# EARLY DETECTION OF DEOXYNIVALENOL IN WHEAT GRAIN

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## OBJECTIVE

Evaluate the potential of pre-harvest sampling of wheat fields to provide estimates of deoxynivalenol in the harvested grain.

## INTRODUCTION

Epidemics of Fusarium head blight (FHB) on wheat and barley occur when rain and flowering coincide (Hart, et al, 1984; McMullen, et al, 1997). The resulting DON contamination of the grain is uneven from field to field and within a field**.** In 2000 FHB epidemics occurred in several Midwest states including Michigan, New York, Ohio, Indiana, and also Canada. Although the 2000 FHB epidemic in Michigan appeared to be mild, wheat processors (General Mills, Kelloggs, Jiffy Mix) indicated that 50% of the wheat they normally use from Michigan had to be imported because of high DON levels. In 2001, FHB was again widespread in Michigan, but DON levels were lower than were predicted from the incidence and severity. The poor correlation between FHB incidence and DON levels in both years suggested a need to develop in field sampling protocols to reliably estimate DON prior to harvest. Statistics can provide us with confidence levels that can be applied toward the implementation of FDA guidelines for DON. Estimates of DON based on non-statistical parameters are not acceptable where the issue of consumer food safety is concerned.

In previous studies spatial trends in deoxynivalenol concentration on truckloads of grain were not evident (Hart and Schabenberger, 1998; Hart, et al 1999). The presence of kernels with high or low concentration at a particular location in the truck yielded no information about concentrations nearby. The distribution of toxin appeared to be completely spatially random. In the study reported here, wheat fields were sampled and the grain analyzed for DON prior to harvest.

## METHODS AND MATERIALS

The research objective in 2000 was to determine if there were spatial relationships for DON concentrations between sampling sites within wheat fields prior to harvest. A relatively small, but intense sampling pattern was chosen to evaluate these relationships (Figure 1). This W pattern had four transects, and samples were collected every fifteen feet along each transect. Every thirty feet additional samples were collected on either side of the transect line. Twenty to twenty-five heads were collected at each sampling point, and the heads kept separate between sampling points. The grain was threshed from the heads using a small gasoline powered thresher.

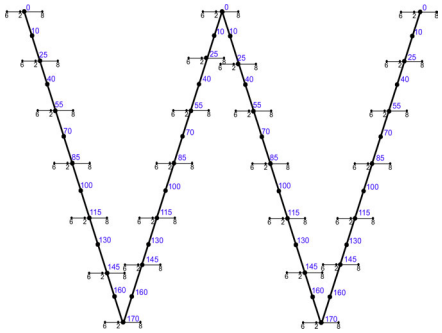

**Figure 1.** W sampling pattern used to study spatial distribution of DON in pre-harvest wheat in 2000. Each dot on the grip represents a sampling point where wheat heads were collected for DON analysis. Numbers represent distance in feet between sampling points.

In 2001 sampling in-field prior was expanded to harvest to cover entire fields. The sampling pattern was in the shape of an hourglass (Figure 2). Twenty to twenty-five heads were collected every fifty feet along each of the transect lines, and the heads kept separate between sampling points (Figure 2). The grain was threshed from the heads using a small gasoline powered thresher. For both 2000 and 2001, as the fields were harvested the grain in the trucks was sampled using commercial probes as described previously (Hart and Schabenberger, 1998), and the grain was analyzed for DON using ELISA as described previously (Hart, et al, 1998).

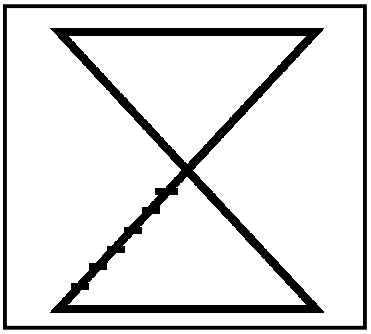

**Figure 2.** Whole field hourglass sampling pattern used in 2001. Samples were collected at 50-foot intervals along grid lines.

## RESULTS

Average DON levels in 2000 ranged from 1.4 to 8.0 ppm, and from 1.7 to 2.1 ppm in 2001 (Table 1). Randomly selecting DON data from between four and twenty of the sampling points in both years indicated that twenty samples/field could provide an estimate of average field DON levels (Table 2). Table 2 shows the 90% confidence intervals for DON levels from pre-harvested wheat. The 90% confidence interval is calculated as the range from the $5^{th}$ to the $95^{th}$ percentiles. Observations were randomly re-sampled to create samples of size 4, 6, 8, 10, 15, and 20. This process was repeated approximately one thousand times for each sample size. The percentiles of the distribution of DON sample means are then estimated from the empirical distribution of the sample means. When 20 samples per field

were randomly selected and used to calculate a field DON mean, the predicted DON mean is withn +/- 0.5 ppm of the mean calculated from all of the samples (Table 1). The 90% confidence interval decreases as the mean level of DON in the field decreases, but a larger confidence interval at high levels of DON is less important since these fields would be rejected as being outside of acceptable levels based on the FDA guidelines for food.

**Table 1**. Descriptive Statistics for Field Samples. A W sampling pattern was used in 2000. An hourglass sampling pattern was used in 2001.

| Field | Number of Observations | Mean DON ppm | Standard Deviation | Min[a] DON | Max[b] DON | Mean DON from probes |
|---|---|---|---|---|---|---|
| Field 1 2000 | 118 | 8.07 | 4.13 | 1.35 | 29.1 | 8.17 |
| Field 2 2000 | 117 | 1.48 | 1.06 | 0.15 | 5.00 | 1.47 |
| Field 3 2000 | 76 | 1.46 | 1.08 | 0.20 | 6.16 | NA |
| Field 1 2001 | 154 | 2.17 | 1.40 | 0.2 | 5.4 | 3.15 |
| Field 2 2001 | 144 | 1.93 | 1.43 | 0.2 | 5.0 | 2.6 |
| Field 3 2001 | 88 | 1.72 | 0.97 | 0.2 | 4.0 | 2.8 |

[a] Lowest level of DON from a single sample. [b] Highest level of DON from a single sample

**Table 2.** 90% Empirical Confidence Intervals for DON levels in fields sampled before harvest. See Table 1 for details.

| Sample Field | Sample size *n* = | Sample Mean | Lower Bound[a] | Upper Bound[b] | Interval Width |
|---|---|---|---|---|---|
| Field 1 2000 | 20 | 8.03 | 6.54 | 9.68 | 3.14 |
| Field 2 2000 | 20 | 1.48 | 1.13 | 1.87 | 0.74 |
| Field 3 2000 | 20 | 1.47 | 1.11 | 1.91 | 0.80 |
| Field 1 2001 | 20 | 2.17 | 1.7 | 2.7 | 1.0 |
| Field 2 2001 | 20 | 1.93 | 1.4 | 2.5 | 1.1 |
| Field 3 2001 | 20 | 1.72 | 1.4 | 2.1 | 0.7 |

[a] 5% of the DON means from randomly selected observations would be below this concentration. [b] 5% of the DON means fro the randomly selected observations would be above this concentration.

The hourglass sampling pattern (Figure 2) used in 2001 gave a better view of the spatial distribution of DON throughout a field compared to the W sampling pattern used in 2000 that represented only small-scale conditions (Figure 1). The scale of the 2000 study did not take into account the conditions across entire fields as did the 2001 study. A kriging map of DON in ppm from one of the fields sampled in 2001 is shown as Figure 3**.** In the center of the field the distribution of DON appears fairly homogeneous with a few areas showing low concentrations. Toward the edges of the field, the toxin concentrations rise sharply.

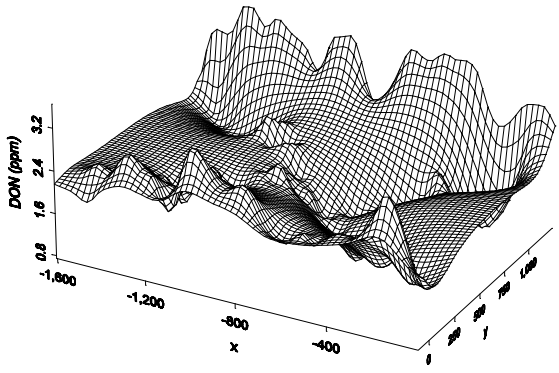


**Figure 3.** Spatial prediction of deoxynivalenol (ppm) across a Michigan field in 2001.

DON levels were similar between pre-harvest samples and probe samples in the 2000 study, but the truck probe samples tested about 1 ppm higher than the field samples in 2001 (Table 1). The results may be attributed the use of different research scale grain threshers between the two years. Small particles, it turns out, were more likely to be removed by threshing of the field samples using the new thresher in 2001, and may have contributed to the difference. Since small kernels are often associated with high deoxynivalenol concentration, this raises the important question as to "what constitutes a sampling unit?" Regardless, our studies in 2000 and 2001 suggested that infield sampling prior to harvest can be used to obtain an estimate of DON corresponding to DON levels in the harvested grain (Table 1).

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# ASSESSING THE RISK OF WHEAT CONTAMINATION BY DEOXYNIVALENOL IN BELGIUM

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## ABSTRACT

The cereals crops in Belgium are not safe from serious contaminations by mycotoxins. The years 1997 and 1998 for example were very favourable to the development of Fusarium head blight. On that account, the project main objective consists in assessing the risk of mycotoxins contamination and studying the environmental factors that contribute to increase the rate of wheat contamination and integrating them in a forecasting model. A competitive direct enzyme-linked immunosorbent assay (CD-ELISA) for the semi-quantitative analysis of deoxynivalenol (DON) has been used on 130 samples of wheat grains of the harvest 2000. In addition, a HPLC multi-residue method is in development to separate various mycotoxins. Samples, mentioned above, were contaminated mainly by *Microdochium nivale* (96%), a fungi inducing scab symptoms but not suspected to produce mycotoxins, *F. culmorum* (22%) and *F. graminearum* (15 %). A small amount of analyzed samples were contaminated by *F. poae* and *F. avenaceum*. PCR analyzes were used to verify the *Fusarium* species microscopic identification. The DON concentration determined by immunological analysis has provided values ranging from 0 to 1,2 ppm (92%: 0-0,5 ppm; 6%: 0,6-1 ppm; 2% > 1 ppm). In the other hand, two types of artificial contaminated field trials were established: 1) A multi-variety trial where different varieties of wheat were inoculated separately with *F. graminearum* and *F. culmorum*. 2) A one-variety trial where 20 *Fusarium* strains belonging to the four *Fusarium* species, above mentioned, were inoculated. For the first trial, we observed differences in both infection rates in the field and DON accumulation between varieties but not between *Fusarium* species inoculated. For the second one, differences in DON production were observed between *Fusarium* species and between some strains of the same species. Microbiological analyses and field observations show a good correlation but they were not related to the DON concentration.

# PHYSICAL TREATMENTS FOR PREVENTING THE POST-HARVEST GROWTH OF *FUSARIUM* IN MALTING BARLEY

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## ABSTRACT

We evaluated the effect of three physical treatments (hot water, UV-C radiation, and electron-beam radiation treatments) on the *Fusarium* infection rates (FIR) and germinative energy rates (GER) in Fusarium head blight infected malting barley. For hot water treatments, four different temperatures (45, 50, 55, and 60°C) for four time periods (1, 5, 10, 15 minutes) were evaluated. For UV-C radiation, three irradiation times (5, 10, and 15 minutes) were evaluated. For electron-beam radiation treatments, dry barley was irradiated at five different doses (2, 4, 6, 8, and 10 kGy). Hot water treatments caused significant reduction in the FIR and GER of barley. The decrease in FIR and GER was more pronounced with increased temperature and time of treatment. Significant reduction (compared to the untreated control) in FIR started at 1 minute for all the temperatures (45, 50, 55, and 60°C). The corresponding reductions on average were found to be 40%, 95%, 99%, and 98% at 1 minute respectively. Longer times at higher temperatures over 45°C eventually caused complete reduction (100%) of FIR. At 45°C, reductions in FIR of 97% and 96% were seen at 10 and 15 minutes respectively, with no significant reductions in GER. Significant reduction (compared to the untreated control) in GER occurred at 50°C after 5 minutes. For temperatures 55 and 60°C, significant reductions (48% and 95% respectively) in GER were seen at 1 minute. Compared to the untreated control, there were no significant reductions in the FIR with increase in UV-C irradiation times. Also, decreases in GER in the UV-C irradiated samples were not significantly different from the untreated sample. For electron-beam radiation, FIR decreased significantly with increase in the dosage used. Significant reduction in the FIR started between 2-4 kGy. Higher doses (8 kGy, and 10 kGy) achieved complete reduction (100%) of FIR. GER also decreased with increase in the electron-beam doses used. Significant decrease (7%) in GER started at a dose of 4 kGy. Higher dosage (10 kGY) caused a larger reduction (32%) in GER relative to the other dosages used. Based on the results we have obtained, further research will be done to evaluate additional treatments and combinations of treatments. Effective treatments will be evaluated further for effect on malt quality and mycotoxigenesis of surviving *Fusarium*.

# RELATIONSHIP BETWEEN FUSARIUM HEAD BLIGHT INFECTION AND THE MALTING QUALITY OF BARLEY

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## ABSTRACT

Maltsters and brewers avoid the use of *Fusarium* infected barley because of concerns over mycotoxins and gushing in the resultant beer, and the US industry has set very tight levels on DON (deoxynivalenol) as a means of regulating entry of FHB (Fusarium headbBlight) infected grain into the market. However, infection with FHB also damages barley and malt quality. These factors are likely to become more of a concern as treatments are identified that are able to eliminate mycotoxins and gushing potential (from beer), and thus encourage the use of some FHB infected grain for malting. The objective of this study was to determine the relationships between the level of FHB infection and specific barley, malt and wort quality parameters. Commercial samples of Robust barley (125) were collected in eastern North Dakota during the 1996-2000 crop years. Samples were malted (N=2) and standard quality parameters determined. Plate count (% infected kernels), DON, ergosterol, xylanase and proteinase were determined as markers of FHB infection.

# UPDATE ON DON DIAGNOSTIC SERVICES IN 2000/2001

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## OBJECTIVES

To provide Fusarium head blight (FHB) research projects with analytical services for deoxynivalenol (DON) and other mycotoxins.

## INTRODUCTION

DON analysis has been an important part of cooperative efforts to fight FHB. In 2000 and 2001, US Wheat and Barley Scab Initiative provided grants to four DON Diagnostic Centers. The centers provided free DON analysis services for all US Wheat & Barley Scan Initiative research projects. The contact information for the DON Diagnostic Centers is listed as the following:

L. Patrick Hart, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824; sample type: wheat; phone: (517) 353-9428, fax: (517) 353-5598, e-mail: hart@msu.edu

Weiping Xie, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; sample type: wheat, barley, special samples (single kernels, small stem and leaf segments etc.); phone (612) 625-2751, fax: (612) 625-9728, e-mail: weipingx@umn.edu

Beth Tacke, Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, ND 58105; sample type: wheat, barley, durum; phone: (701) 231-8309, fax: (701) 231-7514, e-mail: Beth.Tacke@ndsu.nodak.edu

Paul B. Schwarz, Department of Cereal Science, North Dakota State University, Fargo, ND 58105; sample type: barley, malt; phone: (701) 231-7732, fax: (701) 231-7723, e-mail: Paul.Schwarz@ndsu.nodak.edu

## MATERIALS AND METHODS

The analytical methods used by the centers included ELISA (P. Hart), GC/ECD (B. Tacke and P. Schwarz) and GC/MS (W. Xie). The sample preparation method used by GC/ECD and GC/MS analyses was developed by Tacke (1).

Each center conducted its own intralab quality control (QC) throughout the analysis period during the year to ensure the quality of the analysis. A collaborative quality assurance (QA) program was also carried out among the centers. Each month, from Sept. to Dec. in 2000

and Apr. to Oct. 2001, a wheat sample and a barley sample collected by the coordinator (B. Tacke) were sent to each center. Each center performed analyses in two different days and reported the replicate data to the coordinator within one week. The collected data was then sent to the centers. The QA program allowed each center to evaluate the accuracy and precision of their system.

## RESULTS AND DISCUSSION

Table 1 summarized the scale of DON analysis services conducted by the 4 centers in 2000 and 2001. Since most of samples came to the centers starting from August in each year, the data was reported as of 2000/2001 (08/01/00 to 07/30/01) and 2001/2002 (08/01/01 to 07/30/01). The total number of samples analyzed by the 4 centers was 19,672 in 2000/2001. In 2001/2002, a total of 13,136 sample have been analyzed by Nov. 10, 2001. An estimated total of 19,500 to 20,500 samples will be analyzed by the 4 centers in the 2001/2002 year.

The intralab QC information from the 4 DON diagnostic centers is shown in Table 2. The coefficients of variation (CV) varied from 7 to 16% in 2000/2001 and from 5 to 16% in 2001/2002. The average of CV of 8 QC sample pools from the 4 centers was 9.1% in 2001/2002 showing an overall improvement of precision in comparison with 11.4% of 200/2001. The Minnesota diagnostic center experienced a higher coefficient of variation for 2001/2002 (14%) than 2000/2001 (12%). The possible causes will be evaluated.

The interlab QA data of Sep. through Dec. 2000 is shown in Table 3. The average CV of the 5 tests was18% for the wheat check samples and 17% for the barley check samples. Table 4 gives the interlab QA data of Apr. through Oct. 2001. The average CV of the 7 tests was 21% for wheat and 17% for barley. The data shows that there was no major difference between the DON diagnostic centers.

Since samples sent to the DON diagnostic centers were usually in batches and the DON data was usually used to compare samples with a batch, the precision of analysis at each center in a period of a few days is important to the scab researchers. The variation between the two replicate analyses of each interlab QA sample reflects this short-term precision. Table 5 shows the average CV of two replicate QA samples from each center in 2000/2001 and 2001/2002. The data in Table 5 indicates that the reproducibility of each center within one week was quite good.

The interlab QA program will continue through December, 2001 or until most of samples for 2001/2002 year are proceed.

**Table 1.** Number of samples analyzed by DON diagnostic centers.

| Center | PI number | | State number | | Sample number | | Estimated total number of 01/02 |
|---|---|---|---|---|---|---|---|
| | 00/01 | 01/02 | 00/01 | 01/02 | 00/01 | 01/02* | |
| MI-P. Hart | 9 | 17 | 8 | 11 | 2,481 | 3,371 | |
| MN-W. Xie | 11 | 12 | 2 | 1 | 7,533 | 2,970 | 8,000 - 9,000 |
| ND-P. Schwarz | 4 | 3 | 2 | 2 | 5,222 | 4,612 | |
| ND-B. Tacke | 23 | 10 | 6 | 4 | 4,436 | 2,183 | ~4,600 |
| Total | 47 | 42 | 18 | 18 | 19,672 | 13,136 | 19,500-20,500 |

*Numbers as of Nov. 10, 2001.

**Table 2.** Intralab quality control data for Aug-Dec, 2000 and Apr-Nov, 2001.

| Center | Grain | 2000/2001 | | | 2001/2001 | | |
|---|---|---|---|---|---|---|---|
| | | Number | Mean (ppm) | CV (%) | Number | Mean (ppm) | CV (%) |
| MI-P. Hart | Wheat | 56 | 1.6 | 7 | 94 | 2.3 | 5 |
| MN-W. Xie | Wheat | 34 | 12.8 | 12 | 38 | 9 | 14 |
| ND-P. Schwarz | Barley | 120 | 6.3 | 14 | 108 | 6.3 | 11 |
| | Barley | 112 | 1.6 | 16 | 104 | 1.5 | 16 |
| | Barley | 124 | 5.3 | 15 | 104 | 5.1 | 10 |
| ND-B. Tacke | Whaet | 83 | 1.8 | 9 | 31 | 1.7 | 5 |
| | Barley | 83 | 3.1 | 9 | 31 | 2.9 | 5 |
| | Corn | 83 | 5 | 9 | 31 | 4.6 | 7 |

**Table 3.** Interlab quality assurance data of 2000/2001 (Sep. through Dec. 2000).

| Center | Grain | DON results (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| MI-P. Hart | Wheat | 10.8/10.8 | 1.5/NA | 0.6/0.6 | 7.2/7.2 | 2.4/2.5 |
| MN-W. Xie | Wheat | 11.5/11.2 | 0.6/0.7 | 0.3/0.3 | 5.9/5.3 | 2.4/2.3 |
| ND-P. Schwarz | Wheat | 11.5/14.8 | 0.8/0.9 | 0.6/0.5 | 4.0/4.5 | 2.1/2.1 |
| ND-B. Tacke | Wheat | 10.5/10.8 | 0.8/0.9 | 0.5/0.6 | 5.5/6.0 | 2.5/2.5 |
| Ave. | | 11.5 +/- 1.4 | 0.9 +/- 0.3 | 0.5 +/- 0.1 | 5.7 +/-1.1 | 2.4 +/- 0.2 |
| MI-P. Hart | Barley | 2.0/2.1 | 6.6/NA | 7.4/8.4 | 15.0/14.0 | 27.5/28.4 |
| MN-W. Xie | Barley | 2.1/1.9 | 5.6/5.5 | 7.6/7.3 | 14.5/15.3 | 28.0/27.2 |
| ND-P. Schwarz | Barley | 1.4/0.7 | 4.3/4.6 | 9.4/9.5 | 10.1/9.9 | 20.6/21.6 |
| ND-B. Tacke | Barley | 1.8/2.1 | 5.7/5.7 | 7.6/8.0 | 13.7/14.6 | 31.2/29.8 |
| Ave. | | 1.8 +/- 0.5 | 5.4 +/- 0.8 | 8.2 +/- 0.9 | 13.4 +/- 2.2 | 26.8 +/- 3.8 |

**Table 4.** Interlab quality assurance of 2001/2002 (Apr. through Oct. 2001).

| Center | Grain | DON results (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 |
| MI-P. Hart | Wheat | 1.4/1.2 | 12.0/11.2 | 6.0/6.1 | 8.8/9.0 | 3.8/4.2 | 0.4/0.4 | 7.0/9.0 |
| MN-W. Xie | Wheat | 0.8/0.9 | 4.6/8.4 | 2.4/2.5 | 6.8/7.2 | 7.0/7.4 | 0.3/0.3 | 5.9/6.2 |
| ND-P. Schwarz | Wheat | 0.8/0.8 | 7.9/8.3 | 4.0/3.7 | 6.9/6.7 | 4.9/6.0 | 0.2/NA | 7.7/7.6 |
| ND-B. Tacke | Wheat | 1.2/1.3 | 9.1/8.2 | 4.1/4.3 | 6.8/7.5 | 5.7/5.8 | 0.4/0.3 | 6.0/6.3 |
| Ave. | | 1.1+/- 0.3 | 9.1+/-1.6 | 4.1+/-1.4 | 7.5+/-0.9 | 5.6+/-1.3 | 0.3+/-0.1 | 7.0+/-1.1 |
| MI-P. Hart | Barley | 4.0/4.4 | 0.8/1.0 | 6.9/7.5 | 10.2/10.8 | 0.8/0.8 | 3.6/3.4 | 10.0/12.0 |
| MN-W. Xie | Barley | 3.0/3.4 | 0.5/0.6 | 6.1/6.1 | 8.6/9.4 | 7.0/7.4 | 3.5/3.4 | 10.3/10.6 |
| ND-P. Schwarz | Barley | 3.2/2.4 | 0.5/0.6 | 5.9/5.0 | 10.4/10.2 | 0.8/0.8 | 4.2/NA | 8.6/16.4 |
| ND-B. Tacke | Barley | 4.1/3.8 | 0.7/0.7 | 6.1/7.2 | 10.1/10.6 | 1.0/0.9 | 3.4/3.7 | 9.6/9.4 |
| Ave. | | 3.5+/-0.7 | 0.7+/-0.2 | 6.3+/-0.8 | 10.0+/-0.7 | 0.8+/-0.1 | 3.6+/-0.3 | 10.9+/-2.4 |

**Table 5.** Average CV of replicate QA samples from each center in 2000/2001 and 2001/2002.

| Center | 00/01 | | 01/02 | |
|---|---|---|---|---|
| | Ave. CV (%) | | Ave. CV (%) | |
| | Wheat | Barley | Wheat | Barley |
| MI-P. Hart | 2.9 | 4.9 | 6.2 | 7.0 |
| MN-W. Xie | 4.7 | 3.4 | 4.3 | 5.8 |
| ND-P. Schwarz | 9.5 | 11.5 | 4.4 | 16.3 |
| ND-B. Tacke | 4.5 | 6.3 | 6.9 | 5.1 |

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