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Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of *Fusarium*

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Abstract

The phytotoxicity of the *Fusarium* trichothecene and fumonisin mycotoxins has led to speculation that both toxins are involved in plant pathogenesis. This subject has been addressed by examining virulence of trichothecene and fumonisin-nonproducing mutants of *Fusarium* in field tests. Mutants were generated by transformation-mediated disruption of genes encoding enzymes that catalyze early steps in the biosynthesis of each toxin. Two economically important species of *Fusarium* were selected for these studies: the trichothecene-producing species *Fusarium graminearum*, which causes wheat head blight and maize ear rot, and the fumonisin-producing species *F. verticillioides*, which causes maize ear rot. Trichothecene-non-producing mutants of *F. graminearum* caused less disease than the wild-type strain from which they were derived on both wheat and maize, although differences in virulence on maize were not observed under hot and dry environmental conditions. Genetic analyses of the mutants demonstrated that the reduced virulence on wheat was caused by the loss of trichothecene production rather than by a non-target mutation induced by the gene disruption procedure. Although the analyses of virulence of fumonisin-non-producing mutants of *F. verticillioides* are not complete, to date, the mutants have been as virulent on maize ears as their wild-type progenitor strains. The finding that trichothecene production contributes to the virulence of *F. graminearum* suggests that it may be possible to generate plants that are resistant to this fungus by increasing their resistance to trichothecenes. As a result, several researchers are trying to identify trichothecene resistance genes and transfer them to crop species.

Introduction

The genus *Fusarium* produces numerous and chemically diverse mycotoxins. The harmful effects of many of these toxins on animal health are well documented, however, their effects on plant health are poorly understood. Because some *Fusarium* mycotoxins are phytotoxic and many *Fusarium* species are aggressive plant pathogens, it seems likely that some of these mycotoxins contribute to the ability of *Fusarium* to cause plant disease. Such a role for *Fusarium* mycotoxins in plant pathogenesis seems even more likely given the critical roles that fungal toxins play in the pathogenicity of fungi such as *Cochliobolus* (Yang et al., 1996).

At the National Center for Agricultural Utilization Research, work on mycotoxins and plant pathogenesis has focused on two groups of mycotoxins, the trichothecenes and the fumonisins. Although these toxins are produced by a number of *Fusarium* species, our efforts have dealt primarily with two economically important species: *F. graminearum*, which produces the trichothecenes deoxynivalenol (DON) and acetylated DON, and *F. verticillioides*, which produces fumonisins. Our general approach has been, first, to identify genes involved in early steps of mycotoxin biosynthesis, second, to generate mycotoxin-non-producing mutants of the fungi by specifically inactivating one of the biosynthetic genes, and third, to determine whether

the mutants are less virulent than the mycotoxin-producing strains from which they were derived.

Trichothecenes and *Fusarium graminearum*

Fusarium graminearum (teleomorph *Gibberella zeae*) causes head blight of wheat and barley and ear rot of maize. Over the past decade severe head blight epidemics have occurred in North America, particularly in some Midwestern and eastern states of the US and in the Canadian provinces of Manitoba, Ontario, and Québec. It has been estimated that during the 1990s head blight caused losses in excess of $3.5 billion (US) in the USA and Canada (Windels, 2000). These losses result from reduced quality and yield of grain and from contamination of grain with trichothecenes, particularly DON. Trichothecenes are of concern because they inhibit protein synthesis and as a result can induce an array of health problems in animals and humans (Joffe, 1986).

Biochemistry and genetics of biosynthesis

Trichothecenes are sesquiterpenoids and their biosynthesis diverges from general isoprenoid metabolism with the formation of trichodiene via the cyclization of farnesyl pyrophosphate (Figure 1). Trichodiene undergoes a series of oxygenations, cyclizations, isomerizations, and esterifications to yield bioactive trichothecenes such as DON and acetylated DON (Desjardins et al., 1993). Over the past decade, the genes encoding enzymes that catalyze most of these steps have been identified and found to be located within a gene cluster (Hohn et al., 1993; Keller and Hohn, 1997). At least eight genes form the trichothecene biosynthetic gene cluster in *F. graminearum* and in the closely related species *F. sporotrichioides* (Brown et al., 2001). These genes include *TRI5*, which encodes trichodiene synthase (Hohn and Beremand, 1989); *TRI3*, which encodes an acetylase (McCormick et al., 1996); *TRI4* and *TRI11*, which encode cytochrome P450 mono-oxygenases (Alexander et al., 1998; Hohn et al., 1995); *TRI6* which encodes a transcription factor (Hohn et al., 1999), and *TRI12*, which encodes a major facilitator transport protein (Alexander et al., 1999a). At least, one trichothecene biosynthetic gene, *TRI101*, is located outside the trichothecene gene cluster. The *TRI101* protein acetylates the oxygen attached to carbon atom 3 (C-3) of trichothecenes and this markedly reduces their toxicity (Alexander et al., 1999b; Kimura et al., 1998; McCormick et al., 1999).

Trichothecene production and virulence

To examine the role of trichothecenes in the virulence of *F. graminearum* in wheat head blight and maize ear rot, the *TRI5* gene was inactivated via transformation-mediated gene disruption. We chose to disrupt *TRI5* because it encodes the enzyme that catalyzes the first committed reaction in trichothecene biosynthesis, that is, the cyclization of farnesyl pyrophosphate to trichodiene (Figure 1). Thus, disruption of *TRI5* results in mutants that cannot carry out this first biosynthetic step and therefore cannot produce DON, acetylated DON or any trichothecene biosynthetic intermediates (Proctor et al., 1995). To disrupt *TRI5*, we employed a standard protoplast transformation protocol with the hygromycin resistance gene, *HygB*, as a selectable marker (Proctor et al., 1995). Several types of disruption mutants were obtained. However, only two mutants were used in most analyses (Desjardins et al., 1996). The first disruption mutant, GZT33, had two incomplete copies of the *TRI5* coding region, one of which was interrupted by the *HygB* marker. As a result, the *TRI5* gene was inactive in this mutant. The second mutant, GZT40, had a single copy of the *TRI5* coding region that was interrupted by *HygB* and as a result was also inactive.

In wheat field tests, the *TRI5*-disrupted, trichothecene-non-producing mutants caused less severe head blight symptoms and smaller reductions in yield than the trichothecene-producing progenitor strain from which they were derived (Figure 2) (Desjardins et al., 1996). In addition, grain harvested from plants inoculated with the mutants had lower levels of infection and higher rates of germination than seed from plants inoculated with the producing progenitor strain. The reduced virulence of the mutants was observed over several years, at different locations (Illinois and Indiana), and in six different wheat cultivars (Desjardins et al., 1996). These data provide strong evidence that trichothecene production contributes to the ability of *F. graminearum* to cause wheat head blight.

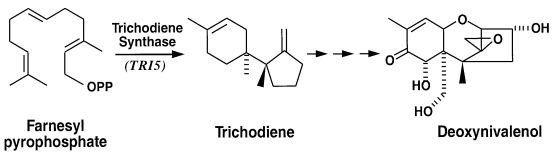

Figure 1. The biosynthesis of the trichothecene deoxynivalenol (DON) from the isoprenoid intermediate farnesyl pyrophosphate via the trichothecene parent compound, trichodiene.

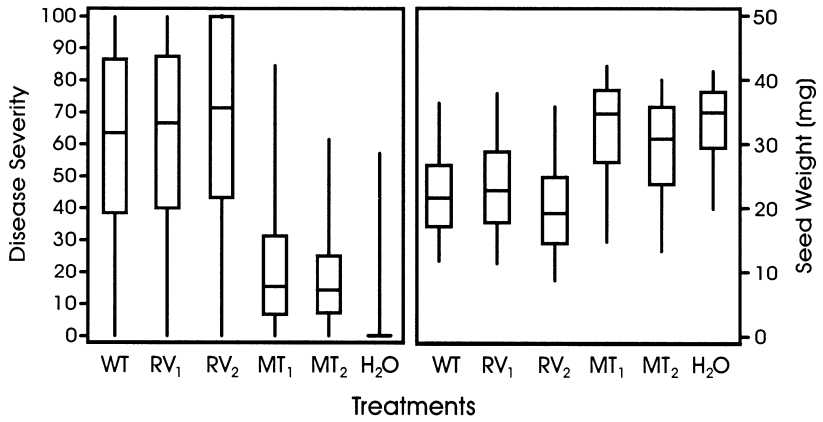

Figure 2. Head blight severity and yield (seed weight) of wheat inoculated with trichothecene producing and non-producing strains of *F. graminearum*. The data are presented as Box-Whisker plots. A box represents the range in which 50% of the data lie, the horizontal line within each box represents the median, and the vertical lines represent the range within which 95% of the data lie. WT, trichothecene-producing progenitor strain; RV_1 and RV_2, trichothecene-producing *TRI5* revertant strains; MT_1 and MT_2, trichothecene-non-producing *TRI5* disruption mutants GZT33 and GZT40; and H_2O, water inoculation. Disease severity was measured as the percent blighted spikelets per head at 19 days after inoculation, and seed weight was measured as the weight of seeds from individual heads. This figure was adapted from (Desjardins et al., 1996).

In a field experiment conducted with two maize cultivars in Ontario, Canada, the trichothecene-non-producing mutants caused significantly less severe ear rot symptoms and lower reductions in grain yield than the trichothecene-producing progenitor strain (Figure 3) (Harris et al., 1999). In the same experiment conducted in Illinois, the mutants induced less severe symptoms than the wild-type strains on one cultivar, but not on the other. In addition, reductions in grain yield caused by mutant and wild-type strains did not differ significantly in Illinois (Harris et al., 1999). The lack of differences in disease induced by the mutant and wild-type strains in Illinois was attributed to relatively low levels of disease induced by the wild-type strain under the hot and dry environmental conditions that prevailed during the experiment. In Ontario, environmental conditions were cooler and the wild-type strain consistently caused high levels of disease. Despite the experimental problems in Illinois, the maize field experiments indicate that trichothecene production contributes to the ability of *F. graminearum* to cause maize ear rot.

Non-target effects of transformation on virulence

The protoplast transformation protocol that was used to disrupt *TRI5* can induce mutations in genes other than those targeted for disruption, and it is possible that the reduced virulence of *TRI5* disruption mutants was caused by such a non-target mutation(s) rather than the inability of the mutants to produce trichothecenes (Desjardins et al., 2000a; Proctor et al., 1997). To address this concern, three types of experiments were done: a reversion analysis, a genetic analysis, and a complementation analysis. In the reversion analysis, the self-fertility of *F. graminearum* was exploited to induce the disrupted *TRI5*, in mutant GZT33, to revert to wild type (Proctor et al., 1997). This reversion was possible because the disrupted *TRI5* in GZT33 included two incomplete copies of the *TRI5* coding region. During meiosis, homologous regions of these two copies could recombine to generate a wild-type *TRI5* coding region and this, in turn, would restore trichothecene production. One progeny obtained by self-fertilizing GZT33 was found to have the wild-type *TRI5* gene and to be restored to trichothecene production. When tested in wheat head blight and maize ear rot assays, this revertant progeny was significantly more virulent than GZT33 and was as virulent as the original wild-type progenitor strain from which GZT33 was derived (Figures 2 and 3) (Desjardins et al., 1996; Harris et al., 1999). The fact that the restoration of trichothecene production restored high virulence in *F. graminearum* indicates that the low virulence of the *TRI5* disruption mutant GZT33 was due to its inability to produce trichothecenes rather than a non-target effect of the transformation procedure.

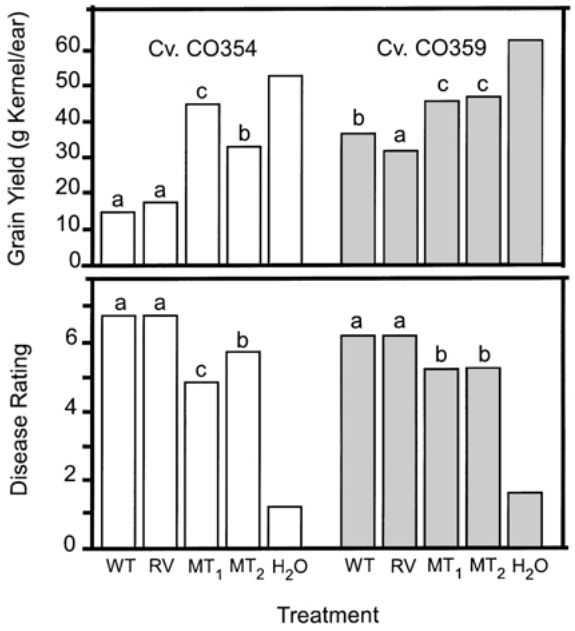

Figure 3. Mean ear rot ratings and grain yield for the two maize cultivars CO354 and CO359 grown in Ontario and inoculated with trichothecene producing and non-producing strains of *F. graminearum*. WT, trichothecene-producing progenitor strain; RV, trichothecene-producing *TRI5* revertant strain; MT_1 and MT_2, trichothecene-non-producing *TRI5* disruption mutants GZT33 and GZT40; and H_2O, water inoculation. A semi-subjective scale was used to measure ear rot ratings (Harris et al., 1999). For a given combination of cultivar and disease measurement (yield or symptoms), bars labeled with different letters are significantly different ($P < 0.05$). Data were taken from Harris et al. (1999).

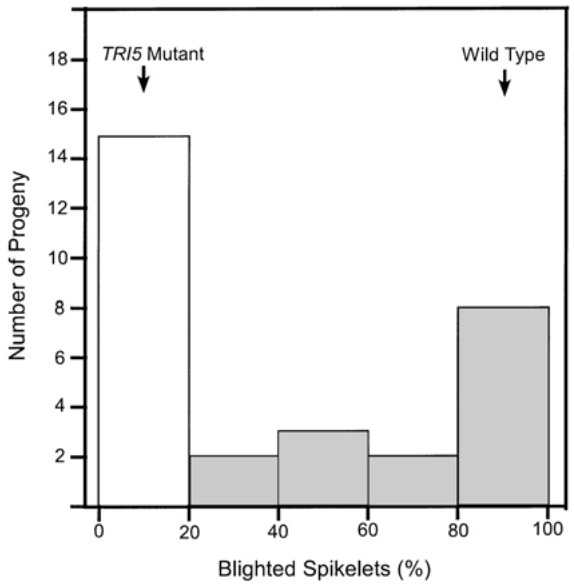

Figure 4. Distribution of head blight symptoms among trichothecene-producing (shaded) and trichothecene-non-producing (not shaded) progeny from a cross between *TRI5* disruption mutant GZT40 and a wild-type strain. Arrows indicate the level of head blight caused by the parental strains. Data were taken from Desjardins et al. (2000).

In the genetic analysis to assess the potential relationship of non-target mutation(s) and reduced virulence, disruption mutant GZT40 was crossed with a trichothecene-producing, highly virulent strain of *F. graminearum* (Desjardins et al., 2000a). Strain GZT40 was chosen for the genetic analysis because the structure of its disrupted *TRI5* allele was such that it could not revert to the wild-type allele. Also, GZT40 and the wild-type parental strain were genetically marked with nitrate-non-utilizing mutations to facilitate the selection of progeny that resulted from a cross between the two parents rather than from self-fertilization. The progeny of this cross were analyzed to determine whether they had the wild-type or disrupted *TRI5* allele, whether they produced trichothecenes, and whether they had high or low virulence on wheat heads. All progeny that had the disrupted *TRI5* allele did not produce DON and exhibited low virulence on wheat heads, whereas progeny with the wild-type *TRI5* allele produced DON and exhibited high virulence (Figure 4) (Desjardins et al., 2000a).

In the complementation analysis to assess the possible relationship of non-target mutation(s) and reduced virulence, a functional *TRI5* allele (*TRI5-4*) was transformed into *TRI5* disruption mutant GZT40 (Proctor et al., 1997). Although the *TRI5-4* allele restored trichothecene production to the mutant it did not restore high virulence on wheat heads (Desjardins et al., 2000a). However, a subsequent genetic analysis revealed that the low virulence of the *TRI5-4*-complemented GZT40 was caused by a non-target mutation that had been induced when the *TRI5-4* allele was transformed into GZT40 rather than by the initial transformation used to disrupt *TRI5* (Desjardins et al., 2000a).

The results from the reversion, genetic, and complementation and subsequent genetic analyses were all consistent and indicated that the reduced virulence of the *TRI5* disruption mutants was not caused by a non-target mutation from the transformation process used to disrupt *TRI5*. Thus, the three analyses confirmed that the reduced virulence of the *TRI5* disruption mutants was caused by their inability to produce trichothecenes.

Fumonisins and *Fusarium verticillioides*

Fusarium verticillioides (syn. *F. moniliforme*, teleomorph *G. moniliformis*, syn *G. fujikuroi* mating population A) causes ear and stalk rot of maize, but it is also frequently present in healthy maize tissue (Munkvold and Desjardins, 1997). Although, the fungus does not typically cause severe epidemics, it is of considerable importance because of its almost ubiquitous distribution in maize-growing regions of North America combined with its ability to produce fumonisins. Fumonisins cause the formation of cancerous tumors in laboratory rodents and there is an epidemiological correlation between the consumption of fumonisin-contaminated grain and human esophageal cancer in some regions of the world (Howard et al., 1999; Nelson et al., 1993). Fumonisins also cause several fatal livestock diseases, such as lung edema in swine and leukoencephalomalacia in horses (Nelson et al., 1993). At the cellular level, fumonisins disrupt sphingolipid metabolism and, because sphingolipids function in a number of cellular processes, it is likely that this disruption leads to the various fumonisin-induced animal diseases (Howard et al., 1999; Wang et al., 1991). Given their carcinogenicity and their widespread occurrence in maize, fumonisins have the potential to severely impact maize producers in North America.

Biochemistry and genetics of biosynthesis

Most strains of *F. verticillioides* produce fumonisin B_1 (FB_1) in highest abundance with smaller amounts of the less oxygenated fumonisins, fumonisins B_2 (FB_2) and B_3 (FB_3) and B_4 (FB_4) (Figure 5) (Nelson et al., 1993). Precursor-feeding experiments with labeled acetate indicate that these fumonisins are synthesized from a linear 20-carbon polyketide that undergoes carbonyl reduction, hydroxylation, condensation with alanine, and esterification with two tricarboxylic acids (Blackwell et al., 1994; Proctor et al., 1999a). The order in which the hydroxylations, condensation, and esterification occur is not clear. However, feeding studies with FB_2, FB_3, and FB_4 indicate that hydroxylation of C-10 must occur before hydroxylation of C-5 in the formation of FB_1 (Proctor et al., 1999a). In addition, the presence of a methyltransferase-encoding region in a polyketide synthase gene (see below) required for fumonisin biosynthesis indicates that the methyl groups at C-12 and C-16 of fumonisins are added during the synthesis of the polyketide (Figure 5).

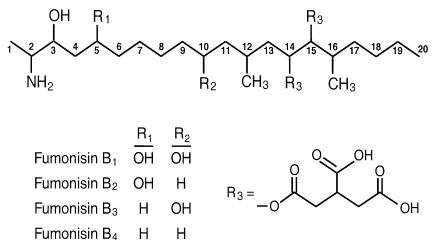

Figure 5. Structures of Fumonisins B_1, B_2, B_3, and B_4.

The fumonisin polyketide synthase gene was identified via PCR using degenerate polyketide synthase primers and a cDNA template that was prepared from a fumonisin-producing liquid culture of *F. verticillioides* (Proctor et al., 1999b). The amplification product from this PCR was used to isolate an 8-kb gene (*FUM5*) that encodes a polyketide synthase. Disruption of *FUM5* in *F. verticillioides*, via essentially the same method used to disrupt *TRI5* in *F. graminearum*, reduced fumonisin production by over 99% and thus demonstrated that *FUM5* participates in fumonisin biosynthesis (Proctor et al., 1999b). It is hypothesized that the polyketide synthase encoded by *FUM5* synthesizes the putative 20-carbon linear polyketide that forms C-3 through C-20 of the fumonisin backbone.

FUM5 is the first fumonisin biosynthetic gene to be characterized at the molecular level and presents an opportunity to begin critical studies on the molecular genetics and biochemistry of fumonisin biosynthesis as well as studies on the role of these mycotoxins in the ecology of *F. verticillioides*. Already, sequence analysis of regions up and down stream of *FUM5* have revealed the presence of other genes. Disruption and expression analyses have revealed that four genes (*FUM6*, *FUM7*, *FUM8*, and *FUM9*) immediately downstream of *FUM5* also participate in fumonisin biosynthesis (Seo et al., 2001). These data, along with those from classical genetic analyses of natural variants of *F. verticillioides* with altered fumonisin production phenotypes (Desjardins et al., 1996), indicate that fumonisin biosynthetic genes are clustered in a manner similar to trichothecene biosynthetic genes.

Fumonisin production and virulence

FUM5 is analogous to *TRI5* in that it encodes an enzyme that catalyzes an early step in mycotoxin biosynthesis. As a result, *FUM5* disruption mutants should be useful tools in the analysis of the role of fumonisins in the virulence of *F. verticillioides*

on maize. Although these analyses are not complete, preliminary results indicate that *FUM5* disruption mutants cause high levels of ear rot, similar to those caused by the fumonisin-producing strains from which the mutants were derived (Desjardins et al., 2000b). These preliminary results are consistent with another set of experiments in which a natural variant of *F. verticillioides* that does not produce fumonisins caused the same levels of maize ear rot as a wild-type fumonisin producing strain (Desjardins and Plattner, 2000). Thus, all results to date indicate that fumonisin production does not have a major effect on the ability of *F. verticillioides* to cause maize ear rot.

Mycotoxin resistance genes and plant disease control

One of the reasons to study the role of mycotoxins in plant pathogenesis is to identify weak links in the producing fungi that can be exploited to control plant disease and mycotoxin contamination problems. The finding that trichothecenes contribute to the virulence of *F. graminearum* has revealed such a weak link. That is, it may be possible to incorporate trichothecene resistance into wheat and maize to render these crops resistant to diseases caused by *F. graminearum*. Reduction of these diseases should, in turn, result in less trichothecene contamination in the crops. Already, researchers have identified three genes that confer resistance to trichothecene: the *Fusarium TRI101* gene, which encodes an enzyme that reduces the toxicity of trichothecenes (Kimura et al., 1998; McCormick et al., 1999); the rice *Rpl3* gene, which encodes the ribosomal protein L3 and the wild-type form of which is a likely target of trichothecenes (Harris and Gleddie, 2000; 2001); and the yeast *PDR5* gene, which encodes an ABC transporter (Balzi et al., 1994). Researchers have already shown that these genes confer trichothecene resistance when expressed in tobacco (Harris and Gleddie, 2001; Muhitch et al., 2000). Whether such genes can confer resistance to trichothecenes and *F. graminearum* in wheat and maize is currently under investigation (Okubara et al., 2000).

If fumonisin production does not have a major effect on the ability of *F. verticillioides* to cause maize ear rot, as preliminary results suggest, it is unlikely that fumonisin resistance would be an effective way to control this disease or fumonisin contamination in maize. However, *F. verticillioides* produces several other toxins (e.g. fusaric acid and the fusarins) and probably other factors (Desjardins and Proctor, 1999) that may contribute to the virulence of this fungus. It is possible that methods to control *F. verticillioides*-induced maize ear rot and fumonisin contamination will become apparent if the relationships of these toxins and virulence are critically examined.

Conclusions

Analyses of *TRI5* disruption mutants have revealed that trichothecene production contributes to high levels of virulence of *F. graminearum* on wheat and maize. How the toxins enhance virulence of *F. graminearum* is not clear, however, it seems likely that the inhibitory effects of trichothecenes on protein synthesis could impair plant defenses. The analyses also indicated that trichothecenes are virulence factors rather than pathogenicity factors. That is, trichothecenes enhance the severity of disease caused by *F. graminearum*, but in the absence of their production the fungus can still cause low levels of disease. The low levels of disease caused by the trichothecene-non-producing mutants demonstrate that other factors contribute to the ability of the *F. graminearum* to cause disease.

If fumonisins are not involved in maize ear rot, it is possible that they enhance the virulence of *F. verticillioides* on some other host plant(s). Such a situation exists with trichothecenes, which enhance the virulence of *F. sporotrichioides* and *F. sambucinum* on parsnip root but not on potato tubers (Desjardins et al., 1993). Furthermore, the lack of a role for fumonisins in virulence does not preclude a role for them in some other aspect of the ecology of *F. verticillioides*.

While transformation-mediated gene disruption has been a crucial tool in studying the role of mycotoxins in plant diseases, one drawback of the technique is that the transformation process can induce non-target mutations that reduce virulence. Thus, gene disruption should not always be considered sufficient to demonstrate the function of a gene involved in complex physiological processes such as virulence. As described above, the presence of non-target mutations can be assessed by reversion, genetic and complementation analyses, although caution must be exercised with transformation-mediated complementation analyses because they can also induce non-target mutations. Alternative transformation protocols, such as gene gun and *Agrobacterium*-mediated methods, are currently being developed to disrupt genes in fungi. These methods may reduce or eliminate non-target mutations

because they employ intact cells rather than protoplasts and, therefore, may be less stressful to fungi.

If trichothecene production contributes to the virulence of *F. graminearum*, it follows that plants that are resistant to the toxins should also be resistant to the fungus. Thus, studying the role of trichothecenes in virulence has revealed a strategy that could control the crop diseases and the resulting mycotoxin contamination problems caused by *F. graminearum*. This should lend support for future experiments to examine the role of other mycotoxins and other potential virulence factors in plant diseases caused by *Fusarium*.

References

Alexander NJ, Hohn TM and McCormick SP (1998) The *TRI11* gene of *Fusarium sporotrichioides* encodes a cytochrome P450 monooxygenase required for C-15 hydroxylation in trichothecene biosynthesis. Applied and Environmental Microbiology 64: 221–225

Alexander NJ, Hohn TM and McCormick SP (1999a) TRI12, a trichothecene efflux pump from *Fusarium sporotrichioides*: Gene isolation and expression in yeast. Molecular and General Genetics 261: 977–984

Alexander NJ, McCormick SP and Ziegenhorn SL (1999b) Phytotoxicity of selected trichothecenes using *Chlamydomonas reinhardtii* as a model system. Natural Toxins 7: 265–269

Balzi E, Wang M, Leterme S, van Dyck L and Goffeau A (1994) PDR5, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator PDR1. Journal of Biological Chemistry 269: 2206–2214

Blackwell BA, Miller JD and Savard ME (1994) Production of carbon 14-labeled fumonisin in liquid culture. Journal of AOAC International 77: 506–511

Brown DW, McCormick SP, Alexander NJ, Proctor RH and Desjardins AE (2001) A Genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genetics and Biology 32: 121–133

Desjardins AE, Bai G, Plattner RD and Proctor RH (2000a) Analysis of aberrant virulence of *Gibberella zeae* following transformation-mediated complementation of a trichothecene-deficient (*Tri5*) mutant. Microbiology 146: 2059–2068

Desjardins AE, Hohn TM and McCormick SP (1993) Trichothecene biosynthesis in *Fusarium* species: Chemistry, genetics, and significance. Microbiological Reviews 57: 595–604

Desjardins AE and Plattner RD (2000) Fumonisin B1-nonproducing strains of *Fusarium verticillioides* cause maize (*Zea mays*) ear infection and ear rot. Journal of Agricultural and Food Chemistry 48: 5773–5780

Desjardins AE, Plattner RD and Proctor RH (1996) Linkage among genes responsible for fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. Applied and Environmental Microbiology 62: 2571–2576

Desjardins AE, Plattner RD and Proctor RH (2000b) Maize ear rot and systemic infection by fumonisin B1-nonproducing mutants of *Gibberella fujikuroi* mating population A. Phytopathology 90 (Suppl): 596

Desjardins AE and Proctor RH (1999) Biochemistry and genetics of *Fusarium* toxins. In: B Summerell B, Leslie JF, Backhouse D, Bryden WL and Burgess LW (ed) Fusarium: Paul E. Nelson Symposium APS Press, St. Paul, USA.

Desjardins AE, Proctor RH, Bai G, McCormick SP, Shaner G, Buechley G and Hohn TM (1996) Reduced virulence of trichothecene antibiotic-non-producing mutants of *Gibberella zeae* in wheat field tests. Molecular Plant–Microbe Interactions 9: 775–781

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH and Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Disease 83: 954–960

Harris LJ and Gleddie SC (2000) Developing plant tolerance to trichothecene mycotoxins using a transgenic approach. Phytopathology 90 (Suppl): 596

Harris LJ and Gleddie SC (2001) A modified *Rpl3* gene from rice confers tolerance of the *Fusarium graminearum* mycotoxin deoxynivalenol to transgenic tobacco. Physiological and Molecular Plant Pathology 58: 173–181

Hohn TM and Beremand P (1989) Isolation and nucleotide sequence of a sesquiterpene cyclase gene from the trichothecene-producing fungus *Fusarium sporotrichioides*. Gene 79: 131–138

Hohn TM, Desjardins AE and McCormick SP (1995) The *Tri4* gene of *Fusarium sporotrichioides* encodes a cytochrome P450 involved in trichothecene biosynthesis. Molecular and General Genetics 248: 95–102

Hohn TM, Krishna R and Proctor RH (1999) Characterization of a transcriptional activator controlling trichothecene toxin biosynthesis. Fungal Genetics and Biology 26: 224–235

Hohn TM, McCormick SP and Desjardins AE (1993) Evidence for a gene cluster involving trichothecene-pathway biosynthetic genes in *Fusarium sporotrichioides*. Current Genetics 24: 291–295

Howard PC, Eppley RM, Stack ME, Warbritton A, Voss KA, Lorentzen RJ, Kovach R and Bucci TJ (1999) Carcinogenicity of fumonisin B_1 in a two-year bioassay with Fischer 344 rats and $B6C3F_1$ mice. Mycotoxins Supplement 1999: 45–54

Joffe A (1986) *Fusarium* Species: Their Biology and Toxicology. John Wiley and Sons, New York

Keller NP and Hohn TM (1997) Metabolic pathway gene clusters in filamentous fungi. Fungal Genetics and Biology 21: 17–29

Kimura M, Kaneko I, Komiyama M, Takatsuki A, Koshino H, Yoneyama K and Yamagushi I (1998) Trichothecene 3-*O*-acetyltransfease protects both the producing organism and transformed yeast from related mycotoxins. Journal of Biological Chemistry 273: 1654–1661

McCormick SP, Alexander NJ, Trapp SC and Hohn TM (1999) Disruption of *TRI101*, the gene encoding trichothecene 3-*O*-acetyltransferase, from *Fusarium sporotrichioides*. Applied and Environmental Microbiology 65: 5252–5256

McCormick SP, Hohn TM and Desjardins AE (1996) Isolation and characterization of *Tri3*, a gene encoding 15-*O*-acetyltransferase from *Fusarium sporotrichioides*. Applied and Environmental Microbiology 62: 353–359

Muhitch MJ, McCormick SP, Alexander NJ and Hohn TM (2000) Transgenic expression of the *TRI101* or *PDR5* gene increases resistance of tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol. Plant Science 157: 201–207

Munkvold GP and Desjardins AE (1997) Fumonisins in maize: Can we reduce their occurrence? Plant Disease 81: 556–565

Nelson PE, Desjardins AE and Plattner RD (1993) Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. Annual Review of Phytopathology 31: 233–252

Okubara PA, Hohn TM, Berka RM, Alexander NA, Wang Z, Hart LP and Blechl AE (2000) Optimizing the expression of candidate anti-*Fusarium* protein genes in hexaploid wheat. Proceedings of the 2000 National Fusarium Head Blight Forum pp 39–43

Proctor RH, Desjardins AE and Plattner RD (1999a) Biosynthetic and genetic relationships of B-series fumonisins produced by *Gibberella fujikuroi* mating population A. Natural Toxins 7: 251–258

Proctor RH, Desjardins AE, Plattner RD and Hohn TM (1999b) A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. Fungal Genetics and Biology 27: 100–112

Proctor RH, Hohn TM and McCormick SP (1995) Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Molecular Plant–Microbe Interactions 8: 593–601

Proctor RH, Hohn TM and McCormick SP (1997) Restoration of wild-type virulence to *Tri5* disruption mutants of *Gibberella zeae* via gene reversion and mutant complementation. Microbiology 143: 2583–2591

Seo J-A, Proctor RH and Plattner RD (2001) Characterization of four clustered and coregulated genes associated with fumonisin biosynthesis in *Fusarium verticillioides*. Fungal Genetics and Biology 34: 155–165

Wang E, Norred WP, Bacon CW, Riley RT and Merrill AH (1991) Inhibition of sphingolipid biosynthesis by fumonisins: Implications for diseases associated with *Fusarium moniliforme*. Journal of Biological Chemistry 266: 14486–14490

Windels C (2000) Economic and social impacts of Fusarium Head Blight: Changing farms and rural communities in the northern Great Plains. Phytopathology 90: 17–21

Yang G, Rose MS, Turgeon BG and Yoder OC (1996) A polyketide synthase is required for fungal virulence and production of the polyketide T-toxin. The Plant Cell 8: 2139–2150