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The phytotoxicity of selected mycotoxins on mature, germinating *Zea mays* embryos

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Abstract

Mature maize (*Zea mays*) embryos were exposed to 5, 10 and 25 μg ml^{-1} of deoxynivalenol (DON), zearalenone (ZEA), ochratoxin A (OA) and a mixture of zearalenone and deoxynivalenol (ZEA/DON) for 9 days. DON and the ZEA/DON combination were consistently more inhibitory of the measured parameters than either ZEA or OA. Based on the predicted additive values, it would appear that, in combination, ZEA and DON act synergistically to inhibit root and shoot growth. For ZEA alone, a concentration of 5 μg ml^{-1} ZEA was generally inhibitory of root and shoot elongation and fresh mass accumulation, while at 10 and 25 μg ml^{-1}, this toxin had a stimulatory effect on these parameters. For OA, the measured effects on root and shoot growth at 5 and 25 μg ml^{-1} were stimulatory, while at 10 μg ml^{-1} OA, an inhibitory effect was observed. For all toxins, inhibitory/stimulatory effects were generally more marked for root parameters than for shoot elongation or mass.

Key words: Deoxynivalenol, Embryo, Mature, Ochratoxin, Plantlet, Zearalenone, *Zea mays*

Abbreviations: ADON, acetyldeoxynivalenol; AFB_1, aflatoxin B_1; DAS, diacetyoxyscirpenol; DON, deoxynivalenol; FB_1, fumonisin B_1; FHB, *Fusarium* head blight; MON, moniliformin; NIV, nivalenol; OA, ochratoxin A; ZEA, zearalenone

Introduction

Under field conditions, plants and developing and maturing seeds are frequently subjected to attack by a variety of micro-organisms, from viruses, through bacteria to fungi. Traditionally, the fungi that invade seed crops have been described as 'field' fungi (e.g. *Cladosporium, Fusarium, Alternaria* spp.), which reputedly gain access to seeds during plant development, and 'storage' fungi, (e.g. *Aspergillus; Penicillum* spp.), which proliferate during storage [1, 2]. It is now, however, generally considered that most of these 'storage' fungi are capable of invasion under field conditions, and the prevalence of one or other species will depend on local environmental factors, the storage regime and interfungal relationships [3]. It is well documented that while *Aspergillus* and *Pencillium* species generally predominate during storage, *Fusarium* species are considerably more active under field conditions. Many *Fusarium* species may, however, persist for several months of storage at low temperature [4]. Some of these fungi elaborate mycotoxins (secondary metabolites), many of which are considered a potential risk to human and animal populations (e.g. aflatoxin B_1 and hepatocellular carcinoma [5])

Several of the Fusaria are regarded as plant pathogens, causing fusarial head blight of wheat (FHB) and ear rot of maize, amongst other diseases [6, 7]. The rôle of the secondary metabolites of *Fusarium* spp. in plant diseases is, however, circumstantial and controversial. *Fusarium* pathogenicity has been related to trichothecene production [7–13], with reduced yield and kernel size being reported [7, 10–14]. On the other hand, Adams & Hart [15] have found that several strains of highly virulent *Gibberella zea* (teleomorph of *F. graminearum*) did not produce DON or ADON, and concluded that toxins were not virulence factors in disease development. The results of these studies have been inconsistent, and it has been suggested that the apparent contradiction regarding the involvement

of *Fusarium* toxins in plant disease may arise, in part, from differing abilities of plants to metabolise the toxins [16].

The present investigation utilises an *in vitro* assay of excised, mature germinating *Zea mays* embryos to assess the phytotoxic effects of DON, ZEA and OA on the growth and development of the plantlets. Since *F. graminearum* may produce several metabolites, of which DON, ADON and ZEA are the most important [6], a combination of ZEA/DON was also investigated.

Materials and methods

Aflatoxin exposure. Mature *Zea mays* seeds (PNR 6363, Pannar Seed Company, Greytown, Kwazulu/Natal, South Africa) were surface-sterilised in 2% Hibitane (v/v) (ICI Pharmaceuticals; South Africa) for 15 min and, in order to facilitate aseptic embryo excision, caryopses were soaked overnight in sterile distilled water. Following excision, embryos were surface-sterilised in 2% Hibitane (15 min), soaked for 15 min in a 10 mg ml^{-1} penicillin/streptomycin mixture (Highveld Biological, South Africa), followed by a 15 min immersion in 2% NaOCl. Embryos were rinsed three times in sterile distilled water and plated aseptically onto a maize embryo germination medium (pH 5.8) [17] to which toxins from stock solutions of toxin (OA, ZEA or DON) were added after autoclaving of the medium to give final toxin concentrations of 5, 10 and 25 μg ml^{-1}. In the case of the ZEA/DON combination, toxins were added in equal volumes, such that final combined toxin concentrations were 5, 10 and 25 μg ml^{-1} (i.e. in 25 ml^{-1} toxin, DON = 12.5 μg ml^{-1} and ZEA = 12.5 μg ml^{-1}). Appropriate controls incorporating methanol (in which toxins had been dissolved) were used.

Measurements. Embryos germinated and established for nine days on the medium (25 ± 3 °C; 16 h photoperiod; 200 μmoles ml^{-1} sec^{-1} photon flux density). Three replicates of twenty or twenty-five embryos were assessed for each treatment. During the incubation period, roots (2, 4 and 6 days) and shoots (4 and 6 days) exceeding 20 mm were recorded. At the termination of the experiment, primary root and shoot length; root and shoot fresh mass and plantlet fresh and percentage dry mass were determined. Dry mass was determined by placing plantlets at 60 °C until constant mass was attained.

Statistics. Data were compared with control values (ANOVA, LSD, $p \leq 0.05$) [Tables 1 and 2]. In addition, measurements were expressed as a percentage inhibition of the mean control value. Inhibitory effects of 5, 10 and 25 μg ml^{-1} toxin concentrations were compared statistically (Figures 1 and 2a–e). For any one toxin treatment (e.g. ZEA) and any one parameter measured (e.g. root fresh mass), different alphabetical letters indicate statistically significant differences (see tables and figures).

Results

Root and shoot emergence

A dose range of 5–25 μg ml^{-1} of toxin had no negative effect on embryo germination (i.e. 100% germination). Differences were, however, observed between the different dose levels for any one toxin regime (Table 1). The percentage of plantlets with primary roots attaining a length greater than 20 mm following DON and ZEA/DON exposure was consistently lower than for ZEA or OA. In most instances, values reported for 25 μg ml^{-1} DON and ZEA/DON were significantly lower than for 5 or 10 μg ml^{-1}. For OA, at 10 μg ml^{-1}, the number of roots exceeding 20 mm in length was significantly lower than for 5 or 25 μg ml^{-1} toxin (Table 1). For shoots, differences between toxin concentrations for any one toxin were less marked than for root measurements. Statistically lower values were recorded for ZEA/DON and DON at 10 and/or 25 μg ml^{-1} when compared with control and plantlets exposed to 5 μg ml^{-1} (Table 1).

Primary root and shoot measurements; fresh and dry mass

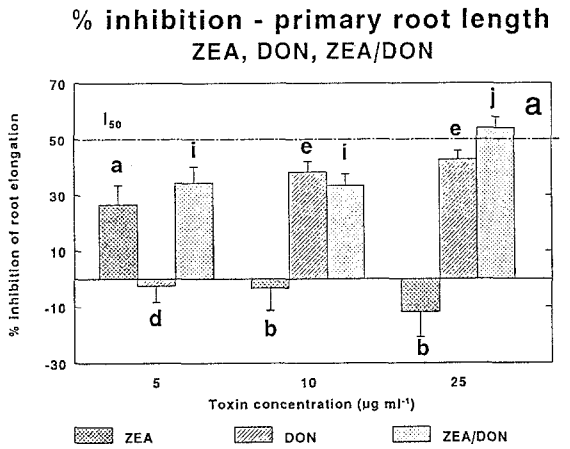
Of the four toxin regimes investigated, DON and ZEA/DON were consistently more toxic than either ZEA or OA (Table 2, Figures 1 and 2a–e). The inhibitory effects on root and fresh mass accumulation were greater than was measured for shoots (Figures 1 and 2a – e).

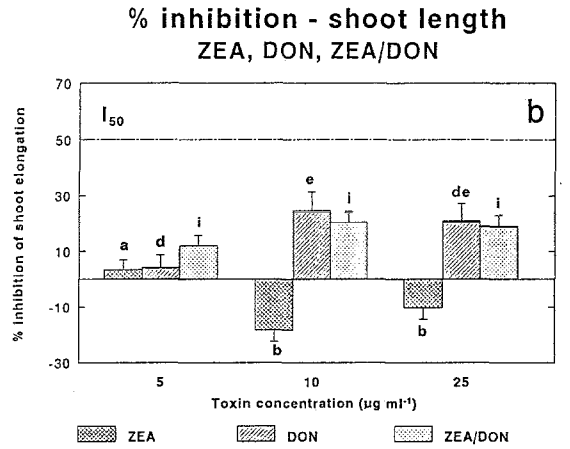
DON. Values for primary root length and root fresh mass of 10 and 25 μg ml^{-1} DON-treated plantlets were significantly lower than control and 5 μg ml^{-1}-treated material (Table 2). At 5 μg ml^{-1}, a slight stimulatory effect on primary root length was recorded (Figure 1a, Table 2). The inhibitory effects (expressed as a %

Table 1. Percentage of root and shoots attaining a length in excess of 20 mm in plantlets of *Zea mays* exposed to 5–25 μg ml^{-1} ZEA, DON, OA and a ZEA/DON combination for 9 days

Parameter	Control	Toxin concentration (μg ml^{-1})		
		5	10	25
% roots > 20 mm				
2 days				
DON	26.6 ± 4.4	24.5 ± 8.3	17.8 ± 9.9	19.0 ± 11.8
ZEA	39.2 ± 4.9	46.7 ± 9.6	48.7 ± 0.7	38.3 ± 2.9
ZEA/DON	38.0 ± 4.3^{h}	20.0 ± 0.6^{h}	23.7 ± 2.0^{i}	6.7 ± 1.8^{i}
OA	43.4 ± 2.4^{m}	48.0 ± 4.9^{n}	29.5 ± 2.3^{n}	43.8 ± 9.2^{mn}
4 days				
DON	73.9 ± 4.9^{a}	75.8 ± 9.2^{a}	62.8 ± 12.5^{a,b}	46.3 ± 12.5^{b}
ZEA	90.6 ± 2.0	87.0 ± 4.0	93.3 ± 3.3	89.3 ± 4.3
ZEA/DON	85.4 ± 2.9^{h}	80.7 ± 8.6^{h}	83.7 ± 5.4^{h}	56.0 ± 8.1^{i}
OA	83.8 ± 3.4^{m}	80.3 ± 5.7^{mn}	62.3 ± 10.9^{n}	80.5 ± 4.6^{mn}
6 days				
DON	81.1 ± 3.4	84.3 ± 7.7	72.3 ± 14.1	60.8 ± 17.6
ZEA	90.3 ± 2.3	91.7 ± 4.9	95.7 ± 2.3	94.7 ± 3.2
ZEA/DON	91.3 ± 2.7^{h}	87.3 ± 5.4^{h}	93.0 ± 3.5^{i}	71.0 ± 2.0^{i}
OA	87.5 ± 2.8^{m}	85.8 ± 5.9^{mn}	69.0 ± 9.9^{n}	84.3 ± 2.8^{mn}
9 days				
DON	93.5 ± 1.4^{a}	91.5 ± 5.0^{ab}	81.0 ± 12.4^{ab}	72.0 ± 3.7^{b}
ZEA	93.8 ± 2.5	91.0 ± 5.5	95.7 ± 2.3	94.7 ± 3.2
ZEA/DON	92.9 ± 2.3^{h}	88.7 ± 6.3^{h}	94.3 ± 3.0^{h}	72.0 ± 17.1^{i}
OA	90.3 ± 2.4^{m}	87.0 ± 5.2^{mn}	77.8 ± 6.2^{n}	87.0 ± 3.2^{mn}
% shoot > 20 mm				
4 days				
DON	32.3 ± 9.9	24.8 ± 14.7	16.5 ± 11.8	7.0 ± 7.0
ZEA	34.6 ± 5.2	29.3 ± 8.4	48.7 ± 1.3	43.7 ± 12.3
ZEA/DON	53.1 ± 5.6^{h}	37.7 ± 2.7^{hi}	33.7 ± 10.5^{hi}	17.7 ± 6.4^{i}
OA	47.0 ± 4.0	52.3 ± 5.3	42.0 ± 11.0	45.3 ± 1.1
6 days				
DON	63.6 ± 8.7	61.5 ± 17.3	63.3.± 19.3	47.8 ± 17.0
ZEA	84.7 ± 4.7	89.3 ± 3.7	93.3 ± 4.4	90.0 ± 5.8
ZEA/DON	90.4 ± 1.8^{h}	87.7 ± 3.7^{h}	75.3 ± 1.2^{i}	73.7 ± 1.3^{i}
OA	84.7 ± 1.8	88.0 ± 4.3	78.5 ± 2.1	84.6 ± 2.7
9 days				
DON	96.3 ± 1.4^{a}	96.3 ± 2.4^{ab}	77.5 ± 13.0^{b}	76.3 ± 13.8^{b}
ZEA	97.4 ± 2.0	100	97.3 ± 2.7	98.3 ± 1.7
ZEA/DON	99.6 ± 0.4	100	98.7 ± 1.3	100
OA	98.3 ± 0.7	98.5 ± 1.5	100	97.8 ± 2.3

$^{a;b;c,\ etc.}$ Different alphabetical characters for any one toxin treatment (control and 5–25 μg ml^{-1} for 2, 4, 6 or 9 days) indicate a statistical difference. The absence of characters indicates that no statistical differences were measured for that toxin treatment. ZEA, DON, ZEA/DON and OA were considered separately.





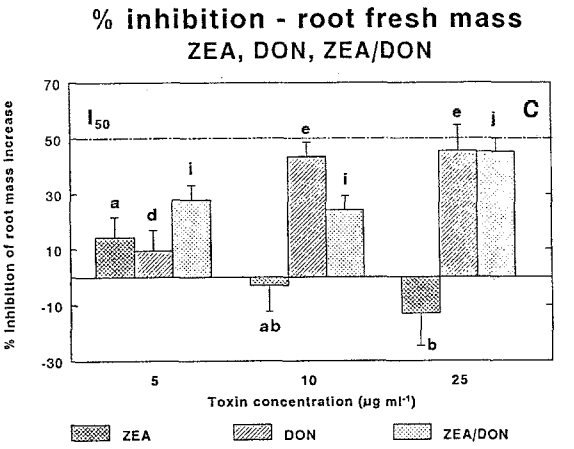
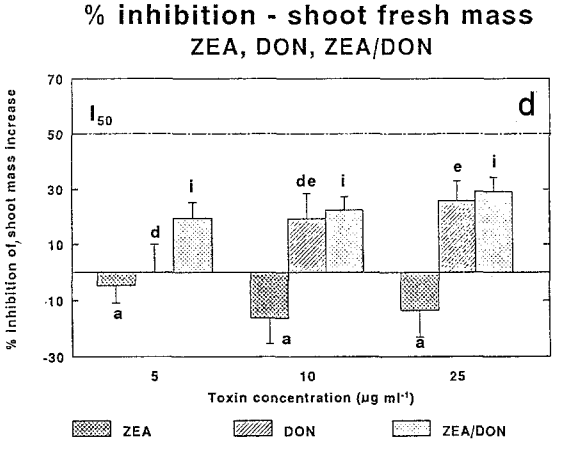


Figure 1a–c. Percentage inhibitory response (with respect to controls) of zearalenone, deoxynivalenol and a ZEA/DON combination on plantlets of *Zea mays*. (a) Primary root length; (b) shoot length; (c) root fresh mass.



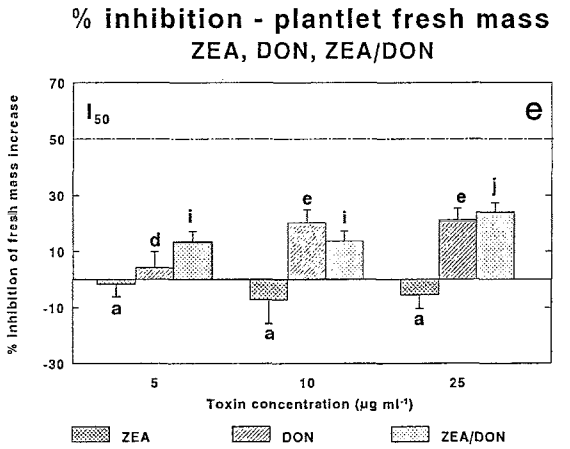


Figure 1d–e. Percentage inhibitory response (with respect to controls) of zearalenone, deoxynivalenol and a ZEA/DON combination on plantlets of *Zea mays*. (d) Shoot fresh mass; (e) plantlet fresh mass.

inhibition with respect to the control) of 10 and 25 μg ml^{-1} DON on root length (38 – 43% inhibition) and root mass (43 – 46%) were significantly higher than at 5 μg ml^{-1} (–3% and 9% inhibition for root length and mass, respectively) (Figures 1a,c). For most of the parameters measured, the greatest inhibitory effect was between 5 and 10 μg ml^{-1} toxin (Figures 1a,e).

The inhibitory effects of DON on shoot development were less marked than for root growth (Figures 1a,d). Mean shoot length, shoot mass and plantlet fresh mass of 10 and 25 μg ml^{-1}-treated plantlets were significantly lower than those measured for 5 μg ml^{-1} or control plantlets (Table 2). Over the 5–25 μg ml^{-1} toxin regime, inhibition of shoot elongation (4–25%), shoot fresh mass (0.05–26%) and plantlet fresh mass

Table 2. The effects of DON, ZEA, OA and a ZEA/DON combination on root and shoot length and mass, plantlet fresh mass and % dry mass of germinating *Zea mays* embryos exposed to toxin for 9 days

Parameter	Control	Toxin concentration (μg ml^{-1})		
		5	10	25
Primary root length				
DON	6.79 ± 0.30^{a}	6.96 ± 0.40^{a}	4.19 ± 0.23^{b}	3.88 ± 0.22^{b}
ZEA	8.95 ± 0.42^{d}	6.59 ± 0.63^{e}	9.24 ± 0.72^{d}	10.01 ± 0.79^{d}
ZEA/DON	7.33 ± 0.37^{h}	4.81 ± 0.48^{i}	4.87 ± 0.35^{i}	3.37 ± 0.29^{i}
OA	6.76 ± 0.33^{mn}	6.85 ± 0.60^{mn}	5.45 ± 0.50^{m}	7.88 ± 0.74^{n}
Root fresh mass				
DON	0.048 ± 0.004^{a}	0.043 ± 0.004^{a}	0.027 ± 0.003^{b}	0.026 ± 0.004^{b}
ZEA	0.076 ± 0.005^{de}	0.065 ± 0.006^{d}	0.079 ± 0.007^{de}	0.086 ± 0.009^{e}
ZEA/DON	0.062 ± 0.003^{h}	0.045 ± 0.003^{ij}	0.047 ± 0.003^{i}	0.034 ± 0.003^{j}
OA	0.065 ± 0.003^{m}	0.062 ± 0.005^{mn}	0.051 ± 0.005^{n}	0.066 ± 0.006^{m}
Shoot length				
DON	5.90 ± 0.18^{a}	5.66 ± 0.28^{a}	4.44 ± 0.40^{b}	4.67 ± 0.38^{b}
ZEA	6.64 ± 0.18^{d}	6.43 ± 0.24^{d}	7.85 ± 0.28^{e}	7.32 ± 0.27^{e}
ZEA/DON	7.96 ± 0.17^{h}	7.00 ± 0.30^{i}	6.33 ± 0.29^{ij}	6.09 ± 0.29^{j}
OA	6.56 ± 0.15^{m}	6.79 ± 0.25^{m}	6.18 ± 0.25^{m}	6.71 ± 0.25^{m}
Shoot fresh mass				
DON	0.105 ± 0.006^{a}	0.105 ± 0.011^{ab}	0.085 ± 0.01^{ab}	0.078 ± 0.008^{b}
ZEA	0.107 ± 0.005^{d}	0.112 ± 0.007^{d}	0.125 ± 0.010^{d}	0.122 ± 0.010^{d}
ZEA/DON	0.121 ± 0.004^{h}	0.097 ± 0.007^{i}	0.094 ± 0.006^{i}	0.086 ± 0.006^{i}
OA	0.105 ± 0.003^{m}	0.109 ± 0.006^{m}	0.101 ± 0.006^{m}	0.102 ± 0.006^{m}
Plantlet fresh mass				
DON	0.261 ± 0.010^{a}	0.251 ± 0.001^{ab}	0.208 ± 0.012^{bc}	0.206 ± 0.011^{c}
ZEA	0.297 ± 0.009^{d}	0.303 ± 0.013^{d}	0.318 ± 0.017^{d}	0.314 ± 0.015^{d}
ZEA/DON	0.294 ± 0.007^{h}	0.255 ± 0.011^{i}	0.254 ± 0.011^{i}	0.224 ± 0.010^{i}
OA	0.280 ± 0.006^{m}	0.281 ± 0.010^{m}	0.265 ± 0.011^{m}	0.261 ± 0.011^{m}
% dry mass DON	14.37 ± 0.31^{a}	14.40 ± 0.41^{a}	16.35 ± 0.31^{b}	16.31 ± 0.60^{b}
ZEA	13.55 ± 0.18^{d}	13.20 ± 0.26^{d}	13.11 ± 0.33^{d}	13.36 ± 0.25^{d}
ZEA/DON	13.84 ± 0.16^{h}	14.14 ± 0.22^{h}	14.98 ± 0.39^{i}	15.14 ± 0.30^{i}
OA	14.51 ± 0.15^{m}	14.10 ± 0.28^{m}	14.06 ± 0.26^{m}	13.96 ± 0.22^{m}

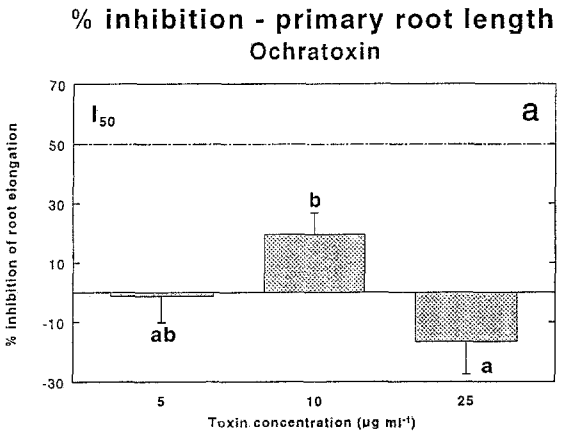
[a,b,c, etc.] Different alphabetical letters for any one toxin treatment indicate a statistical significance.

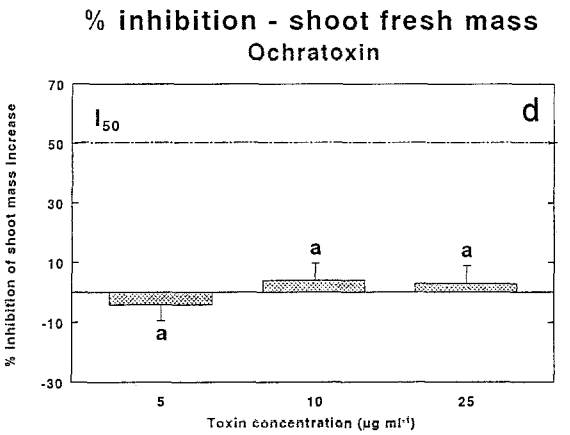
(4–21%) were similar (Figures 1b,d,e). The percentage dry mass of DON-treated plantlets exposed to 10 and 25 μg ml^{-1} DON was significantly higher than control and 5 μg ml^{-1}-treated plantlets (Table 2).

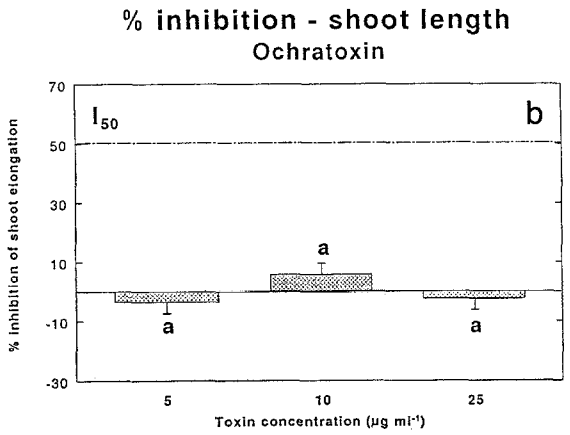
ZEA. For ZEA, 5 μg ml^{-1} was generally inhibitory of the parameters assessed, while at 10 and 25 μg ml^{-1}, a stimulatory effect was recorded (Table 2, Figures 1a–e). The inhibitory effect of 5 μg ml^{-1} ZEA was significant for primary root elongation, while the stimulatory effect was statistically significant for shoot elongation (Table 1). When percentage inhibition for the three toxin concentrations was compared, differences between 5 and 10 or 25 μg ml^{-1} were significant for root and shoot elongation and for root fresh mass (Figures 1a–c). Stimulatory effects of ZEA at 10 and 25 μg ml^{-1} were similar for root and shoot measurement (Figures 1a–c).

Although plantlet fresh mass at all ZEA concentration exceeded (2–7% increase) that of control plantlets, these values were not statistically significant (Table 2). ZEA had little effect on plantlet dry mass (Table 2).

ZEA/DON. Statistically lower values for root and shoot measurements and fresh mass were measured at all ZEA/DON concentrations (Table 2) when compared with controls. The greatest inhibitory response was measured between 5 and 10 μg ml^{-1} toxin (Figures 1a–e). Statistically significant differences were observed between 5/10 and 25 μg ml^{-1} for root elon-







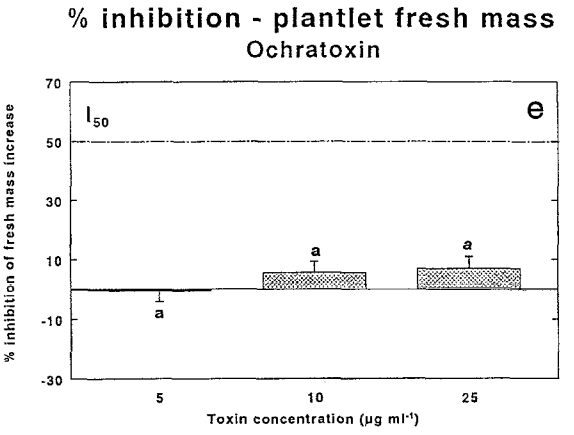


Figure 2d–e. Percentage inhibitory response (with respect to controls) of ochratoxin on plantlets of *Zea mays*. (d) Shoot fresh mass; (e) plantlet fresh mass.

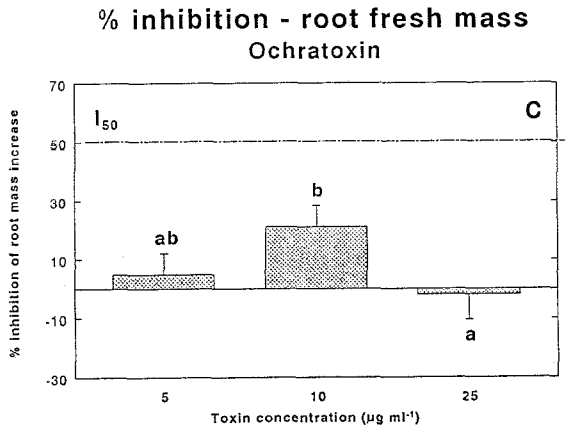


Figure 2a–c. Percentage inhibitory response (with respect to controls) of ochratoxin on plantlets of *Zea mays*. (a) Primary root length; (b) shoot length; (c) root fresh mass.

gation (Figure 1a), root mass (Figure 1c) and plantlet fresh mass (Figure 1e). The greatest inhibitory response (54%) for all toxin treatments in the present investigation was measured for primary root lengths of embryos exposed to 25 μg ml^{-1} ZEA/DON. The inhibitory effects of 25 μg ml^{-1} ZEA/DON on root fresh mass were slightly lower (45%). The inhibitory effects of 25 μg ml^{-1} on shoot mass (19–29%), shoot length (12–21%) and fresh mass (13–24%) were similar, and less inhibitory than measured for root length (34–54% inhibition) or root mass (28–45% inhibition) (Figures 1a–e). Inhibition at 5 μg ml^{-1} was always greater than for ZEA or DON alone (Figures 1a–e). At 10 and 25 μg ml^{-1}, the inhibitory responses of DON and ZEA/DON were similar. At 10 μg ml^{-1}, the inhibitory responses for DON generally exceed-

ed those of ZEA/DON, but this was reversed at 25 μg ml^{-1} (Figure 1d). The percentage dry mass of 10 and 25 μg ml^{-1} ZEA/DON-treated plantlets was significantly greater than control and 5 μg ml^{-1}-treated plantlets (Table 2).

OA. For most of the parameters measured, 5 and 25 μg ml^{-1} OA had a stimulatory effect, while 10 μg ml^{-1} was inhibitory (Table 2). These differences were, however, statistically significant for root length and root fresh mass only (10 μg ml^{-1}). When the inhibitory responses of the three toxin doses were compared, 5 and 25 μg ml^{-1}g compared statistically (Figures 2a–e).

Although both plantlet fresh and dry mass decreased with increasing OA concentration, differences were not statistically significant (Figures 2d, e).

Discussion

DON and ZEA/DON generally had an inhibitory effect on the parameters measured, while for ZEA and OA, these effects were negligible or stimulatory. Since ZEA alone stimulated root and shoot development, and DON was inhibitory, one could predict that, in combination, the effects of ZEA and DON may counteract each other (i.e. an additive response), resulting in less severe phytotoxic effects than DON alone. When comparing predicted additive effects with measured responses for ZEA/DON, the measured responses for ZEA/DON were generally greater (Table 3). At predicted responses for 50 μg ml^{-1} (25 μg ml^{-1} ZEA + 25 μg ml^{-1} DON), the inhibitory response of the measured 25 μg ml^{-1} ZEA/DON combination exceeded these values, suggesting that when ZEA and DON occur simultaneously, their phytotoxic effects may be synergistic (Table 3). These results have important implications regarding the presence in developing cereal crops of strains of *F. graminearum* producing more than one toxin (i.e. DON, ZEA, NIV, and/or ADON) [7], particularly since some of these metabolites are regarded as virulence factors in disease development [7, 11–14]. In wheat, even low incidences of *F. graminearum* and *F. culmorum* cause FHB, resulting in significant yield losses and mycotoxin contamination of grain [18, 19].

Several researchers have investigated the phytotoxic effects of *Fusarium* metabolites. In this regard, Wakuliński [20] found that of six *Fusarium* metabolites (T-2, ZEA, DAS, DON, ADON, MON), DON and 3-ADON (1–100 μg ml^{-1}) were the most inhibitory of wheat seed germination and subsequent root and leaf mass increases. ZEA was the least toxic [20]. Root development generally appeared more sensitive than shoot development to the toxins, as was observed in the present investigation with *Zea mays*. Shimada and Otani [21] found the I_{50} value (50% inhibitory effect) for DON in seven wheat varieties to be $\pm$ 10 μg ml^{-1} for shoot growth, and five-fold lower, at $\pm$2 μg ml^{-1} for root growth [21], suggesting wheat may be more sensitive than presently being reported for maize. The I_{50} value for *Z. mays* root elongation for DON was marginally below 25 μg ml^{-1} (54% inhibition at 25 μg ml^{-1}), while for the ZEA/DON combination, this was just above 25 μg ml^{-1} (43% inhibition at 25 μg ml^{-1}) (Figure 1a). For shoot length, the I_{50} value exceeded 25 μg ml^{-1} for both DON and ZEA/DON (21% and 20% inhibition, respectively, at 25 μg ml^{-1}). Interestingly, the effect of DON alone on shoot elongation was greater than for the ZEA/DON combination (Figure 1b). Bottalico et al. [22] have reported DON to be more inhibitory of root growth than of shoot/leaf growth in tomato seedlings. In wheat seeds, 50 μg ml^{-1} DON completely inhibited germination, while 10–25 μg ml^{-1} significantly reduced plantlet growth [23].

The phytotoxic effects of DON on tissues other than seeds/seedlings have also been investigated [18, 21, 24]. Calli of three wheat genotypes exhibited differing sensitivities to DON, as measured by callus regeneration (calli forming shoots) [25 μg ml^{-1} – 0–63% regeneration] [24]. A dose of 100 ppm DON proved lethal to most calli [24].

There is evidence that DON may exert it effects on nucleic acids. Packa [25] found a decrease in the mitotic index and an increase in chromosome abnormalities in cells of roots of germinated caryopses of rye, wheat, triticale and field bean treated with 10 μg ml^{-1} DON [25]. Similarly, in dividing root tip cells of DON-treated onion seedlings, mitotic activity (MI) and relative division rate (RDR) decreased [26].

In eukaryotic cells, although all 12,13-epoxytrichothecenes (DON, DAS, NIV, T-2 toxin) are reported to be cytotoxic and inhibit protein synthesis, this was dependent on the number and position of the hydroxyl groups and the type of esterifying acids [27]. DON is known to influence protein synthesis by acting on peptidyl transferase [28], and many yeasts, mammalian cells, fungi and the plant, *Baccharis megapotamica*, contain modified peptidyl transferase enzyme systems tolerant to trichothecenes [29, 30]. In wheat seedlings sensitive to both *F. culmo-*

Table 3. Predicted (unshaded) additive values (based on measured results) of percentage inhibition of root and shoot measurements of *Zea mays* embryos exposed to DON and ZEA, and measured inhibitory (shaded) responses of ZEA/DON combinations

Toxin concentration	% inhibition				
	Root length	Root fresh mass	Shoot length	Shoot fresh mass	Plantlet fresh mass
5 μg ml^{-1} ZEA + 5 μg ml^{-1} DON[a] (≡10 μg ml^{-1} toxin)	24.03	23.84	7.18	−4.66	2.42
10 μg ml^{-1} ZEA/DON[b]	33.46	24.31	20.53	22.45	13.79
10 μg ml^{-1} ZEA+ 10 μg ml^{-1} DON (≡20 μg ml^{-1} toxin)	35.07	40.53	6.36	2.77	13.01
25 μg ml^{-1} ZEA + 25 μg ml^{-1} DON (≡ 50 μg ml^{-1} toxin)	30.91	32.58	10.48	12.1	15.61
25 μg ml^{-1} ZEA DON	54.03	45.24	18.99	29.25	23.97

[a]Unshaded area represents predicted additive values (% inhibition), based on the sum of measured responses following exposure to individual toxins (i.e. ZEA + DON).
[b]Shaded area represents a measured value for the ZEA/DON combination.

rum and DON, free proline level increases have been reported, but were influenced by cultivar and DON concentration [23]. The increased proline levels have been interpreted as a plant response to pathogen attack [23]. It is possible that elevated proline levels may reflect an inhibitory effect on protein synthesis (i.e. failure to incorporate proline into proteins).

It is the opinion of Snijders & Kretching [12] that DON is essential for *F. graminearum* colonisation of plants. DON, as an inhibitor of protein synthesis, would inhibit the production of host enzymes (normally elaborated in response to fungal presence), thereby allowing fungal spread [13]. Additionally, DON being water-soluble, may be distributed to the chaff and kernel by phloem vessels, thus promoting fungal proliferation by inhibiting the plant response [12]. In this regard, Wang & Miller [6] have reported an inhibition of DON translocation in a FHB-resistant wheat line, thereby impairing fungal spread through the plant. In extensive screening for FHB resistance in wheat, it has been concluded [18, 21] that seedling response to DON does not correlate with field resistance. Additionally, Wang & Miller [6] have suggested that in tolerance/resistance of wheat (laboratory and field trials) to DON and 3-ADON (10 to 1,000-fold that of susceptible cultivars), more than one mechanism of resistance is operating.

Only a few researchers have investigated the phytotoxic effects of ZEA, a resorcyclic acid lactone frequently co-produced with DON and 3-ADON by *F. graminearum*, the pathogen causing root rot, other seedling diseases [31], head and kernel blight of wheat [32] and stalk and ear rots of maize [33]. Generally, the results of these investigations have been variable. ZEA was the least toxic of six metabolites to wheat seedlings, without significantly affecting germination or subsequent root and leaf development, even at 50 μg ml^{-1} [20]. Interestingly, in two of the three varieties, ZEA (1 and 10 μg ml^{-1}) had a slight stimulatory effect on root and leaf mass. In the present investigation, ZEA initially (at 5 μg ml^{-1}) inhibited most parameters measured, followed by a stimulatory effect at 10 and 25 μg ml^{-1} (Table 2). Since little is known about the mode of action of this mycotoxin on plant cells, the results are difficult to interpret. One must assume, then, that the stimulatory response must arise as a result of an increase in cell number, and/or an increase in cell size. One must also assume that the cells are able to metabolise and/or compartmentalise the toxin, in order to overcome the initial inhibitory response measured for *Z. mays*. It is hoped that an ultrastructural investigation of the primary root tips will shed some light on the mode of action of ZEA. This non-steroidal oestrogenic mycotoxin acts as a hormone in several *Fusarium* species, regulating the sexual stage of development (i.e. formation of perithecia) [34]. In many animal species, ZEA has oestrogenic and anabolic activities, competing with β-estradiol for

receptor binding sites in animal [35] and human tissue [36]. Interestingly, α-zearalenol (a metabolite of ZEA, particularly in man and the pig [37]), is several fold more active than the parent molecule [38]. In *Z. mays* cell suspensions, ZEA was metabolised to the α- and β-zearalenol and the β-D-glucosides of zearalenone and α- and β-zearalenol [39]. Up to 50% of the mycotoxin became bound to starch, hemicellulose, and in particular, lignin fractions, suggesting binding of zearalenone metabolites to cell wall components [39]. Scheel & Sandermann [40] have postulated that the incorporation of xenobiotic metabolites into lignin, followed by deposition in the cell wall, is a means of 'local excretion' and detoxification by plant cells. This might explain a possible 'compartmentalisation' of ZEA, but does not explain the observed stimulatory effect of ZEA on maize plantlets.

At low doses, ZEA does not appear to interfere with nucleic acids to any great extent. Packa [25] found a variable effect of 10 μg ml^{-1} ZEA on germinating seeds of wheat, rye and field bean: the mitotic activity increased for rye, but decreased for wheat and field beans. Since no abnormal metaphase chromosomes were observed [25], it would suggest that at this ZEA concentration minimal disruption of DNA had occurred.

In the present investigation, the results for OA are as difficult to interpret as for ZEA: an initial stimulatory effect (5 μg ml^{-1}), an inhibitory effect (10 μg ml^{-1}), followed by a stimulatory effect (25 μg ml^{-1}) (Table 2, Figures 2a–e). Despite the numerous reports involving the contamination of plants and plant products with OA [41, 42], there is a paucity of information regarding the phytotoxic effects of this toxin. A decline in OA has been observed during storage of cereals [43], and it is assumed that metabolic conversion may take place in plant cells, as has been reported for animals [44]. In wheat and maize cell suspensions treated with a sublethal dose of OA (5 ppm), several metabolites of OA were recovered: hydroxylated metabolites and β-glucosides of hydroxyochratoxin A [45], indicating metabolic conversion of the parent molecule. Based on their results, Ruhland et al [45] are of the opinion that OA derivatives produced by plants may be as toxic as the parent molecule, as found in human microsomes and rat hepatocytes [46]. It must be remembered, however, that the assumptions regarding toxicity of OA metabolites are based on the findings for animal cells. Plants have the ability to compartmentalise noxious substances [40], and hydroxylation and glucosidation of OA may indeed represent a detoxification mechanism. The formation of glucosides of OA by the wheat and maize cells is similar to the findings of Zill & coworkers for ZEA [39].

The apparent recovery of plantlets exposed to 25 μg ml^{-1} OA following the observed inhibitory response at 10 μg ml^{-1} could indicate the existence of mechanisms (metabolism; detoxification; compartmentalisation) to overcome the phytotoxic effects. Since Ruhtand & coworkers [45] have demonstrated that wheat and maize cells are able to metabolise OA, it is possible that the metabolites produced are rendered less toxic, or may somehow be sequestered away from the toxin site/s of action. In one of the few reports on the possible mode of action of OA albeit (in the animal literature), it has been proposed that OA is able to influence lipid peroxidation [47]. If this is indeed the case, and OA exerts minimal phytotoxic effects in comparison with other mycotoxins, then it must be assumed that harmful free radicals that may be generated in plant tissues are adequately scavenged.

While, in the present investigation, DON and the ZEA/DON combination proved to be the most phytotoxic (Figures 1a–e), these mycotoxins were less toxic than has been reported for AFB_1, using the same *Z. mays* embryo bioassay [48, 49] (Table 4). In that investigation, the I_{50} value of AFB_1 for root elongation was $\pm$7.5 μg ml^{-1} toxin. The maximum inhibitory response for any root parameter measured in the present investigation was 54% (root elongation at 25 μg ml^{-1} ZEA/DON), as compared with 81% for AFB_1 (Table 4). The results for DON and ZEA/DON for shoot parameters were somewhat lower than for AFB_1, suggesting that the phytotoxic effects of DON and ZEA/DON may be more localised (i.e. at the root level) than for AFB_1 . It is not known, however, whether DON and ZEA are transported to higher plant parts, but based on the literature (i.e. that DON is water-soluble), it is assumed that toxin may (to some extent) reach aerial plant parts. AFB_1 was indeed able to do so, and so some of the inhibitory effects on shoot development may have resulted directly from toxin exposure following translocation [49].

The increase in plantlet dry mass following exposure to the more phytotoxic mycotoxins (DON, ZEA/DON) is interesting. This increase may arise either as a result of the incorporation of toxin molecules into the cellulose and hemicellulose of the wall, and/or as a result of cell wall thickening as a possible defence mechanism. Zill et al. [39] found that in maize suspension cultures exposed to ZEA, the metabolites produced were incorporated into plant cell walls. Thick-

Table 4. Comparison of maximum inhibitory response of aflatoxin B_1 [49] and toxins used in the present investigation on root and shoot development

Parameter	Maximum inhibitory response (% inhibition)	
	Present investigation	Aflatoxin B_1 [49]
Root length	54.03 ± 3.90 (ZEA/DON – 25 $\mu g\ ml^{-1}$)	81.00 ± 1.33
Shoot length	24.60 ± 6.81 (DON – 10 $\mu g\ ml^{-1}$)	37.68 ± 4.69
Root fresh mass	45.57 ± 7.41 (DON – 25 $\mu g\ ml^{-1}$)	54.32[a]
Shoot fresh mass	29.25± 5.40 (ZEA/DON – 25 $\mu g\ ml^{-1}$)	54.22-[a]

[a]SE not determined (seedlings pooled).

ening of cell walls has frequently been reported in the literature as a response to fungal or toxin presence (i.e. maize callus exposed to FB_1) [50]. Cell wall thickening has been described in cells of maize leaves innoculated with *Puccinia graminis* f.sp. *tritici* or treated with exudates of germinating urediospores [51]. Wheeler [52] has suggested that wall deposits in disease or toxin-treated plants may function as a protective barrier over damaged areas of the plasma membrane.

The apparent synergistic effect of DON and ZEA in combination reported in the present investigation emphasizes the importance of considering toxin combinations in studying the role of mycotoxins in plant disease. This is of particular relevance since a number of the *Fusarium* species implicated in plant disease may produce more than one mycotoxin (e.g. *F. graminearum* is capable of producing DON, NIV, ADON and/or ZEA). It may then be that symptoms associated with a particular disease (e.g. FHB) in the affected plant result from the presence of more than one toxin.

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