Quality Risks Associated with the Utilization of Fusarium Head Blight Infected Malting Barley

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ABSTRACT

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Fusarium head blight (FHB) has adversely affected the quality of barley grown in the northern Great Plains of the United States and the eastern Prairie Provinces of Canada since 1993. Objectives of this study were to document the occurrence of deoxynivalenol (DON) on barley within North Dakota and Minnesota, investigate relationships among FHB, DON, and malt quality, and to determine at what level FHB/DON-contaminated barley can be safely utilized for the production of quality malt. Since 1993, mean DON levels have ranged from 10.3 to 0.4 µg/g, with a corresponding 81 to 32% of the regional barley crop in excess of 0.5 µg/g. Strong relationships were not observed between either kernel size or kernel weight and DON. As a consequence, cleaning is unlikely to achieve significant reductions in DON levels in most cases. In terms of barley and malt quality, the strongest relationships were observed between barley DON and malt DON and malt DON and wort color. However, malt DON levels could not be reliably predicted from barley at <1.0 µg/g. Barley with a DON level of <1.0 µg/g produced acceptable malt.

Keywords: Assortment, Cluster analysis, Deoxynivalenol, Kernel weight, Malt quality, Mycotoxin

RESUMEN

Riesgos de Calidad Asociados con la Utilización de Cebada Malteada Infectada con Destrozo de la Dabeza de Fusarium

El destrozo de la cabeza de Fusarium (FHB) ha afectado adversamente la calidad de la cebada cultivada en los Grandes Llanos norteños de Estados Unidos y las Praderas Provinciales del este de Canadá desde 1993. Los objetivos de este estudio eran documentar la ocurrencia de deoxinivalenol (DON) en cebada dentro Dakota Norte y Minnesota, investigar relaciones entre FHB, DON, y calidad de malta, y determinar hasta que nivel puede ser utilizada con seguridad la cebada contaminada con FHB/DON en la producción de malta de calidad. Desde 1993, el nivel medio de DON se ha extendido de 10.3 a 0.4 µg/g, con el correspondiente 81 a 32% de la cosecha regional de cebada en exceso de 0.5 µg/g. Relaciones fuertes no fueron observadas entre el tamaño de grano, peso de grano y DON. Por consiguiente, es poco probable que la limpieza alcance reducciones significativas de niveles de DON en la mayoría de los casos. En términos de calidad de cebada y malta, las relaciones más fuertes fueron observadas entre DON de cebada y DON de malta y DON de malta y color de mosto. Sin embargo, los niveles de DON en malta no se pudieron predecir seguramente en cebada con <1.0 µg/g. La cebada con un nivel de DON <1.0 µg/g produjo malta aceptable.

Palabras claves: Análisis de grupo, Calidad de malta, Deoxinivalenol, Micotoxina, Peso de grano, Surtido

Over the past decade, Fusarium head blight (FHB), incited by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch), has been responsible for devastating economic losses to wheat and barley producers in the northern Great Plains of the United States and the eastern Prairie Provinces of Canada (11,12,14,29). Both the yield and quality of barley have been affected, but the major concern with the utilization of FHB-infected barley has been the presence of the tricothecene mycotoxin, deoxynivalenol (DON). DON is the primary mycotoxin produced by the pathogen *F. graminearum* (18) and has been found on as much as 86% of regional barley in some crops years.

The U.S Food and Drug Administration (USDA) has issued advisory levels for DON in wheat, wheat-derived products, and other grains destined for animal feed (9). The advisory level for all wheat products intended for human consumption is 1.0 µg/g. While no advisory limits have been established for malting barley, the U.S. malting and brewing industries carefully monitor DON levels in FHB-impacted areas. The amount of DON contamination accepted in malting barley varies slightly between purchasers, and to a degree, with crop year. However, price discounts for barley contaminated with DON generally start at 0.6 µg/g (14,29). DON levels of ≤0.5 µg/g are often referred to as "non-detectable" within the regional grain trade. While this label is erroneous because the actual analytical limits of detection are in the microgram per kilogram range, it reflects a limit of determination that is frequently employed in testing and the fact that price discounts are often imposed when in excess of this value.

Since the mid 1990s, acreage devoted to barley production in the northern Great Plains has declined nearly 50% (USDA National Agricultural Statistics Service, http://www.usda.gov/nass/pubs/estindx1.htm#barley [verified September 29, 2004]). While this decline is due in large part to changes in the U.S. Farm Bill, heavy discounting of malting barley with DON levels of >0.5 µg/g has limited the profitability of malting barley as compared with other crops. The reduction in malting barley production in the northern Great Plains has resulted in difficulties for the malting and brewing industries in obtaining sufficient amounts of six-rowed malting barley in several years since 1993.

Avoidance of DON-contaminated barley and malt is prompted by concerns over public safety and the public's perception of these issues. The tricothecene toxins are associated with inhibition of DNA and protein synthesis, and DON can be acutely lethal when consumed in large amounts (8). DON has been found to carry through malting and brewing into finished beer (20) and has been reported in commercial beers at levels of 0.3 to 569 ng/mL (15,25). Aside from DON, FHB infection can cause a number of processing and product quality problems, with beer gushing being the most infamous (21). The effects of FHB on malt quality that have been observed in several studies were recently reviewed by Schwarz (19). In general, pronounced effects on germination, soluble nitrogen, free amino nitrogen (FAN), wort color, and β-glucan levels were reported and many of the changes likely resulted from enzymes produced by the pathogen (23). However, previous studies were limited by the use of barley artificially inoculated in the field, greenhouse, or malthouse. In many cases, the grain used was so damaged that it would not normally have been purchased or utilized for malt production.

The primary objectives of this study were to document the occurrence of DON on barley grown in North Dakota and Minne-

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sota, investigate relationships among FHB infection, DON, and malt quality in a commercial sample population displaying more acceptable barley quality, and determine at what level of FHB-infected DON-contaminated barley can be safely utilized for the production of quality malt. Commercial samples of a single malting cultivar (Robust) from four consecutive crops years (1996–2000) were utilized to eliminate differences in cultivar response.

EXPERIMENTAL

Barley

Barley samples were collected at harvest throughout all barley-growing regions of North Dakota and Minnesota as part of regional crop surveys from 1993–2003 (3). A portion of all samples collected within a given year were analyzed for DON. Sampling was based on production, with a greater number of samples being collected in counties with higher projected barley production. For the years 2001–2003, every sample collected was tested for DON, for an average testing of one sample for each 350,000 bushels (bu) (159 metric tons) of production. In years previous to 2001, every second to third sample collected was tested for DON, for an average testing frequency of one sample for each 790,000 bu (358 metric tons) of production. The DON data were used to estimate the proportion of the crop within each crop district that fell within the following DON ranges: ≤0.5, 0.5–0.9, 1.0–2.9, and >3.0 μg/g. Estimates of production (bu) at each DON range were obtained by multiplying the proportion of samples in each district at each DON range by the production for that district. All production data were obtained from the USDA National Agricultural Statistics Service (http://www.usda.gov/nass/pubs/histdata.htm [verified September 30, 2004]). Data were then summed across districts and states to calculate the regional production for each DON range.

Subsets of approximately 25 samples of the six-rowed malting barley cv. Robust were selected for further analysis each year (1996–2000) (3–7). Samples were selected from eastern North Dakota since this region was particularly impacted by epidemics of FHB. Selection of samples in each subset was based on DON levels and was intended to provide a range from <0.5 μg/g to the maximal levels observed. Moisture was determined with a Motomco moisture meter (Model 919ES; Seedburo Equipment Co., Chicago, IL). Samples with moisture levels in excess of 13.5% were allowed to air dry (<13.5%) prior to subsequent analyses and storage. Dried Samples were cleaned on a Carter-Day Dockage Tester (Seedburo Equipment Co.).

Mycoflora and Disease Severity

Mycoflora assays were done according to the methods described by Salas et al (18). FHB incidence was visually assessed on 100 or 200 randomly selected kernels from each harvested sample. Kernels with greater than 25% of their surface covered with lesions were considered blighted. FHB incidence was calculated according the formula: %FHB incidence = (number of blighted kernels/total number of kernels) × 100].

Micromalting

Thin kernels passing through a sieve with 1.98- × 19.00-mm slotted openings were removed prior to malting. Micromalting was performed in duplicate on the plump fraction (>1.98 mm) of each barley sample according to our standard method (10). Time required to reach 45% steep-out moisture was first determined by pilot-steeping a 10 g sample. Steeping of 80 g samples was at 16°C with a 1 hr air rest included with each 12 hr of steeping. The steep water was aerated 6 min/hr. Germination was for 4 days at 16°C and ~95% relative humidity. Samples were turned daily by hand to prevent matting, and sample weight was adjusted to 45% moisture with distilled water. Kilning was conducted in a forced-air laboratory kiln. Total kiln time was 24 hr, during which temperatures were ramped from 49 to 85°C. Rootlets were removed from the kilned malt prior to analysis.

DON Determination

DON analysis of the ungraded samples was conducted immediately after harvest each crop year. DON concentration was determined by column cleanup and gas chromatography with electron-capture detection (GC-ECD) according to Tacke and Casper (27). The limit of quantitation (LOQ) was 0.1 μg of DON per g of barley.

Single-kernel analysis of DON was performed according to a modification of the method of Mirocha et al (13). Single seeds were crushed with a mortar and pestle and carefully transferred to preweighed 13- × 100-mm screw-cap test tubes. Acetonitrile/water (2 mL) was added and the tubes were shaken for 1 hr. The extract (1.5 mL) was applied to a C18:alumina SPE column (Alltech Associates, Deerfield, IL). The tubes were rinsed with an additional 1.0 mL of acetonitrile/water and this was applied to the column. Filtrate (2.0 mL), collected in a 12- × 75-mm culture tube was transferred to a screw-cap test tube and evaporated to dryness under nitrogen. Determination of DON by GC-ECD was according to Tacke and Casper (27). The LOQ for this procedure was approximately 60 ng of DON per single seed.

TABLE I
Levels of Deoxynivalenol (DON) in the North Dakota and Minnesota Malting Barley Production Region[a]

		Deoxynivalenol (μg/g)							
					Samples (%)			Estimated Production (1,000 bu)	
Crop Year	No. of Samples Tested	Mean	Min	Max	0.5–0.9	1.0–2.9	≥3.0	Total	<0.5 μg/g DON
2003	243	0.5	<0.5	5.6	21.4	12.8	1.2	117,893	76,169
2002	224	0.7	<0.5	12.8	16.1	12.1	5.4	57,752	38,415
2001	247	2.8	<0.5	61.9	12.2	19.8	32.8	75,204	26,489
2000	134	2.2	<0.5	29.0	25.4	26.9	25.4	99,910	24,453
1999	153	1.1	<0.5	10.6	28.1	24.8	7.9	57,525	22,559
1998	142	2.9	<0.5	28.6	14.1	26.8	32.4	115,866	31,006
1997	156	5.5	<0.5	44.1	7.7	16.0	47.4	113,732	32,807
1996	180	3.2	<0.5	25.8	16.7	25.6	32.2	161,899	41,374
1995	132	6.4	<0.5	34.6	7.6	18.9	59.1	118,399	17,042
1994	144	10.3	<0.5	60.0	6.9	16.0	58.3	140,928	26,424
1993	147	3.7	<0.5	17.2	5.4	21.1	53.1	132,832	27,109

[a] Includes Minnesota crop reporting districts (CRD) 1 and 4, and North Dakota CRDs 1, 2, 3, 5, 6, and 9. See Barr et al (3) for a district map.

Barley Quality Analyses

Barley 1,000-kernel weight and kernel assortment were determined according to standard methods of the American Society of Brewing Chemists (ASBC) (2). Barley protein was determined by near-infrared reflectance (NIR) with an Infratec1226 grain analyzer (Foss North America, Eden Prairie, MN) or by combustion analysis on a Leco Model FP-528 nitrogen determinator system (LECO Corporation, St. Joseph, MI). Protein determination by NIR or combustion analysis were cross-checked against the Kjeldahl method of AACC International (1) using the catalyst described by Williams (30). The above analyses were conducted immediately after harvest for each crop year.

Malt Quality Analyses

Malt extract, wort protein, FAN, wort color, and wort viscosity were determined according to standard or modified methods of the ASBC (2). Extract was determined on a 25-g sample rather than the 50-g sample described in the official method. All weights and volumes were proportionally reduced (10).

Statistical Methods

The association between individual traits was determined using simple linear correlation. First, correlation between all traits was done for each environment. To determine if calculation of pooled correlation values across environments was appropriate, homogeneity of the values from each location was tested (26). When appropriate, the pooled correlation values across environments were calculated as outlined in Steel et al (26). Correlation values were deemed significantly different from zero at $P \leq 0.05$. Cluster analyses were done for barley DON cutoffs of <0.6, <1.0, and <2.0 µg/g to study the multivariate interrelationships between barley DON and malt quality. All analyses were done using PC-SAS (SAS Institute, Inc., Cary, NC).

RESULTS

Survey of DON Levels in the Regional Six-Rowed Malting Barley Crop

Although the economic and social impacts of the regional FHB epidemics have been well documented (11,14), little information has been published on the actual occurrence of mycotoxins (22). To demonstrate the magnitude of this problem, regional crop survey data were used to prepare estimates of the mean DON levels and extent of DON contamination that were observed over the past 11 years in the North Dakota and Minnesota six-rowed malting barley crops (Table I). These data represent, perhaps, one of the most extensive and long-term surveys of mycotoxins in small grains conducted to date. During this 11-year time period, mean DON levels ranged from 10.3 µg/g (1994) to 0.5 µg/g (2003). The average percentage of the regional malting barley crop contaminated with DON in excess of 0.5 µg/g was estimated at 67% and has ranged from a low of 33% in 2002 to a high of 86% in 1995. Given these levels of incidence, regional malting barley producers have clearly faced a high risk of DON contamination, and in turn, substantial risks of receiving discounted prices for the barley they produced. From the perspective of some growers, the risk of DON combined with existing limits for grain protein, kernel plumpness, and other market factors has further reduced the chances of receiving malting barley prices, and perhaps, the incentive to produce malting barley.

The widespread incidence of DON has also caused hardship to the malting and brewing industries. During these years, the brewing industry in the United States utilized approximately 140 million bu of malt on an annual basis (1 bu malt = 34 lbs; statistical data available from Alcohol and Tobacco Tax and Trade Bureau, http://www.ttb.gov/alcohol/stats/index.htm [verified October 28, 2004]). Malting barley requirements would have been in excess of the malt figure given cleaning and malting losses. Total barley production in the United States ranged from 398 million bu in 1993 to 227 million bu in 2002, with North Dakota/Minnesota on average accounting for 36% (±6%) of this production (production data available from USDA, National Agricultural Statistics Service, http://www.nass.usda.gov:81/ipedb/grains.htm [verified October 28, 2004]). While vigilant and routine screening for DON by the grain industry since 1993 has greatly reduced the risk of accepting malting barley with unacceptable levels of DON, this has also served to greatly limit available acceptable stocks. Estimates shown in Table I suggest that the amount of barley with DON levels of <0.5 µg/g has been below 30.0 million bu in six of the past 11 years. One also must consider that the amount of acceptable malting barley within these stocks was further reduced by varietal, protein, and kernel plumpness limitations. As such, the industry has been forced to procure more of their annual

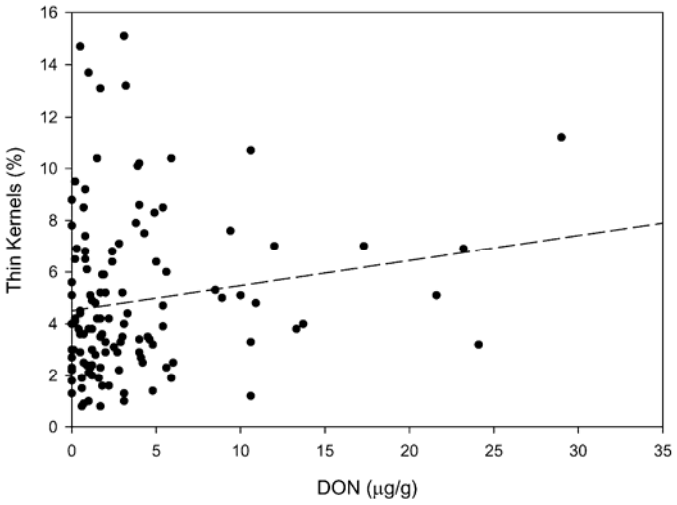

Fig. 1. Relationship between barley deoxynivalenol (DON) and thin-kernel (<1.98 mm) content in 125 samples of cv. Robust barley from eastern North Dakota and northwestern Minnesota (1996–2000 crops) ($r = 0.158$).

TABLE II
Impact of Kernel Sizing on Deoxynivalenol (DON) Levels

DON Range (µg/g)	No. of Samples	Mean Thin Kernels (<1.98 mm) (%)	Mean DON Level of Kernel Size Fractions (µg/g)			Consequences of Removing Thin Kernels		
			Unsized	≥1.98 mm	<1.98 mm	Mean DON Reduction (µg/g)	Mean DON Removed (%)	No. Samples Reduced to <0.5 µg/g DON
0.5–0.9	19	4.7	0.7	0.6	3.0	0.1	24.0	9 (47%)
1.0–2.9	39	4.3	1.4	1.1	7.8	0.3	23.6	5 (18%)
3.0–4.9	22	5.6	3.4	2.5	20.9	1.2	28.3	0
5.0–29.0	24	5.6	9.1	6.3	57.3	3.3	35.7	0

malting barley requirements from other states and from outside the United States. Shipping costs from Canada or elsewhere outside the United States add significantly to the per bushel price paid by industry.

Chronological examination of data in Table I seems to suggest a trend toward lower DON levels and incidence in recent years (2002 and 2003). Empirically, the reduction in the percentage of the crop contaminated with DON may reflect the shift in barley production from eastern North Dakota and Minnesota, where FHB has been more prevalent, to northwestern North Dakota, where disease is less prevalent. However, this would be difficult to prove conclusively without an extensive examination of production, disease, and environmental data.

Relationship of Grain Size and Weight to DON

Descriptions of FHB of cereals frequently refer to *Fusarium*-damaged kernels as being thinner and smaller (28). This would suggest that cleaning operations that remove thin and lightweight seed might be effective in reducing overall DON levels, and in turn, could reduce risk and make a much larger amount of regional grain available for malting. In a previous study, Perkowski (16) separated 48 barley samples, with DON levels ranging from 0.1–157 μg/g, into four kernel size fractions (>2.8, ≤2.8–2.5, >2.5–2.2, and <2.2 mm). On average, 80% of the total sample DON was found in kernels <2.5 mm, and Perkowski (16) suggested that it should be possible to remove a significant amount of DON by rejecting the smallest kernels. However, empirical evidence over the last 11 years has suggested that cleaning is only of limited effectiveness in reducing DON levels in Midwestern six-rowed barley. Isolation of *F. graminearum* from healthy looking kernels is not unusual (Bacilio Salas, *unpublished data*).

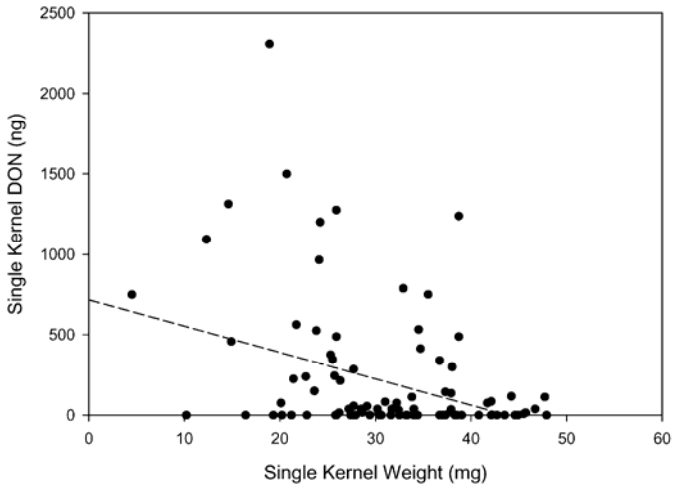

Fig. 2. Relationship between single-kernel weight and deoxynivalenol (DON) (N = 100) in a sample of North Dakota cv. Robust barley (2001 crop) (r = 0.357). The DON content of bulk sample was 11.9 μg/g.

In the current study, thin kernels (<1.98 mm, $^5/_{64}$ in.) were removed from the 125 samples by laboratory screening. DON determinations were performed on the thin and intermediate-plump (≥1.98-mm fractions) as well as on the original unsized sample. The level of DON on the thin kernel fractions was in fact considerably higher than that seen on the unsized samples (Table II). This suggests that thin kernels contain a disproportionate amount of the DON. However, the overall relationship between DON present in the original sample and the amount of thin kernels was quite poor (r = 0.16) and is shown in Figure 1. Perkowski (16) observed much stronger correlations between DON concentration and kernel size, and the discrepancy between the two studies might be explained by the type of samples utilized. In the study by Perkowski, barley was inoculated with *F. graminearum* or *F. culmorum.* In a study by Schwarz et al (24), a pronounced reduction in kernel size was observed when barley was inoculated with *F. graminearum* or *F. poae* (24). However, in the current study, barley was from commercial fields and the timing of natural infection can be extremely variable. Infection of the grain by the pathogen and increases in FHB severity and DON concentration can occur any time from heading to maturity (17). When infection occurs after significant kernel development, the impact on kernel size would be expected to be less pronounced. It is also likely that Perkowski (16), working in Poland, was using two-rowed barley, while six-rowed barley was used in the current study. Two-rowed barley generally has larger and more uniformly sized kernels than does the six-rowed barley. Response to FHB by the two types of barley may not be identical.

The kernel size data also were examined over specific DON ranges since it is possible that removal of thin kernels could be effective for economically significant DON reductions at some levels. In samples where DON levels ranged from 5.0 to 29.0 μg/g, removal of the thin kernels resulted in an average removal of

TABLE III
Quality and Fusarium Head Blight (FHB) Parameters of 1996–2000 cv. Robust Barley Samples

Parameters	No.	Mean	Standard Deviation	Minimum	Maximum
Barley (FHB parameters)					
FHB incidence (%)	119	12.1	16.5	0.0	67.0
Fusarium graminearum incidence (%)	83	14.8	10.5	0.0	56.5
Barley deoxynivalenol (DON) (μg/g)	125	3.6	5.0	0.0	29.0
Barley (quality parameters)					
Kernel plumpness (%)	125	71.3	11.3	39.1	91.9
Test weight (lb/bu)	125	46.2	2.4	41.3	51.5
Kernel color (1–10)[a]	125	6.6	1.5	2.0	9.0
Protein (%)	125	13.0	0.9	10.5	15.1
Malt (disease parameters)					
DON (μg/g)	125	0.9	1.8	0.00	12.0
Malt (quality parameters)					
Extract (%)	122	80.0	1.2	77.0	82.5
Viscosity (cP)	122	1.5	0.0	1.4	1.6
Wort color (°SRM)	122	2.4	0.7	1.7	8.6
Wort protein (%)	122	5.7	0.6	4.3	8.0
Soluble/total protein (%)	122	44.4	4.5	36.0	60.8
Free amino nitrogen (FAN) (mg/L)	122	230.8	26.7	177.0	315.0

[a] *L*-value converted to numeric 1–10 scale with a score of 1 indicating bright barley and a score of 10 representing heavily stained barley.

35.7% of the DON. However, in no case did screening result in a reduction of DON in the intermediate-plump fraction to ≤0.5 µg/g (Table II). The same was true for the samples in the 3.0–4.9 µg/g DON range. The largest reduction in DON seen by removal of thin kernels was 23.2 to 5.0 µg/g. Yet this reduced level would still be problematic for malting. In samples displaying <1.0 µg/g DON, removal of thin kernels resulted in reductions to ≤0.5 µg/g DON in almost 50% of the cases. Approximately 20% of the samples in the 1.0 to 2.9 µg/g DON range were reduced to levels of ≤0.5 µg/g DON with removal of thins. This suggests that producers, grain handlers, or grain processors may have success in reducing DON to ≤0.5 µg/g with some samples. Growers, however, must balance costs of seed cleaning against price discounts for DON.

The relationship between DON and kernel weight was investigated by performing 100 single-kernel DON analyses on each of three bulk samples (cv. Robust, 2001 North Dakota crop) with DON contents of 7.4, 9.7, and 11.9 µg/g. In all cases, a poor relationship between kernel weight and DON was observed (r = 0.13–0.36). The relationship for the sample exhibiting 11.9 µg/g DON is shown in Figure 2. The poor relationships that were observed between both kernel weight and size and DON are not completely surprising since a number of other environmental factors, aside from plant disease, will impact grain fill and thus kernel weight/size. The single-kernel data from all three samples showed that the distribution of DON was very non-uniform, with no DON being detected on 28, 52, and 67% of the kernels, respectively. Individual contaminated kernels contained from ~10 ng to >3,000 ng DON. In two of the samples, approximately 50% of the total sample DON could be attributed to a small number of kernels (≤10%), each containing more than 1,000 ng of DON. In all cases, approximately one-half of these very high DON (>700 ng/kernel) kernels were less than 2.5 mg in weight. Calculations to simulate the removal of kernels <2.5 mm in weight demonstrated large reductions in DON. However, in no case were these of economic significance (<0.5 µg/DON). Examination of Figure 2 clearly demonstrates that relatively high levels of DON are also found on some kernels with weights in excess of 30 mg.

Interpretation of the kernel weight data is somewhat limited by the fact that only three samples of the same cultivar and crop year were studied. The expense and time associated with the number of tests required to get a reasonable population estimate limited the number of samples that could be evaluated in the current study. However, we are currently evaluating a wider range of cultivars and locations as part of study on the relation of FHB disease symptoms to DON levels. Preliminary results from this work confirm the observations that DON is very non-uniformly distributed on barley samples (spikes), and that kernels with very high DON levels make a disproportionate contribution to total sample DON (P. Schwarz, *unpublished*). Results from the current study suggest that there is probably not a strong relationship between seed weight and DON levels in six-rowed malting barley, and that gravity or density separations are unlikely to result in DON reductions of economic significance in moderate to heavily contaminated samples. However, as was the case with kernel sizing, removal of light kernels from samples with low DON (<3.0 µg/g) will be of economic significance in some cases.

Relationship of Barley and Malt Quality to DON

Grain quality of samples used in this study was representative of the barley available for purchase from 1996–2000 and varied from poor to excellent (Table III). Approximately 60–70% of all samples would have been judged as acceptable malting quality on the basis of protein and kernel plumpness limits of ≤13.5 and ≥70%, respectively.

The correlations between barley DON and malt DON, FHB incidence and incidence of *F. graminearum* were all significantly different from zero ($P \leq 0.05$); however, the significance from zero should not be confused with the strength of the associations (Table IV). Moderate associations were observed between barley DON and FHB incidence (r = 0.65) and barley DON and malt DON (r = 0.70). The strength of these relationships suggests that barley with high DON levels would likely produce malt with high DON levels. However, due to the large amount of unexplained variation, this relationship needs to be investigated in greater detail. The correlations between barley DON and barley quality and malt quality traits were generally weak. The only correlation with $r > 0.50$ was between barley DON and wort color (r = 0.59). Overall, this indicates that the level of DON in the barley is not a good predictor of barley, malt quality, or both.

The associations between malt DON and the FHB-related traits and barley and malt quality parameters were similar or slightly weaker than those observed for barley DON (Table IV). A moderately strong association was observed between malt DON and wort color (r = 0.74). This observation is not surprising because DON is an indicator of FHB infection, and *Fusarium* is thought to produce significant amounts of proteolytic enzymes during infection (23). Darker wort color is presumably due to an increased level of color precursors resulting from the proteolytic action of the pathogen.

To gain a better understanding of the relationship between barley and malt DON, correlations were calculated between these two parameters for barley that fell into the acceptable class (i.e., barley DON ≤0.5 ppm) and the unacceptable class. Additionally, correlations between barley and malt DON were calculated for barley DON cutoff values of <1.0, <2.0, and <3.0 µg/g (Table V). On average, the associations between barley and malt DON were

TABLE IV
Correlations Between Barley Deoxynivalenol (DON) and Malt DON and Other Barley and Malt Quality Parameters.

Parameters	Barley DON	Malt DON
Barley (Fusarium head blight [FHB] parameters)		
FHB incidence (%)	0.65**	0.57**
Fusarium graminearum incidence (%)	0.49**	0.36**
Barley (quality parameters)		
Kernel plumpness	–0.14	0.04
Test weight	–0.29*	–0.19
Color	0.18	0.25*
Protein	0.18	0.21*
Malt (FHB parameters)		
Malt DON	0.70**	…
Malt (quality parameters)		
Extract	–0.09	0.14
Viscosity	–0.05	–0.05
Wort color	0.59**	0.74**
Wort protein	0.14	0.36**
Soluble/total protein	0.10	0.16
Free amino nitrogen (FAN)	0.25*	0.45**

[a] * and ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

TABLE V
Correlations Between Barley and Malt Deoxynivalenol (DON) Using Different Barley DON Cutoff Values

Barley DON Cutoff	DON Below Cutoff	DON Above Cutoff
<0.6 ppm	0.21	0.72
<1.0 ppm	0.28	0.70
<2.0 ppm	0.56	0.79
<3.0 ppm	0.43	0.76

much weaker for the acceptable barley DON class ($r = 0.37$) than for the unacceptable class ($r = 0.74$). In fact, the associations were weakest ($r < 0.30$) for barley DON cutoff values <1.0 µg/g. This suggests that malt DON levels cannot be reliably predicted from barley with DON <1.0 µg/g.

To study the relationships between barley FHB, and barley quality and malt quality parameters, three cluster analyses were performed using traits of FHB incidence, barley DON, percent plump kernels, percent thin kernels, test weight, barley protein, kernel color, malt DON, extract, viscosity, wort color, wort protein, soluble/total (S/T) protein, and FAN. Also, a new parameter called barley quality class was included in the analyses. Barley quality class was either "1" for acceptable or "2" for unacceptable. To fall into the acceptable class, a barley sample had to have the following criteria: FHB incidence <10.0%, plump kernels ≥65.0%, thin kernels ≤5.0%, test weight ≥46.0 lb/bu, protein ≤13.5%, and a barley DON cutoff of ≤0.05, 1.0, or 2.0 µg/g. Different cluster analyses were done for each of the barley DON cutoff levels. Results from all analyses indicate that barley DON was no more important in predicting malt quality than was the barley quality parameters of kernel plumpness, test weight, and grain protein. To ensure a high likelihood ($P \geq 0.95$) of having acceptable malt quality, the barley malt needs to have acceptable quality for all barley parameters. A weakness in any one of the barley quality parameters increased the likelihood of having unacceptable malt quality. Barley with unacceptable values for kernel plumpness, test weight, and/or protein could result from a crop stressed by unfavorable growing conditions or disease. Thus, barley DON should not be weighed any differently as a discounting factor as the protein or kernel plumpness.

The running of multiple cluster analyses using different barley DON cutoff values allowed us to determine if raising the cutoff value from ≤0.5 ppm would have an affect on overall malt quality. Results from our analysis indicate that raising the barley DON cutoff to ≤1.0 ppm would not result in malt with DON >0.5 ppm or other unacceptable characteristics. It was only when the barley DON was >1.0 ppm that malt with unacceptable malt DON, wort color, wort protein, or S/T protein was produced.

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