were higher than NIV levels in crops in both years, but especially in 1998/99. In North America, DON tends to be far more common than NIV, with *F. graminearum* isolates mostly producing DON rather than NIV (Abramson et al. 1993). NIV and DON-producing isolates of *F. graminearum* from New Zealand cereal grains have previously been isolated at similar frequencies, with isolates producing one or other, but not both (Lauren et al. 1992). In the data presented here there were few instances of only one mycotoxin occurring in crops, and in these instances overall levels were very low. In all situations where total mycotoxin levels were over 0.1 mg/kg, both NIV and DON were present. This and the fact that *F. graminearum* was by far the most common species isolated in the present study, indicates that both the NIV and DON-producing chemotypes of *F. graminearum* were present in most crops.

Two North American studies that compared DON and NIV production in culture found that *F. poae* was the predominant NIV-producing species there (Abramson et al. 1993; Salas et al. 1999). Salas et al. (1999) stated that the presence of NIV in a grain sample may be suggestive of *F. poae*. In our study, when NIV levels were higher than DON, *F. poae* was usually present in the sample. Also, in one Swedish study, *F. poae* was the only NIV producer isolated from NIV-containing grains (Pettersson 1991). Some New Zealand isolates of *F. poae* are known to produce NIV in culture (H. Pettersson pers. comm.), although many have been shown to be poor producers of mycotoxins (Lauren et al. 1992). Therefore, the presence of NIV does not appear to necessarily indicate the presence of *F. poae* in New Zealand grain samples. In some cases, *F. culmorum* was also present in NIV-containing samples. New Zealand isolates of *F. culmorum* are predominantly NIV-producing (Lauren et al. 1992), and so high NIV levels may also be indicative of *F. culmorum* in some instances.

Although FHB incidence and levels of *Fusarium* infection and mycotoxins in grain were closely related in samples from a particular crop, the relationships were much less apparent between crops. Whereas the same factors will influence each parameter to some extent, the overall level of each will also be affected by site factors, such as the sources of inoculum, nature of inoculum, and timing of *Fusarium* growth in the ears. It would be unwise, therefore, to make assumptions on likely mycotoxin contamination based on FHB severity in a crop or *Fusarium* levels in grain.

Biosynthesis of the mycotoxins depends on many factors, especially the strain of the pathogen, the substrate, the period of colonisation of the substrate, temperature, moisture, and competing organisms (Sutton 1982). Overall, mycotoxin levels in the crops we sampled were lower in grain harvested in 1999 than in 2000. However, there were similar proportions (13 and 14%) in the 2 years respectively of samples where combined NIV + DON levels exceeded 1 mg/kg. This level is the US Food and Drug Administration guideline for DON in human food components, and above some recommendations for animal food.

In New Zealand, *Fusarium* species associations with wheat grains differ between regions and between years. Two species, *F. graminearum* and *F. culmorum*, tend to predominate in North Island samples (Sayer & Lauren 1991; Cromey et al. 2001b). *F. graminearum* was considerably more common than *F. culmorum* in our study, although Sayer & Lauren (1991) found the reverse in 2 years out of 3 in their survey. *F. culmorum* predominates in cooler maritime regions of the world (Parry et al. 1995) and so its relative importance in North Island crops may be affected in part by seasonal differences in environment. *F. avenaceum*, at a mean incidence of 11%, was also common in our North Island samples, but is often the predominant species in South Island wheat grain (Sayer & Lauren 1991; Cromey et al. 2001b). This species is reported to be an important pathogen of wheat head blight in cool climates (Stack & McMullen 1985).

Despite the range of *Fusarium* spp. associated with FHB around the world, three species predominate: *F. graminearum*, *F. culmorum*, and *F. avenaceum* (Parry et al. 1995). Inoculation experiments have shown *F. graminearum* and *F. culmorum* to cause much more severe FHB than other species isolated from blighted grains (Stack & McMullen 1985; Wilcoxson et al. 1988). Sturz & Johnston (1983) identified *F. poae*, and to a lesser

extent *F. avenaceum*, as early colonisers of wheat ears in Canada, although they were not believed to be the primary cause of FHB. It was thought, however, that early infection by these species may have predisposed ears to later infection by the primary FHB pathogens, *F. graminearum* and *F. culmorum*. In our study, both *F. poae* and *F. avenaceum* were common in grain, although *F. graminearum* contributed about half of the isolates.

Highest overall levels of *Fusarium* were recorded in grain samples from crops that followed maize, but this is related to *F. graminearum* levels rather than to other species. In North America, FHB caused predominantly by *F. graminearum* is most severe in crops that follow maize (Parry et al. 1995). The other predominant species in our study, *F. avenaceum*, *F. poae*, and to a lesser extent *F. culmorum*, were more common in samples from crops that did not follow maize. It is possible that movement of *F. avenaceum* and *F. poae* into grains following their early colonisation of ears (Sturz & Johnston 1983) is hindered by later colonisation by *F. graminearum*.

Cultivar susceptibility clearly has a major influence on the severity of FHB and mycotoxin concentrations in North Island wheat, but other factors are also likely to contribute to overall levels. Host debris is the principal reservoir of *F. graminearum*, although soil, seeds, and susceptible plants are also inoculum sources (Sutton 1982). Thus, previous cropping history, and residue management practices are likely to affect the incidence of FHB. In our survey, almost all crops with high levels of FHB and mycotoxins followed maize crops. Although no field trials have been carried out in New Zealand to examine the effects of crop rotation on FHB, the survey results strongly support the results of field trials in the United States (Dill-Macky & Jones 2000). Other factors, such as disease levels in the previous crop, crop residue management, and seasonal or local environment, may also be important. The preponderance of heavily infected crops in southern Manawatu is in part the result of the maize-wheat rotation, but an association remains even after the rotation and cultivar effects are removed. This difference may be environmental or reflect differences in crop management practices.

Levels of FHB and mycotoxins varied widely between crops surveyed. Seasonal environmental conditions are difficult to predict, and crops will be at most risk of infection when there is rain during anthesis. Other factors influencing relative risk of infection in a particular year appear to be primarily cultivar and previous crop. Fungicide applications

have the potential to reduce the risk, but not eliminate it. For instance we recorded mycotoxin levels over 0.5 mg/kg in some instances where triazole fungicides were applied but conditions favoured FHB. These other factors, along with residue management techniques, can be used by growers to minimise the risk of serious infection. Under current management systems and environmental conditions experienced, avoidance of highly susceptible cultivars alone will mostly provide adequate control of FHB and associated increased mycotoxin levels. In particular, susceptible cultivars should be avoided where the previous crop is maize. Under higher risk conditions, use of appropriate fungicides at anthesis will provide some control. If there is a move to more intensive maize/cereal cropping or to conservation tillage methods, the risk of FHB could increase through increased levels of inoculum, especially under favourable environmental conditions. A move to more resistant cultivars will reduce the risk of FHB and high mycotoxin levels.

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