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# Rapid Fluorescence Polarization Immunoassay for the Mycotoxin Deoxynivalenol in Wheat

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The fungus *Fusarium graminearum*, a pathogen of both wheat and maize, produces a toxin, deoxynivalenol (DON), that causes disease in livestock. A rapid test for DON in wheat was developed using the principle of fluorescence polarization (FP) immunoassay. The assay was based on the competition between DON and a novel DON-fluorescein tracer (DON-FL2) for a DON-specific monoclonal antibody in solution. The method, which is a substantial improvement over our previous DON FP immunoassay, combined a rapid (3 min) extraction step with a rapid (2 min) detection step. A series of naturally contaminated wheat and maize samples were analyzed by both FP immunoassay and liquid chromatography (HPLC−UV). For wheat the HPLC−UV and FP methods agreed well (linear regression $r^2 = 0.936$), but for maize the two methods did not ($r^2 = 0.849$). We conclude that the FP method is useful for screening wheat, but not maize, for DON.

**KEYWORDS: Deoxynivalenol; vomitoxin; fluorescence polarization; immunoassay**

## INTRODUCTION

Deoxynivalenol (DON or vomitoxin) is produced by *Fusarium graminearum*, a fungal pathogen of wheat that causes a disease known as head scab or *Fusarium* head blight. This mycotoxin has been found in wheat, barley, and maize worldwide (*1*) and is capable of causing disease in domestic animals such as swine (*2*). In the United States the advisory level is 1 $\mu$g DON/g for grain intended for human consumption, while the Scientific Committee on Food of the European Communities has advised a tolerable daily intake of 1 $\mu$g/kg body weight (*3*).

A variety of techniques have been developed to detect DON in foods at concentrations relevant to the advisory levels. Chromatographic methods such as TLC, HPLC, and GC are commonly used and generally rely upon either the ability of DON to form a fluorescent or volatile derivative, the absorbance of the toxin at 220 nm, or mass spectrometry (*4*). For screening purposes antibody-based methods such as enzyme-linked immunosorbent assays (ELISAs) are widely used. Most of the antibodies that bind DON also cross-react with one or more of the acetylated derivatives of DON (*5*−*11*).

Fluorescence polarization immunoassay (FP) has two important differences from ELISA: the detection does not involve an enzymatic reaction, and separation of the bound and free label is not required. As a result, FP assays do not require the wash step essential to many ELISAs, and they do not require waiting for an enzyme to produce a colored product. In FP the intensity of the fluorescence of a fluorophore is measured along two axes: horizontal ($I_H$) and vertical ($I_V$). The polarization value is defined by the equation $P = (I_V - I_H)/(I_V + I_H)$. $P$, which is typically expressed in millipolarization units (mP), is therefore a measure of the orientation of the fluorescence emission (i.e., horizontal, vertical) rather than a direct measure of fluorophore concentration. Because of this, FP has the useful property of being minimally affected by solution opacity or color, which affect the intensity more than the orientation of the fluorescence. The observed orientation of the fluorescence is related to the rate of rotation of the fluorophore in solution, which in turn is related to the size of the fluorophore. Small molecules have higher rates of rotation and lower polarization than larger molecules. FP immunoassay utilizes the interaction of a toxin-specific antibody with a toxin−fluorophore conjugate (tracer) to effectively decrease the rate of rotation of the tracer. Binding of the antibody to the tracer increases polarization. In the presence of free toxin less of the antibody is bound to the tracer, reducing polarization. The result is that, with this format, the signal is inversely proportional to toxin content.

Previously we have developed monoclonal antibodies for DON and used them in ELISA and FP immunoassays (*11, 12*). Three antibodies, from reference clones #1, #4, and #22 were sensitive for DON using the ELISA format, with $IC_{50}$s of 23, 13, and 8 ng/mL respectively (*11*). These antibodies were also tested in the FP immunoassay format where two of the antibodies (#1, #4) interacted with the tracer to yield functional assays. However, the most sensitive of the three antibodies by ELISA (#22) did not react (*12*). Furthermore, the sensitivities of the previous assays were dramatically affected by the length of the incubation with the tracer. As a result, a lengthy (10 min) incubation was required. The objective of the present work was to improve upon the speed and utility of the FP immunoassay by developing a new DON-fluorescein tracer that would interact

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with our most sensitive DON antibody. Herein we report the development of a novel DON-fluorescein tracer (DON-FL2) and an FP immunoassay based upon antibody #22. Using this antibody/tracer combination the kinetics of the interaction were improved, resulting in a shorter incubation time and a faster assay than reported previously. In addition, the assay was combined with a rapid (3 min) extraction technique to substantially improve sample throughput. The combination of rapid extraction and assay steps substantially improved the utility of the assay. The new techniques were tested against spiked and naturally contaminated wheat and maize and compared to a widely used HPLC method.

## MATERIALS AND METHODS

**Safety.** Deoxynivalenol and related trichothecene mycotoxins have been causally related to animal disease and should be handled with appropriate caution. As such, wheat or maize samples should be handled so as to minimize exposure to contaminated dust during collection, grinding, and extraction.

**Reagents.** Except where noted otherwise, deionized water (Nanopure II, Sybron/Barnstead) was used in the preparation of all reagents. All solvents were HPLC grade. Triacetyl-DON, isotrichodermin, 8-hydroxyisotrichodermin, trichothecolone, and sambucinol were prepared at the USDA National Center for Agricultural Utilization Research (courtesy of Dr. Susan McCormick). DON standard was obtained from Sigma Chemical (St. Louis, MO), as were the standards 3-acetyl-deoxynivalenol (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON), diacetoxyscirpenol, scirpentriol, trichothecin, T-2 toxin, T-2 triol, T-2 tetraol, HT-2 toxin, neosolaniol, roridin A, verrucarin A, and verrucarol. Nivalenol and fusarenon-X standards were purchased from Wako Pure Chemical Industries, Inc. (Japan). 1,1′-carbonyldiimidazole was purchased from Aldrich (Milwaukee, WI). 4′-(Aminomethyl) fluorescein hydrochloride was purchased from Molecular Probes (Eugene, OR). All other chemicals and solvents were reagent grade or better and purchased from major suppliers.

**Preparation of DON−Fluorescein Tracer.** DON (5.75 mg) was dissolved in 0.56 mL of dry acetone, and 160 mg of 1,1′-carbonyldiimidazole was added with vigorous mixing. The mixture was kept at ambient temperature for 90 min and 0.02 mL of water was added followed by 11.5 mg of 4′-(aminomethyl) fluorescein as 1.15 mL of a 10 mg/mL solution in DMF. This reaction was held at 4 °C and tested periodically over a period of 18 days to measure the extent of the reaction using HPLC with fluorescence detection. On the fourth day 0.2 mL of 0.1 M sodium bicarbonate, pH 8.2, was added. On the eighth day 1 mL of the reaction mixture was removed and an additional 0.2 mL of sodium bicarbonate solution was added to the removed portion. On the 18th day a portion of this solution was subjected to semipreparative HPLC to isolate the reaction products.

A portion of the reaction mixture (0.8 mL) was diluted with 0.4 mL of deionized water, and aliquots were injected onto the HPLC under the following conditions. The HPLC system consisted of a Spectra-System P4000 pump (Thermo Separations Products, San Jose, CA), a Rheodyne 9125 injector with a 200-μL loop, a Prep Nova-Pak HR C18, 6 μm, 60 Å, 10 mm × 25 mm i.d. reversed-phase guard column (Waters Corporation, Milford, MA), a Prep Nova-Pak HR C18, 6 μm, 60 Å, 100 mm × 25 mm i.d. reversed-phase semipreparative column (Waters Corp.), a Spectra Physics FL2000 detector (488 nm excitation, 520 nm emission, range set to 20 FU), and a SP4270 integrator (Thermo Separations). The mobile phase was a binary gradient of methanol and 1% v/v acetic acid in water (pH adjusted to 5.0) with a flow rate of 5 mL/min. The initial condition was 45% methanol, which was ramped to 55% methanol at 20 min, and then to 80% methanol at 30 min. At 40 min the methanol content was decreased to 45%, and the column was allowed to equilibrate before the next injection. Three fractions (A, B, and C) were collected from the column, and the mobile phase was removed by a combination of vacuum-evaporation and lyophilization. Fractions of A and B were solubilized in water (A, 5.5 mL; B, 20 mL), whereas fraction C was solubilized with methanol and water (7.5 mL). The fractions were re-distributed into 0.5-mL portions and lyophilized. A sample of each fraction was also injected onto the HPLC as a measure of purity. The fractions were also tested in a competitive-direct ELISA (*11*) to determine whether the contents could compete with a DON−horseradish peroxidase conjugate for binding to DON antibodies. For FP immunoassay the fractions were reconstituted with deionized water and diluted 1:2,000 in phosphate buffered saline (PBS: 10 mM sodium phosphate, 0.85% sodium chloride, pH 7.4) containing 0.1% sodium azide (PBS-A). These working solutions were prepared daily.

**Extraction of Samples for FP Assays.** Wheat or maize was ground using a Stein mill to pass a 20-mesh screen, then mixed, and a 25-g portion was removed for extraction with 100 mL of PBS. To determine the optimum extraction conditions a comparison was made between blending for times ranging from 30 s to 10 min and shaking for 2 h using naturally contaminated wheat. The wheat contained 4.5 μg DON/g as determined by HPLC−UV (see below). Extracts were filtered through a Whatman 2V filter (Whatman International Ltd., Maidstone, U.K.), and the filtrate was used in the FP immunoassay without further treatment.

Once the optimum extraction conditions were determined with the naturally contaminated samples, a series of spiking and recovery studies were conducted. Wheat or maize containing less than 0.1 ppm DON (by HPLC−UV) was spiked with DON at levels ranging from 0.5 to 10 ppm. Samples (25 g each) were spiked at each level in triplicate and extracted with 100 mL of PBS as described above. Each extract was tested by FP immunoassay in triplicate, giving a total of 9 assays per spiking level. DON content of spiked samples was determined by FP immunoassay relative to DON standards in PBS-A as described below. A series of 34 naturally contaminated wheat samples and 15 maize samples were also extracted and tested by this method, with triplicate portions of each sample extracted and triplicate analyses of each extract.

**Fluorescence Polarization Immunoassay.** The fluorescence polarization instrument was a Sentry FP portable unit (Diachemix Corporation, Grayslake, IL) and was described previously (*13*). DON standards were prepared by diluting the DON stock solution with PBS-A. Antibody working solution was prepared by diluting DON monoclonal antibody #22 1:600 in PBS containing 0.1% bovine serum albumin (BSA).

Glass culture tubes (10 mm × 75 mm) (VWR Scientific, West Chester, PA) were used as cuvettes for the assays. PBS-A (0.9 mL) was added to each tube followed by 0.1 mL of the antibody working solution and 30 μL of the sample or standard. After thorough mixing, the test solution was placed in the instrument and used as the blank. Tracer, 25 μL of the DON−FL2 working solution, was then added and mixed. The test solution containing tracer was then returned to the fluorometer, and the signal (mP) was measured. For experiments to elucidate the kinetics of the reaction, measurements were made after holding at ambient temperature for times ranging from 3 s to 15 min. In all other experiments the holding time was 1 min. Unless noted otherwise the DON content of naturally contaminated maize and wheat samples was estimated relative to a standard curve of DON in PBS-A (TableCurve software, Jandel Scientific, San Rafael, CA).

**Cross-Reactivity of FP Immunoassay.** Stock solutions of 21 trichothecene mycotoxins were prepared in either acetonitrile, acetonitrile/water, or methanol depending upon the solubility characteristics of the toxin. Once in solution (generally at 1−2 mg/mL) the toxins were diluted to 20 μg/mL with PBS-A, and 30 μL was tested as described above. Trichothecenes that showed reactivity with the assay at this level were further tested over the concentration range of 1 ng/mL to 100 μg/mL. For comparison, the raw FP data (in units of mP) were normalized to fit the range of 0 to 1 using the equation $Y_{obs} = (mP_{obs} - mP_0)/(mP_1 - mP_0)$, where $mP_{obs}$ is the signal from the test sample, $mP_0$ is the signal from a control that does not contain antibody, $mP_1$ is the signal from a control that does not contain toxin, and $Y_{obs}$ is the normalized result for the test sample (*13*).

**HPLC−UV Assay of Wheat and Maize.** Wheat and maize were tested by HPLC−UV essentially by the method of Trucksess et al. (*14, 15*) with a different mobile phase containing the ion-pair reagent tetrabutylammonium dihydrogen sulfate (TBAHS). Samples, 25 g, were extracted with 100 mL of acetonitrile/water (84 + 16, v/v) by shaking

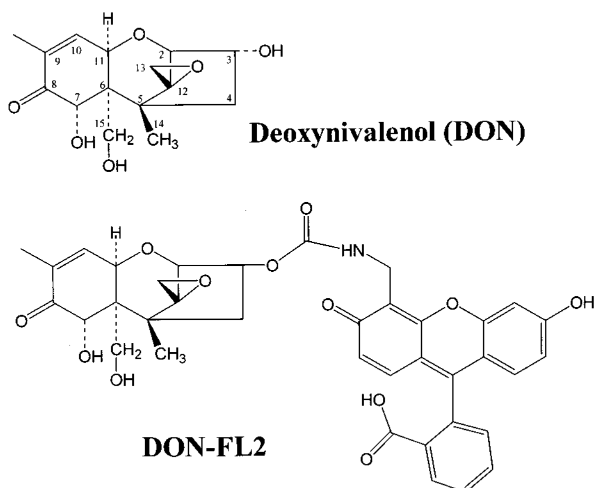


**Figure 1.** Structure of DON and structure of DON-fluorescein tracer (DON-FL2). Linkage shown at the C-3 hydroxyl of DON; linkage is also possible through the C-15 hydroxyl, see text.

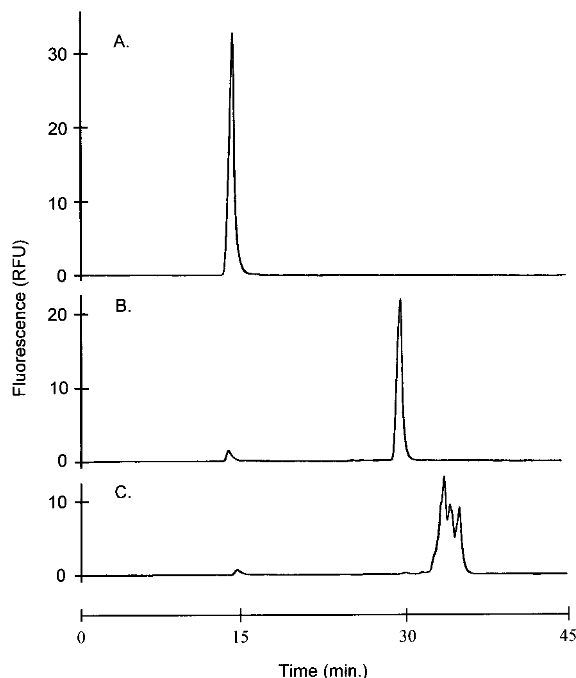


**Figure 2.** HPLC chromatograms of three fractions from the preparation of DON-FL2 tracer. Fraction "B" was further concentrated and used as the tracer in the FP immunoassay described in the text.

for 1 h at ambient temperature, then filtered (Whatman 2V). The filtrate was cleaned-up by passing approximately 4.5 mL through a Romer #225 column (Romer Laboratories, Union, MO). A 4-mL portion of the eluate, equivalent to 1 g of grain, was transferred to a silane-treated vial and dried under a stream of nitrogen gas at 50 °C for 1 h. The dried extract was reconstituted with 0.3 mL of water/methanol (4:1 v/v), passed through a 0.2-micron syringe filter, and 20 $\mu$L was injected for separation by reversed-phase HPLC with detection at 220 nm. The HPLC equipment was the same as that described above. The columns were a NewGuard RP-18 15 mm × 3 mm i.d., 7-$\mu$m reversed-phase guard column (Applied Biosystems, Inc., Foster City, CA) and a ODS-120T, 25 cm × 4.6 mm i.d., 2-$\mu$m reversed-phase analytical column (TosoHaas, Montgomeryville, PA). The detector was a Spectra System UV2000 (Thermo Separations) connected to a computer for data acquisition (AllChrom Plus software, Alltech Associates, Inc., Waukegan, IL). The mobile phase was a binary gradient of methanol and acetonitrile/water with TBAHS at a flow rate of 0.7 mL/min. The latter solution was prepared by adding 10 mL of concentrated ion-pair reagent to 1 L of 8% (v/v) acetonitrile in water with adjustment of the pH to 6.5 with 5 N potassium hydroxide. The concentrated ion-pair reagent consisted of 0.114 g/mL TBAHS and 0.107 g/mL (anhydrous) monobasic potassium phosphate in water. For HPLC the initial condition was 0% methanol, which was ramped to 5% methanol at 17 min, and then increased to 40%. At 25 min the mobile phase was returned to the initial condition and the column was allowed to equilibrate for 20 min before the next injection. Under these conditions DON eluted at 17 min. The HPLC method was not capable of distinguishing between 3-Ac-DON and 15-Ac-DON, which eluted in the 40% methanol "wash".

## RESULTS AND DISCUSSION

Previously we have developed fluorescence polarization immunoassays for the fumonisin mycotoxins and DON (*12, 13*). This report describes significant improvements to the DON assay that enhance the utility of the method by combining a quicker extraction step with a more rapid FP immunoassay. The most sensitive of three monoclonal antibodies which we developed for DON ELISAs, from clone #22 (*11*), did not interact with the DON-FL tracer developed in our original report (*12*). We prepared and isolated a new DON-fluorescein tracer that was able to interact with antibody #22. Unlike the previous DON-FL, the current tracer was linked to 4′-(aminomethyl) fluorescein rather than fluoresceinamine isomer II (6-aminofluorescein). The new tracer, DON-FL2 (**Figure 1**), was isolated by reversed-phase HPLC. Three fractions were collected and concentrated (**Figure 2**). The fractions were tested in the FP format for increased polarization in the presence of the three DON antibodies, indicative of binding of the tracer to the antibody. The FP of fractions A and C did not increase in the presence of the DON antibodies, while the FP of fraction B increased substantially in the presence of antibody #22, but not with antibodies #1 or #4 (data not shown). As a result, we further explored the use of the combination of antibody #22 and fraction B (DON-FL2) in development of an FP assay. The excitation (485 nm) and emission (531 nm) maxima of the DON-FL2 were similar to those of fluorescein.

**FP Immunoassay.** When tracer was incubated in the presence of antibody the FP signal increased. In general, the rate of the increase depends on the kinetics of the interaction between the antibody and the tracer. The presence of the unlabeled toxin in the reaction mixture can influence the rate at which the antibody and tracer reach equilibrium. The order of addition of reagents may also play a role. For example, if the antibody has a strong affinity for DON and the sample is added before the tracer solution, then the antibody binding sites may already be occupied with toxin before the tracer is added. The competition of the DON and tracer for the antibody is therefore skewed somewhat, as the DON must dissociate from the antibody before the antibody is free to interact with the tracer. This effect was observed in our previous FP assay for DON, where the time required for the signal to reach equilibrium was dependent upon the DON concentration (*12*). As a result, in the earlier assays, the incubation time was relatively long (10−12 min) to ensure the system was near equilibrium.

The impact of incubation time upon the calibration curve was studied with the new tracer and antibody combination. In **Figure 3** it is apparent that the assay was most sensitive with a short (15 s) incubation time. However, the nearly identical calibration curves observed with incubation times ranging from 1 to 12 min suggest that equilibrium was achieved within 1 min. For this reason the tracer incubation used in the remainder of the experiments was 1 min, a substantial improvement over the previous tracer incubation of 12 min.

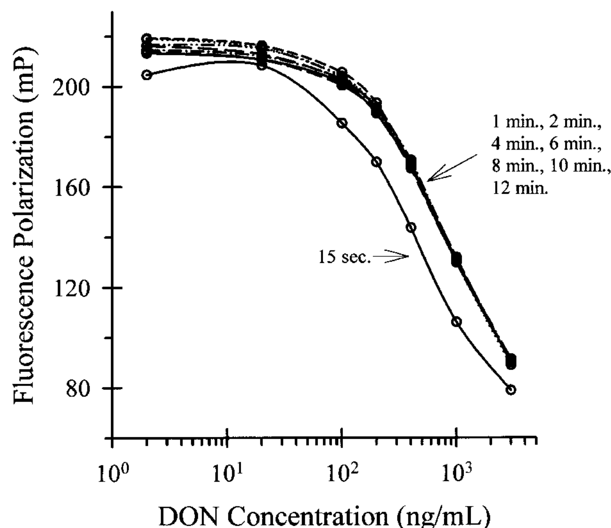


**Figure 3.** Kinetics of the DON FP immunoassay. Calibration curves were obtained at various times using a tracer incubation of 15 s to 12 min and DON as described in the text.

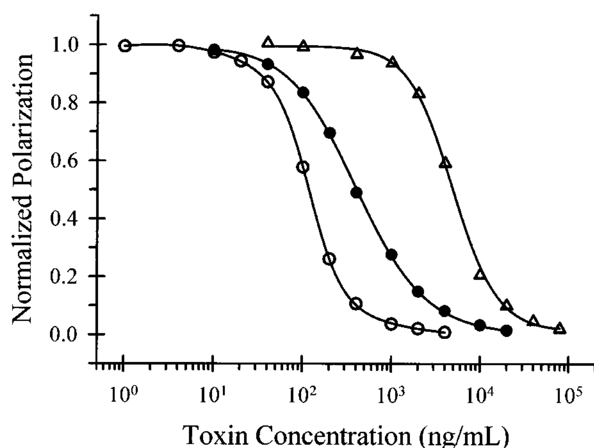


**Figure 4.** Cross-reactivity of the DON FP immunoassay. The concentrations of toxins at the midpoint for the calibration curves were 120 ± 2 ng/mL, 407 ± 8 ng/mL, and 4990 ± 240 ng/mL for 3-Ac-DON (○), DON (●), and 15-Ac-DON (△), respectively.

The antibody used (#22) was shown previously to cross-react with 3-Ac-DON (632%) and 15-Ac-DON (3.3%) in a competitive ELISA format (*11*). The same antibody was tested with 21 trichothecenes in the FP immunoassay format. Toxins tested included DON, 3-Ac-DON, 15-Ac-DON, triacetyl-DON, nivalenol, fusarenone-X, diacetoxyscirpenol, T-2 toxin, T-2 triol, T-2 tetraol, HT-2 toxin, neosolaniol, roridin A, verrucarin A, verrucarol, isotrichodermin, 8-hydroxy-isotrichodermin, sambucinol, trichothecolone, trichothecin, and scripentriol. Response curves for 3-Ac-DON, DON, and 15-Ac-DON, the only three toxins to affect the assay, are shown in **Figure 4**. The concentrations shown in **Figure 4** are the concentrations of the standard solutions before dilution with the antibody/buffer mixture. Because the volume of the standard (30 $\mu$L) was much less than the total volume of the mixture (1055 $\mu$L), the toxin concentrations in the final (measured) mixture are substantially less than those represented in **Figure 4**. For example, the midpoint of 407 ng/mL for DON corresponds to a concentration of 11.6 ng/mL DON in the measured solution. This level of sensitivity is similar to that seen with the same antibody in an ELISA format (*11*). Cross-reactivity relative to DON (100%) was 339% for 3-Ac-DON and 8% for 15-Ac-DON. The cross-reactivity therefore showed a pattern similar to that observed with the ELISA format, although quantitatively different. This result was not unexpected, since a similar effect was seen with the fumonisin FP assay when it was compared to a fumonisin ELISA (*13*).

**Table 1.** Effect of Extraction Time upon Estimated DON Content

| blending time (min) | [DON] ± SD ($\mu$g/g)[a] |
|---|---|
| 0.5 | 5.7 ± 0.4 |
| 1 | 6.0 ± 0.1 |
| 2 | 6.4 ± 0.5 |
| 3 | 6.6 ± 0.4 |
| 5 | 6.6 ± 0.4 |
| 10 | 6.1 ± 0.5 |

[a] DON concentration as measured by FP immunoassay in a sample of wheat naturally contaminated with 4.5 ppm DON as determined by HPLC–UV. The same sample when shaken for 2h, rather than blended, contained 6.8 ± 0.5 ppm by FP immunoassay. Data are for triplicate subsamples with each subsample assayed three times ($n$ = 9).

The cross-reactivity of the assay may provide insights into the location of the linkage of DON to fluorescein. The poorer sensitivity of the assay to 15-Ac-DON relative to 3-Ac-DON indicates that modification of DON at the C-15 position inhibited binding, while modification at the C-3 position did not. Because the DON-FL2 tracer bound readily to the antibody we speculate that the fluorescein was attached predominantly at the C-3 position of DON, as shown in **Figure 1**. This would also explain why the DON-FL2 was not recognized by the antibodies #1 and #4 (which recognize 15-Ac-DON preferentially over 3-Ac-DON). Furthermore, the antibodies that bound the original tracer (DON-FL) were more reactive to 15-Ac-DON, and the original tracer was not bound by the 3-Ac-DON reactive clone (#22).

**Recovery of DON from Wheat and Maize.** The sensitivity of the FP immunoassay for DON in buffer was sufficient that experiments were conducted with spiked and naturally contaminated wheat and maize. In preliminary experiments to determine optimal extraction conditions, naturally contaminated samples were extracted either by blending with buffer for 30 s to 10 min or by shaking for 2 h. DON was quickly extracted from wheat using the blending procedure (**Table 1**). Recovery of DON was not improved by blending for greater than 3 min, therefore a 3-min extraction was used for subsequent spiking/recovery experiments and for testing of naturally contaminated samples.

Wheat or maize containing less than 0.1 $\mu$g/g was spiked with DON over the range of 0.5 to 10 $\mu$g/g. Recovery from maize using the aqueous extraction and testing by FP was excellent, averaging 94.6% (**Table 2**). Recovery from wheat was poorer but adequate, averaging 71.2%. Recovery from wheat and maize using acetonitrile/water (84:16) as the extraction solution for the HPLC analyses was excellent, averaging 91.8 and 94.8%, respectively (**Table 2**).

**Comparison of FP Immunoassay and HPLC−UV for Naturally Contaminated Grains.** A series of 34 naturally contaminated wheat samples and 15 naturally contaminated maize samples were tested by both the FP and the HPLC−UV methods for comparison. There was good agreement between the two methods with naturally contaminated wheat (**Figure 5a**). The data in **Figure 5a** fit the linear regression of DON by FP = −0.54 + 1.33 [DON by HPLC], with an $r^2$ of 0.936. Although the intercept of the regression line was −0.54, the inference that the FP assay was underestimating DON content at low levels of contamination is incorrect. A visual examination of the data at levels less than 2 ppm indicates that the FP assay

**Table 2.** Recovery of Deoxynivalenol from Spiked Wheat and Maize

| commodity | spiking level (μg/g) | percentage recovery ± SD HPLC–UV[a] | FP immunoassay[b] |
|---|---|---|---|
| maize | 0.5 | 91.3 ± 6.7 | 80.0 ± 3.6 |
| | 1 | 98.9 ± 2.8 | 90.2 ± 6.7 |
| | 2 | 89.3 ± 1.8 | 102.2 ± 6.1 |
| | 5 | 89.8 ± 1.4 | 102.5 ± 3.7 |
| | 10 | 104.8 ± 1.8 | 98.3 ± 5.5 |
| | overall average | 94.8 ± 9.7 ($n$=15) | 94.6 ± 9.9 ($n$=15) |
| wheat | 0.5 | 109.5 ± 0.4 | 74.1 ± 1.2 |
| | 1 | 94.1 ± 2.4 | 64.9 ± 5.9 |
| | 2 | 90.2 ± 4.6 | 71.2 ± 7.4 |
| | 5 | 84.2 ± 1.4 | 72.4 ± 8.5 |
| | 10 | 86.9 ± 0.5 | 74.2 ± 4.3 |
| | overall average | 91.8 ± 8.5 ($n$=14) | 71.2 ± 5.4 ($n$=14) |

[a] Extraction using acetonitrile/water, isolation, and detection as described in the text. Control samples contained less than 0.1 μg/g before spiking. [b] Extraction using water with blending for 3 min as described in the text. FP immunoassay data were corrected for the amount of DON detected in the control samples by FP immunoassay (0.6 ppm in corn, 0.27 ppm in wheat).

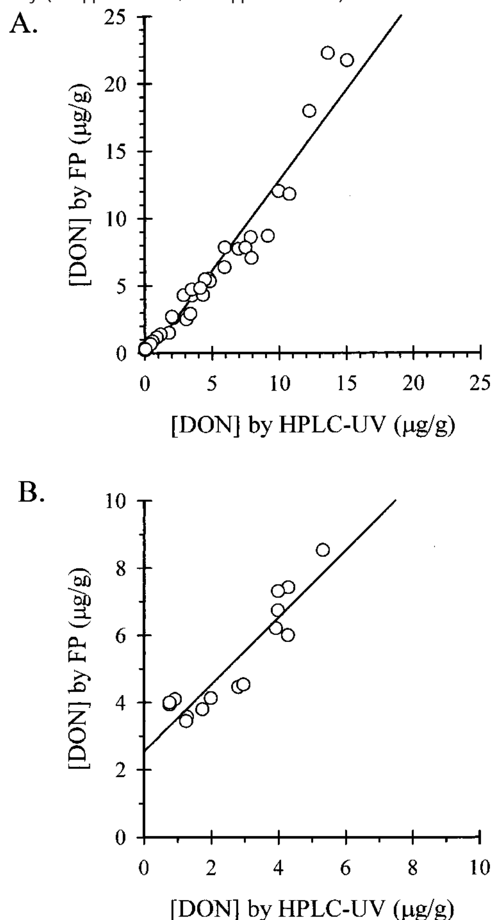


**Figure 5.** Comparison of DON in naturally contaminated samples of wheat (A) and maize (B) analyzed by both HPLC–UV and FP.

was slightly overestimating, not underestimating, DON content in this range (**Figure 5a**). The slope of the regression line (1.33) also indicates a systematic bias for the FP assay toward overestimation of DON content over the entire range tested. The cause for this bias is unknown. We speculate that it may result from fungal metabolites that cross-react with the DON antibody. The HPLC method which we used for quantitation of DON could not be used to quantitate 3-Ac-DON, therefore, we do not know if it was 3-Ac-DON, which cross-reacts highly with the antibody, or some other fungal product that was responsible for the bias.

Whereas the overestimation observed with the wheat samples was fairly minor, with the maize samples it was substantial (**Figure 5b**). Interestingly, while the intercept of the regression line in maize was very high (2.54 μg/g), the slope was better (0.996), and the correlation coefficient was worse ($r^2 = 0.849$) than in wheat. That is, the bias observed with the maize samples was of a different form than that observed with the wheat samples. With the maize samples, we speculate that the bias is resulting primarily from a matrix effect (which would affect the intercept) rather than the presence of cross-reacting fungal metabolites (which would affect the slope). In several experiments (data not shown) we tried unsuccessfully to overcome the matrix affect by preparing the DON calibration curve with standards diluted in extract from a DON-free maize sample. Therefore, we recommend that this assay be used for screening wheat, but not maize, for DON.

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## LITERATURE CITED


(1) Jelinek, C. F.; Pohland, A. E.; Wood, G. E. Worldwide occurrence of mycotoxins in foods and feeds – an update. *J. – Assoc. Off. Anal. Chem.* **1989**, *72*, 223–230.

(2) Rotter, B. A.; Prelusky, D. B.; Pestka, J. J. Toxicology of deoxynivalenol (vomitoxin). *J. Toxicol. Environ. Health* **1996**, *48*, 1–34.

(3) Scientific Committee on Food. Opinion on Fusarium Toxins. Part 1: Deoxynivalenol. In *Annex VI to the Minutes of the 119th Plenary Meeting*; European Commission: Brussels, Belgium, 1999.

(4) Sydenham, E. W.; Shephard, G. S. Chromatographic and allied methods of analysis for selected mycotoxins. In *Progress in Food Contaminant Analysis*; Gilbert, J., Ed.; Blackie Academic & Professional: London, 1996; pp 65–146.

(5) Casale, W. L.; Pestka, J. J.; Hart, L. P. Enzyme-linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. *J. Agric. Food Chem.* **1988**, *36*, 663–668.

(6) Xu, Y. C.; Zhang, G. S.; Chu, F. S. Enzyme-linked immunosorbent assay for deoxynivalenol in corn and wheat. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 945–949.

(7) Mills, E. N.; Alcock, S. M.; Lee, H. A.; Morgan, M. A. An enzyme-linked immunosorbent assay for deoxynivalenol in wheat, utilizing novel hapten derivatization procedures. *Food Agric. Immunol.* **1990**, *2*, 109–118.

(8) Usleber, E.; Märtlbauer, E.; Dietrich, R.; Terplan, G. Direct enzyme-linked immunosorbent assays for the detection of the 8-ketotrichothecene mycotoxins deoxynivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol in buffer solution. *J. Agric. Food Chem.* **1991**, *39*, 2091–2095.

(9) Nicol, M. J.; Lauren, D. R.; Miles, C. O.; Jones, W. T. Production of a monoclonal antibody with specificity for deoxynivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol. *Food Agric. Immunol.* **1993**, *5*, 199–209.

(10) Sinha, R. C.; Savard, M. E.; Lau, R. Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. *J. Agric. Food Chem.* **1995**, *43*, 1740–1744.

(11) Maragos, C. M.; McCormick, S. P. Monoclonal antibodies for the mycotoxins deoxynivalenol and 3-acetyl-deoxynivalenol. *Food Agric. Immunol.* **2000**, *12*, 181–192.

(12) Maragos, C. M.; Jolley, M. E.; Nasir, M. S. Fluorescence polarization as a tool for determination of deoxynivalenol in wheat. *Food Addit. Contam*., in press.
(13) Maragos, C. M.; Jolley, M. E.; Plattner, R. D.; Nasir, M. S. Fluorescence polarization as a means for determination of fumonisins in maize. *J. Agric. Food Chem.* **2001**, *49*, 596−602.
(14) Trucksess, M. W.; Ready, D. E.; Pender, M. K.; Ligmond, C. A.; Wood, G. E.; Page, S. W. Determination and survey of deoxynivalenol in white flour, whole wheat flour, and bran. *J. AOAC Int.* **1996**, *79*, 883−887.
(15) Trucksess, M. W.; Wood, G. E.; Cho, T.-H. Determination of deoxynivalenol in white flour, whole wheat flour, and bran by solid-phase extraction/liquid chromatography: interlaboratory study. *J. AOAC Int.* **1998**, *81*, 880−886.

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