Current Review

Mycotoxins in Plant Pathogenesis

Anne E. Desjardins¹ and Thomas M. Hohn²

¹Bioactive Agents Research and ²Mycotoxin Research, National Center for Agricultural Utilization Research, USDA/ARS, 1815 N. University Street, Peoria IL 61604 U.S.A.

Received 11 November 1996. Accepted 13 December 1996.

The study of fungal toxins in plant pathogenesis has made remarkable progress within the last decade. Prior to the mid 1980s there was indeed a long history of research on fungal toxins. Fungal cultures provided a bewildering array of low molecular weight metabolites that demonstrated toxicity to plants. But although it was easy to demonstrate that fungal cultures contained toxic substances, it proved far more difficult to establish their causal role in plant disease (Yoder 1980). Critical analysis of the role of toxins in pathogenesis had to wait for the development of laboratory methods to specifically eliminate a toxin from a biological system. The development of DNA-mediated transformation of fungal species during the 1980s provided the essential tool to rigorously test the role of toxins, and other factors, in plant pathogenesis.

Beginning in the 1960s, biochemical and classical genetic analyses provided strong evidence that toxins produced by three *Cochliobolus* spp. are important in plant pathogenesis. These classic systems are (1) HC-toxin, a cyclic tetrapeptide, and *C. carbonum*, which causes Northern leaf blight of maize; (2) T-toxin, a linear polyketide, and *C. heterostrophus*, which causes Southern leaf blight of maize with Texas male-sterile cytoplasm; and (3) victorin, a chlorinated cyclic pentapeptide, and *C. victoriae*, which causes Victoria blight of oats. Recently, mutants of *C. carbonum* and *C. heterostrophus* with gene disruptions that block the biosynthesis of their characteristic toxins have been produced by DNA-mediated transformation (Panaccione et al. 1992; Yang et al. 1996). These toxin-nonproducing mutants were greatly reduced in virulence, thus firmly establishing the importance of HC-toxin and T-toxin in pathogenesis on susceptible genotypes of maize. The success of these pioneering studies of *Cochliobolus* spp. has encouraged the application of gene disruption techniques to other, less well-established, fungal systems. In this paper we will discuss recent progress toward applying gene disruption to testing the role of mycotoxins in plant pathogenesis.

Mycotoxins are defined as low molecular weight fungal metabolites that are toxic to vertebrates. Mycotoxins can have dramatic adverse effects on the health of farm animals and humans that eat contaminated agricultural products. Mycotoxicology has not been a traditional field of plant pathological research. Mycotoxin research has historically been performed by natural product chemists, mycologists, animal toxicologists, and human disease epidemiologists. The apparent lack of specificity of mycotoxins has hindered the acceptance of a role for mycotoxins in plant pathogenesis. In addition, mycotoxin contamination was perceived to be a post-harvest problem of stored grain. But it is now well established that many mycotoxin-producing fungal species cause plant disease under field conditions. It thus becomes logical to ask whether mycotoxins themselves play a role in plant pathogenesis in addition to their role in animal diseases.

A wide variety of fungal metabolites are both mycotoxic (toxic to animals) and phytotoxic (toxic to plants). This paper will focus on four classes of mycotoxins of continuing importance in animal and human diseases worldwide: ergot alkaloids, aflatoxins, trichothecenes, and fumonisins. For each mycotoxin class, we will present a brief toxicological history, and an update on the current status of toxin pathway genetic analysis and its application to the role of each toxin in plant pathogenesis.

Ergot alkaloids.

The most notorious mycotoxicosis in human history is ergotism, which is caused by consumption of grain, usually rye, contaminated with sclerotia of *Claviceps purpurea*. Ergotism has been known for more than 2,000 years, and was responsible for numerous epidemics of the disease called St. Anthony's Fire, which included gangrene of the extremities, convulsions, psychoses, and death, in Europe during the Middle Ages. Outbreaks of ergotism are now rare in human populations, largely because modern grain-cleaning procedures remove most sclerotia. In 1918, the alkaloid ergotamine was isolated from sclerotia of *C. purpurea* and proven to be a potent vasoconstrictor. Sclerotia can contain a complex mixture of biologically active alkaloids, which are the principal causative agents of ergot poisoning (Marasas and Nelson 1987; Beardall and Miller 1994).

Ergot alkaloids represent a large family of mycotoxins that are derived from both amino acid and isoprenoid precursors, and include clavines, simple derivatives of lysergic acid, and structurally complex ergopeptines such as ergotamine. The core structural feature of ergot alkaloids is the ergoline nu-

Corresponding author: Anne E. Desjardins
E-mail: desjarae@ncaur1.ncaur.gov

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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cleus, which is formed from 4-(γ,γ -dimethylallyl)tryptophan. Synthesis of 4-(γ,γ -dimethylallyl)tryptophan, the branch point step in ergot alkaloid biosynthesis, is catalyzed by the prenyl-transferase, 4-(γ,γ -dimethylallyl)tryptophan (DMAT) synthase, from dimethylallyl diphosphate and tryptophan. The gene encoding DMAT was recently isolated from *C. purpurea* (Tsai et al. 1995), but no information is available concerning the possibility that additional ergot alkaloid pathway genes may be closely linked, as occurs in other fungal toxin pathways.

Biosynthesis of the ergopeptines is catalyzed by a non-ribosomal peptide synthetase that employs D-lysergic acid, D-proline, and two additional amino acids as substrates. For ergotamine, these unspecified amino acids are L-alanine and L-phenylalanine. Genes encoding specific peptide synthetases involved in ergopeptide biosynthesis have not been identified in *C. purpurea*. However, portions of genes for putative peptide synthetases have been amplified by polymerase chain reaction from *C. purpurea* and from the closely related species *Acremonium coenophialum* (Panaccione 1996).

Outbreaks of ergotism occur in animals that eat grain contaminated with *C. purpurea* and other *Claviceps* spp. Similar mycotoxicoses occur in livestock that graze on pastures of certain fescue and ryegrass species that are infected with various *Acremonium* endophytes. These endophytic fungi appear to enhance growth, disease resistance, and drought tolerance of their grass hosts, but also contaminate them with ergot alkaloids that produce gangrene, convulsions, and other neurological disorders in animals that graze infected pastures. Endophyte-free pastures of fescue and ryegrass can be established to control mycotoxicoses, but such pastures show enhanced susceptibility to insect damage (Marasas and Nelson 1987; Scott and Schardl 1993). Little is known concerning the phytotoxicity of ergot alkaloids. The availability of the *C. purpurea* gene encoding DMAT synthase provides an opportunity to investigate the involvement of ergot alkaloid biosynthesis in fungal-plant and insect-plant interactions.

Aflatoxins.

The modern era of mycotoxicology began in England in 1960 with Turkey X disease and the discovery of aflatoxins. Toxicity of animal feeds containing contaminated peanut meal led to the deaths of more than 100,000 turkeys, and of other farm animals, by acute liver necrosis. Scientists in England quickly identified the toxin-producing organism as *Aspergillus flavus* and the toxic agents as a group of related bisfurano-coumarins that were named aflatoxins B₁, B₂, G₁, G₂, etc. Subsequent studies have shown that aflatoxins are potent liver toxins and liver carcinogens in a wide variety of animals, causing hepatocellular carcinomas in some species at dietary levels below 1.0 μg per kg of feed. Human exposure to aflatoxins can result from consumption of contaminated peanuts, corn, and other agricultural commodities, but also from consumption of meat, milk, and eggs from animals that have consumed contaminated feeds. The occurrence of aflatoxins in milk is of particular concern worldwide (Marasas and Nelson 1987).

During the past 35 years there have been extensive efforts to associate human liver cancer with aflatoxin consumption. These studies are complicated by the fact that human liver cancer is also associated with hepatitis B infection, which is common in many parts of the world where aflatoxin exposure is high. Long-term epidemiological studies of more than

18,000 men in Shanghai, China, have provided the strongest evidence to date that aflatoxins themselves increase the risk of human liver cancer and that aflatoxins interact synergistically with hepatitis B virus (Scholl and Groopman 1995). A particularly critical aspect of these studies was the measurement of aflatoxin adducts in urine and serum as a means of accurately relating aflatoxin metabolism to an individual's risk of developing hepatocellular carcinoma. The biologically effective dose of aflatoxins is determined by aflatoxin metabolism as well as by dietary intake, because the aflatoxin B₁ parent compound is not harmful prior to metabolic activation. The liver Phase I detoxification pathway forms aflatoxin B₁-8,9-epoxide, which is believed to cause site-specific mutations in the tumor suppressor gene p53 that lead to carcinogenesis (Scholl and Groopman 1995).

Aflatoxins are produced by *A. flavus* and *A. parasiticus*; whereas a wide range of *Aspergillus* spp. produce the aflatoxin precursor sterigmatocystin, which also is an animal toxin and carcinogen. The aflatoxin/sterigmatocystin pathways of *Aspergillus* spp. are perhaps the most thoroughly studied fungal polyketide pathways. The first step in the biosynthesis of sterigmatocystin/aflatoxin is catalyzed by a type I polyketide synthase (Chang et al. 1995; Feng and Leonard 1995). In contrast to most polyketide synthases, which utilize acetate as a starter unit, the starter unit for the aflatoxin/sterigmatocystin enzyme is hexanoate (Brobst and Townsend 1994). The synthase reaction product and first stable intermediate in the pathway is norsolorinic acid, which undergoes a complex series of modifications to yield sterigmatocystin and, finally, aflatoxin. Studies of the sterigmatocystin pathway in *A. nidulans* have shown that the gene encoding the polyketide synthase (*pksST*) is part of a gene cluster containing at least 25 pathway-related genes (Brown et al. 1996). This gene cluster occupies a 60-kb region and contains genes for regulatory factors in addition to all of the required pathway enzymes. The genes for the aflatoxin pathways in *A. flavus* and *A. parasiticus* are similarly organized (Yu et al. 1995) and in most cases appear to contain closely related homologs of the sterigmatocystin pathway genes (Trail et al. 1995; Brown et al. 1996).

Both *A. flavus* and *A. parasiticus* are pathogenic on a variety of plant species, although *A. flavus* predominates on most hosts except peanuts. Aflatoxin production is widespread in both species; field strains of *A. parasiticus*, in particular, rarely lose the ability to produce aflatoxins (Payne 1983). Although both aflatoxins and sterigmatocystin have been reported to be phytotoxic (Stoessl 1981; McLean et al. 1992), the potential role of these toxins in plant pathogenesis appears to have received little study during the past 20 years. Field studies have shown that naturally occurring strains of *A. flavus* that produce little or no aflatoxin in vitro can colonize cotton bolls (Cotty 1994). The availability of numerous aflatoxin biosynthetic pathway genes provides an opportunity to investigate the role of aflatoxins in fungal-plant interactions.

Trichothecenes.

For more than 100 years, both acute and chronic mycotoxicoses in farm animals and in humans have been associated with consumption of wheat, rye, barley, oats, rice, and maize contaminated with *Fusarium* spp. that produce trichothecene toxins. Experiments with chemically pure trichothecenes at

low dosage levels have reproduced many of the features observed in moldy-grain toxicoses in animals, including anemia, immunosuppression, hemorrhage, emesis, and feed refusal. Historical and epidemiological data from human populations indicate an association between certain disease epidemics and consumption of grain infected with *Fusarium* spp. that produce trichothecenes. In particular, outbreaks of a fatal disease known as alimentary toxic aleukia, which has occurred in Russia since the nineteenth century, have been associated with consumption of over-wintered grains contaminated with *Fusarium* spp. that produce the trichothecene T-2 toxin. In Japan, outbreaks of a similar disease called akakabi-byo or red mold disease have been associated with grain infected with *Fusarium* spp. that produce the trichothecene deoxynivalenol and related compounds. There is more direct evidence that trichothecenes were responsible for recent human disease outbreaks in India and Japan, where trichothecenes were detected in the toxic grain samples themselves (Marasas et al. 1984; Beardall and Miller 1994). In addition, symptoms produced by the trichothecene diacetoxyscirpenol in clinical trials conducted with terminally ill cancer patients were similar to reported symptoms of alimentary toxic aleukia and akakabi-byo (Anonymous 1983). Following severe animal disease epidemics in the U.S. and in Japan in the early 1970s, scientists from both countries independently isolated and identified trichothecene toxins from the suspect feeds. The continuing natural occurrence of trichothecenes in grains worldwide has prompted study of their chemistry, genetics, and toxicology.

Trichothecenes constitute a large family of sesquiterpene epoxides that inhibit eukaryotic protein synthesis. The biosynthesis of trichothecenes by *Fusarium* spp. proceeds from the hydrocarbon trichodiene through a complex series of steps to trichothecenes such as diacetoxyscirpenol, deoxynivalenol, and T-2 toxin. The details of trichothecene biosynthesis have been established through experiments with a number of *Fusarium* spp. in several laboratories in the U.S., Canada, and England (Desjardins et al. 1993). In common with biosynthetic genes for aflatoxins and many other microbial antibiotics, trichothecene pathway genes in *Fusarium* are closely linked and constitute a gene cluster (Hohn et al. 1995). The characterization of the trichothecene gene cluster continues in our laboratory. To date, 10 genes involved in trichothecene biosynthesis have been localized to a 25-kb region of chromosomal DNA in *F. sporotrichioides*. The cluster contains *Tri5*, the gene encoding trichodiene synthase, which catalyzes the first step in trichothecene biosynthesis. Transformation-mediated disruption of *Tri5* blocks the biosynthesis of trichodiene and all trichothecenes in *F. sporotrichioides*, *Gibberella pulicaris*, and *G. zae* (Hohn and Desjardins 1992; Proctor et al. 1995).

Trichothecenes are produced by a number of *Fusarium* spp., including *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum* (*G. zae*), *F. lateritium*, *F. poae*, *F. sambucinum* (*G. pulicaris*), *F. solani*, and *F. sporotrichioides* (Marasas et al. 1984; El-Banna et al. 1984; Clark et al. 1995). Trichothecene-producing *Fusarium* spp. are destructive pathogens and attack a wide range of plant species. The acute phytotoxicity of trichothecenes and their occurrence in plant tissues also suggest that these mycotoxins play a role in the pathogenesis of *Fusarium* on plants. Our research group has investigated the role of trichothecenes in a number of plant diseases by generating trichothecene-nonproducing mutants

through the disruption of *Tri5*. This approach has been particularly successful because *Fusarium* is haploid, and because *Tri5* occurs as a single copy.

Diacetoxyscirpenol biosynthesis was blocked by disruption of *Tri5* in *G. pulicaris*, which causes dry rots of a variety of plants. The virulence of trichothecene-nonproducing mutants was significantly reduced on parsnip root, but was not changed on potato tuber. To determine whether the reduced virulence of the mutants was due specifically to *Tri5* disruption or to nontarget effects of the transformation process, a *Tri5*⁻ mutant was crossed to a *Tri5*⁺ wild-type strain (*G. pulicaris* is heterothallic). Tetrad analysis resulted in either cosegregation of hygromycin resistance, trichothecene nonproduction, and reduced virulence on parsnip, or in the simultaneous loss of all three traits (Desjardins et al. 1992). These results were consistent with an earlier finding that production of trichothecenes is important for virulence of *F. sporotrichioides* on parsnip root (Desjardins et al. 1989). This apparent effect of the host on the importance of trichothecenes in virulence is still unexplained, but suggests that the importance of trichothecenes in disease may differ from one plant species to another.

Deoxynivalenol biosynthesis also was blocked by disruption of *Tri5* in *G. zae*, which causes seedling blights, root rots, and ear and head blights of wheat, barley, rye, maize, rice, and other grains. The virulence of two trichothecene-nonproducing mutants was significantly reduced in tests in the growth chamber of wheat seedling blight and head scab (Proctor et al. 1995). Virulence was also assessed under field conditions in 1994 (one test site) and 1995 (two test sites) by controlled inoculation of spore suspensions into flowering wheat heads. Trichothecene-nonproducing (*Tri5*⁻) mutants were less virulent than the trichothecene-producing (*Tri5*⁺) parent in their ability to cause head scab. Although trichothecene-nonproducing strains colonized wheat heads, the infected heads showed less disease according to several parameters we tested, including head bleaching symptoms, seed weight, seed viability, and trichothecene contamination (Desjardins et al. 1996b).

To determine whether reduced virulence of *Tri5*⁻ mutants was due specifically to *Tri5* disruption or to nontarget effects caused by the transformation process, we generated a revertant from a *Tri5* disruption mutant by allowing the mutant to pass through the sexual phase of its life cycle (*G. zae* is homothallic) (R. H. Proctor, T. M. Hohn, and S. P. McCormick, *unpublished*). To facilitate strain tracking during the field test, a revertant was marked by transformation with a plasmid conferring resistance to the antibiotic hygromycin B. Virulence of two trichothecene-producing revertants was assessed under field conditions in 1995, and both revertants were indistinguishable from the *Tri5*⁺ parent in their ability to cause head scab on field grown wheat (Desjardins et al. 1996b). This evidence confirms that trichothecene toxins are virulence factors in wheat head scab. Preliminary data from 1996 field tests indicate that trichothecene-nonproducing mutants of *G. zae* also demonstrate reduced ability to cause maize ear rot (L. J. Harris, A. E. Desjardins, R. D. Plattner, R. H. Proctor and T. M. Hohn, *unpublished*).

Fumonisin.

The toxicity of maize contaminated by *F. moniliforme* has been well documented for more than 100 years. A disease of farm animals known as moldy corn poisoning or blind stag-

gers was first described in the U.S. in 1850. The most dramatic manifestation of moldy corn disease is leucoencephalomalacia, a fatal brain disease of horses, donkeys, mules, and rabbits. In 1988, a South African research group reported the isolation of fumonisin B₁ from cultures of *F. moniliforme* (sexual stage, *G. fujikuroi* mating population A), and they subsequently demonstrated that either an oral dose or an intravenous injection of pure fumonisin B₁ could produce leucoencephalomalacia in horses (ELEM) and liver cancer in rats (Marasas 1995; Munkvold and Desjardins, in press). This research was the starting point for worldwide efforts to describe the chemistry, biology, and toxicology of this new group of mycotoxins.

Fumonisin is acutely toxic to the liver and kidney of a wide range of experimental animals. Consumption of feed contaminated with fumonisins or an intravenous injection of pure fumonisin B₁ can produce a fatal lung edema in pigs. Although the role of fumonisins in some moldy corn diseases of livestock has now been well established, their role in human diseases and, most particularly, their carcinogenic potential in humans are much more difficult to determine. The search for causes of the high rate of esophageal cancer in the Transkei region of South Africa and in central China led to the discovery of unusually high levels of fumonisins in maize that was being used for human consumption in these regions (Marasas 1995). The number of maize samples that was analyzed in these studies, however, was too small for a conclusive epidemiological analysis.

Fumonisin is an amino polyalcohol and is structurally similar to the long-chain base backbones of sphingolipids. Fumonisin inhibits the activity of sphingosine N-acetyltransferase, which leads to the accumulation of toxic sphingoid bases. Although current models link the biological activities of fumonisins to sphingolipid metabolism, we still have much to learn about the mechanisms by which fumonisins are toxic and carcinogenic. Treatment with fumonisins induces apoptosis (programmed cell death) in several types of cultured human and animal cells, and in experimental animals (Merrill et al. 1996). The role of sphingolipids and of sphingolipid-analog mycotoxins in apoptosis is a fast-developing field of research that should provide insights into the diseases caused by consumption of fumonisins.

Fumonisin is produced by several members of the *G. fujikuroi* species complex, including serious pathogens of maize, sorghum, millet, and rice, but the most consistent and important producer of fumonisins is *G. fujikuroi* mating population A (Munkvold and Desjardins, in press). The high frequency (>95%) of fumonisin production among strains of *G. fujikuroi* mating population A from maize and the high frequency of fumonisin contamination in maize raise the possibility that fumonisins play a role in pathogenesis on maize. Further indirect evidence is provided by the structural similarity of fumonisins to AAL-toxin, which plays a role in pathogenesis of *Alternaria alternata* f. sp. *lycopersici* on certain genotypes of tomato (Winter et al. 1996). In addition, pure fumonisins at low concentrations have been shown to cause necrosis and other symptoms in maize seedlings, tomato seedlings, and other plants (Lamprecht et al. 1994). Preliminary genetic analysis in *G. fujikuroi* mating population A has also shown an association between production of fumonisins and high levels of virulence on maize seedlings (Desjardins et al. 1995).

The structural similarity of fumonisins to the long chain sphingolipid bases suggests that fumonisin biosynthesis may be similar to sphingolipid biosynthesis. The latter begins with the condensation, catalyzed by serine palmitoyltransferase, of an amino acid with a fatty acyl-CoA. If fumonisin B₁ is synthesized in a similar manner, then alanine would replace serine and an 18-carbon fatty acyl CoA would replace palmitoyl CoA. Isotope-feeding studies determined that alanine is a biosynthetic precursor of fumonisin B₁, and that the polyalcohol moiety is derived from acetate (Blackwell et al. 1996). However, the number and order of the steps of the biosynthetic pathway are unknown, and whether the polyalcohol is synthesized by a fatty acid synthase or by a polyketide synthase has yet to be determined. To date, no fumonisin biosynthetic enzymes have been purified, and no pathway genes have been cloned. Genetic analysis indicates that some genes responsible for fumonisin production are closely linked and may constitute a gene cluster on chromosome 1 of *G. fujikuroi* mating population A (Desjardins et al. 1996a). The recent identification of DNA markers closely linked to fumonisin biosynthetic genes should facilitate map-based cloning strategies and gene disruption to conclusively determine the importance of fumonisins in pathogenesis on maize (Xu and Leslie 1996; Proctor et al. 1995).

Other mycotoxins.

In addition to the well-known mycotoxins discussed above, a number of other mycotoxins warrant closer scrutiny with respect to their role in plant pathogenesis. These include a diverse array of metabolites produced by *Fusarium*, *Aspergillus*, and *Penicillium* spp. With few exceptions, molecular genetic analysis of these mycotoxin biosynthetic pathways is not very far advanced.

Fusarium mycotoxins of interest include zearalenones, the strongly estrogenic polyketides produced by *F. graminearum* and related species. Consumption of feeds contaminated with zearalenones causes severe reproductive and fertility problems in animals (Marasas et al. 1984). Phytotoxicity of zearalenones has not been well studied. The enniatins and beauvericins constitute a family of cyclic depsipeptides that are produced by many *Fusarium* spp. and demonstrate toxicity to both vertebrates and plants. While the importance of enniatins and beauvericins as mycotoxins has yet to be convincingly demonstrated, the production of enniatins by *F. scirpi* was recently implicated as a virulence factor in potato tuber dry rot (Herrmann et al. 1996). Moniliformin is an unusual cyclobutane derivative with phytotoxicity and mycotoxicity, especially to avian species, and has been reported to inhibit mitochondrial oxidative enzymes (Cole et al. 1973; Leslie et al. 1996). Strains of *G. fujikuroi* associated with the Baka disease of rice produce particularly high levels of moniliformin (Marasas et al. 1986).

Aspergillus and *Penicillium* mycotoxins such as patulin and ochratoxins are also candidates for further investigation of their roles in plant-fungal interactions. Patulin is a cyclic tetraketide with phytotoxic activity (McKinley and Carlton 1991). Although patulin can be produced by a wide range of fungi, including numerous *Aspergillus* and *Penicillium* spp., the major source of patulin in the food supply is juice from apples infected with *P. expansum*. Toxicity of patulin to a wide range of experimental animals has been reported, but

human and animal disease epidemics have not been associated with patulin consumption. The gene encoding 6-methylsalicylic acid synthase, a polyketide synthase involved in patulin biosynthesis, has been isolated from *P. patulum* and *P. urticae* (Beck et al 1990; Wang et al. 1991). Ochratoxins are chlorinated cyclic pentaketides with potent liver and kidney toxicity and carcinogenicity (Prelusky et al 1994). Ochratoxins are produced by several *Aspergillus* and *Penicillium* spp. and can occur in a wide variety of grains and other plant products, and also in the meat of animals consuming contaminated feeds. There is considerable epidemiological evidence that consumption of ochratoxins is associated with a chronic, fatal kidney disease of humans in the Balkan region of Eastern Europe. Human exposure has been confirmed by measurement of ochratoxin and its metabolites in serum (Fink-Gremmels et al. 1995). Ochratoxins have been reported to be carcinogens and protein synthesis inhibitors, but their phytotoxicity is not known.

Some qualifications.

The use of gene disruption to block toxin biosynthetic pathways has proven to be a powerful tool for investigating the role of toxins in complex biological processes such as plant disease. Caution is needed in the interpretation of these experiments, however, for two reasons. First, it is important to exclude changes in virulence or pathogenicity that may result from the transformation process or other experimental manipulations that accompany gene disruption experiments. This problem can be addressed, for example, by generating revertants of mycotoxin-deficient mutants and testing these revertants to confirm that they regain virulence when they regain toxin production. Choosing among a variety of reversion strategies depends on the type of gene disruption used and on whether the fungal pathogen can undergo meiosis. Additive types of gene disruption, although less stable than gene replacement, have the advantage of frequently undergoing reversion during meiosis. A second, and more troublesome, complication in interpreting the results of toxin pathway gene disruption experiments is evaluating the effect of a pathway block on fungal metabolism and on pathogenic fitness. Blocking a mycotoxin pathway may alter fungal metabolism in ways that are too subtle to detect by analysis of parameters such as growth rate, but that still alter fitness in the fungal-plant interaction. One might address this problem by comparing mutants blocked at different steps of the toxin biosynthetic pathway, but interpretation of these data would be complicated if pathway intermediates differed in the biological activity under study.

In principle, the identification of toxins as probable virulence factors by fungal genetic analysis should be confirmed by plant genetic analysis. If production of a toxin increases pathogen virulence, then increased host resistance to the toxin should increase host resistance to the disease. Such rigorous proof of function has been achieved in only two fungal toxin systems: *C. carbonum* and HC-toxin, and *C. heterostrophus* and T-toxin. Genetic analyses have shown that resistance of maize to *C. carbonum* is associated with the presence of the *HMI* gene encoding the enzyme HC-toxin reductase, which inactivates HC-toxin by reduction of an essential carbonyl group (Johal and Briggs 1992). Susceptibility of maize to *C. heterostrophus* is caused by the presence of a T-toxin binding

protein encoded by *T-urf 13*, a mosaic gene unique to the mitochondrial chromosome of T-cytoplasm maize (Levings and Siedow 1992). Application of plant genetic analysis to *Fusarium*-trichothecene toxin systems is not likely to be so straightforward, in large part because host cultivars do not show monogenic differences in susceptibility to these pathogens.

Prospects.

Although mycotoxins are an old problem, mycotoxicology is a young science. Despite difficult problems, a good beginning has been made in the study of the biosynthesis and biology of ergot alkaloids, aflatoxins, trichothecenes, and fumonisins. A beginning has already been made, but much remains to be done. We encourage our plant pathologist colleagues to investigate mycotoxins, and mycotoxin-producing fungi.

On the credit side, we note that the plant pathologist often need not invest time and effort in complex toxin isolation procedures because many mycotoxins are commercially available in highly purified form. In addition, the importance of mycotoxins in animal disease has stimulated the development of sensitive, accurate, and inexpensive toxin assays, and antibody-based detection kits for a number of mycotoxins are commercially available.

On the debit side, we recognize that mycotoxins and the fungi that produce them are, by definition, health hazards. Safe handling of mycotoxins in research laboratories requires development and enforcement of guidelines that cover routine handling and decontamination procedures, as well as medical monitoring of laboratory personnel. Safeguarding human health is a major responsibility in the mycotoxin research laboratory. Safeguarding human and animal health is the goal of mycotoxin research. To accomplish this goal, we must not only understand the chemistry and toxicology of mycotoxins, but we must also be able to understand why some plant pathogenic fungi produce them.

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