Deoxynivalenol: Known Facts and Research Questions

**Summary:**

DON (deoxynivalenol) is a damaging toxin produced by the fungus *Fusarium graminearum* in the heads of small grains.  In addition to DON, *F. graminearum* strains may also produce modified forms of DON called 3-ADON and 15-ADON.  Surveys of naturally infected grain in the U.S. and Canada indicate that 3-ADON and 15-ADON are found only at low levels, and only when high levels of DON are present.  Genetic markers can be used for preliminary identification of strains that make relatively more 3-ADON or more 15-ADON, but the markers do not necessarily predict the amounts of DON, 3-ADON, and 15-ADON that fungal strains produce.  While there are major differences among *Fusarium* strains in aggressiveness, or ability to cause disease in plants, there is no evidence that these differences are associated with production or lack of production of 3-ADON or 15-ADON.   DON, 3-ADON, and 15-ADON appear to have similar toxicities to mammals, although the toxicology data are limited.   No evidence currently exists that *Fusarium* is responding to recommended control practices – use of moderately resistant cultivars, and timely application of fungicide if indicated – by becoming more aggressive.

**What is DON and what related forms are associated with *Fusarium*-infected grain?**

Deoxynivalenol (also called DON or vomitoxin) is one of an array of trichothecene mycotoxins produced by *Fusarium graminearum (*sexual stage = *Gibberella zeae)* and several other species of *Fusarium* that cause Fusarium head blight (also called FHB or scab) of wheat, barley, and other grasses and ear and stalk rot of corn.  Surveys to date indicate that DON is the major trichothecene in naturally infected grain in the USA and Canada, usually accounting for more then 90% of the total trichothecenes present.

The U. S. Food and Drug Administration (http://www.cfsan.fda.gov/~dms/graingui.html) has established advisory levels for DON-contaminated grain and grain-based products as follows:

- *1 ppm DON on finished wheat products, e.g. flour, bran, and germ, that may potentially be consumed by humans. The FDA has not established an advisory level for wheat intended for milling because the manufacturing practices and technologies available to millers can substantially reduce DON levels in the finished wheat products from those found in raw wheat. Because of significant variability in manufacturing processes, an advisory level for raw wheat is not practical.*

- *10 ppm DON on grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens, with the added recommendation that these ingredients not exceed 50% of the diet of cattle or chickens.*

- *5 ppm DON on grains and grain by-products destined for swine with the added recommendation that these ingredients not exceed 20% of their diet.*

- *5 ppm DON on grains and grain by-products destined for all other animals, with the added recommendation that these ingredients not exceed 40% of their diet.*

Deoxynivalenol belongs to the family of chemical compounds known as trichothecenes. DON, like many chemicals in nature, is produced via a complex biochemical pathway. In North American grain, surveys to date indicate that DON is the predominant toxin. However, acetylated versions of DON, such as 3-acetylDON (3-ADON) and 15-acetyl DON (15-ADON), are produced as steps along the biosynthetic pathway in *F. graminearum*, and may also be detected in *Fusarium*-infected grain. Significant amounts of 3-ADON and 15-ADON can be produced in laboratory cultures and under conditions where extremely high levels of trichothecenes are produced. In naturally infected grain in the US and Canada, however, surveys to date indicate that 3-ADON and 15-ADON are only present as minor components in grain contaminated with DON. The best information available to date indicates that all *F. graminearum* strains have the ability to produce DON, 3-ADON, and 15-ADON, but that strains can differ in the relative amounts of 3-ADON and 15-ADON they produce. Strains that produce DON and 3-ADON, with little or no 15-ADON, have been called “3-ADON chemotypes" (i.e. chemical phenotypes). Strains that produce DON and 15-ADON, with little or no 3-ADON, have been called “15-ADON chemotypes”. Although the 3-ADON and 15-ADON "chemotypes" are readily distinguishable, both produce mainly DON in naturally infected grain in the US and Canada.

**How are the 3-ADON and 15-ADON genotypes (a.k.a. "chemotypes") determined?** The determination of genotype is conducted using a genetic analysis. This determination does not allow the prediction of the level of any toxin (DON, 3-ADON or 15-ADON) produced by a given isolate, though it does provide an indication of the relative levels of these toxins that *F. graminearum* will produce in the laboratory.

**What are 3-ADON and 15-ADON genotypes?** DON biosynthesis is known to require at least 14 genes. Ten of these genes are located next to each other in a trichothecene (TRI) gene cluster in *F. graminearum*. In some studies, differences in DNA sequences (called genotypes) in some of these TRI-cluster genes have been associated with differences in 3-ADON and 15-ADON production. Because of this association, these TRI-cluster genotypes have even been used as markers for these chemical phenotypes and have been referred to as "chemotypes" in some literature. This terminology has led to some confusion, because the traditional definition of a chemotype is an actual chemical phenotype, not a genotype. Thus, although they are useful markers for analysis of *F. graminearum* populations, TRI-cluster markers are not able to predict the chemical phenotype (i.e. the actual amounts of DON, 3-ADON and 15-ADON) produced by a given strain of *F. graminearum*.

**What is known about the toxicity of DON and these genotypes?** There are few comparative studies in the published literature of the relative toxicities of DON, 3-ADON and 15-ADON, and in several of these studies, the data were based on a very small number of experimental animals. Based on the three largest toxicology studies in mice, published in the 1970s and 1980s, DON, 3-ADON, and 15-ADON have similar acute toxicities after oral ingestion, intraperitoneal injection, and subcutaneous injection. Websites such as www.biopure.at/biopure-index/datasheets/mdc.htm provide fact sheets on DON and the two acetylated forms of DON. These fact sheets indicate that 3-ADON is similarly toxic to DON, whereas 15-ADON is less toxic than DON.

**Is there evidence that fungal isolates that produce 3-ADON are more effective pathogens of wheat than isolates that produce 15-ADON, or than isolates that produce only DON?** Preliminary results from a Canadian greenhouse study of inoculated wheat heads indicated no significant differences in amount of disease caused by 3-ADON compared to 15-ADON "chemotypes" (Gilbert et al., 2005 National Fusarium Head Blight Forum proceedings, p. 160). Although the 3-ADON "chemotypes" produced more DON than the 15-ADON "chemotypes", the DON levels produced by both "chemotypes" far exceeded desirable limits, rendering the difference unimportant from a practical perspective. It is not yet known if 3-ADON "chemotypes" produce more DON in field-grown plants than do 15-ADON "chemotypes." We do know that individual isolates of *F. graminearum* differ greatly in their aggressiveness on wheat and in the level of DON they produce in the grain of *Fusarium*-infected wheat plants. While the capacity of some isolates to produce DON (the most prevalent trichothecene) might help explain differences in aggressiveness, there are insufficient data to support this hypothesis.

**If an isolate produces 3-ADON or 15-ADON in the field, does it also produce regular DON?** Absolutely. Dr. Marcia McMullen, NDSU extension plant pathologist, examined the toxins present in grain samples from naturally infected wheat grown in the 2005 North Dakota wheat crop that was devastated by FHB. Her findings (presented at the 2005 North Central American Phytopathological Society regional meeting) were:

- DON was present in all commercial wheat samples tested from eastern ND
- 15-ADON and 3-ADON were rarely detected
- 15-ADON and 3-ADON were found ONLY in samples which also had high levels of DON
- 3-ADON was found only in susceptible wheat cultivars, not in cultivars with improved resistance to FHB (such as those wheat cultivars with resistance derived from the Chinese wheat Sumai-3)

- 15-ADON was found only in samples from fields that had not been treated with fungicides or treated too early (before heading) for the fungicide to have been effective.

These results are supported by unpublished findings of Dr. Ruth Dill-Macky, research plant pathologist at the University of Minnesota. She collected 150 *F. graminearum* isolates from 50 wheat fields in Minnesota and cultured them on autoclaved wheat grain in the laboratory. In those samples in which 3-ADON or 15-ADON could be detected, the amount of DON present was 20 to 25 times as great as the amount of either 3-ADON or 15-ADON (DON:15-ADON = 25:1, and DON:3-ADON = 21:1).

In a Canadian study of corn in the early 1980s (Miller et al., 1983, Can. J. Bot. 61:3080-3087), researchers report that a *F. graminearum* isolate that produced far more 15-ADON than DON in the laboratory did just the reverse in the field. They detected far more DON than 15-ADON in inoculated corn ears, and the difference increased as the season progressed. It is known that plants and microorganisms, including but not limited to *Fusarium* spp., can convert 15-ADON into DON. Thus, making assumptions on the field performance of a "chemotype" based on laboratory analyses of the fungus would be fraught with hazards. Additional field-based studies are needed to understand the relationship between the toxin production ability of individual isolates, be they 3-ADON or 15-ADON "chemotypes", and the accumulations of DON, 3-ADON and 15-ADON in the kernels of *Fusarium*-infested wheat.

**Do current recommended control practices reduce the risk posed by DON in any of its forms?** The control practices advocated by the US Wheat and Barley Scab Initiative (`http://www.scabusa.org/`) and by many land grant universities, i.e., the use of resistant wheat and barley varieties and timely application of appropriate fungicides, will reduce the level of infection by the fungus and consequently reduce the levels of all forms of DON. There appears to be no evidence to indicate that the fungus is responding to these control practices by becoming more aggressive and/or by producing more toxic forms of DON. Theory tells us that while it is not impossible for *F. graminearum* to adapt in response to wheat resistance or fungicides, there is an important obstacle to such adaptation. *F. graminearum* generally goes through only one cycle on wheat per year, and thus the population would likely be subjected to the selection pressures of the plants' active defense responses for a maximum of 40-50 days a year. During the remainder of the year (300+ days), these *Fusarium* species live on decaying plant debris. Much of the *Fusarium* population likely never encounters a wheat plant, and would be even less likely to encounter wheat with a high level of resistance, as the fungus has a broad host range and can complete its life cycle on living cereal crops, corn, and other wild grasses that serve as hosts, or on the residues of soybeans and other crops considered to be non-host species. This contrasts with obligate pathogens such as the rusts and mildews, which may reproduce many times per growing season and exclusively on one host species. Clearly, more research is required to understand whether planting resistant varieties is a factor in the reported shift from the 15-ADON to the 3-ADON "chemotype" of *F. graminearum*.

The occurrence of DON in commercial fields is well established and its economic consequences are widely documented. For producers, the presence of DON means lower prices and reduced marketability of grain, while processors and end-users struggle to find low-DON or DON-free grain in heavy disease years. Further studies are necessary to determine the relevance of the different genotypes (e.g. 3-ADON and 15-ADON) of *F. graminearum* to the epidemiology and management of FHB.

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