Trichothecenes: from Yellow Rain to Green Wheat

Natural trichothecenes are a chronic problem in agriculture, but revived concerns extend to their potential use as agents of bioterrorism.

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More than 20 years ago, trichothecene mycotoxins made their controversial debut on the world stage. Alexander Haig, who was U.S. Secretary of State and visiting the western sector of then-divided Berlin, alleged in 1981 that trichothecenes had been used as biological warfare agents in Southeast Asia. He cited statements from villagers in the region, who claimed that they became ill after “yellow rain” fell from the sky.

Subsequent chemical and biological tests on samples that were smuggled by soldiers and refugees from Laos and Cambodia to Thailand and brought to the United States for analysis indicated several trichothecenes were present within some of the samples, including T-2 toxin, deoxynivalenol (DON), and nivalenol (NIV). However, although many species of the genus Fusarium produce such chemicals, biological analysis showed that the yellow spots on some samples contained high amounts of pollen and were likely deposited by Asian honeybees. On the basis of such findings, Matthew Meselson of Harvard University in Cambridge, Mass., and his colleagues concluded that the trichothecenes contained in those smuggled samples derived from natural sources and not from biowarfare agents.

During the past 20 years, individuals from several additional nations, including Afghanistan and northern Iraq, have alleged that trichothecenes were being used in biological weapons. Although proof for those allegations is scarce, officials with the United Nations Special Commission on Iraq (UNSCOM) obtained confirmation during the early 1990s after the Persian Gulf War from the Iraqi government of its biological weapons program, which included trichothecene production. According to UNSCOM documents, a team of Iraqi microbiologists began research on large-scale fermentation and purification of trichothecenes for weapons development around 1987. UNSCOM officials further concluded that there was no evidence that Iraq used trichothecenes or other mycotoxins as weapons.

Trichothecenes in Agriculture

The potential inclusion of trichothecenes in weapons developed for biological warfare purposes is a relatively recent concern. However, the occurrence of natural trichothecenes is an old and chronic problem in agricultural settings, especially in temperate regions of the world, where Fusarium species infect maize, wheat, barley, and other cereal plants (Fig. 1). Fusarium head blight and ear rot remain serious concerns for wheat, barley, and maize growers, who have few markets for trichothecene-contaminated grain due to national and international restrictions on its use for human food and animal feed.

More than a century ago, for example, plant pathologists in Europe and the United States associated wheat head blight with infections by F. graminearum, which produces DON and NIV. During World War II, consumption of over-wintered grain contaminated by F. sporotrichioides and related species caused alimentary toxic aleukia and deaths of hundreds of thousands of people in the former Soviet Union. In the 1970s, Russian microbiologists isolated T-2 toxin from strains of F. sporotrichioides and...
F. poae from grain associated with a fatal outbreak of alimentary toxic aleukia.

Meanwhile, during the 1970s in Japan, *F. graminearum* caused severe epidemics of akakabi-byo (red mold disease) on green wheat and other grains. People who ate products containing such contaminated grains typically developed nausea, vomiting, diarrhea, hemorrhaging, anemia, and other symptoms of trichothecene toxicity. In 1972, Japanese scientists were successful in identifying DON and NIV in grain infected with *F. graminearum*.

**Complex Biosynthesis for Extensive Family of Trichothecenes**

Trichothecenes are a family of chemicals containing dozens of sesquiterpenes that share a tricyclic nucleus and an epoxide at C-12 and C-13, which is essential for toxicity. This toxicity is attributed to the ability to inhibit eukaryotic protein synthesis, with 60S-ribosomal protein L3 being the major site of that inhibitory activity. They are named after the fungus *Trichothecium roseum*, from which the first trichothecene was isolated in 1948. Their chemical structures vary in both position and number of hydroxylations, as well as in position, number, and complexity of esterifications. For instance, the *Fusarium* trichothecenes have relatively simple short-chain esters, whereas trichothecenes of *Myrothecium*, *Stachybotrys*, and other fungi may contain more complex, macrocyclic esters.

Trichothecene biosynthesis proceeds from farnesyl pyrophosphate via the hydrocarbon trichodiene. A sequence of oxygenations, isomerizations, cyclizations, and esterifications leads from trichodiene to the more complex trichothecenes such as T-2 toxin, DON, and NIV. Eleven trichothecene biosynthetic genes, *TRI3* through *TRI13*, have been localized to a core gene cluster, and their functions have been established by targeted gene disruptions in *F. graminearum* and *F. sporotrichioides*.

A twelfth gene, *TRI101*, which is located outside the core gene cluster, encodes a 3-O-acetyltransferase that converts trichothecenes to less toxic, acetylated derivatives. Disrupting *TRI101* blocks the biosynthetic pathway at isotrichodermol, the first tricyclic intermediate, and blocks production of more toxic trichothecenes. *TRI101* thus appears to encode a mechanism for trichothecene self-protection for *Fusarium* species.

**Defining How Fungi Use Trichothecenes when Infecting Plants**

Because trichothecenes are highly toxic to plants and many trichothecene-producing *Fusarium* species are virulent plant pathogens, trichothecenes might be virulence factors. One way to test this possibility entails studying mutants that cannot produce trichothecenes—a relatively straightforward task because *Fusarium* is haploid and contains only one copy of *TRI5*, which encodes trichodiene synthase.

During the past 10 years, our research group at the U.S. Department of Agriculture (USDA) laboratories in Peoria, Ill., has conducted a series of experiments to disrupt *TRI5* and investi-
gate the importance of trichothecenes in a number of *Fusarium*-plant interactions. We first obtained TRI5 mutants, using protoplasts to transform virulent, trichothecene-producing *Fusarium* strains, while selecting resistance to the antibiotic hygromycin as a marker.

TRI5 mutants of *F. graminearum* and *F. sambucinum* produce no trichothecenes, but are otherwise indistinguishable from their progenitor strains in terms of morphology, growth rate, and sexual fertility. However, in greenhouse tests and in field tests, the ability of TRI5 mutants of *F. graminearum* to cause wheat head blight and maize ear rot is greatly reduced. Furthermore, the ability of TRI5 mutants of *F. sambucinum* to cause parsnip root rot is greatly reduced. Genetic analysis of *F. graminearum* (sexual stage *Gibberella zeae*) and *F. sambucinum* (sexual stage *G. plicarlis*) confirms that hygromycin resistance, trichothecene nonproduction, and reduced virulence cosegregate. However, the ability of TRI5 mutants of *F. sambucinum* to cause potato tuber rot remains intact, a finding that we cannot yet explain.

**Several Approaches for Rendering Plants Resistant to Fungi**

Knowledge of the biosynthesis and function of trichothecenes suggests new strategies for controlling trichothecene contamination of grains. For example, if a trichothecene enhances virulence of a particular *Fusarium* on a host plant, then altering the trichothecene target site in that plant species could increase resistance to that pathogen.

To this end, maize geneticist Linda Harris and colleagues at Agriculture and Agri-Food Canada in Ottawa, Ontario, can modify a gene encoding ribosomal protein L3 (RPL3) to change amino acid 258 from tryptophan to cysteine, which confers on yeast resistance to trichothecenes. Transgenic expression of this modified Rpl3 in tobacco plants increases resistance of such plants to trichothecenes. Meanwhile, maize, wheat, and barley lines that similarly express modified Rpl3 genes are being constructed and tested for their resistance to *F. graminearum*. Alternative approaches could be based on plants engineered with genes for detoxifying trichothecenes or inhibiting trichothecene biosynthesis.

One such gene, TRI101, encodes a 3-O-acetyltransferase that converts trichothecenes to less-toxic derivatives, while another, PDR5 from yeast, encodes a protein that transports trichothecenes extracellularly. Scientists at USDA laboratories in Peoria and in Albany, Calif., find that transgenic expression of either TRI101 or PDR5 in some plant species increases their resistance to trichothecenes. Specifically and significantly, wheat lines expressing TRI101 show enhanced resistance to wheat head blight caused by *F. graminearum*.

**Evaluating Disrupted-Gene Mutants in Several Contexts**

Disrupting specific genes to block fungal toxin biosynthetic pathways has proved a powerful tool for investigating the role of trichothecenes in complex biological processes such as plant disease. Additional classes of mycotoxins of particular interest to both animal pathologists and plant pathologists include the ergot alkaloids of the genus *Neotyphodium* and related fungi, aflatoxins of the genus *Aspergillus*, and fumonisins of the genus *Fusarium*.

During the past few years, biosynthetic genes for each of these major classes have been disrupted to yield mycotoxin-nonproducing mutants. Gene disruption mutants have now been used to test the roles of three mycotoxin classes in plant pathogenesis and in other aspects of fungal-plant interactions, sometimes with dramatic results.

Consuming cereal grains or pasture grasses that are contaminated by ergot alkaloids gives rise to ergotism, the most notorious mycotoxicosis in history. To learn more about the role of mycotoxins in ergotism, Daniel Panaccione of West Virginia University in Morgantown and his colleagues cloned and disrupted *lpsA*, a peptide synthetase gene required for ergovaline biosynthesis in a *Neotyphodium* species that systemically infects perennial ryegrass. Disrupting this gene in *Neotyphodium* blocks ergovaline biosynthesis but does not affect its virulence as defined by its ability to systemically infect perennial ryegrass.

Fumonisin mycotoxins cause fatal brain lesions in horses, lung edema in pigs, and cancer in experimental rodents. Our research group cloned and disrupted *FUM1*, a polyketide synthase gene required for fumonisin biosynthesis in *F. verticilloides*, which causes maize ear rot.
Disrupting FUM1 in F. verticillioides blocks fumonisins biosynthesis but does not affect the ability of such mutants to infect maize and cause ear rot.

Thus, neither ergot alkaloids nor fumonisins appear to be essential for their respective fungal-plant interactions. In any case, trichothecenes have the dubious distinction of being important both in animal toxicoses and in plant pathogenesis.

Two Trichothecene Toxins Considered Possible Bioterrorism Agents

The terrorist attacks in 2001 in the United States followed by the deadly anthrax incidents reawakened concern about the potential use of trichothecenes or other biological toxins as agents of biowarfare or terrorism. As a follow-up to antiterrorism legislation enacted during 2002, two trichothecene toxins were placed on the “Select Agents List” specifying who may possess or study 36 particular biological agents and toxins. Specifically, officials at the Centers for Disease Control and Prevention designated T-2 toxin and diacetoxyscirpenol as each having the potential to pose a severe threat to public health and safety.

If chemical analyses indicate that these or other trichothecenes are in an environmental sample, identifying the source of those materials can prove extremely difficult—as can proving that they were used deliberately as agents of biowarfare or bioterrorism. For one thing, trichothecene-producing fungi are common in soil and decaying plant matter, and also can occur in house dust. For another, trichothecene-producing fungi are distributed worldwide on a wide variety of host plants, especially cereals and grasses, and as pathogens of trees.

Moreover, in several regions of the world, particularly throughout Asia and Africa, there is scant information on natural occurrences of trichothecenes and on the diversity of Fusarium species and other fungi that produce them. Meanwhile, conducting surveys of trichothecenes and of trichothecene-producing Fusarium species that contaminate agricultural commodities or appear in natural environments would facilitate efforts to determine whether detecting them is due to their accidental or deliberate introduction. Moreover, further studies of global Fusarium populations would facilitate efforts to assess their impact on agriculture, particularly if strains from foreign populations of trichothecene-producing species were introduced into the United States.

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SUGGESTED READING