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Effect of dose rate of azoxystrobin and metconazole on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain

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Abstract

Glasshouse studies were undertaken to determine if fungicides used for the control of Fusarium head blight (FHB) result in elevated concentrations of the trichothecene mycotoxin, deoxynivalenol (DON) in harvested wheat grain. Metconazole and azoxystrobin, at double, full, half or quarter the manufacturer's recommended dose rate, were applied to ears of wheat (cv. Cadenza), artificially inoculated with conidia of either *Fusarium culmorum* or *F. graminearum*. Metconazole demonstrated high activity against both pathogens, reducing significantly the severity of FHB and the DON concentrations at each of the four dose rates tested when compared to untreated controls. Applications of azoxystrobin significantly reduced FHB and DON compared to unsprayed controls. However, their effectiveness was significantly less than that of metconazole and no dose rate response was observed. Quantification of the amount of trichothecene-producing *Fusarium* present in harvested grain was determined using a competitive PCR assay based on primers derived from the trichodiene synthase gene (*Tri*5). Simple linear regression analyses revealed strong relationships between the amount of trichothecene-producing *Fusarium* present in grain and the DON concentrations ($r^2 = 0.72$–0.97). It is concluded that fungicides, applied for the control of FHB, affect DON concentrations indirectly by influencing the amount of trichothecene-producing *Fusarium* species present in wheat grain. There was no evidence that fungicide applications directly increase the concentration of DON in grain.

Introduction

Fusarium head blight (FHB), also known as scab, is a destructive fungal disease of wheat and other small grain cereals throughout the temperate and semi-arid regions in the world. Although 17 casual organisms have been associated with FHB (Parry et al., 1995), *Fusarium avenaceum* (teleomorph, *Giberella avenacea*), *F. culmorum, F. graminearum*, (teleomorph, *G. zeae*), *F. poae* and *Microdochium nivale* (teleomorph, *Monographella nivalis*) are the species most commonly associated with the disease. In recent years, yield loss in small grain cereals due to FHB epidemics has been estimated to be in the order of 10–70% (Apony et al., 1998; Martin and Johnston, 1982; Matthies and Buchenauer, 2000; Munteanu et al., 1972; Sayler, 1998; Windels, 2000). This yield reduction is associated with a decrease of grain number per ear, 1000-grain weight and grain weight per ear (Chelkowski et al., 2000)

Due to the ability of several *Fusarium* species to produce a range of trichothecene mycotoxins such as deoxynivalenol (DON, also known as vomitoxin), nivalenol, 3-acetyl deoxynivalenol (3-ADON), T-2 toxin and also zearalenone (ZON) (Ichinoe and Curata, 1983), the use of grain contaminated by

Fusarium species for animal feed and human consumption may pose serious health threats. For example, during the Second World War in Russia, the syndrome of human toxicosis known as Alimentary Toxic Aleucia (ATA) was associated with the consumption of grain containing T-2 toxin (Joffe, 1978). Human toxicosis associated with *Fusarium* toxins have also been reported in Japan (Yoshizava, 1983) India (Bhat et al., 1989) and China (Li et al., 1999). The occurrence of *Fusarium* mycotoxins in livestock feed supply can result in decreased food consumption, reduced weight gain, vomiting, vaginal prolapses and vulvovaginitis in pigs (Long et al., 1982; Yoshizava, 1983), low egg shell quality (Speers et al., 1971), stunted growth and poor feathering in poultry (Hoerr et al., 1982) and also infertility in cows and sheep (Palti, 1978; Towers and Sprosen, 1993). As a result, several countries have adopted advisory limits to ensure minimum levels of DON in finished products intended for human consumption and for animal feeds (van Egmond, 1989). For example, the Food and Drug Administration (FDA) in the US recommends that DON concentration should not exceed 1000 $\mu g\,kg^{-1}$ in finished wheat products and should not exceed 5000 or 10 000 $\mu g\,kg^{-1}$ for feed intended for swine and cattle, respectively. Although yet to be approved, the proposed advisory limits for trichothecene mycotoxins to be adopted within the European Union are $<500\,\mu g\,kg^{-1}$ for retail products such as breakfast cereals, bread and pasta and $<750\,\mu g\,kg^{-1}$ for grain or any other grain products (Pricket et al., 2000). Although trichothecene mycotoxins are primarily associated with field contamination and development, inappropriate conditions employed during the storage of grain after FHB epidemic years, can result in further increases in mycotoxin content (Birzele et al., 2000; Homdork et al., 2000).

Fungicidal control of FHB has proved inconsistent and conflicting evidence exists regarding the effect of fungicides on the mycotoxin accumulation in grain contaminated by *Fusarium* species. Results from *in vitro* studies have indicated that the presence of certain fungicides can result in elevated concentrations of *Fusarium* toxins. For example, studies on the effect of the fungicide tridemorph on T-2 toxin production by *F. sporotrichioides*, showed that at low concentrations (6–8 $\mu g\,g^{-1}$), the chemical produced a slight increase in the growth of the fungus and inhibited T-2 toxin and diacetoxyscripenol (DAS) production (Moss and Frank, 1985). However, at higher concentrations (30–50 $\mu g\,g^{-1}$), although the fungicide inhibited fungal growth by ca. 50%, T-2 toxin production increased five-fold. Under field conditions, the effect of fungicides on mycotoxin concentration is unclear. For example, during a field trial inoculated with *F. culmorum*, a 16-fold increase in nivalenol concentration in wheat grains was observed after an application of the fungicide product 'Matador' (tebuconazole, 250 g a.i. l^{-1} + triadimenol, 125 g a.i. l^{-1}) to wheat ears, despite a reduction in disease severity (Gareis and Ceynova, 1994). In contrast, other field studies have shown that applications of propiconazole and triadimefon (Boyacioglu et al., 1992), thiophanate-methyl (Ueda and Yoshizava, 1998) and tebuconazole (Suty et al., 1996), all reduced the severity of FHB and concentration of DON. Applications of propiconazole were shown to significantly reduce FHB in wheat but had no effect on DON concentration (Martin and Johnston, 1982). In recent years, an increase in DON concentration has been associated with applications of the strobilurin fungicide azoxystrobin (Ellner and Schroer, 2000; Hart and Ward, 1997).

The aims of this study were to evaluate the effects of a range of dose rates of metconazole and azoxystrobin against (i) the development of FHB (ii) the extent by which grain is colonized by mycotoxin-producing *Fusarium* species and (iii) the concentration of DON in harvested grain, in wheat ears artificially inoculated with *F. culmorum* or *F. graminearum*.

Materials and methods

Storage and production of pathogen inoculum

DON-producing isolates of *F. culmorum* and *F. graminearum* (Table 1) of known pathogenicity were stored in the Harper Adams University College culture collection as a conidial spore suspension in 10% glycerol at $-80\,^{\circ}C$. When required, all isolates were sub-cultured onto Potato Dextrose Agar (PDA) (Lab M, Bury, UK) and incubated under darkness at $20\,^{\circ}C$ for 14 days. Conidial suspensions were obtained for each isolate by washing conidia from sporulating

Table 1. Fusarium isolates used in experimental work

Species	Isolate	Origin
Fusarium culmorum	FC 47/1, FC 95 FC 53, FC 70	Harper Adams University College, Central Science laboratory, York
Fusarium graminearum	Fg F98, Fg NFTP Fg 113, F 145	Harper Adams University College, Central Science laboratory, York

colonies using sterile distilled water (SDW). The suspension obtained was then filtered through two layers of sterile muslin to remove hyphal fragments. Spore concentration was then determined using a haemocytometer (Weber Scientific International Ltd, Teddington, Middlesex, UK) and adjusted to a final concentration of 10^5 spores ml^{-1} of water.

Production of host plants

Fungicide-treated seed (fludioxinil at 25 g a.i. per 100 kg of seed, Beret Gold®, Syngenta Crop Protection) of the winter wheat cv. Cadenza was sown into 15 cm diam plastic pots containing John Innes Number 2 compost at a rate of five seeds per pot. Once seedlings had emerged, plants were placed in the bay of a glasshouse set at $10 \pm 3\,^{\circ}C$ under a photoperiod of 12 h for 30 days in order to encourage efficient tillering. After 30 days, the temperature was increased to $22 \pm 3\,^{\circ}C$ and the photoperiod to 16 h. Plants were watered daily and fed once a week with an application of foliar fertiliser (10% N, 10% P_2O_5, 27% K_2O as Phostrogen®, Phostrogen Ltd, Corwen, Clwyd, UK). When necessary, plants were sprayed with the fungicide Fortress® (quinoxyfen 500 g a.i. l^{-1}, Dow AgroSciences) at a rate of 0.3 l ha^{-1} to prevent infection by powdery mildew. Nicotine shreds (Nicotine 40% Shreds®, Dow AgroSciences) were also used according to the manufacturer's recommendation to fumigate the glasshouse when necessary for eradication of aphid infestations.

Fungicide application

When at growth stage (GS) 59 (full ear emerged) (Zadoks et al., 1974), wheat ears were sprayed with either metconazole or azoxystrobin at concentrations ranging from one quarter to twice the manufacturer's recommended maximum dose rate (Table 2). Metconazole was included in the study since it is known to be effective at controlling FHB caused by *Fusarium* species whilst azoxystrobin has been shown to be ineffective against *Fusarium* species and has been observed to increase concentration of DON in harvested grain. For each fungicide and dose rate, 16 replicate pots were treated. The application of SDW provided untreated controls. All fungicide treatments were applied using a precision pot sprayer carrying Lurmark 110° flat fan nozzles (03-F110, Longstanton, Cambridge, UK) at the rate of 200 l ha^{-1}.

Table 2. Fungicides and their rate of use in the two glasshouse experiments

Fungicide[a]	Dose[b]	Rate[c]
Metconazole	180	2 n
	90	1 n
	45	$\frac{1}{2}$ n
	22.5	$\frac{1}{4}$ n
Azoxystrobin	500	2 n
	250	1 n
	125	$\frac{1}{2}$ n
	62.5	$\frac{1}{4}$ n
Unsprayed control	N/A	N/A

[a]Metconazole (Caramba®) manufactured by BASF plc; azoxystrobin (Amistar®) manufactured by Syngenta Crop Protection.
[b]Grams of active ingredient per hectare.
[c]Double, full, half and quarter of the manufacturer's recommended dose rate (*n*).

Artificial inoculation of wheat ears

When at GS 65 (mid-anthesis), wheat ears were artificially inoculated with either *F. culmorum* or *F. graminearum*. Inoculation was achieved by spraying the prepared conidial suspension onto ears using a hand-held atomiser at a rate of ca. 2.5 ml of spore suspension per ear. Inoculation of ears was organised so that for each fungicide and dose rate tested, eight of the 16 replicate pots were inoculated with *F. culmorum*, whilst the remaining eight replicate pots were inoculated with *F. graminearum*. In order to create conditions conducive for infection by the pathogens, all inoculated ears were immediately covered with polythene bags, which were then removed 48 h later. Pots were arranged according to a randomised block design.

Disease assessment

When plants were at GS 85 (soft dough), all ears were visually assessed and the severity of head blight symptoms recorded as the percentage of spikelets showing disease symptoms. The above procedures were repeated to provide results from two independently performed but identical experiments.

Quantification of Tri*5 DNA and DON in harvested grain*

When ripe (GS 92), wheat ears were harvested and carefully hand threshed in order to avoid loss of any small shrivelled grain. For each treatment,

the *Tri*5 gene, which encodes trichodiene synthase, an enzyme which catalyses the initial reaction in the biosynthetic pathway of all trichothecene mycotoxins, was quantified according to the procedure described by Edwards et al. (2001). DON concentration present in grain was determined for each treatment by using a Ridascreen® DON Fast enzyme immunoassay (supplied by R-Biopharm, Oxford, UK) according to the manufacturer's instructions.

Statistical analysis

Analysis of variance was undertaken on all data to determine if fungicide and dose rate had a significant effect on disease severity, quantity of *Tri*5 DNA and DON concentration in harvested grain. Where data were not normally distributed, appropriate transformations were undertaken prior to analysis. Simple linear regression analysis was also performed in order to determine if significant relationships existed between disease severity, quantity of *Tri*5 DNA and DON concentration. All data analysis was carried out using the statistical software package Genstat 5.4.1 (Lawes Agricultural Trust, Harpenden, UK).

Results

Similar observations were recorded for each fungicide treatment from the two experiments although an F_{max} test revealed significant differences between the two sets of data ($P < 0.001$). Data from the two experiments are, therefore, presented separately. Across all treatments, results obtained from experiment 1 revealed lower concentrations of *Tri*5 DNA and DON in harvested grain when compared to results obtained from experiment 2 (see Tables 4 and 5). This may be explained by the time of year when the two experiments were performed. Experiment 1 was undertaken during September and October of 1999, whilst the repeat experiment 2 was undertaken during March and April of 2000. Although environmental conditions were programmed to be the same for both experiments ($22 \pm 3\,^{\circ}C$), during experiment 2, temperatures in the glasshouse were observed to be as high as $30\,^{\circ}C$ on some days.

Severity of FHB

The effect of fungicide treatment on the severity of FHB caused by *F. culmorum* and *F. graminearum* can be seen in Table 3. In both experiments, applications of either metconazole or azoxystrobin reduced significantly ($P < 0.001$) the severity of disease caused by either of the two fungal pathogens when compared to the untreated controls. Of the two fungicides tested, metconazole proved to be the most effective, providing 98% (experiment 1) and 77% (experiment 2) control for plants inoculated with *F. culmorum*, and 77% (experiment 1) and 17% (experiment 2) control for plants inoculated with *F. graminearum*, when applied at the lowest dose rate. Increasing the dose rate of metconazole applied resulted in an increase in the control of FHB achieved up to the highest dose rate of 180 g a.i. ha^{-1}, where >97% control of disease was recorded whether plants were inoculated with *F. graminearum* or *F. culmorum*. Although applications of azoxystrobin significantly reduced the severity of FHB when compared to unsprayed controls, the percentage control achieved by this fungicide was only between 30% and 55%, even when applied at 500 g a.i. ha^{-1} (double rate). Statistical analysis revealed no evidence of a significant difference between the control of FHB achieved by each of the four dose rates of azoxystrobin used.

Concentration of Tri*5 DNA*

The competitive PCR assay employed to quantify the trichothecene-producing gene *Tri*5 revealed that concentrations of *Tri*5 in harvested grain were significantly lower when plants were treated with either metconazole or azoxystrobin compared to the untreated controls (Table 4). Metconazole reduced *Tri*5 DNA concentration by 68–70% in plants inoculated with *F. culmorum* and by 70–79% in plants inoculated with *F. graminearum* over control plants, even when applied at the lowest dose rate of 22.5 g a.i. ha^{-1} (quarter rate). Increasing the dose rate of metconazole resulted in a concomitant decrease in *Tri*5 concentration until at 180 g a.i. ha^{-1}, a reduction of between 97% and 100% was achieved for experiments 1 and 2, respectively. Azoxystrobin proved less effective at reducing *Tri*5 concentration than metconazole, with reductions of between 20% and 40% for plants inoculated with *F. culmorum*, and between 20% and 52% for plants inoculated with *F. graminearum* being observed in the respective experiments, where the highest dose rate of 500 g a.i. ha^{-1} was applied.

DON concentration

The results for the concentration of DON recorded in harvested grain are presented in Table 5.

Table 3. Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on the severity of Fusarium Head Blight assessed 28 days after artificial inoculation of wheat ears (cv. Cadenza) at GS65 with a conidial suspension (10^5 spores ml^{-1}) of either *F. culmorum* or *F. graminearum*. Numbers in parentheses are back transformed means

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Disease severity (arcsine % spikelets infected)	
			Experiment 1	Experiment 2
F. culmorum	Control	NA	61.01 (76.50)	45.99 (51.72)
	Metconazole	180	00.00 (00.00)	2.160 (0.140)
		90	00.00 (00.00)	1.610 (0.070)
		45	13.44 (5.400)	8.900 (2.390)
		22.5	17.13 (8.670)	20.26 (11.99)
	Azoxystrobin	500	37.98 (37.87)	29.08 (23.62)
		250	31.85 (27.84)	30.96 (26.46)
		125	32.09 (28.22)	29.24 (23.86)
		62.5	29.99 (24.98)	29.06 (23.59)
F. graminearum	Control	NA	83.15 (98.57)	68.22 (86.23)
	Metconazole	180	6.210 (1.170)	8.520 (2.190)
		90	12.53 (4.700)	16.11 (7.690)
		45	24.77 (17.55)	16.00 (7.590)
		22.5	28.47 (22.72)	57.84 (71.66)
	Azoxystrobin	500	56.42 (69.40)	47.81 (54.89)
		250	60.25 (75.37)	64.16 (81.00)
		125	61.21 (76.80)	59.91 (74.86)
		62.5	61.79 (77.65)	57.76 (71.54)
LSD (fungicide)			4.364 ($P < 0.001$)	5.645 ($P < 0.001$)
LSD(dose rate)			4.781 ($P < 0.001$)	6.184 ($P < 0.001$)
LSD (pathogen)			5.855 ($P < 0.001$)	7.573 ($P < 0.001$)
LSD (fungicide $*$ dose rate)			5.520 ($P < 0.001$)	7.140 ($P < 0.001$)
LSD (fungicide $*$ pathogen)			6.172 ($P < 0.001$)	7.983 ($P < 0.050$)
LSD (dose rate $*$ pathogen)			NS ($P > 0.050$)	8.745 ($P < 0.001$)
LSD (fungicide $*$ dose rate $*$ pathogen)			NS ($P > 0.050$)	10.09 ($P < 0.050$)

Results recorded for each treatment were similar to those seen with the concentration of *Tri*5 in harvested grain. Although both fungicides significantly reduced the concentration of DON compared to control plants, metconazole proved the most effective, reducing DON concentration by >95% when applied at dose rates above 45 g a.i. ha^{-1} (half rate), irrespective of the pathogen used. Dose rates of half rate or greater of azoxystrobin were able only to reduce DON concentrations by 20–75% depending on the pathogen used. Regression analysis revealed a significant and strong relationship between the concentration of the *Tri*5 gene in harvested grain and DON concentration (Figure 1). In both experiments, and for each fungicide treatment, it was seen that an increase in *Tri*5 concentration resulted in a concomitant increase in DON concentration. Comparison of linear regression models showed that in both experiments, the relationship between *Tri*5 and DON concentration in grain harvested from ears treated with azoxystrobin was not significantly different to that obtained for untreated controls. However, for plants treated with metconazole, the relationship between *Tri*5 and DON was significantly different to that obtained for untreated and azoxystrobin treated plants with a lower concentration of DON per copy of *Tri*5 DNA.

Discussion

Results from both glasshouse experiments indicate that DON concentration present in harvested grain was correlated with the amount of *Tri*5 gene detected in grain as determined by the competitive PCR assay. For plants sprayed with either azoxystrobin or metconazole, fitting a simple linear model to *Tri*5 and DON data accounted for between 81% and 86% of variance, depending on the fungicide and experiment (Figure 1). Such strong relationships between *Tri*5 and DON concentration suggest that in this study, the fungicide treatments tested did not elevate DON concentration over and above that which was determined by the quantity

Table 4. Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on the quantity of *Tri*5 DNA in harvested grain after artificial inoculation of wheat ears (cv. Cadenza) at GS65 with a conidial suspension (10^5 spores ml^{-1}) of either *F. culmorum* or *F. graminearum*. Number in parentheses are back transformed means

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Log_{10} *Tri*5 DNA (pg ng^{-1})	
			Experiment 1	Experiment 2
F. culmorum	Control	NA	0.552 (3.566)	1.532 (34.04)
	Metconazole	180	0.000 (1.000)	0.065 (1.161)
		90	0.000 (1.000)	0.102 (1.264)
		45	0.029 (1.069)	0.393 (2.471)
		22.5	0.056 (1.137)	0.999 (9.977)
	Azoxystrobin	500	0.330 (2.142)	1.222 (16.67)
		250	0.213 (1.635)	1.346 (22.18)
		125	0.259 (1.816)	1.417 (26.12)
		62.5	0.204 (1.602)	1.263 (18.32)
F. graminearum	Control	NA	0.982 (9.598)	2.227 (168.6)
	Metconazole	180	0.062 (1.155)	0.457 (2.864)
		90	0.066 (1.164)	0.647 (4.436)
		45	0.150 (1.412)	0.565 (3.672)
		22.5	0.299 (1.993)	1.709 (51.16)
	Azoxystrobin	500	0.470 (2.957)	1.793 (62.08)
		250	0.541 (3.477)	2.039 (109.3)
		125	0.505 (3.201)	1.942 (87.49)
		62.5	0.598 (3.968)	1.976 (94.62)
LSD (fungicide)			0.065 ($P < 0.001$)	0.194 ($P < 0.001$)
LSD (dose rate)			0.071 ($P < 0.050$)	0.211 ($P < 0.001$)
LSD (pathogen)			0.087 ($P < 0.001$)	0.259 ($P < 0.001$)
LSD (fungicide $*$ dose rate)			NS ($P > 0.050$)	0.244 ($P < 0.001$)
LSD (fungicide $*$ pathogen)			0.092 ($P < 0.001$)	NS ($P > 0.050$)
LSD (dose rate $*$ pathogen)			0.101 ($P < 0.050$)	NS ($P < 0.050$)
LSD (fungicide $*$ dose rate $*$ pathogen)			NS ($P > 0.050$)	NS ($P > 0.050$)

of trichothecene-producing *Fusarium* present in grain. If an individual treatment had resulted in a cluster of points above the regression line, this would have suggested that such a treatment increased the concentration of DON produced per *Tri*5 copy. The lack of any obvious cluster indicates that neither azoxystrobin nor metconazole influenced DON concentration within grain other than by altering the amount of trichothocene-producing *Fusarium* present. These findings support those recorded under field conditions where grain samples taken from a field trial investigating the effect of a range of fungicides against FHB caused by *F. culmorum*, *F. graminearum* and *M. nivale* revealed that DON concentration was strongly correlated to the concentration of *Tri*5 DNA (Edwards et al., 2001). None of the fungicide treatments used in this study (Edwards et al., 2001), which included metconazole applied at 45 and 90 g a.i. ha^{-1} and azoxystrobin applied at 125 and 250 g a.i. ha^{-1} were shown to elevate DON concentration above that which was determined by the quantity of *Tri*5 DNA grain.

Applications of azoxystrobin applied two days after the artificial inoculation of wheat ears with a mixture of head blight causing pathogens, including *F. culmorum* and *F. graminearum*, have been observed to increased DON concentration per unit of pathogen DNA present in wheat grain (Simpson et al., 2001). Other field studies have also revealed an increase in DON concentration following an application of azoxystrobin when compared to unsprayed controls. Results from the present study are in contrast to these findings. By using a regression analysis approach, it can be seen in the current study, that applications of azoxystrobin, even at dose rates double that of the manufacturer's recommendations, failed to elevate DON concentration over and above that which is determined by the quantity of trichothecene-producing pathogen colonising the grain.

The higher concentration of *Tri5* DNA and DON found in grain across all treatments in experiment 2 may be explained by the fact that the warmer environmental conditions experienced during this experiment may

Table 5. Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on deoxynivalenol (DON) concentration in harvested grain after artificial inoculation of wheat ears (cv. Cadenza) at GS65 with a conidial suspension (10^5 spores ml^{-1}) of either *F. culmorum* or *F. graminearum*

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Deoxynivalenol concentration (mg kg^{-1})	
			Experiment 1	Experiment 2
F. culmorum	Control	NA	25.96	33.28
	Metconazole	180	00.00	00.00
		90	00.00	00.10
		45	0.310	1.340
		22.5	1.410	11.87
	Azoxystrobin	500	10.46	19.61
		250	6.860	25.46
		125	6.610	21.03
		62.5	5.710	18.12
F. graminearum	Control	NA	55.15	63.56
	Metconazole	180	0.350	0.450
		90	1.170	1.950
		45	8.810	2.420
		22.5	8.330	37.70
	Azoxystrobin	500	25.91	36.78
		250	28.77	47.32
		125	31.75	51.03
		62.5	34.62	48.26
LSD (fungicide)			3.537 ($P < 0.001$)	5.742 ($P < 0.001$)
LSD (dose rate)			NS ($P > 0.050$)	6.290 ($P < 0.001$)
LSD (pathogen)			4.745 ($P < 0.001$)	7.704 ($P < 0.001$)
LSD (fungicide $*$ dose rate)			NS ($P > 0.050$)	7.263 ($P < 0.001$)
LSD (fungicide $*$ pathogen)			5.002 ($P < 0.001$)	8.121 ($P < 0.001$)
LSD (dose rate $*$ pathogen)			5.479 ($P < 0.050$)	8.896 ($P < 0.050$)
LSD (fungicide $*$ dose rate $*$ pathogen)			NS ($P > 0.050$)	NS ($P > 0.050$)

have been more favourable for infection and colonisation of grain by *F. culmorum* and *F. graminearum*. Ryu and Bullerman (1999) observed that *F. graminearum* produced greater quantities of DON and ZON when incubated under temperatures cycling between 15 and 30 °C than under temperature cycling between 5 and 30 °C and 10 and 25 °C. It is possible, therefore, that the higher temperatures experienced during experiment 2 may have played some role in the slight elevation in DON concentration recorded when azoxystrobin was applied at the highest dose rate. Further studies on the effect of temperature and its interaction with fungicides on DON accumulation are, therefore required.

The greater reduction in FHB, *Tri*5 DNA and DON in grain achieved by metconazole indicated that this fungicide was very effective against both *F. culmorum* and *F. graminearum*. Such observations are in agreement with those of other studies (Edwards et al., 2001; Jennings et al., 2000; Matthies and Buchenauer, 2000) where the triazole fungicides metconazole and tebuconazole were shown to be effective at reducing both FHB and DON. Dardis and Walsh (2000) also showed that metconazole was the most effective of a range of fungicides tested against FHB caused by *F. culmorum*.

Although azoxystrobin significantly reduced FHB, *Tri*5 and DON compared to unsprayed controls, it was far less effective than metconazole, and unlike metconazole, no significant difference in the level of control was observed between the dose rates used. Such observations are consistent with those of other field studies where applications of azoxystrobin were shown to reduce both FHB and DON concentration in wheat when compared to unsprayed controls (Jones, 2000; Milus and Weight, 1998). The relatively poor efficacy of azoxystrobin against FHB caused by *F. culmorum* and *F. graminearum* in the current study supports previous observations that although the fungicide is effective against FHB caused by *M. nivale*, it has very limited activity against *Fusarium* species (Pirgozliev et al., 2001; Simpson et al., 2001). Jones et al. (2001) compared the effect of azoxystrobin applied to winter wheat plots at 62.5, 125, 187.5 and 250 g a.i. ha^{-1} against the

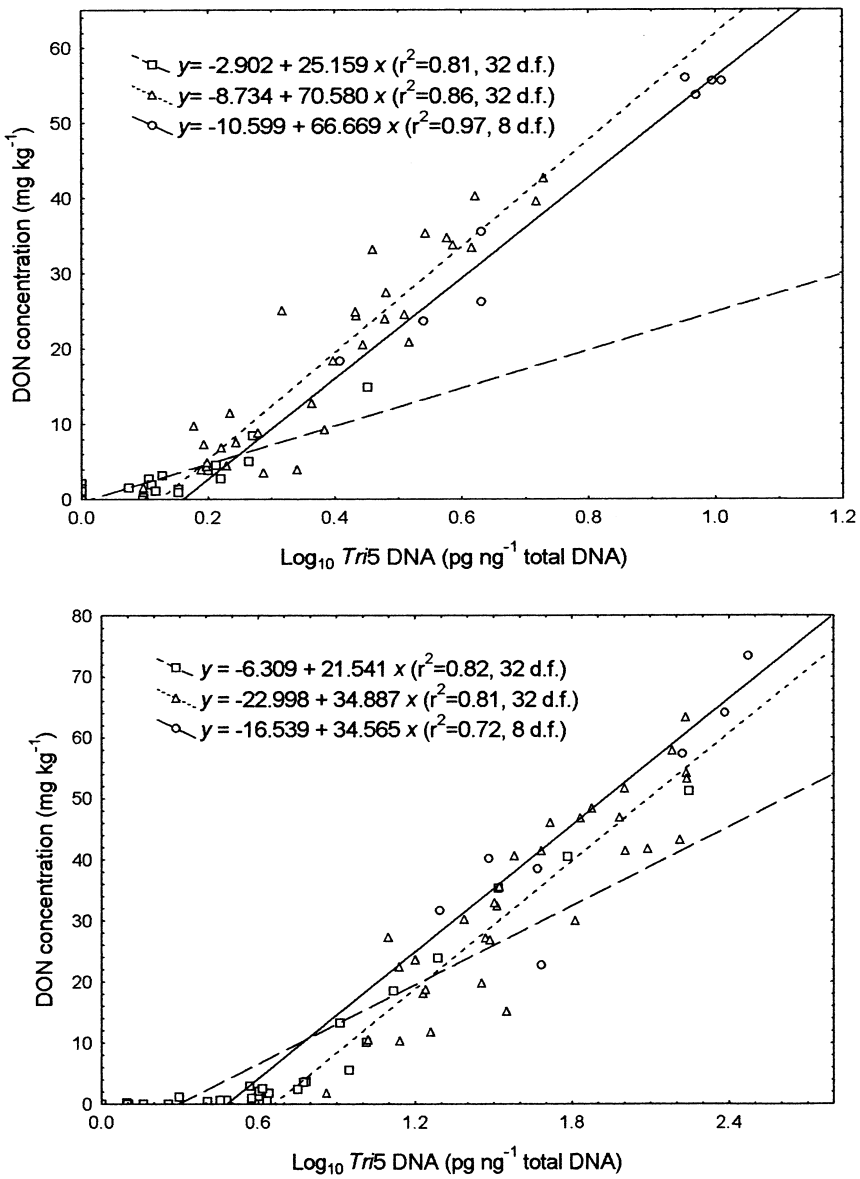

Figure 1. Relationship between quantity of *Tri*5 DNA and DON concentration in grain of winter wheat (cv. Cadenza) harvested from ears treated with a range of dose rates of metconazole (□), azoxystrobin (Δ) or untreated (◯) in experiment 1 (a) and experiment 2 (b).

severity of a range of diseases. Plotting disease severity against fungicide dose rate allowed dose rate response curves to be drawn for each of the diseases assessed. Although dose response curves clearly highlighted a rate response for the majority of diseases, such as in the case of *Septoria tritici*, the rate response curve levelled out and was almost horizontal between the four dose rates of azoxystrobin tested. This would suggest that lower dose rates of azoxystrobin would need to be tested in order to determine a dose rate response for *S. tritici*. Since FHB, *Tri*5 and DON did not respond to dose rate of azoxystrobin applied in these studies, it would appear that the four dose rates used here were above those which could allow a rate response to be determined.

Given the food safety issues associated with wheat grain contaminated with trichothecene mycotoxins, it is proposed that fungicides provide a valuable tool for

reducing DON concentration in wheat by effectively reducing the colonisation of grain by trichothecene-producing *Fusarium* species. However, although DON is by far the most predominant trichothecene identified in wheat-growing areas of temperate climates, other trichothecenes such as nivalenol are frequently identified (Chelkowski, 1989). Further work is therefore required to identify the effect of fungicides on the accumulation of trichothecenes other than DON.

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