Sampling Grain for Deoxynivalenol (DON) Analysis: A Researchers Guide

DON levels present in wheat, barley, and other cereals infected with Fusarium head blight (FHB) may vary according to time of infection, fungal strain, and environmental conditions. This variation is of concern to FHB researchers. For example, researchers may find that the level of DON detected in their grain samples correlate poorly to the level of disease visually observed in experimental plots, including large variation among replicates in individual experiments. The following guidelines may help researchers with obtaining more accurate DON evaluations.

Before deciding how to sample researchers should consider the likely sources of variation and the rationale for their DON analyses. Remember that much of the variability observed in DON levels in grain is related to the biology of the disease, rather than the chemical analysis of the toxins, as the DON accumulation in grain results from a complex host-pathogen interaction which is subject to environmental variability.

Thus before sampling researchers should be aware that:

- Considerable variability exists among fungal strains in their production of DON and related toxins.
- The production of DON, like the visible symptoms of FHB, vary greatly from spikelet to spikelet, spike to spike, and environment to environment.
- The production of DON and its accumulation and retention in plant tissues is influenced by the environmental conditions including temperature and moisture, not only between initial infection and assessment, but also between FHB assessment and harvest.
- Grain sampling greatly affects the accuracy of DON analysis.
- Sampling protocols will likely vary among researchers based on the research objectives and project resources (equipment and labor) - so what others do might not suit your program.

Analytical Variation

The three USWBSI-funded mycotoxin diagnostic laboratories analyze check samples on a monthly basis. As these check samples are ground, blended, and then mailed to each laboratory, the variation in results is largely attributable to analytical variation among the laboratories. The coeffecients of variation (CV) on the analysis of these samples typically range from 5 to 15%, which is considered as acceptable for analytical work in the mg/kg range. Thus at a 10% CV, the expected analytical variation in DON results would be:

Mean DON (ppm)	Standard Deviation	Range of Results (95% CI)
0.50	0.05	0.44-0.56
1.00	0.10	0.89- 1.11
5.00	0.50	4.43-5.57
10.00	1.00	8.87 – 11.13

Collecting a Sample

A representative sample and an appropriate sample size are essential to achieving an accurate DON measurement.

The Grain Inspection, Packers and Stockyards Administration of the U.S. Department of Agriculture (USDA-GIPSA) publishes sampling methods for wheat and barley and specifies that a minimum of 100 g is required for DON testing (1, 2). Freese et al. (4) suggests that a sample size over 100 g does not reduce the variability of DON measurement. Hart (5) showed that the variability of analyzing 50 g subsamples of whole kernels taken from a 500-800 g probe is reasonable small at high DON levels but that variation may be substantial for samples with DON levels less than 2 mg/kg (2 ppm). Very small samples are hampered by the possibility that a single infected kernel with an extremely high levels of DON may have a large impact on the DON.

Sampling Recommendations for Commercial Crops, Storage Facilities and Large Field Plots (e.g. seed increases)

Sampling mechanically harvested grain lots:

1. Obtain a representative sample by combining 1000 ~ 2500 g of seeds collected from several spots (1-4) in the grain container. (NOTE: Avoid collecting a sample from a single spot in a large seed lot)
2. Clean the sample to remove weed seeds and other materials.
3. Obtain ~ 100 g of sample using a grain divider.
4. Send the samples to a testing lab.

Sampling heads directly from the field:

A. The "hundred method" (3)
   1. Collect and combine 10 randomly selected samples of 100 heads from each experimental plot (to get a combined weight of at least 1000 g).
   2. Obtain seeds using a thresher.
   3. Obtain ~100 g of sample using a divider.
   4. Send the samples to a testing lab.

B. The "hourglass sampling pattern" (6)
   1. Collect and combine 20 samples collected along the transect lines of hourglass pattern from each experimental area. Collect twenty to twenty-five heads at each sampling point.
   2. Obtain seeds using a thresher.
   3. Obtain ~100 g of sample using a divider.
   4. Send the samples to a testing lab.

Sampling Recommendations for Small Research Plots (e.g. single row plots from a FHB screening nursery)

Sampling mechanically harvested plots:

1. Harvest the plot.
   NOTE: If harvesting mechanically the air flow on the harvesting equipment should be low to retain the lighter (i.e. FHB damaged) grain - although this will also result in grain that requires further cleaning.
2. Obtain a 100g sample using a divider - the use of a divider is critical to ensure a representative sample.
3. Clean the sample. This may be done by hand or by using seed cleaning equipment.
4. Send the samples to a testing lab.

Sampling plots by hand:

1. Harvest 1-2 feet of row from the center of the plot.
2. Select from the harvested material 30 heads from primary tillers.
3. Thresh these heads. A belt thresher works well for this purpose.
4. Clean the sample. This may be done by hand or by using seed cleaning equipment.
5. If 100 g samples are not available, consider bulking sample (see following paragraph).
6. Send the samples to a testing lab.

When sub-sampling from larger seed lots we advise obtaining a representative sample by using a seed divider. We realize that many researchers may not be able to obtain 100 g of seeds from an experimental plot. In this case, you should consider the value of combining replicates. For example, the Minnesota wheat breeding program harvests the FHB nursery by hand as indicated above and after cleaning using a seed cleaner (Model SLN4, Rationel Kornservice, Denmark) assesses each sample for visually scabby kernels (VSK) and test weight (using a micro volume test weight requiring ~12g of seed) and then bulks the replicates (generally there are two reps in the FHB nurseries) to get ~50 g of seed. This bulked sample is then submitted for DON analysis.

Grinding the Sample

Guidelines from USDA-GIPSA.
The grinding apparatus must be adjusted to produce a particle size that is sufficiently fine enough to obtain a homogeneous blend. Generally, a sufficiently coarsely ground sample of wheat resemble whole wheat flour, while a sample that is too coarsely ground has the appearance of bulgur or semolina.

Some labs will grind samples for researchers while others will request pre ground samples. If a research lab has a good grinder that can provide a ground sample resembling whole wheat flour, it will save our time from grinding samples facilitating the analysis of more samples and faster turn around times.

Labeling and Submitting Samples to USWBSI-Funded Labs

Please check with the lab with which you intend to submit your samples before labeling and sending samples. Individual labs may request that you label samples in a specific manner (e.g. with consecutive numbers) and provide an electronic spreadsheet of your samples to facilitate the timely processing of samples and return of your data.

References

1. DON (Vomitoxin) Handbook, https://www.gipsa.usda.gov/fgis/handbook/MycotoxinHB/MycotoxinHandbook_2016-07-12.pdf
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4. Freese, L. Friedrich R., Kendall, D. and Tanner, S. 2000. Variability of deoxynivalenol measurements in barley, J. AOAC Intl. 83:1259-1263.
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