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Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species

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Abstract

Fusarium head blight (FHB) is a complex cereal disease associated with trichothecene production; these mycotoxins are factors of aggressiveness in wheat. Six species (bread and durum wheat, triticale, rye, barley and oats) were submitted to point inoculations with two isogenic strains of *Fusarium graminearum*; a wild strain (Tri5+) produced trichothecenes and the mutated strain (Tri5−) did not. The trichothecene-producing strain was generally more aggressive than the non-producing strain, but this varied according to crop species. The difference in aggressiveness was less pronounced in rye, a very resistant species. High resistance levels were observed in oats due to the large spacing between florets. In six-row barley, despite the existence of a moderate Type II resistance, the fungus was often observed to move externally from one floret to another within the dense spike, without penetrating the rachis. Bread wheat had low resistance to the trichothecene-producing strain and good resistance to the non-producing strain. Triticale responded to the strains in a similar way but was somewhat more resistant to both: symptoms on the spikelets and rachis of the triticales were restricted to below the point of inoculation. Durum wheat was susceptible to the trichothecene-producing strain and only moderately resistant to the non-producing strain, which was able to cause serious damage only to this species. Our study confirmed that the role of trichothecenes in FHB pathogenesis differs among species. The failure of the trichothecene non-producing *F. graminearum* strain to spread within the inflorescence of wheat, triticale, rye and barley, and the significant reduction of spread in the durum wheat spike strongly suggested that trichothecenes are a major determinant of fungal spread and disease development in *Triticeae*.

Abbreviations: DON – deoxynivalenol; FHB – Fusarium head blight; scab.

Introduction

Fusarium head blight (FHB) is a major problem in many cereal species, and can be devastating during warm and humid seasons in cereal-growing regions of Canada, USA, China and Europe (McMullen et al., 1997). In North America, severe outbreaks of FHB are principally caused by *Fusarium graminearum* (teleomorph = *Gibberella zeae*) (McMullen et al., 1997; Clear and Patrick, 2000). North American FHB epidemics have been documented in 26 states in the USA and five provinces in Canada. Economic losses since 1990 have been estimated at 3 billion US$

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in wheat and 0.4 billion US$ in barley (Windels, 2000). FHB affects yield through premature death of spikes and abnormal grain filling (Desjardins et al., 1996; Ribichich et al., 2000). Several mycotoxins are produced by *F. graminearum* that make the infected grain unsuitable for human and livestock consumption (Ueno, 1977). Mycotoxins produced by *F. graminearum* belong to the zearalenone and trichothecene families. Zearalenone is similar in structure to oestrogen and induces similar responses in mammals (Hidy et al., 1966). Trichothecenes comprise a class of mycotoxins which include deoxynivalenol (DON), the acetylated forms 3-ADON and 15-ADON, nivalenol, diacetoxyscirpenol and the T-2 and HT-2 toxins. They are protein synthesis inhibitors in eukaryotic cells (Ueno, 1977; Miller et al., 1999). Many trichothecenes are associated with FHB, although the predominant one produced by *F. graminearum* is DON (Lemmens et al., 1997).

Up to six types of resistance to FHB have been described (Ban, 2000). Schroeder and Christensen (1963) identified resistance to initial infection (Type I) and resistance to the spread of the infection within a plant (Type II). Type I resistance has been attributed to the constitutive and/or de novo production of biochemical compounds in the infected tissues (Doohan et al., 2000; Mohammadi and Kazemi, 2002; Siranidou et al., 2002). Resistance Type II mechanisms remain poorly described, but spread of infection in wheat was minimal or nil with a trichothecene non-producing (Tri5−) *F. graminearum* strain (Proctor et al., 1995). The ability of the host to degrade and tolerate deoxynivalenol (DON) was considered as the means of Types III and IV resistance, respectively (Miller et al., 1985, 1986; Wang and Miller, 1988). Possible relationships between Types II, III and IV resistance mechanisms have not been examined. Recently, two additional types of resistance were added: Type V resistance to kernel infection (Mesterházy, 1995), which is measured by threshing infected spikes and observing the damage to the kernels, and Type VI, which is tolerance to FHB (Mesterházy, 1989, 1995; Mesterházy et al., 1999), assessed by comparing grain yield in diseased vs. symptom-free spikes or plots. These forms of resistance are race non-specific and likely to be controlled by minor genes (Bai and Shaner, 1994, 1996).

Some researchers have reported a strong association between FHB severity and DON concentration in infected grain (Hart et al., 1984; Wang and Miller, 1988; Snijders and Krechting, 1992; Wong et al., 1994), while others failed to detect an association (Snijders and Perkowski, 1990). The diversity of trichothecene molecules could contributes to Fusarium virulence and genetic variability, and the total trichothecene toxin-producing capacity of the isolates might be the decisive component of pathogenicity (Mesterházy et al., 1999). Arseniuk et al. (1993) showed that inoculum containing a mixture of *Fusarium* spp. produced a more severe FHB reaction than inoculum containing individual species. Since different species of *Fusarium* can produce different trichothecenes, this supports the theory that diversity of trichothecenes may contribute to FHB intensity. The association between aggressiveness of *F. graminearum* and their production of DON in the infected grain was stronger for the nivalenol-producing isolate F89.4 (from France) when the sum of DON and nivalenol content was considered (Mesterházy et al., 1999). Mixtures of *F. graminearum* strains or *Fusarium* spp. often coexist within the same barley head and sometimes in the same seed (McCallum et al., 1999, 2001). The phytotoxicity of trichothecenes, associated with resistance Types III and IV, and DON degradation and tolerance, respectively, were also found with wheat and barley seedlings. Coleoptile and root growth of both species were affected by DON, but wheat seemed to be more sensitive than barley (Wang and Miller, 1988; Eudes et al., 2000; Dahleen and McCormick, 2001).

The trichothecene-deficient mutant GZT40 (Tri5−) was generated by gene disruption of the *Tri5* gene of the trichothecene-producing *F. graminearum* strain GZ3639 strain (Proctor et al., 1995). Those two strains provided a useful tool to study the role of trichothecenes in the interaction of cereals with *F. graminearum*. The trichothecene-producing strain was more aggressive than the trichothecene non-producing strain in a Fusarium seedling blight assay in wheat, oats and winter rye, and in a head blight assay in wheat (Proctor et al., 1995; Desjardins et al., 1996; Eudes et al., 2001; Bai et al., 2001). However, in maize no significant difference was observed between strains (Proctor et al., 1995; Harris et al., 1999). The role of trichothecenes in the development of Gibberella

ear rot of maize was less important since the trichothecene non-producing strain was still very pathogenic and spread within maize ears (Harris et al., 1999). The importance of the role of trichothecenes appeared to be host species-specific, based on a comparison of wheat and maize (Proctor et al., 1995; Harris et al., 1999). Trichothecenes had been proposed as the main virulence factor in FHB in wheat, playing an important role in the spread of *F. graminearum* within the spike, but having a negligible effect on initial infection (Desjardins et al., 1996; Eudes et al., 2001; Bai et al., 2001). Yet, no data were available about the effects of these two *F. graminearum* strains relative to FHB disease in other small-grain cereal species, including major commodities like barley and durum wheat. The research presented here was designed to fill this gap and study the impact of trichothecenes in the pathogenesis of *F. graminearum* during FHB development in six different cereal species.

Materials and methods

Six cereal species were studied in this experiment, common wheat (*Triticum aestivum*), durum wheat (*T. durum*), triticale (*X Triticosecale*), rye (*Secale cereale*), barley (*Hordeum vulgare*) and oat (*Avena sativa*), using four genotypes of each. The cultivars and lines were chosen to represent available resistance levels for each species. The choice of lines and cultivars was based on unpublished information generated by several scientists in Canada, part of the data coming from cereal registration trials (A. Devaux, MAPAQ, St-Hyacinthe; J. Gilbert, CRC, AAFC, Winnipeg; S. Rioux, and Y. Dion, CEROM, St-Bruno-de Montarville; J. Collin, Laval University, Ste-Foy; G. Fedak, ECORC, AAFC, Ottawa). The cereal lines included four Canadian oat cultivars (Capital, Rigodon, Sylva, and Triple Crown); four rye lines including the Canadian cultivars Gazelle and Prolific, a Brazilian landrace, Paulo Frontin, and BR1-A1, a reselection from the Brazilian cultivar BR1; three Canadian barley cultivars (ACCA, Béluga and Chapais) and the FHB resistant barley Chevron (Ma et al., 2000). The durum wheat included two Canadian durum cultivars Kyle and Plenty, plus US cultivar Edmore and Ste-Foy durum line 88 ADD-11.7 derived from a durum/aestivum// durum cross. The bread wheat lines included the Chinese resistant check Sumai 3 (Bai and Shaner, 1996) and three Canadian cultivars (McKenzie, AC Barrie, and Neepawa). Triticales included two Canadian cultivars, AC William and Bura, the CIMMYT triticale Gnu's, and Ste-Foy breeding line QT 12.16.

Nine plants per genotype were grown in 144 mm diam pots set up in a randomized factorial design with three replicates. Plants were grown in a greenhouse with a 16 h photoperiod (1000 W mercuric lamp, 140 μE m-2 s) at 20–26 °C during the day. Plants were watered each day and fertilized (20-20-20) once a week. The experiment was repeated twice.

Two strains of *F. graminearum* were used, the trichothecene non-producing strain GZT40 and the trichothecene-producing strain GZ3639. Macroconidia formation was induced in modified carboxymethyl cellulose (CMC) medium (Cappellini and Peterson, 1965) ($MgSO_4 \cdot 7H_2O$ 0.5 g l^{-1}, NH_4NO_3 1 g l^{-1}, KH_2PO_4 1 g l^{-1}, bacto yeast extract (Difco 212750) 1 g l^{-1}, CMC (Sigma C9481) 15 g l^{-1}, Antifoam (Sigma C9481) 0.5 g l^{-1}), supplemented with 50 ppm of streptomycin sulphate before the fungus was added to the medium. *Fusarium graminearum* strains were cultured 24 days at 25 °C with oxygenation in a growth-room (40 W Sylvania tube, one Blacklight Blue for one Cool White) set for a 12 h photoperiod. After filtration through two layers of cheesecloth, the density of the spore suspension was evaluated using a hemacytometer, and 50 ppm of streptomycin sulphate was again added. The inoculum was kept at 4 °C, and the percentage of viable spores was verified daily and standardized at 1×10^{-4} colony forming units per ml.

Three to five spikes (sampling) per pot (experimental unit) were inoculated with each *F. graminearum* strain at anthesis using the point inoculation method. In the middle of each spike, 10 μl of macroconidial suspension was injected into two florets of opposite and median spikelets. A white-translucent glassine paper bag was placed over the inoculated spikes to prevent cross-infection and to maintain a humid environment.

Twenty days after inoculation, data were recorded from successful infections. Variables recorded included the number of spikelets with symptoms (either discolouration or spikelets with visibly infected grains) from the inoculation point

to the bottom, the length of rachis or floral peduncle discoloured below the inoculation point, and the percent of the spike that showed bleaching above the inoculation point (wilt). Rachis discolouration was measured with a ruler, below the inoculation point; discolouration going lower into the peduncle or stem was included. For the purpose of this research, the pedicels of oats were considered equivalent to rachis and peduncle tissue, and the cluster of sessile flowers at a rachis node of six-row barley were considered to represent an equivalent of a wheat spikelet. ANOVAs and correlations were calculated using SAS Software (SAS Institute Inc., 1988).

Results

Fungal attack became visible on floral parts and grain of the attacked spikelets after 6–12 days. Yet, above the inoculation point, bleaching (with very limited browning) was often observed after the first week, generally without much sign of fungal invasion above the inoculation point. Seeds in the upper part of the spike shrivelled and died young and did not show external signs of fungal attack. Visual observations on the infection process indicated that most of the contamination took the route of the rachis, but direct floret to floret contamination was observed as a significant phenomenon in six-row barley. This route was apparently as rapid, or more rapid than the rachis-related spike invasion only in barley. Correlations among variables observed were high, especially for the length of discolouration in the rachis vs. total number of infected spikelets ($r = 0.972$). Upper spike bleaching percentage also correlated with the disease spread in the rachis ($r = 0.849$) and with the number of infected spikelets ($r = 0.868$ overall, and $r = 0.856$ strictly within the Triticeae). The aggressiveness of the trichothecene-producing strain GZ3639 and of the trichothecene non-producing strain GZT40 was compared within and between species.

Spread to new spikelets

Both strains behaved similarly in oats, showing no difference among cultivars, all of which were quite resistant regardless of fungal strains (Figure 1a). In rye and barley, only Prolific (Figure 1b) and ACCA (Figure 1f) respectively, displayed a reduced spread of the trichothecene non-producing strain to new spikelets. In all other species and respective lines, the trichothecene non-producing strain was significantly less aggressive (Figure 1c–e), except in FHB-resistant cv. Sumai 3. The contrast in aggressiveness was most clearly discerned in durum wheat (Figure 1e), triticale (Figure 1c) and the three Canadian common wheat cultivars (Figure 1d). The trichothecene non-producing strain remained contained within the inoculated spikelet of every species, except in durum wheat. Even in the absence of trichothecenes, there were more than three damaged spikelets per spike in durum wheat lines (Figure 1e).

Durum wheat lines were the most susceptible cereal lines to the trichothecene-producing strain ($P < 0.0001$). Triticale and common wheat were not significantly different ($P = 0.4024$) to each other, but more susceptible than barley ($P < 0.003$). Rye and oats were not significantly different ($P = 0.6825$), but much less susceptible to the trichothecene-producing strain than all other species ($P < 0.001$). When inoculated with the trichothecene non-producing strain, GZT40, durum wheat was significantly more susceptible than other species ($P < 0.0001$). The only other differences detected with this strain were between barley and rye ($P = 0.0062$) or triticale ($P = 0.0199$); in rye and triticale fewer infected spikelets developed disease symptoms, while almost all barley spikelets inoculated with strain GZT40 became discoloured.

In barley and oats, differences among cultivars were not significant with either strains ($P > 0.08$, $P > 0.26$, respectively). In spring rye, the FHB resistant selection BR1-A1 was more resistant to the trichothecene-producing strain than cv. Gazelle ($P = 0.0152$) and cv. Prolific ($P = 0.0057$), while Paulo Frontin was intermediate. Under artificial inoculation with the trichothecene non-producing strain, no differences were reported among rye lines ($P > 0.19$). Rye line BR1-A1 was the most resistant cereal among all tested. In Triticales, cv. Bura was less susceptible than the other triticales ($P < 0.037$) to the wild strain, but triticale lines were not different in tests with the mutated strain ($P > 0.27$). In common wheat, the resistant check Sumai 3 was much less susceptible than the Canadian cultivars in the test with the trichothecene-producing strain ($P < 0.0001$). No

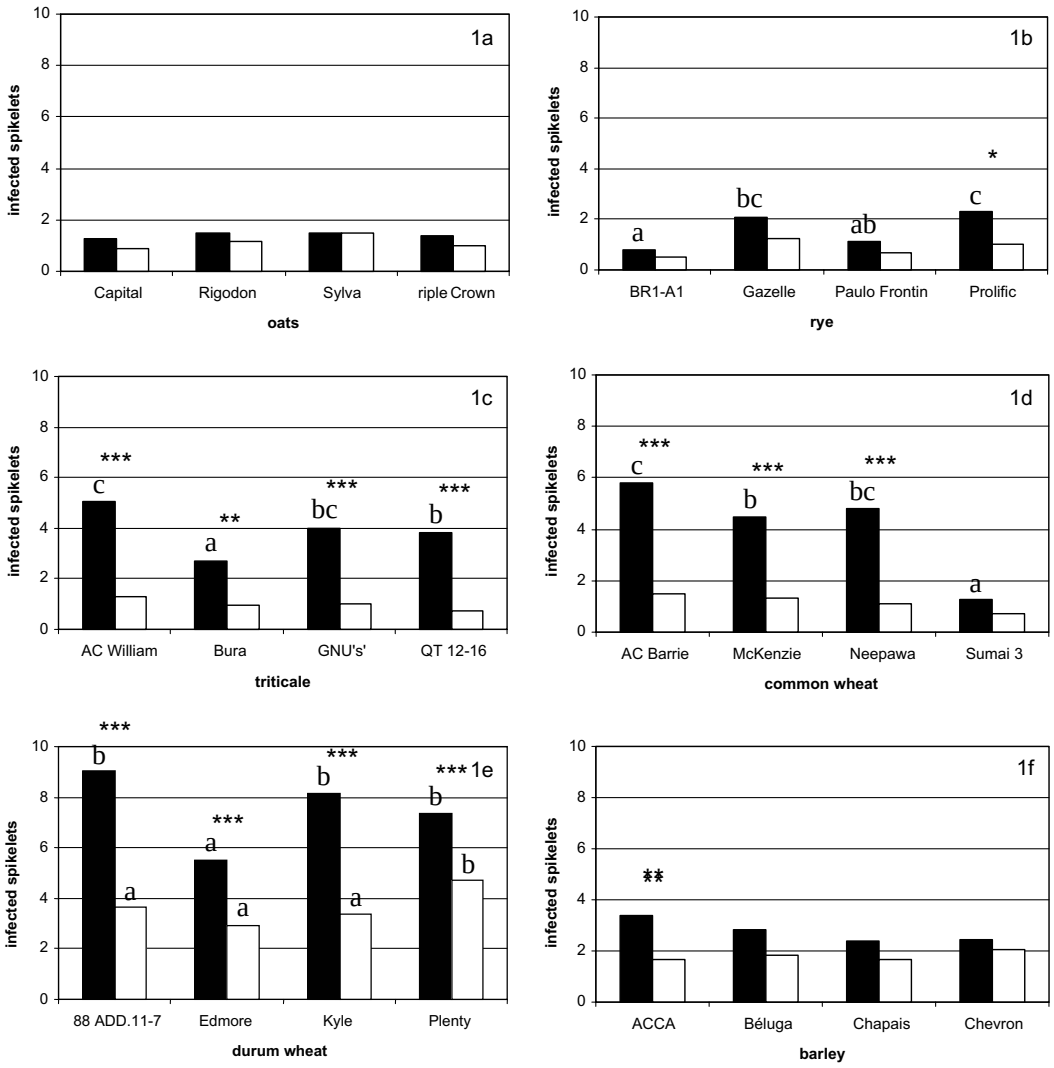

Figure 1. Impact of *Fusarium graminearum* trichothecene-producing and non-producing strains on basipetal spread of the disease in six small-grain cereal species. (■) trichothecene-producing strain GZ3639; (□) trichothecene non-producing strain GZT40. Significant cultivar*strain differences are indicated using stars: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Different letters indicate significant differences ($P < 0.05$) among cultivars, within species and strains.

differences were recorded with GZT40 strain ($P > 0.16$). In durum wheat, which was overall a highly susceptible species, cv. Edmore showed a lower susceptibility than the other three durum lines ($P < 0.001$). With the trichothecene non-producing strain, cv. Plenty was more susceptible than the other durum lines ($P < 0.001$).

Spread in the rachis

The data on symptom progression in the rachis (or floral pedicels in the case of oats) showed a strong contrast between the trichothecene-producing and non-producing strains, and followed a similar trend to the data on infected spikelets. Both strains behaved similarly in oats, rye and barley, showing no differences among cultivars, all being quite resistant to spread in the rachis or floral peduncle regardless of fungal strain (Figure 2a, 2b and 2f). In triticale, common wheat and durum wheat lines, the trichothecene non-producing strain was significantly less aggressive (Figures. 2c–e), except in FHB–resistant bread wheat cv. Sumai 3. The contrast in aggressiveness

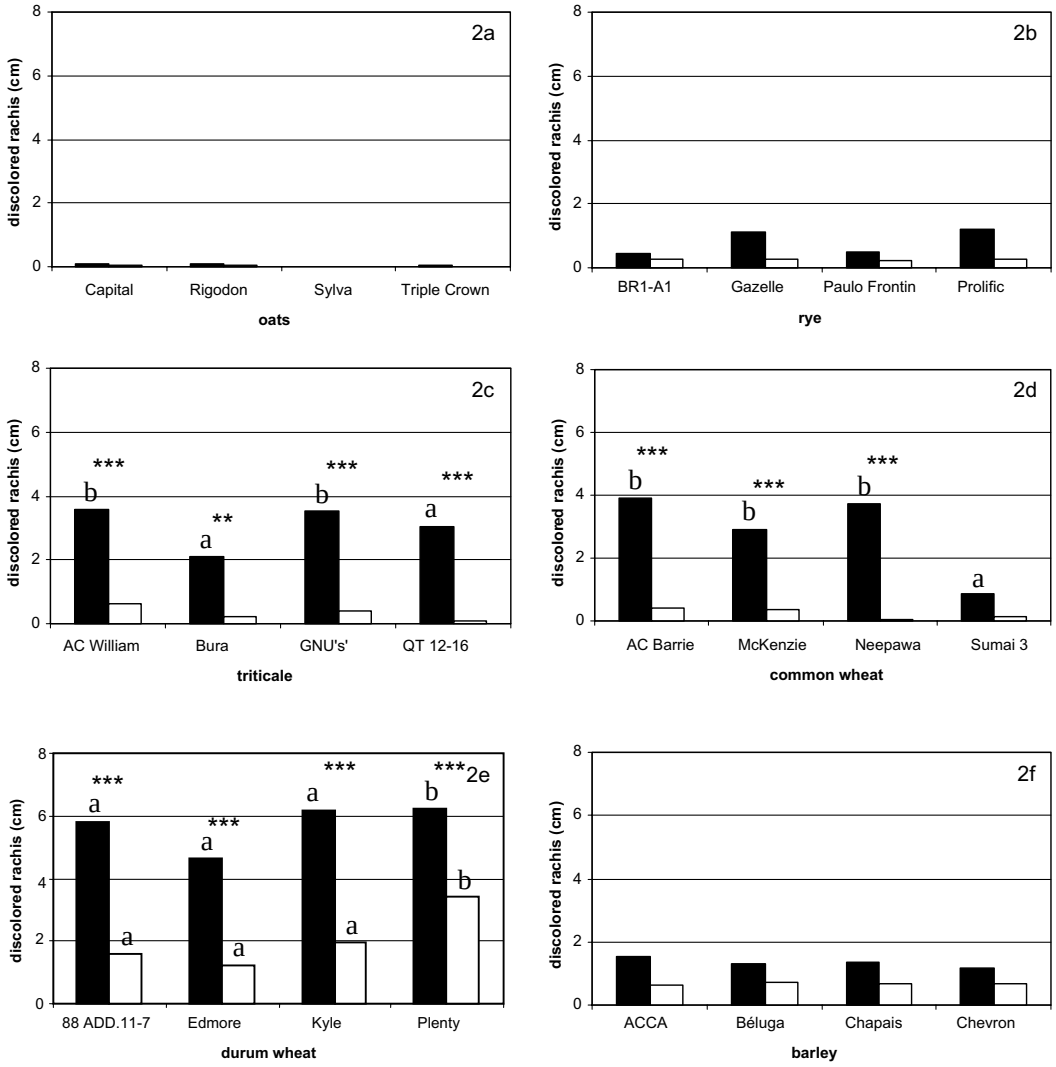

Figure 2. Impact of *Fusarium graminearum* trichothecene-producing and non-producing strains on basipetal spread of the disease in the rachis or floral peduncle of six small-grain cereal species. (■) trichothecene-producing strain GZ3639; (□) trichothecene non-producing strain GZT40. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Different letters indicate significant differences ($P < 0.05$) among cultivars, within species and strains.

was most impressive in durum wheat (Figure 2e), triticale (Figure 2c) and Canadian common wheat cultivars (Figure 2d). Again, the trichothecene non-producing strain was not contained in durum wheat, but induced discolouration on average of more than 3 cm length of the rachis in cv. Plenty.

The trichothecene-producing strain caused more discolouration of the rachis in durum wheat than in other species ($P < 0.0001$). Triticale and common wheat were not significantly different from each other ($P = 0.6578$), but were more susceptible than barley and rye ($P < 0.0001$). The latter species had similar rachis discolouration to barley ($P = 0.0788$) but more than oats ($P < 0.0001$), a panicle inflorescence type species. The trichothecene non-producing strain caused more discolouration of the rachis in durum wheat than in other species ($P < 0.0001$). When inoculated with the trichothecene non-producing strain, durum wheat showed significantly more rachis discolouration than other species ($P < 0.0001$). The only other differences detected with the GZT40 strain were between barley and oats ($P = 0.0475$).

In oats, barley and rye, the differences among cultivars were not significant when inoculated with either the trichothecene non-producing strain or the trichothecene-producing strain ($P > 0.21$). Important disease symptoms in the rachis were seen in triticale, and in *Triticum* species inoculated with the trichothecene-producing strain. The triticale, cv. Bura, was less susceptible than triticales AC William and GNU's ($P < 0.022$), while QT 12–16 was intermediate. The resistant check Sumai 3 was much less susceptible to rachis discolouration than the Canadian cultivars ($P < 0.002$). In the highly susceptible durum wheat species all lines responded with severe symptoms, especially cv. Plenty in which rachis discolouration extended furthest (Figure 2e). No differences in rachis discolouration were observed among triticale lines ($P > 0.38$) and common wheat cultivars ($P > 0.55$).

Head bleaching

Percentage head bleaching of the upper part of the spike showed a strong contrast between the trichothecene-producing and non-producing strains only in common wheat and durum wheat (Figure 3). However, Sumai 3 behaved differently, with no head bleaching induced by either strain.

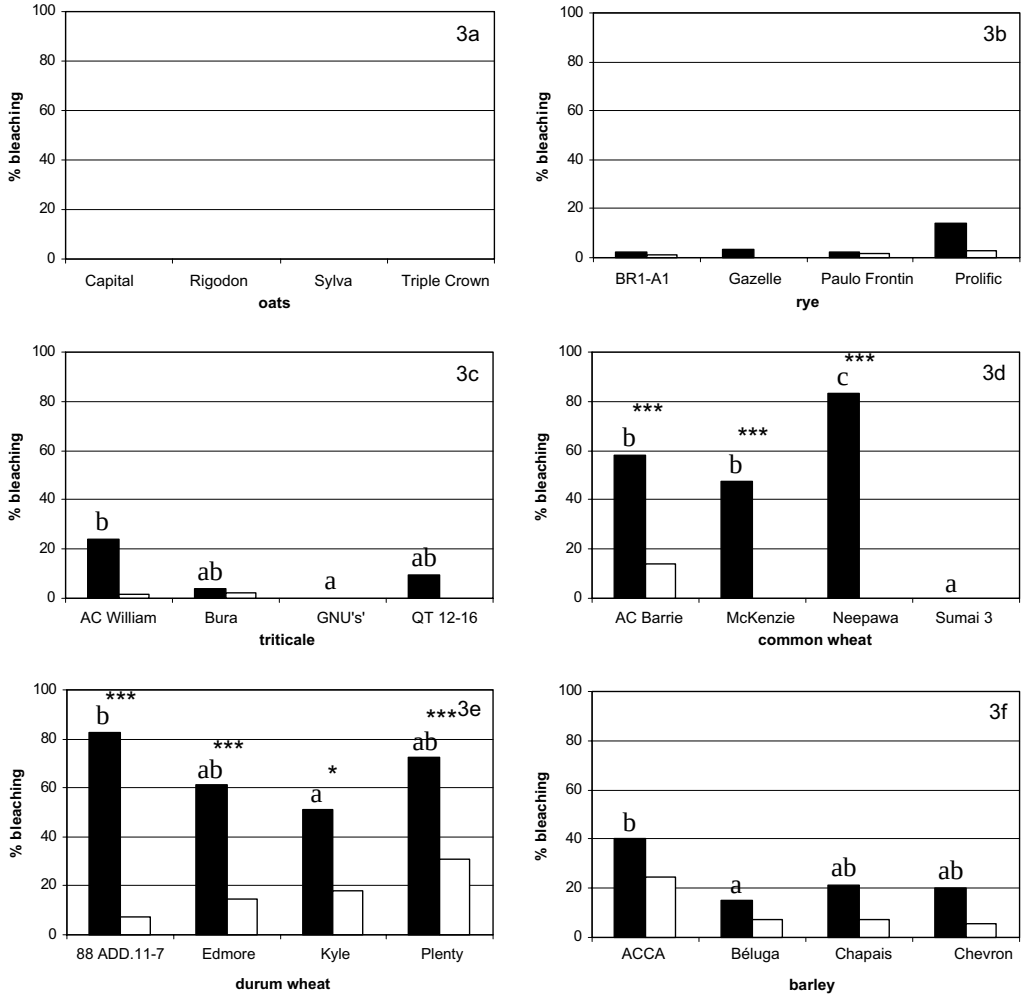

Figure 3. Impact of *Fusarium graminearum* trichothecene-producing and non-producing strains on spike bleaching in six small-grain cereal species. (■) trichothecene-producing strain GZ3639; (□) trichothecene non-producing strain GZT40. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Different letters indicate significant differences ($P < 0.05$) among cultivars, within species and strains.

Cultivars of other species behaved similarly when inoculated with either one of the fungal strains ($P > 0.063$).

Percentage head bleaching related to trichothecenes was very high in the *Triticum* species, intermediate in barley and triticale, low in rye and not visible in oats (Figure 3). Durum wheat showed more head bleaching induced by the trichothecene-producing strain than any other species ($P < 0.005$), although Canadian wheat cultivars were quite similar. The *Triticum* species were more susceptible to bleaching than barley ($P < 0.0001$). Triticale, rye and oats were not significantly different from each other ($P = 0.1544$), but less susceptible than other species ($P < 0.0095$). There was significantly more head bleaching in durum wheat when inoculated with the trichothecene non-producing strain than other species ($P < 0.0001$). Barley was intermediate ($P = 0.2645$). Bleaching in the barley cv. ACCA and wheat cv. AC Barrie was more than 20 and 10%, respectively.

A graph of the infected spikelets vs. percent head bleaching (Figure 4) showed that for a given number of spikelets infected with the trichothecene-producing strain, triticale had less bleaching than expected based on a global regression line for the *Triticeae* species. Besides this, durum wheat infected with the trichothecene non-producing strain showed to some extent a similar phenomenon.

Discussion

The current experimental data come from point inoculation protocols, designed for Type II resistance evaluation, also defined as resistance to spread. The definition leaves room for questions about how symptoms relate to the spread of the fungus above and below the infection point, and about possible correlations with Types III and IV resistance. Accurate assessment of a specific resistance type is difficult because other types can confuse the results and very few mechanisms of resistance have been properly described.

Components of Type II resistance other than the general plant defence systems thus remain poorly defined. However, the ability of resistant plants to degrade or to tolerate higher trichothecene con-

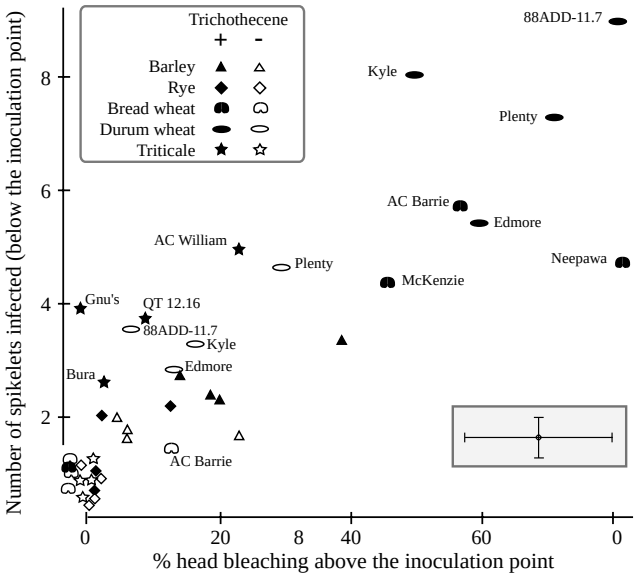

Figure 4. Head bleaching vs spikelets infected, for all cultivars of Triticeae and both strains of fungus. Results from trichothecene non-producing strains are with empty symbols and italicized names. Specific durum wheat, bread wheat and triticale lines are identified when feasible on the graph. The standard error values are in the gray rectangle. Correlation between parameters is $r = 0.856$, yet the triticales inoculated with the wild strain have a higher number of spikelets infected than the bleaching would lead to believe (leftmost gray triangle), and a similar case is seen for some durum lines inoculated with trichothecene non-producing *F. graminearum* (rightmost gray triangle).

centrations is now recognised as being likely to have an impact during fungal spread in the *Triticeae*. The possibility that trichothecenes serve as virulence factors in wheat FHB was suggested (Proctor et al., 1995; Desjardins et al., 1996) and later confirmed by two other groups (Bai et al., 2001; Eudes et al., 2001). In the present study, we reported a difference in aggressiveness between *F. graminearum* GZT40 and GZ3639 in head blight of common wheat, durum wheat, triticale and one barley and one rye cultivar (Figures. 1 and 2). In the absence of trichothecenes, the same cereal lines, except for durum wheat, were restricted *Fusarium* growth to the infected florets. The trichothecene non-producing strain GZT40 grew in the rachis and spread rapidly in spikes of *Triticum durum*, although to a lesser extent than the wild strain GZ3639. The failure of the trichothecene non-producing *F. graminearum* strain to spread within the inflorescence of common wheat, triticale, rye and barley, and the significant reduction of spread in the durum wheat spike suggests that trichothecenes are a major determinant in the ability of the fungus to develop in *Triticeae*. The high susceptibility of durum wheat to both strains may indicate evidence for the absence of some basic Type II resistance mechanism(s) unrelated to trichothecenes. Conversely, this may suggest that barley, rye, triticale and common wheat genotypes possess mechanisms of resistance to fungal spread that are absent in durum wheat. Compared to common wheat species and triticale, the rye and barley lines tested showed a better resistance to spread of the trichothecene-producing strain, with differences of aggressiveness between *F. graminearum* strains only in one cultivar for each species. However, Type II resistance needs to be improved in barley. The resistance in rye is high and could be useful; it is expressed in some of the primary triticale lines created from wheat/rye crosses (Fedak et al., 1997).

The description of genes or markers associated with Type II resistance in wheat and barley is related to the method used to test the plants rather than to the actual mechanism of defence, which might be misleading (Singh and van Ginkel, 1997). It is possible that genes and markers we associated with resistance to spread in common wheat and barley are in fact confounded by mechanisms of trichothecene resistance and tolerance. Interestingly, wheat lines resistant to fungal spread, such as Sumai 3 and Nobeoka Bozu, have the ability to degrade and accumulate very low concentrations of DON (Miller et al., 1985; Atanassov et al., 1994). Trichothecenes are not volatile and must move into plant tissues internally. Visual observations of the spread of the fungus support the hypothesis that trichothecene-related resistance mechanisms might be more effective within the rachis, which is an important transport area for the toxin, and less effective in anthers and glumes.

Relative to other species tested, durum wheat, a species with A and B genomes, had poor resistance to the spread of both strains. The effect of a trichothecene non-producing strain on durum wheat might be due to other substances produced by the genus *Fusarium* such as enniatins, and cyclic depsipeptides, known to be produced by other *Fusarium* species, although not by *F. graminearum* (Hohn, 1997), or be interpreted as being due to a lack of general defence mechanisms in *T. durum*. Bread wheat and triticale also have A and B genomes, but resisted the trichothecene non-producing strain. Although this suggests that trichothecene resistance may be easier to find in genomes D and R, Sumai 3 resistance to FHB was not conferred by the D genome (Gilbert et al., 2000; Anderson et al., 2001). Attempts to improve trichothecene resistance and tolerance, and resistance to fungal spread in durum wheat might benefit from genes from the wild or cultivated relatives of this species. However, the FHB problems of durum are clearly more severe. New questions or hypotheses could be investigated related to the biochemical reasons for a putative weakness of general defense mechanisms in durum wheat.

The frontier point among organs (glumes, seeds, rachis) may represent a temporary barrier to further invasion by mycelium and / or a barrier to toxin movement. In some cases especially in barley, the fungus was observed to bypass the rachis and jump to adjacent florets directly, regardless of the presence or absence of trichothecene production. This was later confirmed by field observations. Anatomical differences between species thus have important effects and must be discussed; such traits have been found well correlated to FHB resistance in barley (Zhu et al., 1999; Ma et al., 2000) and wheat (Couture, 1982; Mesterházy, 1995). In barley, triticale and wheat, the flowers are very close to the rachis and to each other. However, in oats, we see a much longer path and

an aerated inflorescence. *Triticeae* with only one flower per rachis node, like rye, or two-rowed barley, which was not part of the current study but is commonly recognised as more FHB–resistant than six row barley (Zhu et al., 1999), may benefit from a morphological advantage.

Plant tissue that shows symptoms in Type II testing protocols is not necessarily invaded by *F. graminearum*. Bleaching of the upper spike was frequent in wheat, with little DON and fungus above the inoculation point (Savard et al., 2000). Bleaching (wilt) above the inoculation point relates to blocking of phloem and xylem, and grains above that point tend to shrivel and die without being rapidly attacked by the fungus. In the present study, triticales developed levels of disease in the spikelets and rachis that were similar to common wheat, but significantly less bleaching (Figure 4). In field evaluations, symptom ratings were partly based on bleaching, and while some triticales suffered little bleaching, they had 3–10 times higher DON levels than expected according to symptoms (unpublished data, A. Devaux, MAPAQ; Y. Dion and S. Rioux, CEROM). Studies of DON levels in grains and floral organs of many lines of triticale would therefore provide valuable information.

The approach of comparing cereal species has lead to better insight about the nature of FHB resistance and tolerance mechanisms. It is concluded that there is a diversity of resistance mechanisms in different cereal species. A functional trichothecene pathway increases the aggressiveness of the fungus, and trichothecene-related resistance still appears to be an important component of maximal resistance in the *Triticeae*. In a recent study, Mesterházy (2002) concluded that in a given cultivar, the level of resistance is more important in governing DON accumulation than the aggressiveness of an isolate. Frontier points among resistance Types II, III and IV are definitively not obvious, but these resistance types may all reduce the toxic effect of trichothecenes on the spike and grains.

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