USWBSI Workshop on Factors Influencing DON Accumulation in Wheat Heads
Etiology, Epidemiology and Disease Forecasting (EEDF) Research Area
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Marriott Detroit Airport Hotel
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Present:

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Erick DeWolf, Pennsylvania State University
Ruth Dill-Macky, University of Minnesota
Char Hollingsworth, University of Minnesota
Willie Kirk, Michigan State University
Gretchen Kuldau, Pennsylvania State University
Gene Milus, University of Arkansas
Pierce Paul, Ohio State University
Lawrence Osborne, South Dakota State University
Albert Tenuta, Ontario Ministry of Agriculture, Food & Rural Affairs
Frances Trail, Michigan State University

Goals:

1) Improve our understanding of factors influencing DON concentrations, in particular what gives rise to high-DON/low-symptom situations

2) Apply that understanding to possible DON management strategies

3) Identify areas that need investigation and possible projects for collaboration

Reports:

Gary Bergstrom reported on a greenhouse study by Emerson Del Ponte who spray-inoculated wheat heads at different stages of kernel development, and assessed disease symptoms at 6 days following inoculation. All heads were harvested at the same time, at physiological maturity, and visually scabby kernels (VSK), infected kernels (on agar medium), and DON were assayed. Initial FHB symptoms were delayed following inoculation at anthesis as compared to kernel milk stages. Kernel infection (%) remained high through inoculation at hard dough even though late infections did not reduce grain weight. DON at harvest was maximal in heads that had been inoculated at kernel watery-ripe and early-milk stages. Relatively lower kernel symptoms and DON high enough to be problematic coincided in those heads that were inoculated at late-milk and early-dough stages. There was a large variation in DON when VSK percentages were 80-100%, because the extent of damage to each kernel varied.

Christina Cowger reported on a field experiment to determine the effects of post-harvest moisture duration, late infection, and resistance type on FHB symptoms and DON concentrations in different spike tissues. Seven (in 2005) or eight (in 2006) cultivars with apparently different types of resistance were subjected to 0, 10, 20, or 30 days of post-flowering misting. In the first year (2005), a mild epidemic occurred, and there was no effect of post-flowering moisture duration on disease, DON, or percentage of tombstones. Plots receiving the longest duration of post-flowering misting (30 days) had less grain DON. DON was much higher in chaff and rachis tissues than in grain. Single-head inoculations more than 10 d. post-flowering did not lead to significant disease or DON, whereas earlier single-head inoculations did. *Fusarium* biomass (by RT-PCR) was correlated with DON in grain samples, and more weakly correlated in rachis and glume samples. DON fell by about 50% every 10 days during grain-fill. In the second year (2006), a more severe epidemic occurred. Disease increased with up to 20 days' post-flowering moisture and then held steady with 30 days post-flowering moisture. DON, FDK, and *Fusarium* biomass are still being evaluated.

Ruth Dill-Macky's group collected 150 *Fusarium* isolates, three each from 50 fields, and assayed them for toxin production and aggressiveness. They twice soaked and autoclaved 50 g of clean seed per flask, inoculated the flasks with the isolates, allowed 20 days for colonization, dried and ground the materials, and had the toxins analyzed. They found that a few isolates produced huge quantities of one toxin, and many produced the whole suite of toxins: DON, 3-ADON, 15-ADON, nivalenol, and zearalenone. DON was almost always highest by 2-10 times, although sometimes other toxins were. DON is not produced in Mung bean medium. The highest toxin producers were also generally more aggressive. There were some cases of low DON production with high aggressiveness, but no cases of high DON production and low aggressiveness.

In another project, Ruth's group looked at the effect of moisture. Two cultivars were spray-inoculated at anthesis, and irrigation commenced on all plots. At 15 days after inoculation, irrigation was turned off in half the plots, while the other half were irrigated until maturity. Samples were taken at early dough, hard dough, and later stages. DON was found to be lower in the plots misted longer than in those misted for a shorter time. In some manner, DON is being removed from the wettest treatments.

Willie Kirk reported on a fungicide trial with wheat inoculated using *F. graminearum* infested cracked corn, in which no fungicides resulted in significantly reduced DON relative to a susceptible unsprayed check, and the combination of Headline (a strobilurin) at boot stage and Caramba (metconazole) at flowering significantly increased DON levels. Increased DON after strobilurin application might be due to the fact that strobilurins keep plant tissue green longer. We noted this was consistent with Frances' report that DON production appears to occur primarily in green plant tissues. Another hypothesis suggested was that pathogens normally competitive with *F. graminearum* were controlled, allowing infestation of wheat heads without interference.

Gretchen Kuldau and Erick DeWolf are co-investigating the effects of moisture timing; whether late infections can lead to asymptomatic kernels with > 2 ppm DON; and whether late-infection effects differ by cultivar. Mobile field greenhouses enable them to compare four moisture regimes, with each pair of terms referring to the anthesis and grain-fill periods, respectively: ambient-ambient, dry-wet, wet-dry, and wet-wet. They are using three SRWW cultivars: Hopewell (MS), Truman (MR), and Valor (MR). In 2003-04, they saw some suggestion that late infections resulted in significant DON with low symptoms. The experiment is still in progress. They are sorting grain in the dry-wet moisture treatment into asymptomatic and symptomatic categories, in order to assay for DON differences, and may carry out single-kernel analyses.

Gene Milus and his student Peter Horevaj are working on a project funded through the VDUN RAC. They've identified a set of 11 resistant and 1 susceptible SRWW cultivars that flower within 4 days of each other. Corby Kistler is providing them with four well-characterized *F. graminearum* isolates: two nivalenol producers and two DON producers, and within each chemotype one with high levels of toxin production and one with low levels. Their goal is to assess whether the cultivars are resistant to DON, to kernel infection, and/or to "late blighting," or late infection. They are also asking whether resistance to DON is effective against nivalenol, and whether high levels of toxin production can overcome these resistances. They are trying to develop a protocol to test whether resistance to disease spread in the head is the same as resistance to DON. This involves obtaining purified DON.

Frances Trail described the development physiology of *F. graminearum*. In planta, there are two dikaryotic stages. The first is at infection: when the fungus moves through the xylem and pith with its hyphae, dikaryotic hyphae form just behind the infection front. Haploid hyphae grow off the dikaryons laterally. The first symptom, darkening, occurs when these radial hyphae reach the chlorenchyma. Then a second kind of dikaryotic hyphae are produced, and also perithecial initials. At this point, the fungus can go dormant (e.g., for overwintering). Fluffy growth indicates that dormancy has failed. The tissue must dry down for dormancy to occur, and then the fungus is vulnerable (e.g., to surface-sterilization). The dikaryotic hyphae form lots of lipid bodies in order to form perithecia. These hyphae also produce the characteristic red pigment aurofusarin, which is closely associated with saprophytic growth, and is toxic to birds and microbes.

Gene-chip studies also indicate when mycotoxins form during the life cycle of *F. graminearum*. DON biosynthesis genes are expressed when wheat plants are still green, and in barley inflorescences throughout the later stages of grain-fill. They are not expressed in moistened commercial cracked corn. Biosynthesis of DON appears to be exclusively in green tissues, although DON can be produced by mycelia grown in artificial, nutrient rich, saprophytic conditions, such as autoclaved grain.

Zearalenone is modestly synthesized in barley inflorescences.

Issues:

Erick reported that the collaborative FHB modeling effort is shifting its focus to include DON, and is seeking an “ensemble” approach in cooperation with Art Schaafsma’s DON forecasting program. The ensemble approach would allow these modeling groups to combine the prediction models into a single management tool that could be used by growers to evaluate the risk of disease and DON. We discussed the issues associated with DON forecasts:

* Disease and DON levels can vary widely even at a small scale, e.g., within counties;

* Growers would not want grain purchasers to “blackball” regions on the strength of DON forecasts, which might have only general applicability;

* If DON forecasts indicated potential problems, growers should be able to take late-season management measures, including pre-harvest DON tests, early harvest and drying, setting combines to exclude more kernels, etc.

We concluded that DON forecasts could be useful if provided to growers in a manner allowing them to take management steps, and that millers are already using a variety of information sources to identify problem areas. The forecasting model or DON models are just one source of information that millers use to decide from which regions to source grain.

Conclusions and Important Areas of Investigation:

1) Our best current explanation for cases of high DON/low symptoms is that late infections shift the peak of DON production in heads closer to harvest, so that grain with a relatively low incidence of

symptomatic kernels or FDKs has relatively high DON levels. This needs to be further tested experimentally.

2) It would be helpful to develop pre-harvest sampling procedures and weather-based DON forecasts that allow us to give growers timely alerts about DON in general, and the low symptom/high DON scenario in particular.

3) In turn, growers need practical sampling protocols so they can determine ahead of time if they will have a DON problem, and take management steps such as blending the crop with stored grain, adjusting the combines, etc.

4) If DON production occurs strictly in green, pre-senescent plants, how useful is it to recommend early harvest and drying of grain in cases where DON levels are worrisome? Does it actually affect DON levels, or just fungal colonization?

5) Could grain imbibition of rain or dew in the immediate pre-harvest period affect DON levels if glumes are heavily colonized with *Fusarium*? Could this help explain high DON levels in low-symptom grain?

6) We need protocols for distinguishing resistance phenotypes:

    A) resistance to late infection – is there such a thing, is it different from resistance to "anthesis" infections, and thus is it worth adding to uniform FHB nurseries as a separate screening procedure (late inoculations)? Are there developmental differences in cultivar resistance during the grain-fill period?

    B) resistance to/degradation of DON, e.g., can two genotypes have similar degrees of kernel infection, yet different levels of DON?

7) We need to develop methodology for studying the effects of plant health/condition on DON. For example, could certain forms or timings of spring nitrogen increase DON, perhaps by delaying plant maturity? What effect does early, stress-related senescence have on DON levels?

8) We need to do some histology on infected kernels: are there measurable differences in kernel colonization that account for differences in DON content among grain samples with similar external symptom severities?

9) DON seems to be primarily synthesized in green plants. Besides pathogenesis, is there a role for DON vis-à-vis third organisms, e.g., pathogenic or saprophytic competitors?

Collaboration:

National survey: Ruth and Gary are willing to coordinate a national survey at grain intake points to determine DON and *Fusarium* levels in commercial crops. Part of the motivation is to address the perception that grain crops without significant *Fusarium* symptoms (or damage) can nevertheless have problematic DON levels. Survey data could also be useful in the forecasting effort, particularly if at least some of the samples are collected near weather stations. The current forecasting model could be used to target regions and states with different predicted levels of disease, in order to sample there. Of particular interest would be regions with limited symptoms and variability in toxin concentrations. Pacific Northwest samples would also be useful, as they might shed light on DON concentrations in heads of plants with crown rot. We would collect grain samples as they arrive at intake points, and record information about field location and variety. County agents and other collaborators could be enlisted in the sample gathering effort. The samples would be plated for infection incidence, and toxin tests would be conducted.

Other collaboration: Cooperative efforts in addressing questions identified above were encouraged.

Participants of EEDF FHB Workshop, Romulus, MI.