Relationship between Mycotoxin Content in Winter Wheat Grain and Aspirated Dust Collected during Harvest and after Storage

Victor Limay-Rios* and Arthur W. Schaafsma

ABSTRACT: A total of 323 paired grain and grain dust samples (particle size <1650 μm) were collected from combines at harvest (56%), on-farm bins (28%), and experimental minibins seeded with an ochratoxin A (OTA)/Penicillium verrucosum hot spots (15%) of which >98% were soft red winter wheat. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to detect 21 mycotoxins, including deoxynivalenol (DON) and its plant-conjugated form, deoxynivalenol 3-β-D-glucoside (DON 3-Glc). Except for DON 3-Glc, all mycotoxin concentrations found in grain dust were higher than in grain (p < 0.0030). Pearson correlation coefficients and two-variable regression show a significant ($p < 0.0001$) linear relationship between the mycotoxin content in grain and that in grain dust with 19 toxins. In only five mycotoxins (DON, OTA, ochratoxin B, citrinin, and enniatin A1), more than 82% of the variation in the data is explained by the two-variable regression model. Because of its higher mean concentration and detection frequency, only DON produced a strong relationship ($r^2 = 0.949$) with low root-mean-square error (RMSE) (293.41 ng/g). The results suggest that modeling levels in grain based upon levels in grain dust can be used to estimate DON in grain bulk.

INTRODUCTION

Mycotoxin concentrations in whole grains are very difficult to accurately estimate when a small portion of the grain lot is used for analysis.1 Currently, grain sampling for mycotoxin analysis is performed in two main ways: (1) dynamic, when sampling is performed during the movement of grain on a conveyor belt by collecting increments at a fixed time intervals; and (2) static, when sampling is performed on bulk lots, in containers, silos, bins, cargo ships, etc. Static sampling is more difficult because several increments of all layers of the bulk need to be sampled.2 The heterogeneity of mycotoxin distribution in bulk grain makes obtaining a representative sample for analysis difficult and costly. In Ontario, two different types of mycotoxins, deoxynivalenol (DON, 1, Figure 1) produced by Fusarium graminearum Schwabe3 and ochratoxin A (OTA) produced by Penicillium verrucosum Diercks4 (OTA, 22) in the field (former) and during storage (latter), respectively, are of particular concern when grain enters the value chain after leaving the farm. DON (1) is the main mycotoxin associated with F. graminearum infection in winter wheat fields in the province. The grain industry has struggled with how to sample and detect DON in a cost-effective, efficient, and reliable manner. DON is managed routinely by end-users at 1000 ng/g, and this limit is currently at the lower limit of confidence due to persistent uncertainty using the best sampling and analytical practices available.3 For instance, given a 0.454 kg sample with a 25 g test portion, the variance associated with a DON test procedure at 5000 ng/g was reported to be 22.0, 56.0, and 22.0% for sampling, sample preparation, and analytical steps, respectively.5 The Canadian grain industry at the receiving elevator uses a visual estimation of Fusarium-damaged kernels for grading. Still, this method does not always accurately predict DON levels in specific lots.6 Furthermore, the high variability of the DON content was found in grain collected from the same wheat field.7 There is resistance to sampling grain and testing for DON at elevators because of the cost, logistics, and sampling uncertainty. In 2004, Stroka et al. first described a sampling technique to estimate the mycotoxin content in bulk material from a small vacuumed dust fraction.8 Later, in 2014 in Europe, the relationship between aspirated grain dust collected from small field plots,9 or while the whole grain is unloading from trucks to grain elevators,10 was described providing reliable regression between the DON content in the grain with that in the dust.
In storage, mycotoxins, such as OTA (22) produced by *P. verrucosum*, present an even more significant challenge for grain sampling and testing. This challenge is due to their greater heterogeneous distribution, usually distributed in hot spots,
Table 1. Matrix Effect, Limits of Detection and Quantification, and Recovery of Mycotoxins from Spiked Winter Wheat Grain and Grain Dust Samples

| mycotoxin                | LOD grain (ng/g ± s.d.) | LOD dust (ng/g ± s.d.) | LOQ grain (ng/g ± s.d.) | LOQ dust (ng/g ± s.d.) | Recovery (ng/ng ± s.d.) | Recovery (ng/ng ± s.d.) | Matrix effect (mean ± s.d.) |
|--------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|---------------------------|
| DON (1)                  | 3.12 ± 0.09             | 0.87 ± 0.05            | 6.58 ± 0.51             | 1.70 ± 0.09            | 100 ± 88               | 54 ± 4                  | -0.03 ± 0.02               |
| 3-acetyl-DON (2)         | 1.15 ± 0.01             | 0.92 ± 0.29            | 2.35 ± 0.11             | 1.97 ± 0.48            | 100 ± 101              | 54 ± 6                  | -0.20 ± 0.08               |
| 15-acetyl-DON (3)        | 10.75 ± 0.81            | 2.41 ± 0.12            | 18.51 ± 2.14            | 4.05 ± 0.21            | 100 ± 89               | 52 ± 4                  | 0.05 ± 0.10                |
| DON 3-Glc (4)            | 1.44 ± 0.82             | 0.28 ± 0.04            | 3.37 ± 2.34             | 0.60 ± 0.10            | 100 ± 91               | 72 ± 6                  | 0.13 ± 0.17                |
| nivalenol (5)            | 3.01 ± 0.28             | 1.48 ± 0.43            | 6.09 ± 0.74             | 2.80 ± 0.96            | 100 ± 79               | 56 ± 4                  | 0.03 ± 0.20                |
| fusarenon-X (6)          | 9.98 ± 0.14             | 9.65 ± 1.88            | 18.90 ± 0.42            | 19.40 ± 5.60           | 100 ± 84               | 54 ± 10                 | -0.06 ± 0.11               |
| HT-2 (7)                 | 2.88 ± 0.54             | 3.15 ± 0.56            | 4.91 ± 0.78             | 5.64 ± 0.13            | 100 ± 111              | 48 ± 10                 | 0.20 ± 0.07                |
| T-2 (8)                  | 0.06 ± 0.01             | 0.06 ± 0.01            | 0.12 ± 0.01             | 0.18 ± 0.02            | 100 ± 89               | 49 ± 11                 | 0.44 ± 0.14                |
| diacetoxyscirpenol (9)   | 0.07 ± 0.01             | 0.1 ± 0.01             | 0.15 ± 0.04             | 0.22 ± 0.02            | 100 ± 72               | 68 ± 10                 | 0.02 ± 0.04                |
| neosolaniol (10)         | 0.14 ± 0.12             | 0.04 ± 0.01            | 0.26 ± 0.21             | 0.10 ± 0.01            | 100 ± 73               | 45 ± 6                  | -0.15 ± 0.07               |
| fumonisin B$_1$ (11)     | 0.09 ± 0.01             | 0.05 ± 0.02            | 0.22 ± 0.02             | 0.15 ± 0.05            | 100 ± 118              | 62 ± 6                  | -0.04 ± 0.18               |
| fumonisin B$_2$ (12)     | 0.53 ± 0.30             | 0.85 ± 0.88            | 1.33 ± 0.74             | 2.23 ± 2.28            | 100 ± 80               | 45 ± 8                  | 0.19 ± 0.44                |
| fumonisin B$_3$ (13)     | 0.68 ± 0.20             | 0.51 ± 0.36            | 1.70 ± 0.50             | 1.29 ± 0.90            | 100 ± 90               | 60 ± 15                 | -0.05 ± 0.24               |
| moniliformin (14)        | 0.04 ± 0.01             | 0.03 ± 0.02            | 0.07 ± 0.01             | 0.05 ± 0.03            | 10 ± 79                | 45 ± 4                  | 0.23 ± 0.13                |
| zearalenone (15)         | 0.01 ± 0.01             | 0.02 ± 0.01            | 0.02 ± 0.01             | 0.04 ± 0.03            | 10 ± 113               | 87 ± 12                 | 0.45 ± 0.23                |
| beauvericin (16)         | 0.01 ± 0.01             | 0.02 ± 0.02            | 0.01 ± 0.01             | 0.05 ± 0.07            | 10 ± 79                | 41 ± 10                 | 1.68 ± 0.31                |
| enniatin A (17)          | 0.01 ± 0.01             | 0.02 ± 0.02            | 0.01 ± 0.01             | 0.04 ± 0.05            | 10 ± 90                | 64 ± 4                  | 0.01 ± 0.18                |
| enniatin A$_1$ (18)      | 0.01 ± 0.01             | 0.02 ± 0.03            | 0.01 ± 0.01             | 0.05 ± 0.07            | 10 ± 100               | 68 ± 5                  | 0.43 ± 0.18                |
| enniatin B (19)          | 0.01 ± 0.01             | 0.01 ± 0.01            | 0.01 ± 0.01             | 0.02 ± 0.02            | 10 ± 73                | 46 ± 5                  | 1.35 ± 0.38                |
| enniatin B$_1$ (20)      | 0.01 ± 0.01             | 0.01 ± 0.01            | 0.01 ± 0.01             | 0.01 ± 0.01            | 10 ± 105               | 46 ± 5                  | 0.03 ± 0.16                |
| sterigmatocystin (21)    | 0.01 ± 0.01             | 0.01 ± 0.01            | 0.01 ± 0.01             | 0.01 ± 0.01            | 10 ± 86                | 45 ± 5                  | 2.43 ± 0.35                |
| ochratoxin A (22)        | 0.05 ± 0.04             | 0.03 ± 0.06            | 0.10 ± 0.09             | 0.08 ± 0.14            | 10 ± 107               | 84 ± 14                 | 0.10 ± 0.17                |
| ochratoxin B (23)        | 0.03 ± 0.01             | 0.02 ± 0.01            | 0.05 ± 0.01             | 0.05 ± 0.01            | 10 ± 71                | 46 ± 8                  | 0.35 ± 0.11                |
| citrinin (24)            | 0.01 ± 0.01             | 0.01 ± 0.01            | 0.01 ± 0.01             | 0.01 ± 0.01            | 10 ± 70                | 45 ± 2                  | 0.28 ± 0.15                |
| penitrem A (25)          | 0.04 ± 0.01             | 0.11 ± 0.10            | 0.10 ± 0.01             | 0.09 ± 0.25            | 10 ± 86                | 41 ± 6                  | 1.19 ± 0.20                |
| roquefortine C (26)      | 0.04 ± 0.01             | 0.06 ± 0.05            | 0.08 ± 0.01             | 0.10 ± 0.11            | 10 ± 93                | 56 ± 4                  | 0.20 ± 0.05                |
| alternariolmethylether (27) | 0.01 ± 0.01          | 0.02 ± 0.02            | 0.02 ± 0.01             | 0.02 ± 0.02            | 10 ± 99                | 50 ± 13                 | 1.10 ± 0.53                |
| alternariol (28)         | 0.20 ± 0.05             | 0.15 ± 0.02            | 0.45 ± 0.13             | 0.30 ± 0.03            | 100 ± 88               | 45 ± 9                  | 0.29 ± 0.21                |

"LOD (S/N = 3, n = 10) and level of quantitation (LOQ) (S/N = 3, n = 10) were determined using matrix-matched calibration curves for grain and grain dust. Recovery: mycotoxins were spiked in triplicates at 10 or 100 ng/g, equilibrated for 3 days at 40 °C before extraction with a dilution factor of 8× for the recovery test (%) and standard deviation (s.d.) determination; matrix effect was calculated as (peak area of matrix − peak area of solvent)/peak area of solvent (n = 3)."
Mycotoxins and their respective fungi have been identified in dust samples for mycotoxin analysis with less labor and cost. Many dust samples, 12,18 and occupational exposure has been acknowledged.19 However, only recently, methods for obtaining representative dust samples have been reported for 

Fusarium mycotoxins. The objective of this study was to determine whether aspirated grain dust particles, collected during grain transfer at harvest or after storage using a simple vacuum system, could be used for sampling and determination of the mycotoxin content in the corresponding winter wheat grain.

### RESULTS AND DISCUSSION

**Quantity of Dust Collected.** A total of 323 paired grain and grain dust samples (particle size (p.s.) <1650 μm) were collected from January 2015 to April 2018, of which 317 were from soft red winter wheat, five winter wheat durum, and one hard red winter wheat. The samples were classified into three types: field samples collected off the combine at harvest (56%), storage samples collected from on-farm bins at out-loading (28%), and storage samples collected from experimental minibins seeded with an OTA/P. verrucosum hot spots (15%). In this study, we were able to follow 53% of sampled grain lots from combine harvesters to their respective on-farm storage bins, representing 75% of storage samples collected during grain out-loading.

The quantity of dust collected (mean 8.9 ± 9.6 g) was significantly affected by the sample type ($F = 11.14, df_1 = 1, df_2 = 126, p = 0.0011$), time in storage (mean 3.5 ± 4.1 months, $F$...
When the relationship between the quantity of dust and grain was calculated as the ratio of dust collected (mg) per quantity of grain sampled (kg), we found that this relationship was only affected by the sample type (F = 55.68, df_n = 2, df_d = 126, p < 0.0001), grain test weight (F = 3.03, df_n = 50, df_d = 126, p < 0.0001), grain moisture content at harvest (F = 2.05, df_n = 10, df_d = 82, p = 0.0387), and the interaction of both (F = 2.90, df_n = 94, df_d = 121, p < 0.0001). The ratio was significantly higher in samples collected from experimental nonaerated minibins (mean 9.90 ± 7.5) than samples from commercial on-farm storage bins (mean 1.27 ± 1.33) and those collected from the field at harvest (mean 0.40 ± 0.32). Early studies focused on the quantity of grain dust aspirated for environmental control and safety within grain handling facilities as dust in grain can fuel elevator explosions and expose workers to inhalable dust. Martin and Stephens found that grain handling affects the total amount of dust collected. After an initial buildup, fine particles remained constant during repeated handling cycles. Schnake reported seasonal variation on the quantity of wheat grain dust aspirated related to rain just before or at harvest; however, the grain moisture content was not measured. Similar to our results, Spanke and Cherrie found higher levels of inhalable dust at grain loading or unloading than at harvest time.

**Mycotoxin Incidence.** Of 28 mycotoxins studied (Table 1), nivalenol (S) and fusarenon-X (6) were not detected in this study. HT-2 (7), diacetoxyisercirpenol (9), and neosolaniol (10) were not found in grain, and only in 12, 11, and 3% of grain dust samples, respectively. These toxins will be excluded from any pairwise analysis (Table 2). DON was found at higher concentrations than any other mycotoxin from grain or grain dust from out of the field or from storage (Tables 2 and 3). DON concentration means were 460.4 ng/g in grain and 2722.0 ng/g in grain dust, whereas all of the other toxins detected had concentrations lower than 40 and 100 ng/g in grain dust from out of the field or from storage (Tables 2 and 3).

### Table 3. Difference between Mycotoxin Levels Found in Winter Wheat Grain Samples Collected from Three Different Sources from January 2015 to April 2018

| Mycotoxin          | Field (n = 182) | Grain Samples (n = 323) | Grain Samples (n = 92) | Nonaerated Minibins (n = 49) |
|--------------------|---------------|-------------------------|------------------------|----------------------------|
|                    | F^a  | p^a | >LOD (%) | Mean (ng/g) | SD (±) | F^a | p^a | >LOD (%) | Mean (ng/g) | SD (±) | F^a | p^a | >LOD (%) | Mean (ng/g) | SD (±) |
| DON (1)            | 34.54 | <0.0001 | 63 | 29.2 | (106.8) | A | 100 | 1428.8 | (2119.1) | B | 98 | 244.1 | (401.3) | AB |
| 3-acetyl-DON (2)   | 0.34  | 0.761 | 0 | 0.0 | (0.0) | A | 11 | 0.9 | (3.4) | A | 0 | 0.0 | (0.0) | A |
| 15-acetyl-DON (3)  | 48.2  | <0.0001 | 3 | 0.0 | (0.5) | A | 47 | 34.3 | (49.3) | A | 20 | 4.0 | (9.2) | A |
| DON-3-Glc (4)      | 36.39 | <0.0001 | 51 | 5.0 | (15.7) | A | 100 | 1177.7 | (178.9) | B | 37 | 10.3 | (18.4) | A |
| HT-2 (7)           | 14.93 | <0.0001 | 0 | 0.0 | (0.0) | A | 0 | 0.0 | (0.0) | A | 0 | 0.0 | (0.0) | A |
| T-2 (8)            | 13.49 | <0.0001 | 3 | 0.1 | (0.4) | A | 33 | 1.0 | (2.1) | B | 2 | 0.1 | (1.0) | A |
| diacetoxyisercirpenol (9) | 0.06 | 0.9441 | 0 | 0.0 | (0.0) | A | 0 | 0.0 | (0.0) | A | 0 | 0.0 | (0.0) | A |
| fumonisin B1 (11)  | 1.28  | 0.2784 | 5 | 0.0 | (0.1) | A | 33 | 0.1 | (0.2) | A | 76 | 1.2 | (1.3) | A |
| fumonisin B2 (12)  | 0.46  | 0.6345 | 4 | 0.0 | (0.1) | A | 17 | 0.1 | (0.2) | A | 71 | 1.4 | (2.2) | A |
| fumonisin B3 (13)  | 0.16  | 0.8555 | 3 | 0.0 | (0.0) | A | 20 | 0.1 | (0.2) | A | 59 | 2.3 | (2.8) | A |
| moniliformin (14)  | 13.75 | <0.0001 | 16 | 0.1 | (0.3) | A | 36 | 1.1 | (2.5) | B | 57 | 0.4 | (1.0) | A |
| nibбережin (15)    | 25.05 | <0.0001 | 35 | 0.1 | (0.4) | A | 79 | 8.3 | (13.9) | B | 88 | 6.1 | (9.1) | A |
| beaureverin (16)   | 1.47  | 0.2119 | 44 | 0.3 | (0.5) | A | 84 | 0.8 | (1.7) | A | 100 | 4.4 | (4.9) | A |
| enniatin A (17)    | 26.34 | <0.0001 | 46 | 0.2 | (0.4) | A | 85 | 1.6 | (2.4) | B | 78 | 0.6 | (1.1) | A |
| enniatin A (18)    | 30.25 | <0.0001 | 84 | 0.9 | (1.7) | A | 95 | 10.1 | (15.8) | B | 3 | 1.4 | (1.8) | A |
| enniatin B (19)    | 28.48 | <0.0001 | 77 | 1.2 | (1.9) | A | 96 | 31.2 | (53.1) | B | 100 | 4.2 | (5.7) | A |
| enniatin B (20)    | 40.66 | <0.0001 | 75 | 1.8 | (2.6) | A | 95 | 28.5 | (42.6) | B | 100 | 3.7 | (4.0) | A |
| sterigmatocystin (21) | 0.67 | 0.5137 | 0 | 0.0 | (0.0) | A | 17 | 0.5 | (3.2) | A | 61 | 3.0 | (8.4) | A |
| ochratoxin A (22)  | 10.10 | <0.0004 | 0 | 0.0 | (0.0) | A | 3 | 0.3 | (1.7) | A | 63 | 68.4 | (141.5) | B |
| ochratoxin B (23)  | 10.09 | <0.0004 | 0 | 0.0 | (0.0) | A | 1 | 0.0 | (0.0) | A | 49 | 1.5 | (3.0) | B |
| cirratin (24)      | 22.13 | <0.0001 | 46 | 0.2 | (0.4) | A | 85 | 1.6 | (2.4) | A | 20 | 5.9 | (14.9) | B |
| penitrem A (25)    | 2.16  | 0.1170 | 0 | 0.0 | (0.0) | A | 4 | 0.0 | (0.1) | A | 69 | 0.1 | (0.1) | A |
| roquefortine C (26) | 2.82 | 0.0609 | 0 | 0.0 | (0.0) | A | 3 | 0.0 | (0.1) | A | 47 | 0.2 | (0.4) | A |
| alternariol (27)   | 0.06  | 0.9461 | 41 | 0.3 | (0.6) | A | 85 | 1.2 | (3.5) | A | 100 | 1.3 | (1.2) | A |
| alternariol (28)   | 5.62  | 0.0065 | 59 | 1.1 | (2.1) | A | 91 | 2.4 | (2.8) | A | 100 | 6.0 | (3.8) | B |

*Restricted maximum likelihood (REML) variance component summary: type III test of fixed effects. Tukey–Kramer grouping by sample source (α = 0.05), the mean with the same letter is not significantly different; and the values were not corrected for recovery.

https://dx.doi.org/10.1021/acsomega.0c04256
ACS Omega 2021, 6, 1837–1871
grain and grain dust, respectively. Mycotoxins like beauvericin (16), enniatins (17–20), and alternariolmethyl ether (27) were found more frequently than DON in grain, and especially in grain dust. This was due to a lower level of detection (LOD), which was more than 300 and 40 times lower than those of mycotoxins like beauvericin (all paired tests, p < 0.0030). Mean concentration ratios of mycotoxins found in grain vs paired dust grain varied greatly. Mycotoxins like fumonisins (11–13), penitrem A (25), roquefortine C (26), and Alternaria metabolites (27, 28) were found at low concentrations in grain and were 62, 35, 23, 48, 18, 35, and 21 times higher in grain dust. Ratios for all of the other mycotoxins ranged from 1:4 to 1:15 with DON (1) and OTA (22) found 6 and 9 times higher in grain dust than in grain.

Different factors affect the distribution of mycotoxins in the grain and in the surface of the grain that, because of abrasion, is released in the form of grain dust. One consideration is the relative solubility of the individual mycotoxin. We found that zearalenone (15) was, on average, 13 times more concentrated in the dust, compared with DON (1), which is only 6 times. Both toxins are produced by F. graminearum, mainly on the surface of the grain. However, DON is highly soluble in water (57 000 μg/mL at 25 °C), thus it migrates easily to the inner part of the grain. Zearalenone is relatively insoluble in water (4.81 μg/mL at 25 °C). Therefore, it is found highly concentrated in the dust. Our results corroborate previous results by Reichel et al. in wheat. Another consideration is the nature of the fungal infection: Fusarium proliferatum (Matsushita) Nirenberg is considered the predominant species producing fumonisins (11–13) in wheat, albeit in low concentrations. Infection often occurs systemically, as an endophyte, with transmission from seed or roots to kernels. Mostly, the infection is asymptomatic, with intercellular hyphal growth. However, F. proliferatum is also an opportunistic pathogen of wheat that, under stress conditions, can grow intracellularly and produce fumonisins. This infection appears to concentrate in the outer layer of the seed as studies in dry-milled maize fraction suggest. In this study, the accumulation of fumonisin B1 (11), fumonisin B2 (12), and fumonisin FB3 (13) were 62, 35, and 23 times more in the grain dust than in the grain. These results are similar to those in maize, where higher levels of these toxins were found in grain dust collected from aspirators, filters, and after intensive scouring. A third consideration is the mycotoxin conjugation to glucose. Plants accumulate starch (a polymer of glucose) in the seed endosperm vacuoles as an energy reserve. Mycotoxins like DON 3-β-D-glucoside (DON 3-Glc) are also accumulated, as a detoxification product of DON (1), in cell vacuoles of infected spikelets. DON 3-Glc was the only mycotoxin in this study, with concentrations in the grain higher than in the dust, which could be attributed to the lower

Table 4. Difference between Mycotoxin Levels Found in Winter Wheat Dust Grain Samples Collected from Three Different Sources from January 2015 to April 2018

| mycotoxin          | field (n = 182) | on-farm storage (n = 92) | monoaerated münihns (n = 49) |
|--------------------|----------------|-------------------------|-------------------------------|
| DON (1)            | 44.63 ± 0.0001 | 82.0 ± 201.7 (508.5)     | 100 ± 806.39 (10 689.2)       |
| 3-acetyl-DON (2)   | 30.46 ± 0.0001 | 3.0 ± 0.2 (1.5)         | 38 ± 11.3 (18.6)               |
| 15-acetyl-DON (3)  | 54.51 ± 0.0001 | 70 ± 16.1 (67.0)        | 93 ± 202.5 (216.6)             |
| DON 3-Glc (4)      | 22.08 ± 0.0001 | 18 ± 2.6 (7.0)          | 66 ± 66.4 (120.2)              |
| HT-2 (7)           | 24.87 ± 0.0001 | 1 ± 0.4 (4.3)           | 40 ± 11.7 (17.5)               |
| T-2 (8)            | 22.22 ± 0.0001 | 31 ± 0.4 (0.8)          | 70 ± 2.6 (3.6)                 |
| diacetoxyscirpenol (9) | 0.01 ± 0.0001 | 0 ± 0.0 (0.0)          | 2 ± 0.0 (0.3)                  |
| neosolanioi (10)   | 1.28 ± 0.2784  | 0 ± 0.0 (0.2)           | 9 ± 0.0 (0.1)                  |
| fumonisin B3 (11)  | 14.75 ± 0.0001 | 30 ± 1.1 (3.9)          | 92 ± 9.1 (12.7)                |
| fumonisin B2 (12)  | 24.43 ± 0.0001 | 23 ± 0.4 (2.0)          | 68 ± 4.2 (6.9)                 |
| fumonisin B1 (13)  | 27.72 ± 0.0001 | 20 ± 0.2 (1.2)          | 73 ± 5.1 (7.6)                 |
| moniliformin (14)  | 26.75 ± 0.0036 | 23 ± 0.7 (1.8)          | 53 ± 2.6 (5.3)                 |
| zearalenon (15)    | 53.24 ± 0.0001 | 80 ± 5.1 (11.7)         | 100 ± 100.0 (199.2)            |
| beauvericin (16)   | 29.13 ± 0.0001 | 95 ± 9.7 (10.0)         | 100 ± 22.5 (200.0)             |
| enniatin A (17)    | 39.75 ± 0.0001 | 98 ± 3.1 (3.0)          | 100 ± 25.0 (34.8)              |
| enniatin A1 (18)   | 48.36 ± 0.0001 | 97 ± 8.5 (9.1)          | 100 ± 73.2 (91.0)              |
| enniatin B (19)    | 62.02 ± 0.0001 | 96 ± 20.4 (28.0)        | 100 ± 130.9 (140.3)            |
| enniatin B2 (20)   | 57.64 ± 0.0001 | 93 ± 14.8 (19.8)        | 100 ± 94.1 (102.8)             |
| sterigmatocystine (21) | 7.72 ± 0.0007 | 21 ± 0.1 (0.3)         | 96 ± 5.3 (11.8)                |
| ochratoxin A (22)  | 10.13 ± 0.0004 | 21 ± 0.8 (4.2)          | 73 ± 16.4 (30.2)               |
| ochratoxin B (23)  | 10.54 ± 0.0005 | 1 ± 0.0 (0.0)           | 46 ± 0.5 (0.8)                 |
| citrinin (24)      | 9.43 ± 0.0004  | 5 ± 0.1 (0.4)           | 74 ± 3.0 (4.1)                 |
| penitrem A (25)    | 25.26 ± 0.0001 | 0 ± 0.0 (0.0)           | 92 ± 1.1 (1.5)                 |
| roquefortine C (26)| 21.49 ± 0.0001 | 13 ± 0.0 (0.1)          | 43 ± 0.5 (1.2)                 |
| alternariolmethyl ether (27) | 3.02 ± 0.0619 | 91 ± 17.4 (20.9)       | 100 ± 34.8 (38.5)              |
| alternariol (28)   | 6.48 ± 0.0022  | 77 ± 23.7 (32.7)        | 98 ± 50.3 (51.9)               |

remLVariance component summary: type III test of fixed effects. Tukey–Kramer grouping by sample source (α = 0.05), the mean with the same letter is not significantly different; values not corrected for recovery.
content of starch in the bran as grain dust is generated by the friction of the outer layer of seeds or bran during grain transfer. Furthermore, Martin found that wheat dust has a higher fiber and lower starch content than wheat grain. A fourth consideration is that mycotoxins produced during storage, like those produced by *P. verrucosum* (21–23), can arise from contaminated debris from combine and augers and be released as dust contaminated with mycotoxins. For instance, in our study, OTA (22) was detected in dust collected during harvest, but it was not detected in grain going into storage (Tables 3 and 4).

Depending on the mycotoxin, differences were also found between where the samples were sourced. In grain (Table 3), DON, DON 3-Glc (4), T-2 (8), moniliformin (14), enniatins (17–20), and alternariol (28) were found at higher concentrations in samples collected from on-farm storage bins than those obtained directly from the field at harvest (*p < 0.0001*). OTA (22), ochratoxin B (23), and citrinin (24) were found at higher concentrations in samples collected from our experimental bins, which were seeded with a *P. verrucosum* hot spot (*p = 0.0004*) as well as alternariol (28) (*p = 0.0065*). In grain dust, all mycotoxins, except those found at very low frequency (9, 10), were found at higher concentrations in samples from on-farm storage bins than from those collected in the field after harvest. In the experimental nonaerated minibins, fumonisins (11–13), beauvericin (16), penitrem A (25), roquefortine C (26), and alternariol were found at higher concentrations compared with field and on-farm stored grain dust samples (Table 4). These results indicate that different mycotoxin profiling is found in samples collected from the field, managed on-farm storage bins, and nonaerated experimental bins posing a challenge for obtaining a model that covers all three sample sources.

### Mycotoxin Content in Grain vs Grain Dust

Pearson correlation coefficients and two-variable regression show a strong and significant (*p < 0.0001*) linear relationship between the mycotoxin content in grain and that in grain dust with most mycotoxins, except for 3-acetyl-DON (2), beauvericin (16), and alternariolmethylether (27). The most robust relationships were found, in decreasing regression coefficient values, for OTA (22) produced by *P. verrucosum* and DON (1) produced by *F. graminearum* in Ontario with 0.952 and 0.949, respectively. Other toxins related to *P. verrucosum*, citrinin (24), and ochratoxin B (23), as well as enniatin A1 (18) associated with *F.avenaceum* in Canada, had regression coefficient values >0.820 (Table 5). A robust dataset was obtained for enniatin A1 (18) and DON (1), which have the higher detection frequency and the higher mean concentration found in this study, respectively (Table 2). Although mycotoxins related to *P. verrucosum* (22–24) were found frequently in dust from the stored grain, only the experimental bins seeded with *P. verrucosum* generated enough contaminated grain samples to build a robust model as the natural occurrence of OTA (22) and related toxins is low or occurs in hot spots inside storage bins. In all of these mycotoxins (1, 18, 22–24), more than 82% of the variation in the data is explained by the two-variable regression model. The results for all of the other mycotoxins are summarized in Table 5.

In 2013, Sanders et al. reported a linear correlation between DON_{grain} and DON_{dust} in a small set of wheat samples (*r^2 = 0.941*, *n = 12*, dust particle size (p.s) < 50 μm) with a DON_{grain}/DON_{dust} ratio of 1:1.31. A year later, the same group determined a sigmoidal relationship between DON_{dust} vs.

---

**Table 5. Correlation and Two-Variable Regression between the Mycotoxin Content in the Paired Samples of Winter Wheat Grain and Grain Dust Collected from January 2015 to April 2018 in Southwestern Ontario**

| mycotoxin                  | correlation | regression       | intercept  | slope    |
|----------------------------|-------------|------------------|-----------|----------|
|                            | *r*         | *p*              | *r^2*     | *p*      |
| DON (1)                    | 0.974       | <0.0001          | 0.949     | <0.0001  |
| 3-acetyl-DON (2)           | 0.538       | <0.0001          | 0.280     | <0.0001  |
| 15-acetyl-DON (3)          | 0.809       | <0.0001          | 0.564     | <0.0001  |
| DON 3-Glc (4)              | 0.793       | <0.0001          | 0.629     | <0.0001  |
| T-2 (8)                    | 0.893       | <0.0001          | 0.797     | <0.0001  |
| fumonisin B1 (11)          | 0.803       | <0.0001          | 0.645     | <0.0001  |
| fumonisin B2 (12)          | 0.751       | <0.0001          | 0.564     | <0.0001  |
| fumonisin B3 (13)          | 0.772       | <0.0001          | 0.597     | <0.0001  |
| moniliformin (14)          | 0.804       | <0.0001          | 0.646     | <0.0001  |
| zearalenone (15)           | 0.882       | <0.0001          | 0.779     | <0.0001  |
| beauvericin (16)           | 0.373       | <0.0001          | 0.782     | <0.0001  |
| enniatin A (17)            | 0.884       | <0.0001          | 0.820     | <0.0001  |
| enniatin A1 (18)           | 0.911       | <0.0001          | 0.139     | <0.0001  |
| enniatin B (29)            | 0.841       | <0.0001          | 0.707     | <0.0001  |
| stergmatocystin (21)       | 0.825       | <0.0001          | 0.680     | <0.0001  |
| ochratoxin A (22)          | 0.976       | <0.0001          | 0.952     | <0.0001  |
| ochratoxin B (23)          | 0.914       | <0.0001          | 0.836     | <0.0001  |
| citrinin (24)              | 0.931       | <0.0001          | 0.867     | <0.0001  |
| penitrem A (25)            | 0.847       | <0.0001          | 0.718     | <0.0001  |
| roquefortine C (26)        | 0.791       | <0.0001          | 0.626     | <0.0001  |
| alternariolmethylether (27) | 0.665     | <0.0001          | 0.442     | <0.0001  |
| alternariol (28)           | 0.839       | <0.0001          | 0.703     | <0.0001  |

*a = 323 paired samples. *Pearson correlation coefficients. *Two-variable regression. *RMSE: root-mean-square error. *SS: sum of squares.*
log $\text{DON}_{\text{grain}}$ ($r^2 = 0.829$, $n = 40$, p.s. $< 50 \mu g$) with a $\text{DON}_{\text{grain}}/\text{DON}_{\text{dust}}$ ratio of 1.59 in samples with higher DON content from experimental fields with five wheat varieties. They concluded that the relationship is concentration-dependent, and a linear correlation was proposed for concentrations lower than 1250 ng/g and a sigmoidal curve for higher concentrations. In the same year, Reichel et al. reported a linear relationship ($r^2 = 0.85$, $n = 78$, p.s. 100–1000 μg) with a $\text{DON}_{\text{grain}}/\text{DON}_{\text{dust}}$ ratio of 1.75 with samples collected during truck unloading to mills at the beginning of the harvest season. However, they recommend separate linear regression for different concentration ranges or the use of a nonlinear model to fit all levels. The accuracy of the model will depend on individual mycotoxin, recommended maximum limits (MLs), and the regression root-mean-square error (RMSE) of the model. Previous studies did not reveal the mean square error in their regressions or correlations. In our study, a linear relationship is reported ($r^2 = 0.949$, $n = 323$, p.s. $< 1650 \mu g$) with a $\text{DON}_{\text{grain}}/\text{DON}_{\text{dust}}$ ratio of 1:5.4 in samples from the field to storage. The regression equation generated for DON was $Y = 0.1868X - 47.9516$ ($F_{(1,321)} = 596.31, p \leq 0.0001$, $r^2 = 0.949$, RMSE $= 293.41$), where $X = \text{DON}_{\text{dust}}$, and $Y = \text{DON}_{\text{grain}}$. Health Canada’s proposed MLs for DON (1) is 2000 ng/g in uncleaned wheat. According to our results, this model will be adequate for predicting DON in grain as RMSE was approximately 293 ng/g, with 95% of the values falling within 2 standard deviations of their respective predicted values (2 std = 586 ng/g). This value is higher than the cutoff level of 150 ng/g found by Reichel et al. in dust samples with smaller and more restrictive particle size range (100–1000 μm) obtained using a cyclone-based system. In our study, the objective was to develop an easy and farm-friendly method for dust collection. Thus, the particle size was set at 1650 μm. Because of the prediction error (RMSE) of ±293 ng/g, our linear model may not be adequate to predict DON levels in grain with more restrictive MLs, like the 1000 ng/g in uncleaned wheat for use in baby food proposed by Health Canada. Nonlinear regression between $Y$ (DON$_{\text{grain}}$) and $X$ (DON$_{\text{dust}}$) was also investigated using the Marquardt method in the NLIN procedure of SAS. This relationship can be described by the following exponential equation: $Y = (1 + a \exp(b \times X))$ with parameters $a = 378$ and $b = 0.00008 (F_{(1,321)} = 634.71, p < 0.0001, r^2 = 0.870, \text{RMSE} = 618.95)$. In this dataset, RMSE and $r^2$ values were higher than those found in the two-variable regression.

The regression equation obtained for OTA (22) was $Y = 0.1070X - 0.0292 (F_{(1,321)} = 6416.1, p < 0.0001, r^2 = 0.952, \text{RMSE} = 13.10)$, where $X = \text{OTA}_{\text{dust}}$ and $Y = \text{OTA}_{\text{grain}}$. Health Canada’s proposed MLs for OTA (22) is 5 ng/g in raw wheat. The RMSE obtained was ±13.10 ng/g (Table 5). According to these results, this model is not adequate for OTA. There are no proposed MLs for citrinin (24), ochratoxin B (23), and enniatin A$_1$ (18). Further analysis of the factors affecting the accuracy of the method is discussed below.

As mentioned above, the ratio between the mycotoxin content in grain and paired grain dust samples not only varies across different mycotoxins (Table 2) but within each mycotoxin (Table S1). This variability can pose a challenge to modeling. For instance, this ratio in DON (1) varied from undefined (10%), zero (11%), and positive ratios (79%) ranging from 0.001 to 0.725 with a mean of 0.115 or 1:9, while in OTA (22) the same ratio varied from undefined (54%), zero (35%) and positive ratios (11%) ranging from 0.001 to 0.400 with a mean of 0.089 or 1:11. To understand the source of this variability, a stepwise regression analysis was performed after log +1 transformation for each mycotoxin profile, both in grain and corresponding grain dust. Sources of variation included year and farm location nested within years as random

![Table 6a. Stepwise Multiple Regression Analysis of the Mycotoxin Content in 323 Grain and Paired Grain Dust Samples Collected from January 2015 to April 2018 in Southwestern Ontario](https://dx.doi.org/10.1021/acsomega.0c04256)
| Mycotoxin       | TWt \( r^2 \times 100 \) | dust × Wd \( r^2 \times 100 \) | dust × TWt \( r^2 \times 100 \) | W × TWt \( r^2 \times 100 \) | dust × W × TWt \( r^2 \times 100 \) |
|----------------|--------------------------|-------------------------------|-------------------------------|--------------------------|-------------------------------|
| DON (1)        |                          |                               |                               |                          |                               |
| 3-acetyl-DON (2)| 0.1020                   | 31.3                          | ***                           |                          |                               |
| 15-acetyl-DON (3)| -0.0749                 | 8.7                           | ***                           |                          |                               |
| DON 3-Glc (4)  | -0.0840                  | 4.6                           | ***                           | 0.0004                   | 0.8                           | **                           |
| T-2 (8)        |                          |                               |                               |                          |                               |
| fumonisin B1 (11)| -0.00400                | 0.8                           | *                             | 0.0019                   | 45.9                          | ***                           |
| fumonisin B2 (12)|                        |                               |                               |                          |                               |
| fumonisin B3 (13)|                         |                               |                               |                          |                               |
| moniliformin (14)|                         |                               |                               |                          |                               |
| zearalenone (15)| -0.0367                  | 1.5                           | ***                           |                          |                               |
| beauvericin (16)| 0.0049                   | 2.9                           | ***                           | 0.0005                   | 22.1                          | **                            |
| enniatin A (17)| 0.0006                   | 0.3                           |                               |                          |                               |
| enniatin A1 (18)| 0.0011                   | 77.7                          | ***                           |                          |                               |
| enniatin B (29)| -0.0899                  | 4.1                           | ***                           |                          |                               |
| enniatin B1 (20)|                         |                               |                               |                          |                               |
| sterigmatocystin (21)| 0.0328               | 0.8                           | *                             | 0.0044                   | 67.8                          | ***                           |
| ochratoxin A (22)|                         |                               |                               |                          |                               |
| ochratoxin B (23)| -0.0518                  | 0.4                           | **                            |                          |                               |
| citrinin (24)| 0.0378                   | 1.4                           | ***                           |                          |                               |
| penitrem A (25)|                         |                               |                               |                          |                               |
| roquefortine C (26)|                         |                               |                               |                          |                               |
| alternariolmethylether (27)| -0.0132         | 2.2                           | **                            |                          |                               |
| alternariol (28)|                         |                               |                               |                          |                               |

*p < 0.05.
**p < 0.001.
***p < 0.0001.

TWt: grain test weight (kg/hL) at sampling.
W: grain moisture content at sampling (%).

"In the model, all coefficients were obtained after applying transformation (log + 1) to all mycotoxin results (ng/g) in both grain and grain dust; RMSE: root-mean-square error; SS: sum of squares; p < 0.01."
effects, sample type (harvest, storage, minibins), mycotoxin content in grain dust, months in storage, sampling duration (min), grain moisture content (%), and grain test weight (kg/hL) at sampling as fixed effects. The stepwise regression eliminated those variables that did not contribute additional predictive power. For all mycotoxins studied, the mycotoxin content in grain dust, grain moisture content at sampling (W) and/or grain test weight (TWt) and/or their interaction were significant. The stepwise regression analysis of all mycotoxins is summarized in Tables 6a and 6b.

For DON (1), the contribution of grain dust (X = DONgrain) alone to the mycotoxin content in grain (Y = DONgrain) was higher than 80% (83.3%, p < 0.0001), followed by the grain moisture content (W) at harvest (2.3%, p < 0.1000) and the interaction between both variables and test weight in (TWt) grain (0.4%, p < 0.0001). In Ontario, DON is produced by F. graminearum entering the value chain by infecting wheat heads in the field. In this study, DON was found in all sample sources from grain harvested in the field (n = 114, 45%, mean 29.2 ± 106.8 ng/g), on-farm storage (n = 92, 36%, mean 1428.8 ± 2119.1 ng/g), and experimental minibins (n = 48, 19%, mean 244.1 ± 401.3 ng/g). The same was observed for grain dust collected in the field (n = 149, 51%, mean 201.7 ± 508.5 ng/g) on-farm storage (n = 92, 32%, mean 8063.9 ± 10689.2 ng/g) and experimental bins (n = 49, 17%, 2052.6 ± 3109.4 ng/g). The resulting regression equation was log(Y + 1) = −2.4106 + 1.3436 log(X + 1) + 0.1600 × W − 0.0001 × W × TWt log(X + 1) (F(3,313) = 641.0, p < 0.0001, R^2 = 0.859, RMSE = 0.725) (Tables 6a and 6b). Although RMSE was reduced from 293.41 ng/g in two-variable regression to 5.31 ng/g (antilog value) using a stepwise approach, the latter includes the grain moisture content at sampling and grain test weight in the model. These two variables were measured at the source, therefore difficult to obtain once the grain has entered the supply chain. These results may suggest that a simple two-way linear regression would be enough for predicting the DON content in grain using grain dust values (R^2 = 0.952).

Unlike DON, OTA is not distributed widely throughout our sample sources in grain and grain dust. OTA is a mycotoxin associated with stored grain; in our study, this toxin was not found in grains collected during harvest, and the positive grain samples (>LOD, n = 34) were mainly found in nonaerated experimental minibins seeded with P. verrucosum hot spots (n = 31, 91%, 108.2 ± 163.4 ng/g). The natural occurrence of this mycotoxin was low in grain from on-farm storage bins (n = 3, 9%, 7.7 ± 5.4 ng/g). However, OTA was found in grain dust collected in the field but at a lower concentration (n = 39, 26%, 3.7 ± 8.4 ng/g) compared with stored samples from on-farm bins (n = 67, 45%, 22.5 ± 33.2 ng/g) and experimental minibins (n = 43, 29%, 697.7 ± 1348.6 ng/g). Previous studies on the OTA content in airborne grain dust focused on models to predict exposure to workers.12,38,39 The only one comparing OTA concentrations in airborne dust with the corresponding grain sample in barley used a small number of samples (n = 23). Similar to our on-farm storage results, OTA frequency and concentration were higher in the dust (n = 18, range 0.05–9.9 ng/g) than in corresponding grain samples (n = 3, range 0.01–0.51 ng/g).38 Hot spots of OTA contamination in storage are found in areas affected by moisture migration and exposure to moisture from precipitation or condensation.4 Hence, factors like moisture content at sampling (W) and test weight (TWt) are important factors for the prediction of OTA levels in grain (Y) from paired OTA levels in grain dust (X). In this study, the interaction of these three variables (X, W, and TWt) was the most important factor (78.9%, p < 0.0001) for predicting Y = OTAgrain, followed by the interaction of W and TWt (3.2%, p < 0.0001) and OTAgrain alone (2.0%, p < 0.0001) (Tables 6a and 6b). The regression equation was log(Y + 1) = 3.7999 − 0.6516 log(X + 1) − 0.0005 × W × TWt + 0.0002 × W × TWt log(X + 1) (F(3,313) = 554.3, p < 0.0001, R^2 = 0.912, RMSE = 0.244) (Tables 6a and 6b). Similar to DON, RMSE was reduced from 13.10 ng/g in two-variable regression to 0.244 ng/g (antilog value) using a stepwise approach with 84% of the values falling within 2 standard deviations of their respective predicted values (2 std = 0.488 ng/g). According to our results, a stepwise approach may be used for predicting OTA levels in grain from values found in the dust as Health Canada’s proposed MLs for OTA (22)37 is 5 ng/g in raw wheat. However, two potential caveats of this model merit comment. First, samples from the experimental nonaerated minibins, which represented 91% of grain samples positive for OTA, had lower TWt and higher W than samples collected from on-farm storage bins; therefore, this differentiation is then reflected in the model. Second, in this study, each grain and grain dust sample pair were collected for 10 min. Therefore, obtaining precise data on test weight and especially moisture content, at source, in large operations (e.g., whole on-farm storage bins, whole trucks, commercial grain elevators) and/or further up in the grain distribution chain will be operationally challenging and impractical.

Despite the different factors affecting the quantity of aspirated dust (mean = 8.9 ± 9.5 g) obtained per grain collected (mean = 4.5 ± 1.4 kg), in this dataset, a two-variable regression was used successfully for predicting the DON concentration in grain from the DON content in grain dust (particle size <1650 μm). However, there are some limitations of this model and important considerations for future models: (1) sampling error: paired samples were collected for 5–10 min from augers loading and unloading grain from combine harvesters, on-farm storage bins, and small experimental bins. Consequently, this relationship needs to be re-evaluated in larger harvest, storage, or transportation facilities, where the effect of grain sampling error will be more significant, especially if a whole storage bin or truck needs to be sampled for one representative mycotoxin result. Furthermore, a more diverse sample source needs to be considered, as 98% of the samples collected were soft red winter wheat. (2) Recovery rates in grain dust: in this study, we used an extraction method already validated for grain and, for simplicity, extended to grain dust. On average, recovery values of spiked mycotoxin standards in grain dust were 38.6% lower than those obtained in grain. For DON, recovery values were 88% in grain vs 54% in grain dust. Because of this disparity, mycotoxin levels were not adjusted according to recovery rates as they will only modify the slope in the regression model. (3) Plant-conjugated mycotoxins: in our survey, 253-grain samples were positive for DON; of those, 201 were also positive for DON 3-Glc (4), representing on average, 23.5% of DON concentration. DON 3-Glc can be metabolized to free DON during digestion, contributing to an increase in the dietary exposure of DON in humans and animals.12,40 DON 3-Glc was the only mycotoxin in this study, with concentrations in the grain higher than in the aspirated dust. Therefore, posing a challenge for estimating the overall toxicity profile associated with DON.
**MATERIALS AND METHODS**

**Sampling.** Grain samples were collected into a new cotton sack (38 cm wide × 71 cm long) using an high-density polyethylene (HDPE) dip cup (630 mL) fixed on a pole inserted into the moving stream of grain at 1 min intervals for 3–10 min until approximately 5–10 kg of grain was obtained. The cup was cleaned with a methanol-soaked disposable cloth between each lot sampled. The generated grain dust was aspirated into 5.7 L preweighed HEPA vacuum bags (GK-PHP06HEPA, Green Klean, Cary, IL) during the whole period of grain sampling using a modified 6.0 HP drum vacuum cleaner (RIDGID WD14500; 8.0 kW and 53 L; Ridge Tool Company, Elyria, OH) equipped with an in-line air filter housing (Fleetguard OptAir 1100 series; Cummins Filtration, Nashville, TN). To increase the surface area for dust collection, an adapter was attached to the vacuum sampling wand. The adapter consisted of a poly(vinyl chloride) (PVC) friction fitted to an acrylonitrile—butadiene—styrene (ABS) reducing pipe coupling (101.6–50.8 mm diameter, socket type) attached to the suction end of a 50.8 mm diameter vacuum wand. The opening was covered with an aluminum window screen (12 × 12 mesh size or an opening diameter of 1650 μm) fastened in place with a worm gear hose clamp. The collection device was inserted into the most intense part of the dust plume possible near the grain stream, generally near the point where the moving grain was striking the grain pile in the receptacle. The samples from unloading combines were taken downwind as the combine was unloading into a wagon or truck during harvest. The samples from grain storage were collected at the intake or receiving hopper, where grain was being released from the augered and loaded into a truck. The total grain sampled was calculated by multiplying the maximum auger capacity (kg/min) as reported by manufacturers and sampling time (min). All samples were transported on the same day of collection and stored in an air-ventilated cold room at 2 °C, and 40 ± 5% relative humidity (RH) after thousand kernel weight and grain moisture contents were determined.

Individual grain samples were placed in an electric stainless steel rotary drum mixer. Twenty 100 g portions were removed for 5 min from the rotating mixer using a dip cup (200 mL) to form a 2 kg composite subsample. The entire subsample was then ground into three portions using a Romer subsampling mill (Fred Stein Lab, Inc., Atchinson, KS), a 10 g of the resulting finely ground subsample was transferred into a 50 mL polypropylene centrifuge tube for mycotoxin extraction. For dust samples, preweighed filter bags were used to calculate the total amount of dust collected. Individual filter bags were opened with clean surgical scissors, and the dust was then collected into a 200 mL snap cap polypropylene vial; a 1 g dust subsample was transferred into a 50 mL polypropylene centrifuge tube for mycotoxin extraction.

**Minibins Set Up.** Because OTA is difficult to find under natural conditions, we set up several small-scale storage bins with wheat grain, followed by seeding with OTA (22) contaminated grain in artificial hot spots. There were three-round galvanized steel minibins (2.4 m tall, 1.8 m in diameter, or 6700 kg capacity situated at 0.9 m from each other) set up. Each bin was filled with approximately 6100 kg feed grade winter wheat grain and left un aerated for at least eight months. These bins were run for two cycles. The first cycle was loaded and infested in fall 2015 and sampled in spring 2016. The second cycle was loaded in fall 2017 and then seeded with infected grain in spring 2018 and sampled in late spring 2018.

For the first cycle, the hot spot inoculum was prepared by mixing 1 g of ground grain clumps that had been naturally infected with 55 500 CFU/g of P. verrucosum or with a measured concentration of SS2.0 ng/g of OTA, per 0.5 kg of clean grain and tempering to an 18% moisture content. In 2015, infected (3 kg) grain was placed in a polypropylene mesh bag (31.8 × 45.7 cm²) in the center of the bin and buried under the top layer of grain, 2.5 cm from the surface. In addition, for the first cycle, we placed a cone of ice (7.2 cm radius × 25. 4 cm high or a volume of 737.4 cm³) over the infected grain bags at 8 am on February 12, 2016. The bag of inoculum was removed, leaving the infected grain in the bin on June 2, 2016. The minibins were emptied and sampled for grain and dust on June 22, 2016. For the second cycle, the bins were filled in the fall of 2017. Hot spots were created by incubating infected grain at room temperature and 18% moisture content for 12 weeks. Around 6 kg of infected grain was spread over the top of the grain pile in each bin before out-loading (April 26, 2018).

For out-loading after both cycles, a 25.4 × 25.4 cm² galvanized metal hopper, attached to a 15.2 cm diameter spiral auger powered by a 1 hp electric motor (Hutchinson Mfg. Inc., Clay Center, KS) was installed in the aerated floor of each minibin. Each minibin was sampled every 10 min for grain and grain dust following the protocol described above, collecting around 5 kg of grain. Grain and dust samples were handled, prepared, and stored as described above.

**Chemical Reagents and Equipment.** All reagents were LCMS grade or higher and, unless otherwise noted, obtained from Fisher Scientific (Oakville, ON, Canada). Methanol (MeOH) and acetonitrile (MeCN) were obtained from JT Baker (Phillipsburg, NJ). Water was obtained from OmniSolv (Billerica, MA). Glacial acetic acid (AcOH), ammonium acetate, formic acid, and ammonium formate were purchased from Fisher Scientific (Fairlawn, NJ). Deoxynivalenol, 1; 3-acetyl-deoxynivalenol, 2; 15-acetyl-deoxynivalenol, 3; deoxynivalenol 3-β-D-glucoside, 4; nivalenol, 5; fusarenon-X, 6; HT-2 toxin, 7; T-2 toxin, 8; diacetoxyscirpenol, 9; neosolaniol, 10; fumonisin B₁, 11; fumonisin B₂, 12; fumonisin B₃, 13; moniliformin, 14; zearalenone, 15; beauvericin, 16; sterigmatocystin, 21; ochratoxin A, 22; ochratoxin B, 23; citrinin, 24; roquefortine C, 26; alternariolmethylether, 27; and alternariol, 28 were obtained from Biopure standards (Romer Labs, Tulln, Austria). Enniatin A, 17, enniatin A₇, 18, enniatin B, 19, and enniatin B₂, 20 were obtained from Enzo Life Sciences (Farmingdale, NY). Penitrem A, 25 was obtained from Sigma-Aldrich (Milwaukee, WI).

High-performance liquid chromatography (HPLC) was performed using a Nexera XR ultra HPLC with a CTO-20AC column oven, two LC-20ADXR pumps, and a DGU-20A5 degasser attached to a SIL20ACXR autosampler (Shimadzu, Kyoto, Japan) equipped with a 100 μL injection syringe. Mass spectrometry (MS/MS) was performed using a QTRAP 6500+ hybrid triple quadrupole/linear ion trap system equipped with a TurboSoftSpray probe in the electrospray ionization (ESI) source (AB SCIEX, Concord, Canada). Pure nitrogen gas was used as the curtain, drying, and collision gases during MS/MS analysis and was generated using a NitroFlow.
Table 7. Optimized Multiple-Reaction Monitoring (MRM) Conditions Used for HPLC-ESI-MS/MS Analysis

| mycotoxin          | 'R' (min) | precursor ion (m/z) | DP (V) | product ion (m/z) | CE (V) | CXP (V) |
|--------------------|----------|---------------------|--------|-------------------|--------|--------|
| DON (1)            | 6.3      | 355.2 [M + Ac]^-    | −80    | 59/265.2          | −45/−22| −12    |
| 3-acetyl-DON (2)   | 7.7      | 337.1 [M + H]^+     | −40    | 307/265           | −16/−26| −12    |
| 15-acetyl-DON (3)  | 7.7      | 339.4 [M + H]^+     | 18     | 320.7/137.1       | 17/21  | 12     |
| DON 3-Glc (4)      | 6.1      | 517.3 [M + Ac]^-    | −60    | 427.1/246.9       | −28/−36| −12    |
| nivalenol (5)      | 5.5      | 371.1 [M + Ac]^-    | −90    | 311/281.1         | −15/−22| −12    |
| fusarenon-X (6)    | 6.8      | 413.2 [M + Ac]^-    | −90    | 59/353.1          | −52/−15| −12    |
| HT-2 (7)           | 10.7     | 442.1 [M + NH4]^+   | 50     | 263.2/215         | 16/17  | 20/15  |
| T-2 (8)            | 12.1     | 484.2 [M + NH4]^+   | 40     | 305.1/215.2       | 20/27  | 22/14  |
| diacetoxyscirpenol (9) | 9.0  | 384.2 [M + NH4]^+   | 35     | 307.3/199.1       | 15/28  | 18     |
| neosolaniol (10)   | 6.9      | 400.2 [M + NH4]^+   | 30     | 305/245           | 16/15  | 20     |
| fumonisin B1 (11)  | 9.6      | 722.5 [M + H]^+     | 95     | 352.2/334.4       | 50/54  | 23/27  |
| fumonisin B2 (12)  | 12.9     | 706.4 [M + H]^+     | 51     | 363.6/512.2       | 47/43  | 22/12  |
| fumonisin B3 (13)  | 12.9     | 706.3 [M + H]^+     | 51     | 364.6/354.3       | 48/44  | 18/21  |
| moniliformin (14)  | 4.9      | 97.0 [M + H]^+      | −50    | 41                | −20    | −18    |
| zearalenone (15)   | 14.0     | 317.1 [M + H]^+     | −160   | 175.1/131         | −33/−40| −12    |
| beauvericin (16)   | 16.9     | 801.3 [M + NH4]^+   | 73     | 244.1/134         | 41/105 | 20/18  |
| enniatins A (17)   | 17.1     | 699.3 [M + NH4]^+   | 40     | 210.2/228.2       | 40/44  | 15     |
| enniatins A (18)   | 17       | 685.4 [M + NH4]^+   | 49     | 668.5/228/210.2   | 25/39/38| 18/13/15|
| enniatins B (19)   | 16.7     | 657.5 [M + NH4]^+   | 75     | 196.2/213.9       | 41     | 16/25  |
| enniatins B (20)   | 16.8     | 671.4 [M + NH4]^+   | 75     | 210.2/654.6/196.1 | 50/25/45| 15     |
| sterigmatocystin (21) | 14.7  | 325.1 [M + H]^+     | 60     | 310.1/281         | 35/50  | 21/15  |
| ochratoxin A (22)  | 14.26    | 404.1 [M + H]^+     | 40     | 221/239.1         | 47/32  | 12     |
| ochratoxin B (23)  | 12.1     | 370.3 [M + H]^+     | 29/25  | 205/187           | 30/46  | 12/15  |
| cirinrin (24)      | 13.3     | 281.0 [M + H + OH]^-| −60    | 249/205/177       | −23/−30/−37| −15/−12/−12|
| penitrem A (25)    | 16.4     | 653.4 [M + H]^+     | 50     | 540.3/558.3       | 39/27  | 14     |
| roquefortine C (26)| 9.0      | 390.0 [M + H]^+     | 47     | 193/322           | 36/28  | 11/22  |
| alternariomethyl ether (27) | 15.4 | 271.0 [M + H]^+   | −130   | 181.8/155.1       | −70/−59| −12    |
| alternariol (28)   | 11.8     | 257.0 [M + H]^+     | −140   | 212.9/65         | −33/−75| −12    |

aRetention time ('R'). bDeclustering potential (DP). cQuantifier/qualifier ions. dCollision energy (CE). eCell exit potential (CXP).

TG2 gas generator (Parker-Balston, Haverhill, MA). Eluents A and B consisted of H2O/MeOH/AcOH (89:10:1, v/v/v) with 5 mM ammonium acetate buffer and MeOH/H2O/AcOH (97:2:1, v/v/v) with 5 mM ammonium acetate buffer, respectively.

**Sample Preparation, Extraction, and Mycotoxin Determination.** Grain (10 g) and dust (1 g) samples were shaken at 180 osc/min with 40 mL of MeCN/H2O/AcOH (79:20:1, v/v/v) for 90 min and then centrifuged for 2 min at 3000 rpm. The supernatant (200 μL) was transferred to a 400 μL glass vial inserted in a 2 mL amber glass vial, evaporated to dryness, and reconstituted in a 400 μL eluent A/B (50:50, v/v) solution. A 10 μL aliquot of the extract was then injected into the HPLC system, with an eluent flow rate of 0.6 mL/min. The column used was a 150 × 4.6 mm² i.d., 5-μm, Gemini C18, with a 4 × 3-mm² i.d. guard column of the same material (Phenomenex, Torrance, CA). The chromatographic separation was performed using a gradient program (19 min) with a binary mobile phase. The gradient began with 0.1% B and ramped linearly over 11.5 min to 99.9% B. These conditions were held for 2 min and finally for a 4 min equilibration, resetting to the initial conditions of 0.1% B.

The source gas temperature was set to 575 °C, nitrogen curtain gas 35 psi, gas source 1 was 60 psi, gas source 2 was 65 psi, collision gas at medium, and the ionization voltage was 5500 V for positive analytes and −4500 V for negative analytes. All parameters used in the detection and quantitation were obtained via direct infusion of individual analytes (10 ng/μL solutions in 1:1 eluent A/B) into the MS/MS at 10 μL/min using an integrated Harvard syringe (AB SCIEX, Concord, ON) fitted with a 1000 μL Gastight 1750 syringe (Hamilton, Reno, NV). Every sample was injected once and analyzed in positive and negative polarities, with the ESI source temperature kept constant at 550 °C. Nitrogen curtain gas was kept constant at 552 kPa, and nebulizer gas and collision gas were set to 8 arb unit and the column temperature was maintained at 30 °C. Instrument control, parameter optimization, data collection, peak integration, and quantitation were completed using PeakView software version 2.0 and MultiQuant software version 3.0 (AB Sciex, Concord, ON, Canada).

**Analytical Performance.** Specific parameters for each mycotoxin were obtained by direct infusion of the analytical standard (6 ng/μL in eluent A/B (50:50, v/v) solution) into the ESI-MS/MS system at 10 μL/min using a Fusion 100 infusion pump (Chemyx Inc, Stafford, TX) fitted with a 500 μL syringe (Gastight 1750, Hamilton, Reno, NV). Each compound was analyzed in positive or negative ion polarity mode using a multiple-reaction monitoring (MRM) procedure with one precursor ion (Q1) and two product ions (Q3). The most intense peak in Q3 was used for quantification (qualifier ion) and the second peak for confirmation (qualifier ion). MRM parameters were optimized by selecting collision energy and declustering and cell exit potentials, producing the most intense response of the precursor and product ions for each corresponding analyte. The system was operated in a polarity switching mode at 5 ms (±) with a target scan time of 1 s and an MRM detection window of 150 s. Optimized instrument parameters are summarized in Table 7.

Matrix effects are encountered when a component present in the matrix being analyzed coelutes with the analyte of interest.
in HPLC-ESI-MS/MS analysis. In this study, the percentage of grain and dust grain matrix effects were calculated as the peak area of matrix extract minus the peak area of solvent (eluent A/B) divided by the peak area of solvent ($n = 3$). The negative matrix effect or ion suppression was found in DON (1), 3-acetyl-DON (2), and fusaronen-X (6), being slightly larger in grain dust ($-0.14$) than grain ($-0.10$). Sanders et al. also found a minor ion suppression for DON (1) in grain dust compared with the grain matrix. In grain, ion suppression was also found in neosolaniol (10), fumonisin B$_1$ (11), and fumonisin B$_3$ (13) and in grain dust in OTA (22) and DON 3-Glc (4). On average, the grain dust matrix ($-0.22$) suppressed ions more intensively than grain ($-0.09$). Ion enhancement was found for all of the remaining mycotoxins being, on average, more intense in dust grain (2.74) than the grain matrix (0.51). Often, the lower matrix effect is referred to as an advantage for mycotoxin detection in grain dust over grain. However, at the same dilution factor, we found a higher matrix effect in grain dust with either ion suppression or enhancement, depending on the mycotoxins considered (Table 1).

Level of detection (LOD) and quantitation (LOQ) for grain and dust grain were determined using their respective matrix-matched calibration curve at 10 concentration levels from 0.5–256 ng/g (14–27) or 5–2560 ng/g (1–13 and 28) in addition to a double blank (matrix extract) sample, a no-matrix sample [eluent A/B (50:50, v/v)], and a quality control samples in every batch. Blank matrix extracts for grain and grain dust were obtained from two 2016 field samples that tested negative for all of the 28 mycotoxins included in this study, except for traces of enniatins found in the corresponding dust sample (<0.01 ng/g). Calibration curves showed good linearity for all mycotoxins ($R^2 > 0.99$). LOD and LOQ were calculated as the mean peak height that could be detected using the mean height of the noise signal plus 3 and 10 times the standard deviation, respectively, around the analyte retention time at ten concentration levels. LOD was lower than 0.90 ng/g, for both grain and grain dust, in 21 out of 28 mycotoxins (8–28), with an average LOD of 0.10 ng/g for both matrices. Trichothecenes B (1–6) and HT-2 (7) had a higher LOD with an average of 5.19 ng/g in grain and 2.68 ng/g in grain dust. LOD and LOQ of trichothecenes B (1–6) were lower in grain dust than in the grain matrix (Table 1).

Recovery tests were performed simultaneously and in triplicate by spiking a homogenized sample of 1 g of blank ground winter wheat and corresponding dust samples with the appropriate volume of analytical standards to yield 10 ng/g (14–27, LOD < 0.05 ng/g in grain) or 100 ng/g (1–13 and 28, LOD > 0.06 ng/g in grain). Spiked samples were allowed to equilibrate for 3 days at 40 °C in the dark, followed by extraction with a dilution factor of 8X and the standard analysis procedure. Good recoveries were obtained in grain ranging from 70% (24) to 118% (11) with an average of 89%. In grain dust, however, lower recoveries were obtained, ranging from 41% (16, 25) to 87% (15) with an average of 54% (Table 1). Recoveries in grain dust were, on average, 39% lower compared with the respective recovery in grain. All recoveries in grain were considered within the acceptable range. In grain dust, however, only three mycotoxins (HT-2, 7; fusaronen-X, 6; and 15-acetyl-DON, 3, with the recovery of 48, 54, and 52%, respectively) did not comply with the recovery range recommendation of 80–110% at LOD ≤ 10 ng/g according to the Procedural Manual of the Codex Alimentarius Commission. Lower recovery rates in grain dust can be attributed to the following. (1) Binding effect of fiber: grain dust particles consist of trichomes, spherical and thin fragments of the pericarp, and hunks fragments, which results in grain dust having 5–6 times more fiber content than wheat grain. Fiber has been reported to reduce the bioavailability of trace minerals and to adsorb mycotoxin in the gastrointestinal tract of animals through a series of ion exchanges with the dietary fiber carboxyl groups. (2) Particle size and surface area: grain samples were ground to a particle size <850 μm. In contrast, grain dust samples were <1650 μm. Smaller particles have a larger surface area in direct contact with extraction solvents, increasing the mass transfer surface. (3) Bulk density is the ratio of the sample mass and the volume occupied: bulk density is higher in ground whole wheat (0.619 g/cm³) than wheat dust bulk density (0.208 g/cm³). More ground whole wheat mass can be covered, given a similar extraction solvent volume, increasing extraction efficiency.

Statistical Analysis. Linear mix models for the quantity of dust collected, the ratio of the quantity of dust collected to grain sampled and mycotoxin content (ng/g) in dust and grain were obtained using a Gaussian distribution in the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute, Cary, NC). Sources of variation included year and farm location nested within years as random effects, sample types (harvest, storage, minibins), months in storage, sampling duration (min), a grain moisture content (%), and grain test weight (kg/hL) at sampling as fixed effects. Restricted maximum likelihood (REML) was used to estimate the variance components of fixed and random effects with the degrees of freedom of the fixed effects F-test adjusted for statistical dependence using the Kenward—Roger method with a dual quasi-Newton optimization technique. All fixed effect interactions that were not statistically significant were not included in the final model. A paired t-test was conducted, using the PROC TTEST procedure in SAS to compute the difference between paired mean mycotoxin content in grain vs dust. Differences between sample types (field, on-farm storage, and minibins) were determined using Tukey–Kramer tests ($\alpha = 0.05$) with PDFF in the LSMEANS option of the GLIMMIX procedure. Multiple correlations were performed using the PROC CORR procedure to calculate Pearson’s correlation coefficients ($R$) and their associated significance level ($p$). The PROC REG procedure was used to build a simplified two-variable regression model for the mycotoxin content in grain and grain dust. To understand the source of variability, a stepwise regression analysis was performed for each mycotoxin. Mycotoxin data, for both grain and grain dust, were analyzed using the PROC GLIMMIX procedure after applying log($X + 1$) transformation to achieve the assumption of normally distributed residuals. Restricted maximum likelihood procedure, following the random and fixed effects described above, including the mycotoxin content in the dust, was used for variance component estimation. Fixed effects that were significant, to wit: grain moisture at sampling, grain test weight, mycotoxin content in the dust, and their interactions were subjected to a multiple stepwise selection in the PROC REG procedure with $\alpha = 0.05$ for both entry and removal of variables. To simplify interpretation, all results, unless otherwise noted, are reported as arithmetic means ± standard deviation calculated from the untransformed data.
ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04256.

Distribution of the ratio between the mycotoxin content in grain and the mycotoxin content in paired grain dust samples (Table S1); correlation, two-variable regression, and multiple regression results for each mycotoxin (Figure S1); fit diagnostic measurements and results for nonlinear regression between the deoxynivalenol (DON) content in grain and grain dust (Figure S2) (PDF)

AUTHOR INFORMATION

Corresponding Author
Victor Limay-Rios — University of Guelph, Ridgetown, Ontario N0P 2C0, Canada; orcid.org/0000-0002-0866-3568; Phone: +1 519-674-1500x; Email: vlimaryri@uoguelph.ca; Fax: +1 519-674-1515

Author
Arthur W. Schaafsma — University of Guelph, Ridgetown, Ontario N0P 2C0, Canada

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acsomega.0c04256

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Field technical support by Todd Phibbs, Darrell Galbraith, Gurkanwal Arora, and Natun Dasgupta. Laboratory technical support by Bryn Tucker, Blake Morey, Stacie Dobson, Emma Langlois, Julia Lupton, and Hyerin Kim. Investment in this project was provided in part by the Agrinnovation Program of Agriculture and Agri-Food Canada. This research is part of project number AIP-P272 0639, cosponsored by Food and Consumer Products of Canada, Grain Farmers of Ontario, University of Guelph, Ridgetown Campus, PepsiCo Foods, Mondelēz, Kellogg, Prairie Oat Growers, Nestlé/Gerber, Canadian National Millers Association and Western Grains Research Foundation.

ABBREVIATIONS

DON: deoxynivalenol; DON 3-Glc: deoxynivalenol 3-β-D-glucoside; 15-acetyl-DON:15-acetyl-deoxynivalenol; 3-acetyl-DON:3-acetyl-deoxynivalenol; HT2: HT-2 toxin; T2-T: T2 toxin; Ochratoxin A: OTA; MLs: recommended maximum limits; RMSE: regression root-mean-square error; W: moisture content at sampling; TW: test weight; p.s.: dust particle size

REFERENCES

(1) Whittaker, T. B. Sampling Foods for Mycotoxins. Food Addit. Contam. 2006, 23, 50–61.
(2) Sanders, M.; De Boevre, M.; Dumoulin, F.; Detavernier, Cl.; Martens, F.; Van Poucke, C.; Eeckhout, M.; De Saeger, S. Sampling of Wheat Dust and Subsequent Analysis of Deoxynivalenol by LC-MS/MS. J. Agric. Food Chem. 2013, 61, 6259–6264.
(3) Schaafsma, A. W. Economic Changes Imposed by Mycotoxins in Food Grains: Case Study of Deoxynivalenol in Winter Wheat. In Mycotoxins and Food Safety; DeVries, J. W.; Truckssess, M. W.; Jackson, L. S., Eds.; Springer, 2002; Vol. 504, pp 271–276.
(4) Limay-Rios, V.; Miller, J. D.; Schaafsma, A. W. Occurrence of Penicillium verrucosum, ochratoxin A, ochratoxin B and citrinin in on-farm stored winter wheat from the Canadian Great Lakes Region. PLoS One 2017, 12, No. e0181239.
(5) Whittaker, T. B.; Hagler, W. M.; Giesbrecht, F. G.; Johansson, A. S. Sampling, Sample Preparation, and Analytical Variability Associated with Testing Wheat for Deoxynivalenol. J. AOAC Int. 2000, 83, 1285–1292.
(6) Schaafsma, A. W.; Savard, M. E.; Clear, R.; Dexter, J. Methods and issues regarding detection of deoxynivalenol, Fusarium-damaged kernels, and Fusarium spp. in commercial grain in Canada. Can. J. Plant Pathol. 2004, 26, 443–452.
(7) Oerke, E. C.; Meier, A.; Dehne, H. W.; Sulyok, M.; Kriska, R.; Steiner, U. Spatial variability of fusicladium head blight pathogens and associated mycotoxins in wheat crops. Plant Pathol. 2010, 59, 671–682.
(8) Stroka, J.; Spanjer, M.; Buechler, S.; Barel, S.; Kos, G.; Ankalam, E. Novel sampling methods for the analysis of mycotoxins and the combination with spectroscopic methods for the rapid evaluation of deoxynivalenol contamination. Toxicol. Lett. 2004, 153, 99–107.
(9) Sanders, M.; Landschoot, S.; Audenaert, K.; Haesaert, G.; Eeckhout, M.; De Saeger, S. Deoxynivalenol content in wheat dust versus wheat grain: A comparative study. World Mycotox J. 2014, 7, 285–290.
(10) Reichel, M.; Staiser, S.; Biselli, S. Analysis of Fusarium toxins in grain via dust: A promising field of application for rapid test systems. World Mycotox J. 2014, 7, 465–477.
(11) Whittaker, T. B.; Slate, A. B.; Nowicki, T. W.; Giesbrecht, F. G. Variability and distribution among sample test results when sampling unprocessed wheat lots for ochratoxin A. World Mycotox J. 2016, 163.
(12) Tangni, E. K.; Pussemier, L. Ochratoxin A and citrinin loads in stored wheat grains: Impact of grain dust and possible prediction using ergostrol measurement. Food Addit. Contam. 2006, 23, 181–189.
(13) Laca, A.; Mousia, Z.; Diaz, M.; Webb, C.; Pandiella, S. S. Distribution of microbial contamination within cereal grains. J. Food Eng. 2006, 72, 332–338.
(14) Kushiro, M. Effects of Milling and Cooking Processes on the Deoxynivalenol Content in Wheat. Int. J. Mol. Sci. 2008, 9, 2127–2145.
(15) Scudamore, K. A.; Banks, J.; MacDonald, S. J. Fate of ochratoxin A in the processing of whole wheat grains during milling and bread production. Food Addit. Contam. 2003, 20, 1153–1163.
(16) Voigt, C. A.; Schäfer, W.; Salomon, S. A. Secreted lipase of Fusarium graminearum is a virulence factor required for infection of cereals. Plant J. 2005, 42, 364–375.
(17) Palmgren, M. S.; Lee, L. S. Separation of mycotoxin-containing sources in grain dust and determination of their mycotoxin potential. Environ. Health Perspect. 1986, 66, 105.
(18) Krysinska-Traczyn, E.; Kiecana, I.; Perkowski, J.; Dutkiewicz, J. Levels of fungi and mycotoxins in samples of grain and grain dust collected on farms in Eastern Poland. Ann. Agric. Environ. Med. 2001, 8, 269–274.
(19) Jargot, D.; Melin, S. Characterization and validation of sampling and analytical methods for mycotoxins in workplace air. Environ. Sci.: Processes Impacts 2013, 15, 633–644.
(20) Schnake, L. Grain Dust: Problems and Utilization, ESS-6; United States Department of Agriculture, Economic Research Service: Washington, DC, 1981.
(21) Spankie, S.; Cherrie, J. W. Exposure to Grain Dust in Great Britain. Ann. Occup. Hyg. 2011, 56, 25–36.
(22) Martin, C. R.; Stephens, L. E. Broken Corn and Dust Generated During Repeated Handling. Trans. ASAE 1977, 20, 0168–0171.
(23) Feltrin, A. C. P.; Sibaja, K. V.; Tuksi, C.; Caldas, S. S.; Primel, E. G.; Garda-Buffon, J. Evaluation of the Suitability of Analytical Methods in Trichothecene A and B Degradation. J. Braz. Chem. Soc. 2018, 29, 2117–2126.
(24) Lemke, S. L.; Grant, P. G.; Phillips, T. D. Adsorption of zearalenone by organophilic montmorillonite clay. *J. Agric. Food Chem.* 1998, 46, 3789–3796.

(25) Edwards, S. G.; Dickin, E. T.; MacDonald, S.; Buttrler, D.; Hazel, C. M.; Patel, S.; Scudamore, K. A. Distribution of Fusarium mycotoxins in UK wheat mill fractions. *Food Addit. Contam., Part A* 2011, 28, 1694–1704.

(26) Kwon, S.-I.; von Dahlen, C. D.; Anderson, A. J. Gene sequence analysis of an opportunistic wheat pathogen, an isolate of *Fusarium proliferatum*. *Can. J. Bot.* 2001, 79, 1115–1121.

(27) Cendoya, E.; Chiotta, M. L.; Zachetti, V.; Chulze, S. N.; Ramirez, M. L. Fumonisins and fumonisin-producing Fusarium occurrence in wheat and wheat by products: A review. *J. Cereal Sci.* 2018, 80, 158–166.

(28) Shephard, G. S.; van der Westhuizen, L.; Gatyni, P. M.; Katere, D. R.; Marasas, W. F. O. Do Fumonisin Mycotoxins Occur in Wheat? *J. Agric. Food Chem.* 2005, 53, 9293–9296.

(29) Zhiqing, G.; Katharina, P.; Petri, K.; Heinz-Wilhelm, D.; Boran, A. Fumonisin B1 and beauvericin accumulation in wheat kernels after seed-borne infection with *Fusarium proliferatum*. *Agric. Food Sci.* 2016, 25, 138.

(30) Katta, S. K.; Cagampang, A. E.; Jackson, L. S.; Bullerman, L. B. Distribution of Fusarium Molds and Fumonisins in Dry-Milled Corn Fractions. *Cereal Chem. J.* 1997, 74, 858–863.

(31) Pietri, A.; Zanetti, M.; Bertuzzi, T. Distribution of aflatoxins and fumonisins in dry-milled maize fractions. *Food Addit. Contam., Part A* 2009, 26, 372–380.

(32) Lemmens, M.; Scholz, U.; Berthiller, F.; Dall’Asta, C.; Koutnik, A.; Schuhmacher, R.; Adam, G.; Buerstmayr, H.; Mesterházy, A.; Kraka, R.; Ruckenbauer, P. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. *Mol. Plant–Microbe Interact.* 2005, 18, 1318–1324.

(33) Martin, C. R. Characterization of Grain Dust Properties. *Trans. ASAE* 1981, 24, 0738–0742.

(34) Tittlemier, S. A.; Roscoe, M.; Trelka, R.; Gaba, D.; Chan, J. M.; Patrick, S. K.; Sulyok, M.; Krska, R.; McKendry, T.; Grafenhan, T. Occurrence of *Fusarium* toxins and their source organisms in durum wheat harvested in 2010. *J. Agric. Food Chem.* 2013, 61, 5438–5448.

(35) Health Canada. *Health Canada’s Maximum Levels for Chemical Contaminants in Foods; Bureau of Chemical Safety Food Directorate Health Products and Food Branch*, 2018.

(36) Ngo, T. H. D.; La Puente, C. In *The Steps to Follow in a Multiple Regression Analysis*, Proceedings of the SAS Global Forum, Citeseer, 2012; pp 22–25.

(37) Health Canada. *Information Document on Health Canada’s Proposed Maximum Limits (Standards) for the Presence of the Mycotoxin Ochratoxin A in Foods; Bureau of Chemical Safety Food Directorate Health Products and Food Branch*, 2009.

(38) Gareis, M.; Meussdoerffer, F. Dust of Grains and Malts as a Source of Ochratoxin A Exposure. *Mycotoxin Res.* 2000, 16, 127–130.

(39) Mayer, S.; Curtui, V.; Usleber, E.; Gareis, M. Airborne mycotoxins in dust from grain elevators. *Mycotoxin Res.* 2007, 23, 94–100.

(40) Zhang, Z.; Nie, D.; Fan, K.; Yang, J.; Guo, W.; Meng, J.; Zhao, Z.; Han, Z. A systematic review of plant-conjugated masked mycotoxins: Occurrence, toxicology, and metabolism. *Crit. Rev. Food Sci. Nutr.* 2020, 60, 1523–1537.

(41) Kanrar, B.; Mandal, S.; Bhattacharyya, A. Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 2010, 1217, 1926–1933.

(42) Limay-Rios, V.; Schaafisma, A. W. Effect of Prothioconazole Application Timing on Fusarium Mycotoxin Content in Maize Grain. *J. Agric. Food Chem.* 2018, 66, 4809–4819.

(43) World Health Organization. *Codex Alimentarius Commission: Procedural Manual*, WHO, 2013; p 21.