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REVIEW

From Yellow Rain to Green Wheat: 25 Years of Trichothecene Biosynthesis Research

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Trichothecene biosynthesis research at the U.S. Department of Agriculture in Peoria, IL, began in 1984 in response to concerns about the use of trichothecenes in biological warfare, but continued as a long-term research program on the intractable problem of trichothecene contamination of human foods and animal feeds. Over 25 years, the trichothecene biosynthesis research group integrated natural product chemistry with fungal genetics and plant pathology in the laboratory and in the field to understand how and why *Fusarium* species make these complex and highly toxic metabolites. This interdisciplinary research placed trichothecenes in the unique class of fungal metabolites that not only cause mycotoxicoses in animals but also are virulence factors in plant disease.

KEYWORDS: Trichothecene biosynthesis; *Fusarium graminearum*; *Fusarium sporotrichioides*; *TRI* genes; wheat head blight; maize ear rot

INTRODUCTION

In 1984 in Peoria, IL, the U.S. Department of Agriculture (USDA) established a new research program on the biosynthesis of trichothecene mycotoxins (**Table 1**; **Figure 1**), fungal metabolites that are toxic to animals. Over the next 25 years, the trichothecene biosynthesis research group in Peoria became a center of the international community of scientists striving to reduce trichothecene contamination of human foods and animal feeds. Mycotoxicology is an interdisciplinary field, and from its beginnings the trichothecene biosynthesis research group functioned at the interfaces of the scientific disciplines of chemistry, genetics, and biology. This review is an effort to chronicle how this group of USDA scientists conceptualized and conducted research on the chemistry, genetics, and biology of trichothecene biosynthesis. Other mycotoxin research conducted in Peoria has been excluded from this paper; decisions of what to include and exclude and other judgments are of course mine.

25 YEARS OF TRICHOTHECENE BIOSYNTHESIS RESEARCH

The Yellow Rain Controversy. More than 25 years ago, trichothecene mycotoxins made their controversial debut on the world stage (*1*). In 1981, Alexander Haig, who was then U.S. Secretary of State, presented two types of evidence for his allegation that trichothecenes had been used as biological warfare agents in Southeast Asia. Hmong villagers, who had fled from northern Laos to refugee camps in Thailand, claimed that they had become ill after "yellow rain" fell from the sky onto their villages. Chemical analyses in the United States found trichothecenes in some alleged yellow rain samples, such as leaves, rocks, and soil debris, that had been smuggled from Laos to Thailand.

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In 1982, in response to a request from the U.S. Army, the National Research Council formed a Committee on Protection from Mycotoxins. The committee was concerned about exposure to trichothecenes due to their well-documented inhibition of protein synthesis and cell growth and their harmful effects on human health. In 1983, the committee reported a need for research on the detection, natural occurrence, and biosynthesis of trichothecenes (*2*). To this end, Robert Detroy and Shelby Freer at the USDA in Peoria submitted a successful proposal for a new interdisciplinary research program on trichothecene biosynthesis by *Fusarium* species. The project began in 1984 with support for four new research scientist positions that were to be divided between chemistry and biology. The four founding group members were chemists Frank VanMiddlesworth and Anne Desjardins and biologists Thomas Hohn and Marian Beremand.

During the mid-1980s, while the new group was developing a trichothecene biosynthesis research program, a number of studies were seriously weakening the case for the use of trichothecenes in biological warfare in Southeast Asia (*1*). Independent analyses of alleged yellow rain samples failed to detect trichothecenes, and more extensive surveys showed that trichothecene-producing fungi and trichothecene contamination occurred naturally in the region. Biological analysis showed that some alleged yellow rain samples contained high amounts of pollen and were likely to have been deposited by cleansing flights of Asian honeybees. The yellow rain controversy highlights the difficulty of proving that biological warfare is the source of trichothecenes, or any naturally occurring toxin, in an environmental sample.

The potential use of trichothecenes in warfare stimulated initiation of the trichothecene biosynthesis research program in Peoria in 1984, but it soon became clear to the research group that the natural occurrence of these mycotoxins is a more serious problem than any deliberate distribution. Trichothecene contamination of grain is an old and chronic problem, especially in temperate regions of the world, where trichothecene-producing

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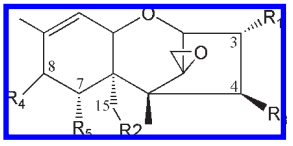
species of the fungus *Fusarium* infect maize (*Zea mays*), wheat (*Triticum aestivum*) (**Figure 2**), and other cereal crops (*3*). During the 1980s, the impact of trichothecenes on agricultural systems and on food safety became and remained the reason that USDA funded the trichothecene biosynthesis research program for the next 25 years.

Take T-2. The first priority in 1984 was to select one of the many trichothecene-producing *Fusarium* species as a model system for biosynthetic studies (*4*). The *Fusarium* trichothecenes comprise a family of more than 40 naturally occurring tricyclic sesquiterpenes with an epoxy group, which is essential for their toxicity. T-2 toxin, diacetoxyscirpenol, nivalenol, and deoxynivalenol are the trichothecenes most frequently found in agricultural commodities infected by *Fusarium* species, and each of these compounds can be produced by more than one species.

Table 1. Structures of Selected Trichothecenes



	substituents[a]				
compound	R_1	R_2	R_3	R_4	R_5
isotrichodermol	OH	H	H	H	H
isotrichodermin	OAc	H	H	H	H
calonectrin	OAc	OAc	H	H	H
scirpentriol	OH	OH	OH	H	H
15-acetoxyscirpenol	OH	OAc	OH	H	H
4,15-diacetoxyscirpenol	OH	OAc	OAc	H	H
T-2 toxin	OH	OAc	OAc	Olso	H
deoxynivalenol	OH	OH	H	=O	OH
nivalenol	OH	OH	OH	=O	OH
4-acetylnivalenol	OH	OH	OAc	=O	OH

[a] Ac, acetyl ester; Iso, isovaleryl ester.

Criteria for selecting the model system were both chemical and biological. Prior to 1984, laboratory production of trichothecenes usually involved fermentation on autoclaved grain or other solid substrates, which were difficult matrices for the isolation of intermediates and enzymes. To facilitate biochemical studies and high-throughput screening, the ideal *Fusarium* species should consistently produce large amounts of trichothecenes in liquid media and also should produce a trichothecene for which a highly specific immunoassay method is already available. In 1984, these criteria eliminated all trichothecenes except T-2 toxin.

Many trichothecene-producing *Fusarium* species have a two-stage life cycle: a *Fusarium* stage in which they produce asexual spores and a *Gibberella* stage in which they produce sexual spores. A model *Fusarium* species should have a *Gibberella* stage amenable to sexual crossing and linkage analysis. The model system also should be amenable to the new techniques of molecular genetics such as DNA-mediated transformation, which were being developed during the 1980s.

After hundreds of strains of more than a dozen *Fusarium* species were screened for trichothecene production and for sexual fertility, none of the species met all of the criteria of an ideal model organism. In 1985, the group selected *Fusarium sporotrichioides* strain NRRL 3299 as the primary model system for elucidation of trichothecene biosynthesis. Strain NRRL 3299 had been isolated as strain T-2 in 1968 from maize ear rot in France and was the original source and eponym for T-2 toxin. This strain was a reliable producer of T-2 toxin in liquid media, and an immunoassay was available for high-throughput screening. The major disadvantage of *F. sporotrichioides* was its sexual nonfertility, without which linkage analysis was not feasible.

F. sporotrichioides was first described in 1915 from dry-rotted potato tubers in New York, but also has been associated with maize ear rot and head blight of small-grain cereals, particularly

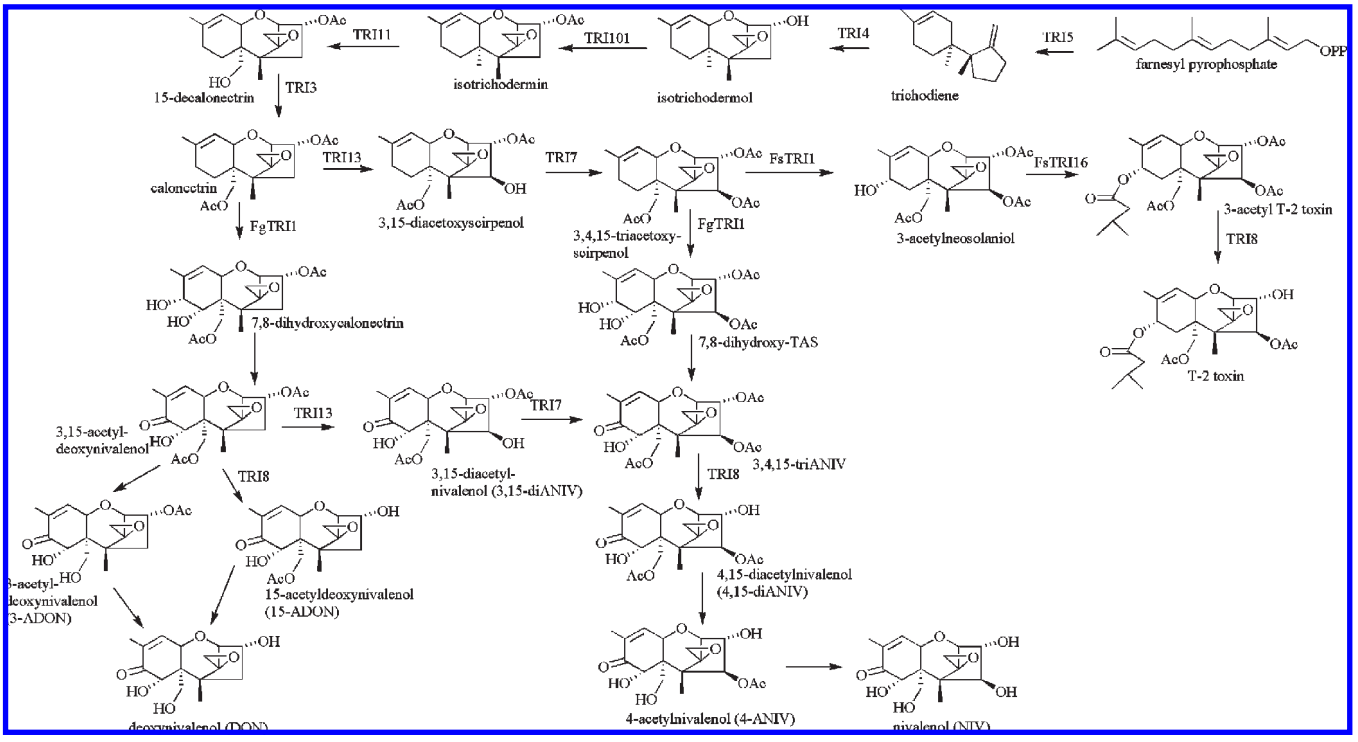


Figure 1. Trichothecene biosynthesis pathways to T-2 toxin, deoxynivalenol, and nivalenol. Unlabeled arrows indicate reactions for which genes/enzymes are unknown.

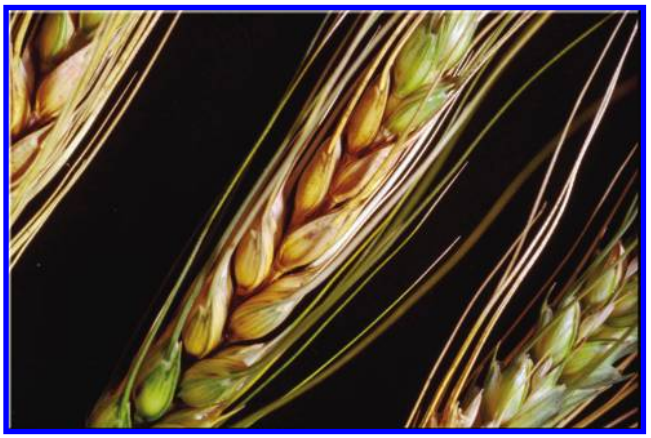


Figure 2. Wheat head infected with *Fusarium graminearum*.

in cooler climates (*4*). During World War II, consumption of overwintered grain contaminated with *F. sporotrichioides* and related species caused alimentary toxic aleukia and the deaths of thousands of people in Russia. Two decades later, T-2 toxin was shown to produce symptoms characteristic of alimentary toxic aleukia: nausea, vomiting, diarrhea, anemia, skin rashes, and gastrointestinal necrosis. T-2 toxin is a strong candidate as a major causal agent of alimentary toxic aleukia outbreaks since the 19th century and also has been associated with outbreaks of hemorrhagic syndrome in farm animals in North America and Europe.

The research group selected *Fusarium sambucinum* strain R-6380 as a second model organism for elucidation of the trichothecene biosynthetic pathway. Strain R-6380 originally was isolated in 1978 from rotted potatoes in Germany and produced high levels of diacetoxyscirpenol in liquid media. Desjardins and M. Beremand developed a method to produce the sexual stage in the laboratory and used sexual crosses between strains with different trichothecene chemotypes to show that trichothecene production is a heritable trait and that some loci affecting trichothecene production are not linked (*5*). This was the first documented genetic system in a trichothecene-producing species.

From the outset, *F. sporotrichioides* strain NRRL 3299 and *F. sambucinum* strain R-6380 were reliable trichothecene producers and excellent model organisms and formed the core of the first years of the trichothecene biosynthesis research program. VanMiddlesworth and Desjardins used both strains to isolate trichodiene, the first intermediate in trichothecene biosynthesis. From strain NRRL 3299, Hohn and VanMiddlesworth isolated the first trichothecene enzyme, trichodiene synthase, and M. Beremand produced the first trichothecene mutants. Both organisms later proved to be amenable to molecular genetics and DNA-mediated transformation and to the development of plant pathogenicity assays that became an important component of trichothecene gene function analysis.

In Search of Trichodiene. In plants and fungi, sesquiterpene synthases catalyze the conversion of farnesyl diphosphate into more than 300 known structurally different sesquiterpenoid products (*6*). During the 1970s, scientists in Japan and England isolated the sesquiterpene trichodiene from the fungus *Trichothecium* and showed that radioactively labeled trichodiene was incorporated into trichothecenes by this fungus (*4*). Thus, trichodiene became a likely candidate as the first intermediate in the trichothecene pathway in *Fusarium*. In Peoria, the search for trichodiene began late in 1984, but was hampered by the chemical complexity of the fungal extracts and their very low levels of trichodiene. For assistance with these problems, VanMiddlesworth and Desjardins enlisted the collaboration of chemist Ronald Plattner, who was well-established in the mass spectrometry of fungal and plant natural products at the USDA in Peoria. Mass spectrometric analysis of *F. sporotrichioides* grown in the presence of $^{18}O_2$ or $H_2{}^{18}O$ showed that each of the oxygenations of trichodiene to produce T-2 toxin required oxygen gas, not water, as a substrate (*7*). These data suggested that trichothecenes were biosynthesized by cytochrome P450 monooxygenase enzymes and, therefore, that biosynthetic intermediates should accumulate upon specific inhibition of this class of enzyme. A screen of putative inhibitors of cytochrome P450 monooxygenases found that the substituted pyrimidine ancymidol caused inhibition of trichothecene biosynthesis and accumulation of trichodiene (*8*). Ancymidol treatment provided access to gram quantities of natural trichodiene for further studies of trichothecene biosynthesis.

Because trichodiene is the first compound in the trichothecene biosynthetic pathway, the enzyme that synthesizes trichodiene is a potentially important regulatory point. In 1986, Hohn and VanMiddlesworth purified trichodiene synthase by chromatographic methods from a subcellular fraction of *F. sporotrichioides* (*9*). Trichodiene synthase was the first trichothecene biosynthetic enzyme to be purified to homogeneity. Isolation of this enzyme was a critical step toward the discovery of the trichothecene biosynthetic gene cluster.

A Few Good Mutants. The structural diversity of the *Fusarium* trichothecenes argued that they were likely to result from a long and complex biosynthetic pathway. Treatment of fungal cultures with monooxygenase inhibitors led to the accumulation of trichodiene, but yielded only trace amounts of later pathway intermediates. To find additional intermediates, M. Beremand used ultraviolet irradiation to mutagenize *F. sporotrichioides* and produce strains that had lost the ability to produce T-2 toxin. Because *Fusarium* is haploid, recessive loss-of-function mutations are readily detectable. M. Beremand used a T-2 toxin immunoassay to screen colonies of *F. sporotrichioides* grown in microtiter plates. The immunoassay was particularly useful because the antibody was specific for the isovalerate ester unique to T-2 toxin and, thus, would not detect any pathway intermediates without this moiety. A laborious screen of more than 10000 mutagenesis-surviving colonies yielded a handful of the first trichothecene biosynthetic mutants (*10*).

In the late 1980s, detailed biochemical studies of three T-2 toxin-non-producing mutants assigned the mutations to specific enzymatic reactions (*4*, *11*). Chemical analyses of the mutants were conducted by Plattner and by Susan McCormick, a natural product chemist who joined the research group in 1987 after the departure of VanMiddlesworth. Mutant MB1716 accumulated trichothecenes that are not oxygenated at C-8; mutant MB2972 accumulated trichothecenes not oxygenated at C-8 or C-4; mutant MB5493 accumulated trichodiene. Mixtures of the mutants in all pairwise combinations produced T-2 toxin, demonstrating that the mutations were complementary and, thus, likely to be mutations in genes encoding different biosynthetic enzymes.

Because *F. sporotrichioides* is asexual, linkage of putative trichothecene gene mutations could not be investigated in this species. Linkage was studied using a collection of *F. sambucinum* from the field, which yielded strains that were sexually fertile and showed natural variation in trichothecene chemotype. In 1985, genetic crosses conducted in this species defined a locus for oxygenation at C-8 (*TRI1*) and suggested that at least two unlinked loci were involved in trichothecene biosynthesis (*4*, *5*). Although the first *TRI* gene to be defined genetically, the *TRI1* gene was not isolated until 2003, by M. Beremand and associates at Texas A&M University after she left the USDA in 1991 (*12*).

TRI, TRI Again. From the beginning in 1984, isolating trichothecene biosynthetic genes was a major goal of the research group in Peoria. Although M. Beremand's mutants were useful for biochemical experiments, her mutagenesis protocol did not include tagging for gene identification. Furthermore, in 1984, Polymerase Chain Reaction strategies now used for gene amplification had not yet been developed. The first fungal genes had been isolated in the mid-1970s by using bacterial plasmids and viruses as vehicles for gene amplification, and this approach was used by Hohn and P. Beremand to isolate the first trichothecene biosynthetic gene, trichodiene synthase (*TRI5*), from *F. sporotrichioides* in 1989 (*13*). This gene was a useful DNA hybridization probe for other trichodiene synthase genes: *F. sambucinum* in 1992, *Fusarium graminearum* in 1995, and the related fungus *Myrothecium roridum* in 1998. Trichodiene synthase also was the first of the terpene cyclase class of genes to be isolated from any fungus or plant. Determination of the X-ray crystal structure of recombinant trichodiene synthase has facilitated a wide range of mechanistic studies of terpene cyclization (*14*).

During the mid-1980s, technology became available for insertional mutagenesis of fungi using plasmid and cosmid transformation vectors with selectable markers. In 1991, Hohn, McCormick, and Desjardins undertook the isolation of trichothecene genes by transformation and mutant complementation of *F. sporotrichioides*. Although it is now well-established that genes for mycotoxins and other fungal secondary metabolites are often clustered, this was not obvious in 1991. Indeed, genetic analysis in *F. sambucinum* had indicated that at least one gene (*TRI1*) was not linked to other trichothecene genes (*TRI5*). However, the first fungal secondary metabolite gene cluster (for penicillin biosynthesis) had been reported in 1990 (*15*), leading Hohn to propose that *TRI5* might be linked to other *TRI* genes. Consequently, *TRI1*, *TRI3*, and *TRI4* mutants were transformed with cosmid vectors that contained the *TRI5* gene and up to 40 kb of flanking DNA. Chemical analysis of the transformants showed that integration of vectors containing *TRI5* restored production of T-2 toxin to *TRI3* and *TRI4* mutants but not to a *TRI1* mutant, indicating that *TRI3*, *TRI4*, and *TRI5* were organized in a gene cluster (*16*).

Chromosome walking, DNA sequencing, gene disruption, and biochemical studies conducted in Peoria from 1992 to 2002 elucidated the trichothecene core gene clusters for T-2 toxin in *F. sporotrichioides* and for deoxynivalenol in *F. graminearum* (*4*). The clusters are highly homologous in gene content and organization, with 12 genes tightly linked within a 25 kb region in these two species. Ten of the genes have been shown by targeted disruption to be required for trichothecene biosynthesis. Along with trichodiene synthase (*TRI5*), the core cluster contains three cytochrome P450 monooxygenases (*TRI4*, *TRI11*, and *TRI13*), two acyltransferases (*TRI3* and *TRI7*), an esterase (*TRI8*), and a transporter (*TRI12*). The cluster also contains two positive regulatory genes, *TRI6*, which coordinates the expression of genes encoding the trichothecene biosynthetic enzymes, and *TRI10*, which controls *TRI6*. The function of *TRI10* was characterized by M. Beremand and associates at Texas A&M University (*17*).

The majority of *F. graminearum* strains from the United States produce deoxynivalenol, but some strains from other geographical regions, especially Asia, produce nivalenol. These trichothecenes differ only by the presence or absence of a C-4 hydroxyl group, a reaction catalyzed by the TRI13 cytochrome P450 monooxygenase. Sequences of trichothecene core clusters of a deoxynivalenol-producing strain and a nivalenol-producing strain were compared by Daren Brown, who joined the research group in 2000. The deoxynivalenol chemotype was due to large insertions and deletions in the DNA sequence of *TRI13* that render the gene nonfunctional; a similar nonfunctionalization was discovered in *TRI7*, which encodes the 4-acetylation enzyme (*18*). Nonfunctionalization of *TRI13* and *TRI7* was discovered also by Lee and associates in Korea (*19*). Thus, nivalenol production is the ancestral trait in *F. graminearum*, and deoxynivalenol production evolved by gene nonfunctionalization.

Although it would seem logical for all of the trichothecene biosynthetic genes to be clustered, at least three of the genes, *TRI101*, *TRI1*, and *TRI16*, are not linked to the core cluster in *F. sporotrichioides* and *F. graminearum*. Indeed, on the genomic map of *F. graminearum* that was first published in 2003, *TRI1* is adjacent to *TRI16* on one chromosome, but *TRI101* and the *TRI5* core cluster are on two other chromosomes (*4*). Because these genes are not linked to *TRI5*, they were found not by chromosome walking but by other strategies. Research groups in Peoria and in Japan isolated *TRI101* by its ability to enable yeast transformants to grow in the presence of trichothecenes (*20*, *21*). The research group in Peoria and Beremand and colleagues in Texas identified the cytochrome P450 monooxygenase *TRI1* and linked acyltransferase *TRI16* by their gene expression patterns (*12*, *22*).

By 2004 it appeared that there was little left to learn about the organization of trichothecene biosynthesis genes in *Fusarium*, but that confidence appears to have been misplaced. In 2007, Proctor discovered that *Fusarium equiseti* has an expanded trichothecene core cluster, in which both *TRI1* and *TRI101* are present (*23*). The cluster organization in *F. equiseti* is significantly different from that in *F. sporotrichioides* and *F. graminearum*. Thus, the trichothecene gene cluster in the genus *Fusarium* appears to have evolved by complex processes of gene recruitment, reorganization, neofunctionalization, and nonfunctionalization.

A Fork in the Road. From 1984 to 1992, the Peoria group had focused its efforts on elucidating the T-2 toxin biosynthetic pathway in *F. sporotrichioides*, which was an ideal system for high-throughput screening for trichothecene mutants and intermediates. In 1985, *F. graminearum* had been rejected as a model system, mainly because this species did not produce high levels of deoxynivalenol in liquid media and because immunoassays for this toxin were not yet available for high-throughput screening. Beginning in 1991, however, epidemics of wheat and barley (*Hordeum vulgare*) head blight caused by *F. graminearum* increased in frequency and severity in the eastern and central United States and, by 1999, the USDA was ranking epidemics of *F. graminearum* head blight of wheat and barley as the worst plant disease outbreaks since the 1950s (*24*). Consequently, in 1992, *F. graminearum* strain GZ3639, which had been isolated from wheat head blight in Kansas, was incorporated as a new model organism for elucidation of the deoxynivalenol biosynthetic pathway.

Grain contamination with *F. graminearum* and deoxynivalenol has been associated with field outbreaks of swine feed refusal and emetic syndromes (*3*, *4*). Furthermore, ingestion of pure deoxynivalenol produces feed refusal and emesis in swine and other symptoms of toxicosis in experimental animals. Since the 19th century, outbreaks of a human disease known as red mold toxicosis have occurred in Japan, China, Korea, and Russia and have been associated mainly with consumption of grain contaminated with *F. graminearum*. Patients experienced nausea, vomiting, and diarrhea, often accompanied by headache, dizziness, trembling, euphoria, and even visual hallucinations. Deoxynivalenol, nivalenol, and related 8-keto trichothecenes were shown to produce symptoms characteristic of the disease and are strong candidates as major causal agents of human disease syndromes associated with the ingestion of red mold-contaminated grain.

Using transformation methods that had been developed for *F. sporotrichioides*, genes for trichodiene synthase and other trichothecene biosynthetic enzymes were disrupted to make mutants of *F. graminearum* that produce biosynthetic intermediates. Armed with an array of such mutants of both *Fusarium* species, McCormick created a library of dozens of trichodiene derivatives, trichothecene intermediates, and related compounds (*4*). To diversify chemical profiles, mutants were grown on solid substrates and in liquid media under a range of conditions and treatments with enzyme inhibitors. Additional compounds were produced by synthetic chemistry and by feeding trichothecene precursors to wild-type and mutant strains. This extensive trichothecene library has proven useful to scientists in Peoria and elsewhere for studies of trichothecene biosynthesis and of structure−activity relationships of trichothecenes in plant and animal systems (*25*, *26*).

By analysis of the compounds that fungal strains produced and incorporated into trichothecenes, McCormick and collaborators established the sequence of oxygenations, isomerizations, cyclizations, and esterifications leading from trichodiene to the more complex trichothecenes (*4*). In both *F. sporotrichioides* and *F. graminearum*, trichodiene is oxygenated and undergoes extensive molecular rearrangements to produce isotrichodermol, the first pathway intermediate with a trichothecene skeleton. Isotrichodermol contains the trichothecene pyran ring, the C-12,13 epoxide, and a C-3 hydroxyl group. The next three pathway steps are acetylation of the C-3 hydroxyl group, C-15 hydroxylation, and acetylation to produce calonectrin, after which the pathway splits into two major branches. One branch leads to diacetoxyscirpenol and T-2 toxin in *F. sporotrichioides* (and *F. sambucinum*), with hydroxylations and acylations at C-4 and C-8. The other branch leads to deoxynivalenol and nivalenol in *F. graminearum*, with hydroxylations and acetylations at C-4 and C-7 and hydroxylation and oxidation to create the keto group at C-8.

Isotope incorporation experiments in 1985 had shown that all six oxygens of the core structure of T-2 toxin are derived from molecular oxygen (*7*), but by 2004 only four cytochrome P450 monoxygenases had been linked to trichothecene biosynthesis. Gene disruption studies indicated that the TRI11 and TRI13 monooxygenases each are responsible for a single hydroxylation, but that the TRI1 and TRI4 monooxygenases are more complex in function. Heterologous expression of *TRI1* and *TRI4* in the trichothecene-non-producing species *F. verticillioides* showed that the TRI4 enzyme from *F. graminearum* catalyzes all four oxygenations required for the conversion of trichodiene into isotrichodermol and is therefore a multifunctional monooxygenase (*27*). The *F. graminearum* TRI1 enzyme is multifunctional, catalyzing hydroxylation at C-7 and C-8, whereas the *F. sporotrichioides* TRI1 enzyme catalyzes hydroxylation at C-8 only (*28*). The reasons for the different product specificities of these TRI1 enzymes are now under investigation. Unexpectedly, *F. verticillioides* was able to oxidize the C-8 hydroxyl group to a keto group. The oxidoreductase responsible for this reaction remains unknown, but the sequencing and publication of the *F. graminearum* genome in 2003 and of the *F. verticiollioides* genome in 2005 should facilitate identification of candidate genes for enzymes that catalyze this reaction.

A Humble Beginning. During the first five years, the trichothecene biosynthesis research group followed a linear route of discovery of trichothecene chemistry and genetics. By 1990, however, the research emphasis began to shift to include the biology of trichothecenes, especially their role in plant pathogenesis. A wide range of plant pathogenic *Fusarium* species produce trichothecenes in planta, and trichothecenes are acutely toxic to plants. Thus, it seemed logical that evolution of the complex trichothecene biosynthetic pathway might benefit the plant pathogenic lifestyles of *Fusarium* species. By the end of the 1990s, determining the role of trichothecenes in wheat head blight and maize ear rot had become a major achievement of the research group. Plant pathology research in Peoria, however, began rather humbly, with parsnips and potatoes.

The road to parsnips (*Pastinaca sativa*) began in 1986 during a survey of plant natural products for inhibition of trichothecene biosynthesis. One of the most efficient inhibitors was xanthotoxin, a furanocoumarin produced by a wide range of plants, including parsnips. Parsnip roots infected with *F. sporotrichioides* or *F. sambucinum* produced xanthotoxin, which blocked trichothecene biosynthesis and caused the accumulation of nontoxic trichodiene in root tissues. At the same time, however, plant production of xanthotoxin was countered by fungal production of enzymes that detoxify xanthotoxin and apparently override the plant response, with a final outcome that trichothecene-producing fungal strains caused root rot (*29*). In contrast, trichothecene-non-producing mutants, obtained by ultraviolet irradiation or by disruption of the *TRI5* gene, were substantially reduced in virulence on parsnip roots (*29*, *30*). The parsnip root assays were the first demonstration that trichothecenes are important for plant pathogenesis in *Fusarium*.

Unexpectedly, virulence of *F. sambucinum TRI5* gene disruption mutants on potato (*Solanum tuberosum*) tubers was indistinguishable from virulence of the wild-type strain, indicating that production of trichothecenes is important for virulence on parsnip but not on potato (*30*). The basis for this host specificity is not known. However, 4,15-diacetoxyscirpenol was deacetylated in potato tubers, but not in parsnip roots or fungal cultures, suggesting that potato enzymes catalyze the deacetylations that yield scirpentriol, which is >100-fold less phytotoxic than 4,15-diacetoxyscirpenol (*26*). The humble parsnip and potato illustrate the complexity and integration of chemical interactions between *Fusarium* species and their host plants. Moreover, the opposite outcomes in the parsnip and potato systems indicate that one should be cautious in generalizing results from one plant species to another when assessing the role of trichothecenes, or any fungal metabolite, in plant disease.

Out Standing in Our Field. In its first decade, the trichothecene biosynthesis research group had integrated chemistry, genetics, and biology into a multidisciplinary research program. The research group had elucidated most of the biosynthetic pathway, discovered a cluster of biosynthetic genes, developed a method for gene disruption in *Fusarium*, and used it to produce an array of mutants. By 1993, evidence was accumulating that production of trichothecenes could affect *Fusarium*−plant interactions, although the outcome appeared to vary between systems. Studies with *TRI5* gene disruption mutants had shown that trichothecene production enhanced the abilities of *F. sporotrichioides* and *F. sambucinum* to cause parsnip root rot. In addition, Robert Proctor, who joined the group in 1990, used *TRI5* gene disruption mutants of *F. graminearum* to demonstrate a role for trichothecenes in wheat head blight (*31*). All of these experiments, however, had been conducted in controlled laboratory environments. The spring and summer of 1993 brought months of rain and disastrous floods to Illinois and much of the midwestern United States. Along with the rains came epidemics of wheat and barley head blight and grain contamination with deoxynivalenol so severe that farmers in some regions burned their crops in the field. This evidence of the economic impact of *F. graminearum* head blight emphasized the need to validate the importance of trichothecenes in wheat head blight under realistic agricultural conditions.

In the spring of 1994, under stringent conditions of permits from the USDA Animal and Plant Inspection Service, the Peoria

group began 10 years of Illinois field tests of *TRI5* gene disuptants and various other genetically modified strains of *F. graminearum*. Additional field tests of trichothecene mutants were conducted with collaborators outside Illinois: on wheat in Indiana in 1995 and on maize in Ontario, Canada, in 1996. The field tests consistently showed that trichothecene production plays a significant role in the spread of *F. graminearum* in wheat heads and maize ears, but is not necessary for initial infection of wheat or maize (*32*, *33*). As far as I am aware, the 1994 test was the first USDA-approved field test of a genetically modified plant pathogenic fungus in the United States.

With the successful completion of field testing, the importance of trichothecenes in wheat head blight and maize ear rot was conclusively proven under realistic agricultural conditions. Prior to this work, their apparent lack of host specificity had hindered the acceptance of a role for trichothecenes in plant pathogenesis. Plant pathological research in the laboratory and in the field has now placed trichothecenes firmly in the unique class of fungal metabolites that not only cause mycotoxicoses in animals but also are virulence factors in plant disease. Indeed, the trichothecenes remain to date the only major class of mycotoxins that has been proven to play a role in plant pathogenesis.

The Road to Resistance. As epidemics of wheat and barley head blight became more severe during the 1990s, USDA and university scientists began to work more closely with each other and with the agricultural industry on research toward controlling *Fusarium* head blight. In particular, because trichothecene production enhances the spread of *F. graminearum* in wheat heads, then interfering with trichothecene biosynthesis or toxicity might reduce head blight and grain contamination with trichothecenes. Thus, identification of genes for trichothecene resistance became a high priority of the research group in Peoria, leading microbiologist Nancy Alexander to join the research group in 1996 to assist Hohn and McCormick in this search. On the basis of the logical assumption that trichothecene producers would have genes for resistance to their own toxins, the search began with the T-2 toxin-producing species *F. sporotrichioides*. In 1998, *TRI101*, a gene for trichothecene 3-O-acetylation, was isolated from *F. sporotrichioides* in Peoria and from *F. graminearum* in Japan, in both cases by its ability to enable yeast transformants to grow in the presence of the trichothecenes diacetoxyscirpenol or T-2 toxin (*20*, *21*).

TRI101 was the first trichothecene resistance gene to be isolated and continues to be a target of efforts worldwide to develop cereal crops resistant to trichothecenes and to *Fusarium* head blight. Transgenic wheat, barley, and rice plants expressing *TRI101* genes have shown some resistance to head blight under greenhouse conditions, but transgenic wheat and barley expressing *F. sporotrichioides TRI101* have not consistently shown increased resistance in field tests (*34*). Structural and functional comparisons of TRI101 enzymes from the two species indicate that, although both enzymes can accommodate a range of trichothecene substrates, the *F. sporotrichioides* enzyme has a decreased catalytic efficiency for deoxynivalenol (*35*). Thus, *F. graminearum TRI101* might be a stronger candidate for development of transgenic crops resistant to deoxynivalenol-producing *Fusarium* species. However, relative phytotoxicities of 3-acetylated trichothecenes and their 3-non-acetylated homologues vary significantly between plant test systems and between trichothecenes (*26*, *34*). Thus, one should again be cautious in generalizing results from one plant species to another, or from one trichothecene to another, when assessing the potential for trichothecene acetylation to be a detoxification and to confer disease resistance.

Resistance to *Fusarium* head blight in wheat has not been associated with 3-O-acetylation, but has been associated with 3-O-glucosylation, of deoxynivalenol (*36*). In populations derived from the Chinese wheat line Sumai 3, this trait has been mapped to the 3BS chromosomal locus *Fhb1* (synonym: *Qfhs.ndsu-3 BS*). In addition, a 3-*O*-glucosyltransferase gene (*DOGT1*) is a likely deoxynivalenol-resistance gene in *Arabidopsis thaliana* (*37*). The product deoxynivalenol 3-*O*-glucoside was less active in the inhibition of protein synthesis, and plants constitutively overexpressing *DOGT1* were less sensitive to deoxynivalenol.

Expression of trichothecene resistance genes in cereal crops has the potential to reduce levels of *Fusarium* head blight and to decrease trichothecene contamination of cereal grains; however, much work remains to be done. A range of studies indicates that modifications of extraskeletal hydroxyl and ester groups may be reversible in vivo, in plants and animals, and may result in only partial detoxification and incomplete resistance. Moreover, isotrichodermol, the first and simplest trichothecene in the pathway, appears to contain all of the structural features necessary for phytotoxicity (*24*). For complete detoxification of trichothecenes, therefore, the highest priority should be discovery of enzymes and compounds that block or undo the 12,13-epoxidation of trichodiene that occurs at the beginning of the biosynthetic pathway. Trichothecene de-epoxidation activities have been demonstrated in bacteria but apparently have not yet been successfully transferred to plants (*4*).

CONCLUSIONS

Over 25 years, the trichothecene biosynthesis research group at the USDA in Peoria has integrated natural product chemistry with fungal genetics and with plant pathology in the laboratory and in the field to conduct groundbreaking research on how and why *Fusarium* species make these very complex and highly toxic metabolites. The ultimate success of the trichothecene biosynthesis research program was facilitated by the long-term commitment of public funds through the USDA and by the sustained collaboration of an interdisciplinary group of scientists. During the past 25 years, the field of trichothecene research has expanded enormously, and today research on trichothecene-producing *Fusarium* species and on trichothecene chemistry, genetics, plant pathology, and resistance is being conducted at dozens of institutions worldwide. It has been my privilege to be a part of this international research effort, which is producing new crop protection agents, postharvest treatments, chemical analytical methods, agronomic practices, plant varieties, and other strategies to reduce *Fusarium* diseases and trichothecene contamination of human foods and animal feeds worldwide.

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