

Natural Occurrence of Mycotoxin-Producing *Fusaria* in Market-Bought Peruvian Crops: a Food Safety Threat for Andean Populations

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1. Supplementary Methods: Tri5-TCTA and Tri5-TCTB primer design

Multiple sequence alignment was performed using nucleotide sequences of *Tri5* gene extracted from *Fusarium* species that can produce either type A trichothecens (TCTA) or type B trichothecens (TCTB). Species and accession numbers are provided in Supplementary Table S1. Primers for Sybr Green-based qPCR assay were subsequently designed in discerning regions using Primer3 [1,2].

Table S1. *Fusarium* species and NCBI sequence accession numbers used to design TCTA and TCTB-discerning primers.

Toxins Produced	Species and Strain	NCBI Accession Number of <i>Tri5</i> Sequence
TCTA	<i>F. camptoceras</i> NRRL3381	GQ915545.1
	<i>F. sporotrichioides</i>	AY130293.1
	<i>F. langsethiae</i> IBT 9956	AF449792.1
	<i>F. kyushuense</i> NRRL 25349	GQ915548.1
	<i>F. incarnatum</i> NRRL 31160	GQ915550.1
	<i>F. armeniacum</i> FRC R-0933	GQ915543.1
	<i>G. publicaris</i> FRC_R 07843	GQ915552.1
TCTB	<i>F. graminearum</i> NRRL9394	AY102605.1
	<i>F. austroamericanum</i> NRRL 2903	AY102596.1
	<i>F. asiaticum</i> NRRL 28720	AY102590.1
	<i>F. graminearum</i> GZ3639	AF359361.3
	<i>F. graminearum</i> PH-1	supercont3.2 -3376420-3377787 ¹
	<i>F. cortaderiae</i> NRRL 29306	AY102601.1
	<i>F. culmorum</i> NRRL 25475	AY102571.1
	<i>F. acaciae-mearnsii</i> NRRL 26754	AY102577.1
<i>F. cerealis</i> NRRL 25491	AY102572.1	

¹ Sequence source from [3].

2. Supplementary Figures

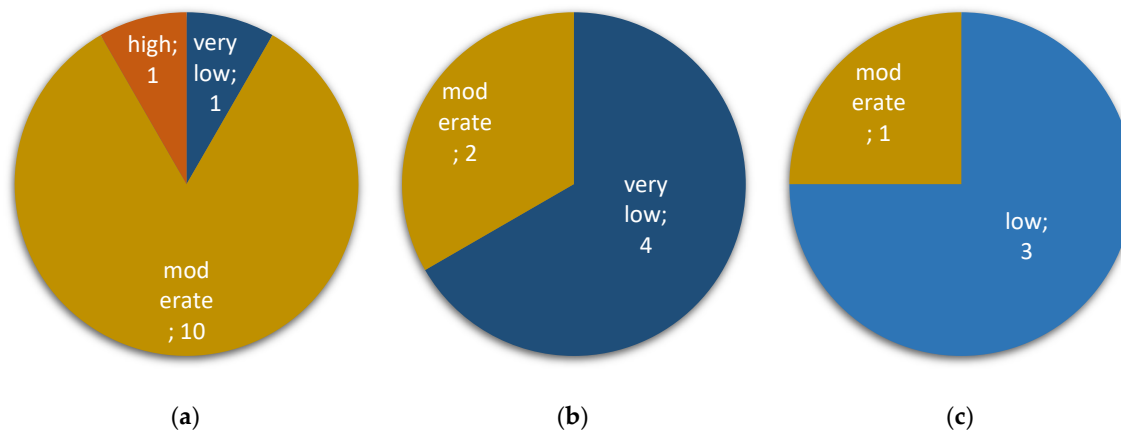


Figure S1. *Fusarium* risk assessment in collected amaranth, barley and oat grain samples. Risk is ranked as *very low*, *low*, *moderate*, *high*, or *very high*. Number after semicolon indicates corresponding sample size. (a) amaranth seeds; (b) barley; (c) oat.

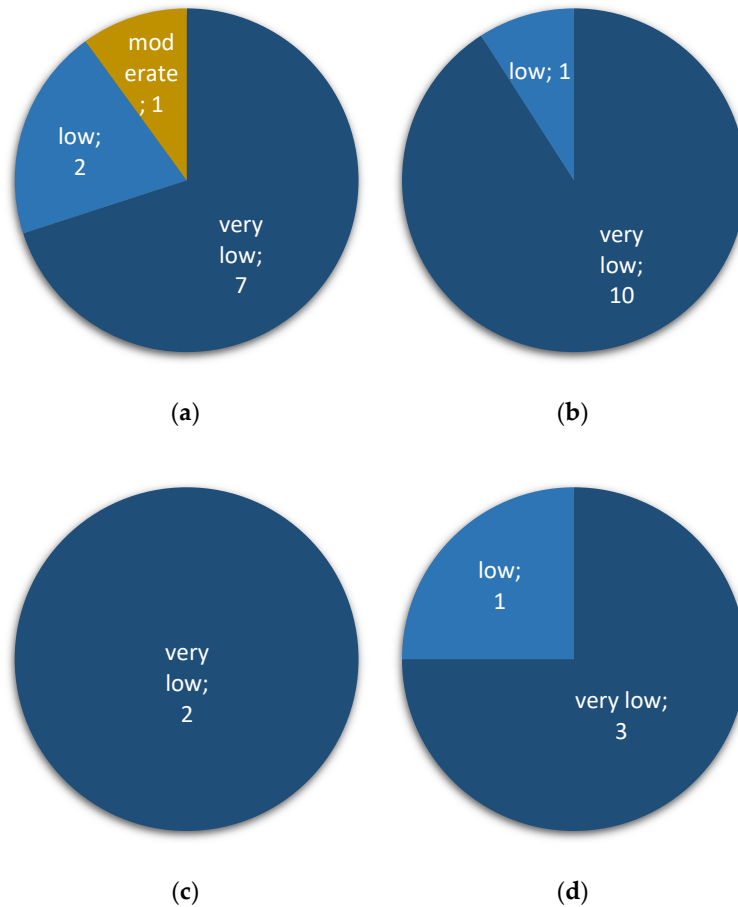


Figure S2. Assessment of the risk associated with the presence of fumonisin producers in cereal and pseudo-cereal grains contaminated with *Fusarium* species. Risk is ranked as *very low*, *low*, *moderate*, *high*, or *very high*. Number after semicolon indicates corresponding sample size. (a) rice; (b) amaranth seeds; (c) barley; (d) oat.

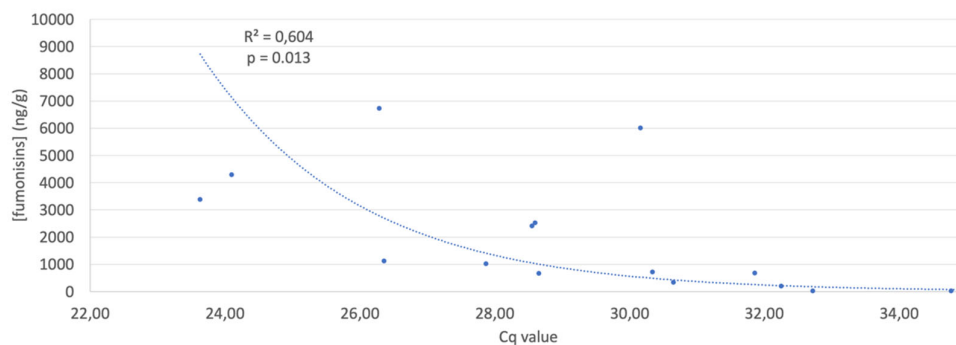
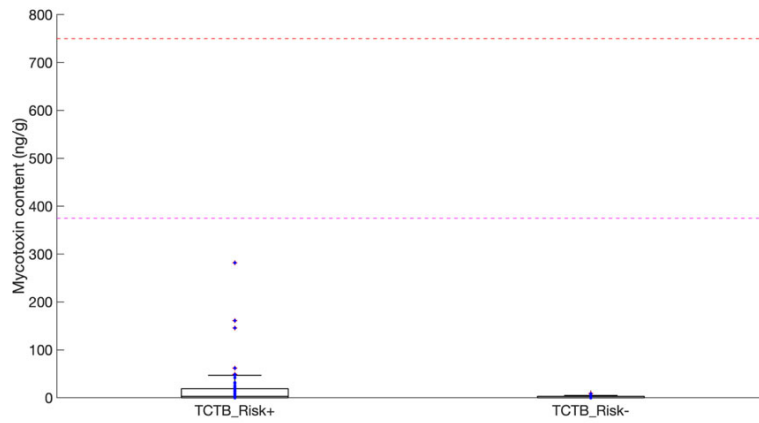
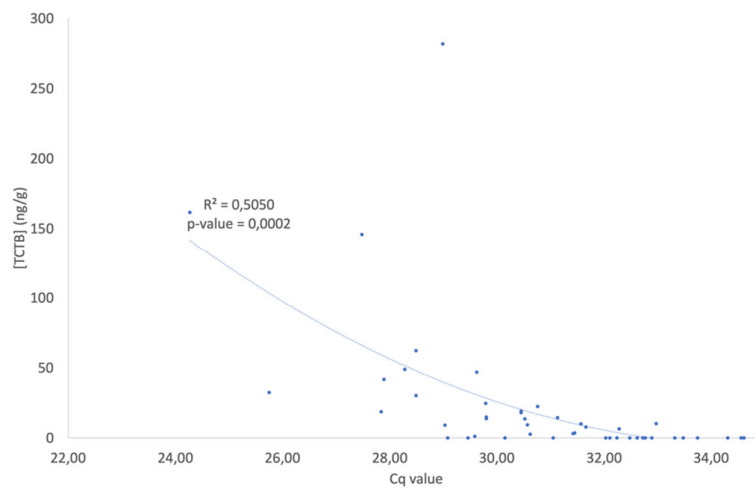


Figure S3. Fumonisin content as a function of the relative abundance of *Fum1*-containing DNA measured by qPCR in cereal samples. Pearson product-moment correlation coefficient (R^2) and associated p -value were computed with Matlab 2020b (MathWorks) and used to estimate the goodness of fit of the monotonic non-linear relationship.



(a)



(b)



(c)

Figure S4. Toxin contamination profiles in cereal and pseudo-cereal grains contaminated with *Fusarium* species that can produce TCTB and zearalenone. (a) Boxplot representation of TCTB contents. “TCTB-Risk+” refers to samples with moderate to very high TCTB-associated risk, and “TCTB-Risk-” refers to 10 randomly selected samples with risk ranked as very low to low. The pink and red dashed lines indicates the 375 and 750 ng.g⁻¹ thresholds, respectively; (b) TCTB content as a function of the relative abundance of *Tri5*-containing DNA measured by qPCR in cereal samples. Pearson product-moment correlation coefficient (R^2) and associated p -value were computed with Matlab 2020b (MathWorks) and used to estimate the goodness of fit of the monotonic non-linear relationship; (c) Boxplot representation of zearalenone contents. “ZEA-Risk+” refers to samples with moderate to very high ZEA-associated risk, and “ZEA-Risk-” refers to samples with risk ranked as very low to low. The pink and red dashed lines indicates the 50 and 100 ng.g⁻¹ thresholds, respectively.

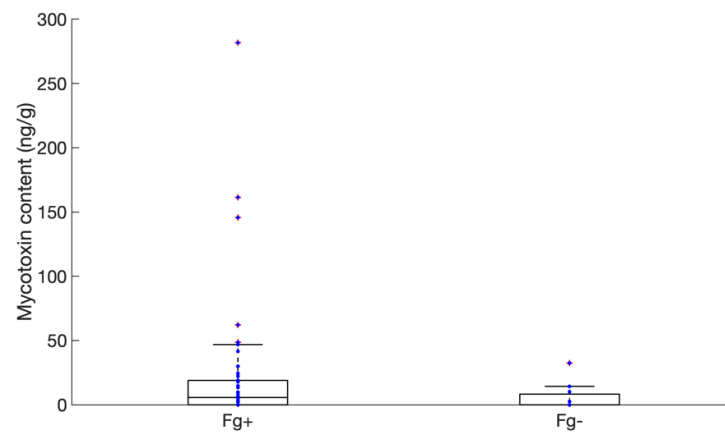
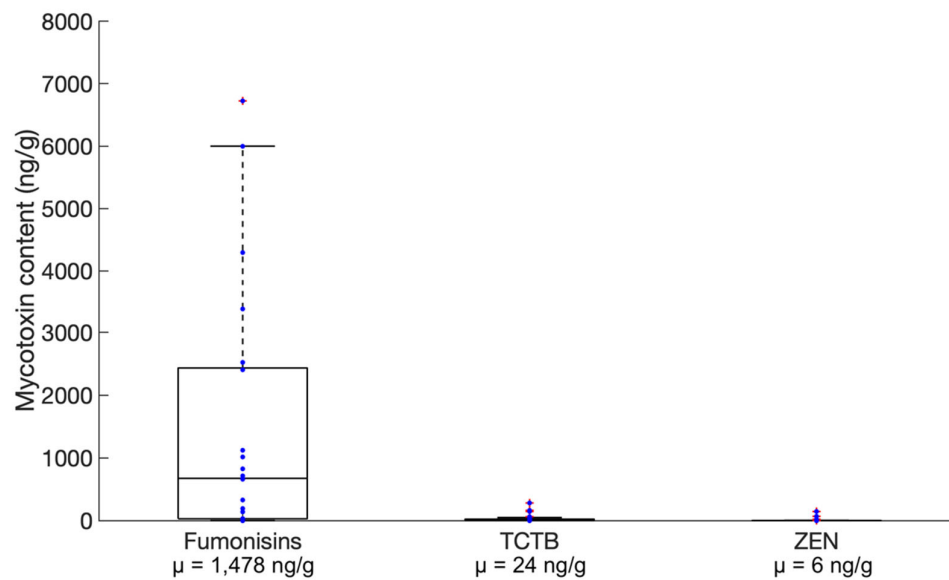


Figure S5. Boxplot representation of TCTB contents of *Tri5*-positive samples that tested positive for *F. graminearum* (“Fg+”) or negative (“Fg-”).



(a)

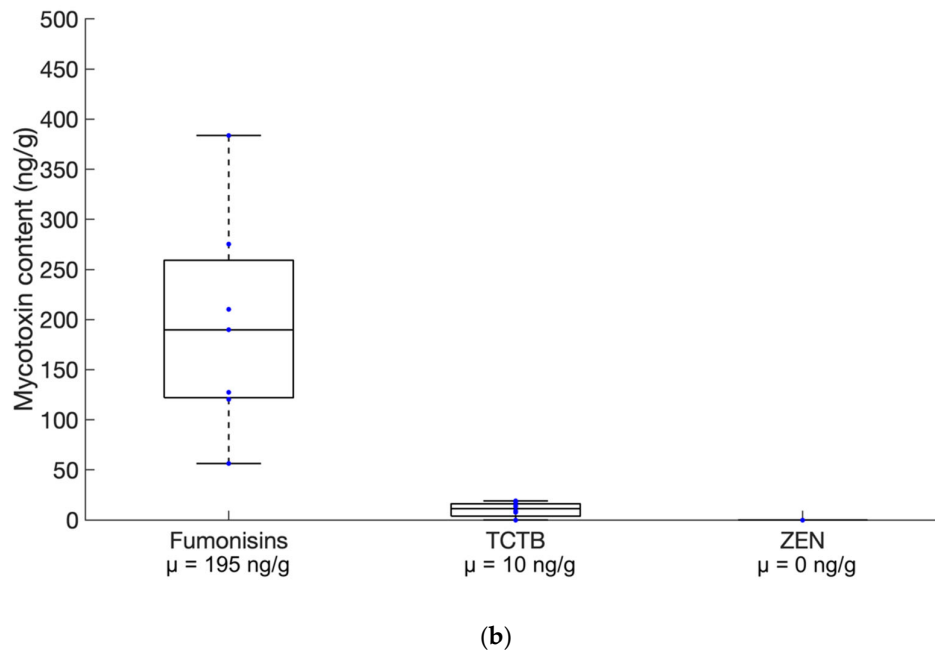


Figure S6. Boxplot representation of mycotoxin content in corn and wheat measured in samples with *moderate to very high* mycotoxin-associated risk. The values μ indicate mean values for the corresponding toxins. $\mu = 0$ means no mycotoxin could be detected. (a) Corn. (b) Wheat. Boxplots and means were not computed for other cereals for which only three samples or less were at *moderate to very high* mycotoxin-associated risk and showing very low or no detectable levels of mycotoxins.

3. Additional references

1. Untergasser, A.; Cutcutache, I.; Koressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. Primer3—New Capabilities and Interfaces. *Nucleic Acids Res* **2012**, *40*, e115, doi:10.1093/nar/gks596.
2. Koressaar, T.; Remm, M. Enhancements and Modifications of Primer Design Program Primer3. *Bioinformatics* **2007**, *23*, 1289–1291, doi:10.1093/bioinformatics/btm091.
3. Wong, P.; Walter, M.; Lee, W.; Mannhaupt, G.; Münsterkötter, M.; Mewes, H.-W.; Adam, G.; Güldener, U. FGDB: Revisiting the Genome Annotation of the Plant Pathogen *Fusarium Graminearum*. *Nucleic Acids Res* **2011**, *39*, D637–639, doi:10.1093/nar/gkq1016.