

Effects of Azoxystrobin on Mycotoxin Production in a Carbendazim-Resistant Strain of *Fusarium sporotrichioides*

J.P. Felix D'Mello, Ann M.C. Macdonald and Rosalie Rinna¹

Carbendazim-resistant (RS) and control (CS) strains of *Fusarium sporotrichioides* Sherb., previously developed in our laboratory, were exposed to graded concentrations of azoxystrobin in broth media under shake-culture conditions for 2, 3, 4 and 8 days. Azoxystrobin concentrations were 0, 1, 10 and 100 mg l⁻¹ broth and cultures were incubated at a constant 25°C. Mycelial growth was significantly affected by strain ($P < 0.01$), azoxystrobin concentration ($P < 0.001$) and incubation time ($P < 0.001$). Combined results for the four incubation times showed that CS yielded higher mycelial mass than RS ($P < 0.01$) only in the absence of azoxystrobin. At fungicide additions of 1, 10 and 100 mg l⁻¹ mycelial growth was reduced ($P < 0.001$) with minimal strain differences ($P > 0.05$) at all three doses of azoxystrobin. Significant ($P < 0.05$ or better) strain–fungicide interactions were recorded in trichothecene production following exposure to azoxystrobin. At 4 and 8 days of incubation, the 10 mg l⁻¹ addition of azoxystrobin stimulated T-2 toxin synthesis ($P < 0.05$) only in RS cultures. In contrast, T-2 toxin enhancement in CS cultures occurred only on day 8 but at a lower level of azoxystrobin (1 mg l⁻¹). Thus, the stimulation of T-2 toxin synthesis depended upon strain and azoxystrobin level. Production of diacetoxyscirpenol (DAS) was affected by a more complex set of interactions. Overall means showed that, in comparison with initial values (on day 2 or 3), DAS output maximized significantly ($P < 0.05$) on day 4 in RS cultures and on day 8 in CS. Marked strain effects were observed on exposure to the 10 mg l⁻¹ level of azoxystrobin. At this level, DAS production was enhanced in RS only after 4 ($P < 0.01$) and 8 ($P < 0.05$) days of incubation, while in contrast, CS reduced DAS production. As with T-2 toxin, DAS production in CS was stimulated ($P < 0.05$ or better) only at low exposure levels of azoxystrobin. In the case of neosolaniol (NEO), however, the main effect of strain was significant ($P < 0.05$), with CS producing consistently more of the mycotoxin than RS on day 4 of the experiment. At this point, the NEO:T-2 toxin ratio was also higher in CS (0.63) than in RS (0.12), a feature reported by us previously. In conclusion, the present investigation has shown for the first time that the development of resistance to one fungicide can affect trichothecene production in *F. sporotrichioides* on exposure to a second fungicide. These results have been incorporated into a new classification scheme for fungicide efficacy which is also presented in this paper.

KEY WORDS: Azoxystrobin; carbendazim-resistance; *Fusarium sporotrichioides*; trichothecene production; fungicide classification.

Received March 15, 2001; received in final form July 24, 2001; <http://www.phytoparasitica.org> posting Oct. 7, 2001.

¹The Scottish Agricultural College, Crop Science Department, Edinburgh, UK [e-mail: f.dmello@ed.sac.ac.uk].

INTRODUCTION

Recent laboratory and field data have highlighted the variable efficacy of fungicides in controlling mycotoxin residues in cereal grains harvested from crops infected with fusarium head blight (FHB) (4). We hypothesized that the acquisition of fungicide resistance by *Fusarium* phytopathogens might impair fungicide control of mycotoxin contamination. Limited evidence for such an effect has emerged from our laboratory studies with pure cultures of *Fusarium culmorum*. Initial work by D'Mello *et al.* (3,5) showed that resistance to difenoconazole induced increased persistence of 3-acetyl deoxynivalenol (3-ADON) production compared to a control strain of the pathogen. These strain differences were apparent at each of three sampling times during a 22-day incubation period. Moreover, there was some evidence of different patterns of 3-ADON synthesis between the two strains. A subsequent study (2) examined the effects of carbendazim resistance on mycotoxin production in *Fusarium sporotrichioides* Sherb. That investigation was designed to test the universality of concepts emerging from the earlier work (3,5). Thus, carbendazim was used instead of difenoconazole as part of this strategy. In addition, *F. sporotrichioides* was selected as the test fungus since, in comparison with *F. culmorum*, it produces a diverse range of trichothecene mycotoxins, including T-2 toxin, diacetoxyscirpenol (DAS) and neosolaniol (NEO). It was concluded that carbendazim resistance also induced genuine differences in mycotoxin production in *F. sporotrichioides*. Combined results for three incubation periods showed dose-related effects in carbendazim inhibition of T-2 toxin production by the control strain (CS). In contrast, depending upon fungicide dose, cultures of the resistant strain (RS) either increased T-2 toxin synthesis or showed lower levels of inhibition than CS isolates. In particular, at the $2 \mu\text{g ml}^{-1}$ addition of carbendazim, T-2 toxin production was markedly enhanced in RS isolates. Furthermore, the ratio of NEO to T-2 toxin production was affected by an interaction involving incubation time, strain and carbendazim dose. It was suggested that the focus of the strain difference might reside in the conversion of NEO to T-2 toxin which, in turn, is probably sensitive to fungicide concentration. However, this implication was based on limited data for the two mycotoxins (2).

It is not known how exposure to other commonly used fungicides might affect mycotoxin production in carbendazim-resistant strains of *Fusarium*. This knowledge would be of practical importance, since mixtures of fungicides are now regularly applied to cereal crops to improve overall efficacy. In the current paper we report the results of such an investigation. The strobilurin analog, azoxystrobin is now recommended for late ear diseases in spring and winter wheat and would appear to be a suitable fungicide to test our hypothesis. The two *F. sporotrichioides* strains (CS and RS) used previously (2) were individually exposed to graded concentrations of azoxystrobin. T-2 toxin, DAS and NEO production were determined in a factorial experiment designed to explore main effects of, and interactions involving strain, incubation time and azoxystrobin concentration in liquid media. An objective of this design was to obtain a complete set of data for DAS and NEO production to assess the validity of previous implications that were based on limited values (2). Finally, the results obtained were considered in the context of other published data to assess the overall efficacy of fungicides for mycotoxin control. In consequence, a novel system of fungicide classification was devised and is presented in this paper.

MATERIALS AND METHODS

Glucose yeast extract medium A glucose–yeast extract–peptone (GYP) broth was used in this study. The broth contained 1% glucose, 0.1% yeast extract and 0.1% peptone. The pH was set at 6.5. Prior to use the medium was autoclaved at 121°C for 15 min.

Azoxystrobin Azoxystrobin was added as Amistar (Zeneca, Agrochemicals, UK), containing 250 g a.i. l⁻¹. Prior to use, and as in the previous study (2), Amistar was diluted with ethanol to provide the desired final concentrations. The diluted fungicide was then added to sterilized GYP media and mixed thoroughly.

Fungal isolates The CS and RS strains of *F. sporotrichioides* were described and characterized elsewhere (2). The two strains showed markedly diverging growth patterns following exposure to graded levels of carbendazim (2). Stock cultures prepared for the previous experiment were maintained in their respective GYP media prior to use in the current experiment as described below.

Experimental design The GYP cultures of CS and RS were used to inoculate separate petri dishes of PDA which were then incubated at 25°C until growth was established. The PDA cultures were used to prepare a spore suspension adjusted to 5 × 10³ spores ml⁻¹ GYP medium for each strain (CS and RS). Each suspension was used to inoculate separate experimental flasks containing GYP. After incubation for 1 day at 25°C on a shaker, Amistar was added to provide azoxystrobin concentrations of 0, 1, 10 and 100 mg a.i. l⁻¹ of GYP in factorial combination. The 0 mg l⁻¹ treatment contained an appropriate volume of ethanol consistent with the level used for the fungicide treatments. Sufficient flasks were incubated at 25°C on a shaker to provide two replicates at each incubation time for each strain and azoxystrobin level. Sixty-four flasks were inoculated for the entire experiment, allowing sampling at 2, 3, 4 and 8 days of incubation. At each of these times, samples were taken for mycelial mass and mycotoxin determinations.

Growth measurements After 2, 3, 4 and 8 days of incubation, entire cultures from two replicates in each treatment were filtered through filter paper (Whatman No. 4) and the mycelia were freeze-dried and weighed. As in the preceding study (2), these weights were used to represent fungal growth. The filtrates arising from this stage were analyzed for three trichothecene mycotoxins.

Determinations of T-2 toxin, diacetoxyscirpenol and neosolaniol T-2 toxin, DAS and NEO were quantified in filtrates arising from both replicates for all experimental treatments and incubation times. Each filtrate was washed with chloroform, filtered through silicone-treated phase separation paper, reduced in volume using a thin-film rotary evaporator, dried under N₂ and stored at -20°C prior to analysis by thin layer chromatography (TLC). For the determination of T-2 toxin, DAS and NEO, the dried extracts were re-suspended in 100 µl chloroform and 5 µl was spotted onto TLC plates (2). The plates were then developed to a predetermined mark (10 cm) in a mixture of chloroform and methanol (volumetric proportions: 93 and 7, respectively). The plates were air-dried, turned through an angle of 90°C and developed in a mixture of toluene, ethyl acetate and formic acid (volumetric proportions: 50, 40 and 10, respectively). With both dimensions, plates were developed in separate equilibrated chambers. Derivatization was accomplished by dipping the plates in 8% sulphuric acid. Plates were then heated at 110°C for 20 min prior to examination under UV light. The presence of the three mycotoxins was assessed visually by comparing

R_f values and color development with respective standards. Quantification was performed with a CAMAG CD60 densitometer. The output from the densitometer also provided the means for positive identification of the three mycotoxins by spectral analysis. Exact details of all protocols and analytical hardware used appear in an earlier paper (2).

Statistical analysis Data were subjected to analysis of variance for a factorial design using Minitab. Significant main effects and interactions were identified by F tests. Significant differences between means were established using t tests according to standard protocols (9).

RESULTS

Mycelial yield The mycelial mass results are summarized in Table 1. The original, replicated data were subjected to analysis of variance which showed that the main effects of azoxystrobin concentration and of incubation time were highly significant ($P < 0.001$). The main effect of strain was also significant ($P < 0.01$). In addition, the interaction between azoxystrobin concentration and duration of exposure was highly significant ($P < 0.001$). Examination of the overall means in Table 1 indicates that the strain difference was apparent only on day 3 of incubation ($P < 0.01$), although the effects of time were significant for both strains ($P < 0.05$ or better). Similarly, within the body of the table, strain differences were significant on day 3 ($P < 0.01$) for cultures without fungicide treatment and on day 8 ($P < 0.05$) for cultures exposed to azoxystrobin at 100 mg l^{-1} of medium. In contrast, both strains were sensitive to azoxystrobin concentration, with significant depressions in mycelial yield occurring even at the 1 mg l^{-1} addition on day 3 ($P < 0.01$ or better), day 4 ($P < 0.01$) and day 8 ($P < 0.05$ or better). The reductions precipitated by this level of azoxystrobin were greater for the CS strain ($P < 0.001$) than for the RS strain ($P < 0.05$ or $P < 0.01$) on days 3 and 8 of incubation. However, higher levels of azoxystrobin in general induced smaller reductions in mycelial yield that in many cases failed to reach significance ($P > 0.05$). In addition, an anomalous value was recorded for the CS sample exposed to azoxystrobin at 100 mg l^{-1} for 8 days.

TABLE 1. Effects of incubation time and azoxystrobin concentrations on mycelial mass in control (CS) and carbendazim-resistant (RS) strains of *Fusarium sporotrichioides*

Azoxystrobin level (mg l^{-1})	Incubation time (days)							
	2		3		4		8	
	CS	RS	CS	RS	CS	RS	CS	RS
	Mycelial mass (mg)							
0	212	135	888	433	464	472	492	381
1	69	30	274	143	209	215	162	160
10	48	26	221	115	237	204	146	137
100	52	98	50	44	91	34	383	175
Overall mean	95	72	358	184	250	231	296	213
LSD ($P < 0.05$)	177							

T-2 toxin production Mean values for T-2 toxin production are presented in Table 2. Analysis of variance of the original replicated data indicated significant main effects of time ($P < 0.001$) and azoxystrobin concentration ($P < 0.01$) on T-2 toxin production. The main

TABLE 2. Effects of incubation time and azoxystrobin concentrations on T-2 toxin production by control (CS) and carbendazim-resistant (RS) strains of *Fusarium sporotrichioides*

Azoxystrobin level (mg l ⁻¹)	Incubation time (days)							
	2		3		4		8	
	CS	RS	CS	RS	CS	RS	CS	RS
	T-2 toxin production (mg l ⁻¹)							
0	282	30	49	200	49	331	41	45
1	0	0	174	72	374	109	528	70
10	0	0	0	0	387	686	428	473
100	0	0	0	0	0	84	25	100
Overall mean	70.5	7.5	55.8	68	202.5	302.5	255.5	172.0
LSD (<i>P</i> <0.05)	339							

TABLE 3. Effects of incubation time and azoxystrobin concentrations on diacetoxyscirpenol (DAS) production by control (CS) and carbendazim-resistant (RS) strains of *Fusarium sporotrichioides*

Azoxystrobin level (mg l ⁻¹)	Incubation time (days)							
	2		3		4		8	
	CS	RS	CS	RS	CS	RS	CS	RS
	DAS production (mg l ⁻¹)							
0	0	0	6	0	12	14	12	0
1	0	0	0	0	36	5	81	14
10	0	0	0	0	6	96	9	40
100	0	0	0	0	0	0	0	0
Overall mean	0	0	1.5	0	13.5	28.8	25.5	13.5
LSD (<i>P</i> <0.05)	33							

effects of strain were not significant (*P*>0.05). However, the interactions between strain and fungicide concentration and between incubation time and fungicide concentration were significant (*P*<0.05). The main effect of time is shown in a comparison of the overall means in Table 2. T-2 toxin production maximized on day 8 in CS (*P*<0.05) and on day 4 in RS (*P*<0.01), although the interaction between strain and time was not significant (*P*>0.05). Significant effects of time may also be seen in the main body of Table 2. Thus, in comparison with initial values on day 2, T-2 toxin production maximized on day 4 for CS (*P*<0.05) and on day 8 for RS (*P*<0.01) following exposure to azoxystrobin at 1 mg l⁻¹ GYP broth. The strain–fungicide interaction is worthy of note in that 8 days after

TABLE 4. Effects of incubation time and azoxystrobin concentrations on neosolaniol (NEO) production by control (CS) and carbendazim-resistant (RS) strains of *Fusarium sporotrichioides*

Azoxystrobin level (mg l ⁻¹)	Incubation time (days)							
	2		3		4		8	
	CS	RS	CS	RS	CS	RS	CS	RS
	NEO production (mg l ⁻¹)							
0	0	0	85	34	211	67	40	0
1	0	0	16	0	192	44	187	32
10	0	0	0	0	104	30	71	90
100	0	0	0	0	0	0	0	0
Overall mean	0	0	25.3	8.5	126.8	35.3	74.5	30.5
LSD (<i>P</i> <0.05)	144							

incubation, azoxystrobin stimulated T-2 toxin production ($P<0.05$) in both strains but only 1 mg l^{-1} was sufficient for this increase in CS, whereas 10 mg l^{-1} was required for RS. Enhancement of T-2 toxin production also occurred on day 4, but significance was observed only with RS exposed to azoxystrobin at 10 mg l^{-1} . However, at 100 mg l^{-1} the fungicide significantly ($P<0.05$) reduced T-2 toxin output equally in both strains.

Diacetoxyscirpenol production Analysis of variance of replicated data indicated that incubation time induced a highly significant effect ($P<0.001$) on DAS production. Examination of the overall means in Table 3 shows that this effect was discernible for both strains. Thus, in comparison with values for day 2 or 3, DAS production maximized ($P<0.01$) on day 4 for RS and on day 8 for CS, although the main effect of strain and the interaction between time and strain were not significant ($P>0.05$). In the analysis of variance, azoxystrobin concentration emerged as a significant factor in respect to main effects ($P<0.01$) and to interactions with strain ($P<0.01$) and duration of exposure to the fungicide ($P<0.05$). In addition, the azoxystrobin–strain–time interaction was significant ($P<0.05$). Thus, azoxystrobin added at 10 mg l^{-1} stimulated DAS production in RS after 4 days ($P<0.01$) and 8 days ($P<0.05$) of incubation. In contrast, enhanced DAS production by CS cultures occurred only on day 8 ($P<0.01$) but at a lower level of azoxystrobin addition (1 mg l^{-1}). The interactions are further exemplified by two other comparisons of data in Table 3. On day 4, RS produced more DAS than CS following exposure to azoxystrobin at 10 mg l^{-1} ($P<0.01$), but on day 8 the difference was reversed ($P<0.01$) but at a lower level of fungicide (1 mg l^{-1}). At the 100 mg l^{-1} addition, DAS production was reduced to undetectable levels ($P<0.05$ or better) in both strains.

Neosolaniol production Analysis of variance indicated significant ($P<0.05$) main effects of incubation time and of strain on NEO production. All other effects were non-significant ($P>0.05$). Inspection of the overall means in Table 4 shows that the effect of time was significant only for CS, which maximized NEO production on day 4 of incubation. That overall mean reflected significantly ($P<0.05$) higher yields in CS compared with initial values and with RS following incubation with azoxystrobin at 0 and 1 mg l^{-1} . On day 8, CS exposed to 1 mg l^{-1} also produced more NEO than RS ($P<0.05$). In addition, this level of azoxystrobin stimulated NEO synthesis in CS ($P<0.05$) but not RS ($P>0.05$) on day 8 of incubation, compared with respective cultures incubated without fungicide.

DISCUSSION

This is the first study with a *Fusarium* phytopathogen indicating that development of resistance to one fungicide might affect trichothecene production on subsequent exposure to a second fungicide. In an earlier study (2) we showed that a carbendazim-resistant strain of *F. sporotrichioides* enhanced its synthesis of T-2 toxin following further exposure to the same fungicide. This strain of *F. sporotrichioides* (RS) was used in the current investigation. Marked differences were recorded in trichothecene production between RS and a control strain (CS) after both were exposed to graded levels of azoxystrobin (Tables 2–4). It is worthy of note that for T-2 toxin and DAS the main effects of strain were not significant ($P>0.05$). However, significant ($P<0.05$ or better) strain–fungicide interactions were observed in synthesis of these two mycotoxins. In the case of DAS (Table 3), incubation time emerged as an additional component in this interaction ($P<0.05$).

TABLE 5. Fungicide efficacy: a tentative classification for trichothecene control^z

Class	Descriptor	Examples of fungicides	Trichothecene affected ^y	Conditions
I	Effective	None	–	–
IIA	Partially effective [growth-dependent inhibition; mycotoxin residues possible]	Tebuconazole Thiophanate-methyl Prochloraz	DON DON and NIV 3-ADON	Field trial Field trial <i>In vitro</i>
IIB	Partially effective [direct inhibition of mycotoxin synthesis; disease/infection/fungal growth possible]	Thiabendazole Dicloran	DON DAS	Field trial <i>In vitro</i>
IIIA	Ineffective	Propiconazole Morpholines	DON 3-ADON	Field trial <i>In vitro</i>
IIIB	Stimulatory and/or inducing resistance	Iprodione Tridemorph Difenoconazole Carbendazim Azoxystrobin	DON T-2 toxin 3-ADON T-2 toxin T-2 toxin, DAS and NEO	Field trial <i>In vitro</i> <i>In vitro</i> <i>In vitro</i> <i>In vitro</i> ^x

^zFor data sources, see text of this paper and reviews by D'Mello *et al.* (4) and D'Mello and Macdonald (1).

^yDON, deoxynivalenol; NIV, nivalenol; 3-ADON, 3-acetyl deoxynivalenol; DAS, diacetoxyscirpenol; NEO, neosolamol.

^xSee Tables 1–4 of this paper.

The strain–fungicide interaction is clearly exemplified by the T-2 toxin results in Table 2. In qualitative terms, it can be seen that after 4 days of incubation, azoxystrobin at 100 mg l⁻¹ inhibited T-2 toxin formation in CS while allowing some synthesis in RS cultures. In addition, three statistically significant comparisons underline the strain–fungicide interaction. At 4 and 8 days of incubation, the 10 mg l⁻¹ addition of azoxystrobin stimulated T-2 toxin synthesis ($P < 0.05$) only in RS cultures. In contrast, T-2 toxin enhancement in CS cultures occurred only on day 8 but at a lower level of azoxystrobin (1 mg l⁻¹). Thus the stimulation of T-2 toxin synthesis depended upon strain and fungicide level. The strain difference in T-2 toxin production occurs only following exposure to low or moderate levels of azoxystrobin.

Production of DAS was affected by a more complex set of interactions, as illustrated by the results in Table 3. Overall means showed that, in comparison with initial values (on day 2 or 3), DAS output maximized significantly ($P < 0.05$) on day 4 in RS cultures and on day 8 in CS, although the strain–time interaction proved to be not significant ($P > 0.05$). More striking strain effects were observed on exposure to the 10 mg l⁻¹ level of azoxystrobin. At this level, DAS production was enhanced only in RS, after 4 ($P < 0.01$) and 8 ($P < 0.05$) days of incubation, whereas CS, in contrast, reduced DAS production. As with T-2 toxin, DAS production in CS was stimulated ($P < 0.05$ or better) only at low exposure levels of azoxystrobin. In the case of NEO (Table 4), however, the main effect of strain was significant ($P < 0.05$), with CS producing consistently more of the mycotoxin than RS on day 4 of the experiment. At this point, the NEO:T-2 toxin ratio was also higher in CS (0.63) than in RS (0.12), a feature which we reported previously (2). The new evidence corroborates further the concept that carbendazim resistance may promote conversion of NEO to T-2 toxin and that this trait is discernible even when RS is exposed to another fungicide.

Another unique feature of this study was the simultaneous enhancement of T-2 toxin, DAS and NEO synthesis on exposure of *F. sporotrichioides* to low or moderate levels of azoxystrobin. It is possible that this effect is associated with azoxystrobin in particular, since other fungicides have been linked specifically with T-2 toxin stimulation (4). The implications for mycotoxin control in cereal production remain to be elucidated.

Validation of the present results under field conditions would not be practically feasible, since the different factors examined and conditions used would be difficult to control in cereal production. However, if these results are replicated under field conditions, then the significance for fungicide efficacy and cereal grain safety would be important. Thus type, level and timing of fungicide applications would be critical. At low to moderate levels after infection has been established, trichothecene contamination may be increased or, more likely, more difficult to control. Higher application levels and different combinations of fungicide mixtures may be required, with attendant consequences of increased costs of cereal production and of environmental damage.

It is fortuitous that in the present study fungal biomass production was depressed to similar extents in both strains following exposure to equivalent concentrations of azoxystrobin (Table 1). Consequently, the differences in mycotoxin production at low to moderate fungicide exposure (Tables 2–4) may be attributed to intrinsic strain effects at the biosynthetic level rather than to discrepancies in growth. Earlier studies (2,3,5) were somewhat inconclusive in this respect.

An aspect worthy of future investigation would be to confirm whether the effects on mycotoxin production would be replicated in different carbendazim-resistant strains of *Fusarium* phytopathogens. The current and previous (2) studies have examined mycotoxin synthesis in just one strain of *F. sporotrichioides*. A program to screen other toxigenic fungal pathogens would be of practical interest given the widespread and relatively rapid development of fungicide resistance among these organisms (4).

The present study is the final in a series of experiments (2,3,5) designed to examine the control of trichothecene production in *Fusarium* species resistant to common fungicides. It therefore appeared appropriate to review the major outcomes and to consider them within a scheme of fungicide efficacy. A summary is presented in Table 5 representing a distillation of this and other evidence (1,4,6-8,10,11). Individual fungicides were placed in three major groups based on the effectiveness of fungicides to control completely FHB infection and mycotoxin production in grain or in culture. Fungicides that satisfied these criteria would appear in class I, while those of intermediate efficacy would be placed within class II. Class III included fungicides with performance characteristics which were inconsistent with the above criteria and which would also pose additional risks. Subdivisions were necessary for class II given that partial efficacy might arise through growth-dependent retardation of *Fusarium* phytopathogens or by direct inhibition of trichothecene biosynthesis. With class IIA fungicides, if fungal growth or infection is not totally inhibited then residual mycotoxin production might be retained with the potential to contaminate cereal grains. With class IIB fungicides, trichothecene production in *Fusarium* species would be prevented but FHB or infection might not be suppressed fully and cereal yield would be compromised. Class III subdivisions were proposed on the basis that inefficacy would be compounded by the propensity of certain fungicides to stimulate fungal growth and/or trichothecene production. With both class II and III fungicides there appeared to be an additional risk that *Fusarium* phytopathogens could develop resistance to these compounds and overall efficacy might be further prejudiced.

It will be noted that no fungicide could be identified which could be considered to be completely effective in the terms identified above. It will also be apparent that tebuconazole, specifically recommended for FHB, has been excluded from class I and indeed designated as a class IIA fungicide. The evidence presented by Suty *et al.* (11) indicated that following tebuconazole application, residual levels of deoxynivalenol (DON) in grain would still exceed advisory guidelines issued by the Food and Drug Administration in the United States (1). More recent work (7) has confirmed that tebuconazole is only partially effective for DON control in wheat kernels. If the data of Gareis and Ceynowa (6) are taken into account then tebuconazole would be accorded a less favorable rating since, in combination with triadimenol, it may enhance nivalenol contamination of grain. Those data have, however, been excluded from the present assessment since corroboration has not appeared and moreover it is not possible to disentangle the effects of the individual components of the fungicide mixture. Propiconazole was designated a class IIIA fungicide due to residual trichothecene contamination of grain even in studies with beneficial outcomes (1). Two additional field trials showed no control of trichothecene contamination of cereal grains following application of propiconazole (8,10). A number of fungicides are proposed for class IIIB since they all show stimulatory effects or have the capacity to induce resistance which, in turn, may affect the pattern and levels of mycotoxin production. The evidence for this class includes data from the current study (Tables 1–4) and from previous papers.

In conclusion, the present investigation has shown for the first time that the development of resistance to one fungicide can affect trichothecene production in *F. sporotrichioides* following subsequent exposure to a second fungicide. A new classification scheme for fungicide efficacy is also presented. It is envisaged that this classification will form the basis for improving fungicide efficacy in the future and for developing alternative strategies for mycotoxin control. However, it is clear that considerable work has yet to be undertaken to develop an effective fungicide for trichothecene control.

ACKNOWLEDGMENTS

The authors acknowledge support from the Scottish Executive Rural Affairs Department (SERAD).

REFERENCES

1. D'Mello, J.P.F. and Macdonald, A.M.C. (1998) Fungal toxins as disease elicitors. pp. 255-291. *in*: Rose, J. [Ed.] Aspects of Environmental Toxicology. Overseas Publishers Association, Amsterdam, the Netherlands.
2. D'Mello, J.P.F., Macdonald, A.M.C. and Briere, L. (2001) Mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides*. *Mycotoxin Res.* 16:101-111.
3. D'Mello, J.P.F., Macdonald, A.M.C. and Dijkema, W.P.T. (1998) 3-Acetyl deoxynivalenol production in a strain of *Fusarium culmorum* insensitive to the fungicide difenoconazole. *Mycotoxin Res.* 14:9-18.
4. D'Mello, J.P.F., Macdonald, A.M.C., Postel, D., Dijkema, W.P.T., Dujardin, A. and Placinta, C.M. (1998) Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. *Eur. J. Plant Pathol.* 104:741-751.
5. D'Mello, J.P.F., Macdonald, A.M.C., Postel, D. and Hunter, E.A. (1998) 3-Acetyl deoxynivalenol production in a strain of *Fusarium culmorum* insensitive to the fungicide difenoconazole. *Mycotoxin Res.* 13:73-80.
6. Gareis, M. and Ceynowa, J. (1994) Influence of the fungicide Matador (tebuconazole/triadimenol) on mycotoxin production by *Fusarium culmorum*. *Z. Lebensm.-Unters. Forsch.* 198:244-248.
7. Jones, R.K. (2000) Assessments of fusarium head blight of wheat and barley in response to fungicide treatment. *Plant Dis.* 84: 1021-1030.
8. Martin, R.A. and Johnston, H.W. (1982) Effects and control of fusarium diseases of cereal plants in the Atlantic Provinces. *Can. J. Plant Pathol.* 4: 210-216.

9. Mead, R. and Curnow, R.N. (1983) *Statistical Methods in Agriculture and Experimental Biology*. Chapman and Hall, London, UK.
10. Milus, E.A. and Parsons, C.E. (1994) Evaluation of foliar fungicides for controlling *Fusarium* head blight of wheat. *Plant Dis.* 78: 697-699.
11. Suty, A., Mauler-Machnik, A. and Courbon, R. (1996) New findings on the epidemiology of fusarium ear blight on wheat and its control with tebuconazole. *Proc. Brighton Crop Protection Conf.* pp. 511-516.