

Supplementary materials: Impact of Environmental Conditions on the Concentrations of Trichothecenes, Their Glucosides, and Emerging Fusarium Toxins in Naturally Contaminated, Irradiated, and Fusarium langsethiae Inoculated Oats

Abimbola Oluwakayode, Brett Greer, Julie Meneely, Franz Berthiller, Rudolf Krska, and Angel Medina

Table S1: The measurement uncertainty for each analyte

| Analytes | 2SD | 3SD | Mean Conc. ($\mu\text{g/kg}$) | U_r (%) | U (%) |
|-------------------------|------|------|------------------------------------|-----------|-------|
| Moniliformin | 22.2 | 33.3 | 174 | 13 | 19 |
| 15- | 30.2 | 45.2 | 114 | 26 | 40 |
| Acetyldeoxynivalenol | | | | | |
| 3-Acetyldeoxynivalenol | 10.1 | 15.1 | 190 | 5 | 8 |
| Beauvericin | 10.2 | 15.3 | 93.8 | 11 | 16 |
| Diacetoxyscirpenol | 20.0 | 30.0 | 187 | 11 | 16 |
| Deoxynivalenol | 12.3 | 18.5 | 191 | 6 | 10 |
| DON-3-G | 6.5 | 9.7 | 69.0 | 9 | 14 |
| Enniatin A | 11.5 | 17.3 | 85.9 | 13 | 20 |
| Enniatin A ₁ | 9.5 | 14.3 | 88.8 | 11 | 16 |
| Enniatin B | 16.8 | 25.2 | 97.5 | 17 | 26 |
| Enniatin B ₁ | 13.4 | 20.1 | 93.8 | 14 | 21 |
| HT-2 toxin | 9.6 | 14.5 | 188 | 5 | 8 |
| HT-2-Glc | 9.9 | 14.9 | 192 | 5 | 8 |
| Nivalenol | 71.7 | 108 | 160 | 45 | 67 |
| T-2 toxin | 9.0 | 13.4 | 187 | 5 | 7 |

SD- Standard Deviation. Conc.- Concentration.

Ur: Relative expanded measurement uncertainty estimated from intra-laboratory validation (intermediate precision) data based on five lots of oats, close to a 95% confidence interval.

U: Relative expanded measurement uncertainty estimated from intra-laboratory validation (intermediate precision) data based on five lots of oats, close to a 99% confidence interval.

Table S2: Statistical differences in the concentrations of each trichothecene at all storage conditions using one-way ANOVA and nonparametric comparisons for each pair using the Wilcoxon method.

| Treatments | | Levels | T-2 toxin | HT-2 toxin | HT-2-Glc | DON | DON-3-G | DAS |
|--|--------|---------------|-----------|------------|----------|---------|---------|--------|
| Naturally contaminated oat control | a_w | One-way ANOVA | 0.3660 | 0.2225 | 0.6449 | 0.0009 | 0.0015 | 0.0198 |
| | | 0.98- 0.95 | 0.6721 | 0.3184 | 0.9575 | 0.0009 | 0.0009 | 0.0135 |
| | T [°C] | One-way ANOVA | 0.4929 | 0.2784 | 0.3600 | 0.2483 | 0.2758 | 0.2031 |
| | | 25- 20 | 0.2237 | 1.0000 | 0.7899 | 0.3717 | 0.3184 | 0.4005 |
| Naturally contaminated oat + <i>F. langsethiae</i> | a_w | One-way ANOVA | 0.2930 | 0.3516 | 0.3834 | <0.0001 | 0.0001 | 0.5608 |
| | | 0.98- 0.95 | 0.1036 | 0.5635 | 0.3706 | 0.0009 | 0.0009 | 0.3184 |
| | T [°C] | One-way ANOVA | 0.547 | 0.2673 | 0.2120 | 0.4350 | 0.2386 | 0.4931 |
| | | 25- 20 | 0.0661 | 0.7132 | 0.6355 | 0.4948 | 0.4309 | 0.6365 |
| Irradiated oat control | a_w | One-way ANOVA | 0.0015 | <.0001 | 0.0072 | 0.0003 | <.0001 | 0.1489 |
| | | 0.98- 0.95 | 0.0122 | 0.0008 | 0.0116 | 0.0004 | 0.0004 | 0.1709 |
| | T [°C] | One-way ANOVA | 0.1362 | 0.5863 | 0.0383 | 0.5082 | 0.9093 | 0.9914 |
| | | 25- 20 | 0.2202 | 0.7091 | 0.1071 | 0.7791 | 0.9553 | 1.0000 |
| Irradiated oat + <i>F. langsethiae</i> | a_w | One-way ANOVA | 0.0662 | <.0001 | <.0001 | 0.2383 | 0.0422 | 0.9257 |
| | | 0.98- 0.95 | 0.0831 | 0.0009 | 0.0009 | 0.2271 | 0.0831 | 0.4309 |
| | T [°C] | One-way ANOVA | 0.0307 | 0.9702 | 0.3185 | 0.1389 | 0.6538 | 0.0337 |
| | | 25- 20 | 0.0313 | 0.6365 | 0.2271 | 0.0074 | 0.9581 | 0.1036 |

DON: Deoxynivalenol. DON-3-G: Deoxynivalenol-3-Glucoside. DAS: Diacetoxyscirpenol. HT-2-Glc: HT-2-glucoside. a_w : water activity. T: Temperature.

Table S3: Statistical differences in the concentrations of trichothecenes at each water activity and temperature for all treatments using one-way ANOVA and nonparametric comparisons for each pair using the Wilcoxon method.

| | | 20 °C | | | | 25°C | | | |
|--|-----------------------|---------------|--------------|---------------|-----------|---------------|--------------|---------------|-----------|
| Treatments | a _w Levels | One-way ANOVA | T-2: HT2-Glc | HT-2: HT2-Glc | T-2: HT-2 | One-way ANOVA | T-2: HT2-Glc | HT-2: HT2-Glc | T-2: HT-2 |
| Naturally contaminated oat control | 0.95 | 0.5718 | 1.000 | 0.4678 | 0.3123 | 0.2139 | 0.3123 | 0.8852 | 0.0304 |
| | 0.98 | 0.1418 | 0.1241 | 0.3836 | 0.1241 | 0.4106 | 0.4705 | 0.6650 | 0.3123 |
| Naturally contaminated oat + <i>F. langsethiae</i> | 0.95 | 0.4139 | 1.0000 | 0.4705 | 0.4705 | 0.1538 | 0.0304 | 0.6650 | 0.0304 |
| | 0.98 | 0.6135 | 1.0000 | 1.0000 | 0.6650 | 0.1682 | 0.0304 | 0.3123 | 0.1939 |
| Irradiated oat control | 0.95 | 0.0131 | 0.0131 | 0.0131 | 0.0131 | 0.0131 | 0.0131 | 0.0131 | 0.0131 |
| | 0.98 | 0.5275 | 0.1832 | 0.1832 | 1.0000 | 0.4426 | 0.8852 | 0.3123 | 0.4705 |
| Irradiated oat + <i>F. langsethiae</i> | 0.95 | 0.0009 | 0.0304 | 0.0304 | 0.0304 | <.0001 | 0.0304 | 0.0304 | 0.0304 |
| | 0.98 | 0.7799 | 0.4705 | 1.0000 | 0.4705 | 0.0071 | 0.6650 | 0.0304 | 0.0304 |

Table S4. Influence of a_w x T on the concentrations of Diacetoxyscirpenol in oats.

| Mycotoxins (ng/g of grains) ^a | | | |
|--|-------|-------|-------|
| Treatments | a_w | 20 °C | 25 °C |
| Naturally contaminated oat control | 0.95 | 12 | 8 |
| | 0.98 | 7 | < LOD |
| Naturally contaminated oat + <i>F. langsethiae</i> | 0.95 | 7 | < LOQ |
| | 0.98 | < LOQ | < LOQ |
| Irradiated oat control | 0.95 | < LOQ | < LOQ |
| | 0.98 | < LOD | < LOD |
| Irradiated oat + <i>F. langsethiae</i> | 0.95 | 19* | 9* |
| | 0.98 | 19* | 9* |

^a Results are a mean of 4 replicates at different storage conditions. a_w -water activity. T- temperature.

*= significant differences (using One-way ANOVA) in the concentrations of DAS for both temperatures in each row. < LOD- below limit of detection. < LOQ- below limit of quantitation.

Table S5: Statistical differences in the concentrations of DON-3-G to DON at each water activity and temperature for all treatments using one-way ANOVA and nonparametric comparisons for each pair using the Wilcoxon method.

| Mycotoxins | NOC | | | NOFL | | IOC | | IOFL | |
|--------------|----------------------|--------|----------|--------|----------|--------|----------|--------|----------|
| | $a_w, T [^{\circ}C]$ | ANOVA | Wilcoxon | ANOVA | Wilcoxon | ANOVA | Wilcoxon | ANOVA | Wilcoxon |
| DON-3-G: DON | 0.95, 20 | 0.3049 | 0.1939 | 0.0013 | 0.0304 | 1.0000 | 1.0000 | 0.0008 | 0.0304 |
| DON-3-G: DON | 0.95, 25 | 0.3709 | 1.0000 | 0.0034 | 0.0304 | 1.0000 | 1.0000 | 0.0024 | 0.0304 |
| DON-3-G: DON | 0.98, 20 | 0.0760 | 0.1939 | 0.0054 | 0.0304 | 0.0079 | 0.0304 | 0.1241 | 0.0304 |
| DON-3-G: DON | 0.98, 25 | 0.0031 | 0.0304 | 0.0124 | 0.0304 | 0.0536 | 0.0304 | 0.0080 | 0.0304 |

NOC- Naturally contaminated oat control. NOFL: Naturally contaminated oat + *F. langsethiae*. IOC: Irradiated oat control. IOFL: Irradiated oat + *F. langsethiae*. a_w : water activity.

T- Temperature. DON: Deoxynivalenol. DON-3-G: Deoxynivalenol-3-glucoside.