


**COMPREHENSIVE REVIEW**

# Mycotoxin contamination in organic and conventional cereal grain and products: A systematic literature review and meta-analysis

Juan Wang<sup>1,2,3</sup> | Enas Khalid Sufar<sup>1</sup> | Aksel Bernhoft<sup>4</sup> | Chris Seal<sup>2</sup> |  
 Leonidas Rempelos<sup>3,5</sup> | Gultekin Hasanaliyeva<sup>6</sup> | Bingqiang Zhao<sup>7</sup> |  
 Per Ole Iversen<sup>8,9</sup> | Marcin Baranski<sup>10</sup> | Nikolaos Volakakis<sup>3</sup> | Carlo Leifert<sup>8,11</sup> 

<sup>1</sup>Department of Clinical Nutrition, College of Health Science and Technology, Shanghai Jiao Tong University School of Medicine, Shanghai, China

<sup>2</sup>Human Nutrition and Exercise Research Centre, Population Health Sciences Institute, Newcastle upon Tyne, UK

<sup>3</sup>Nafferton Ecological Farming Group, Newcastle University, Stocksfield, UK

<sup>4</sup>Section for Chemistry and Toxinology, Norwegian Veterinary Institute, Aas, Norway

<sup>5</sup>Lincoln Institute for Agri-Food Technology, University of Lincoln, Lincoln, Lincolnshire, UK

<sup>6</sup>School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Nottingham, UK

<sup>7</sup>Institute of Agricultural Resources and Regional Planning (IARRP), Chinese Academy of Agricultural Science (CAAS), Beijing, China

<sup>8</sup>Department of Nutrition, IMB, University of Oslo, Oslo, Norway

<sup>9</sup>Department of Haematology, Oslo University Hospital, Oslo, Norway

<sup>10</sup>Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

<sup>11</sup>SCU Plant Science, Southern Cross University, Lismore, NSW, Australia

**Correspondence**

Carlo Leifert, SCU Plant Science,  
 Southern Cross University, Military Rd.,  
 Lismore, NSW 2480, Australia.  
 Email: [carlo.leifert@gmail.com](mailto:carlo.leifert@gmail.com)

Juan Wang, Department of Clinical  
 Nutrition, College of Health Science and  
 Technology, Shanghai Jiao Tong  
 University School of Medicine, Shanghai  
 200025, China.  
 Email: [j.wang28@outlook.com](mailto:j.wang28@outlook.com)

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**Abstract**

There is still considerable controversy about the relative risk of mycotoxin exposure associated with the consumption of organic and conventional cereals. Using validated protocols, we carried out a systematic literature review and meta-analyses of data on the incidence and concentrations of mycotoxins produced by *Fusarium*, *Claviceps*, *Penicillium*, and *Aspergillus* species in organic and conventional cereal grains/products. The standard weighted meta-analysis of concentration data detected a significant effect of production system (organic vs. conventional) only for the *Fusarium* mycotoxins deoxynivalenol, with concentrations ~50% higher in conventional than organic cereal grains/products ( $p < 0.0001$ ). Weighted meta-analyses of incidence data and unweighted meta-analyses of concentration data also detected small, but significant effects of production system on the incidence and/or concentrations of T-2/HT-2 toxins, zearalenone, enniatin, beauvericin, ochratoxin A (OTA), and aflatoxins. Multilevel meta-analyses identified climatic conditions, cereal species, study type,

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and analytical methods used as important confounding factors for the effects of production system. Overall, results from this study suggest that (i) *Fusarium* mycotoxin contamination decreased between the 1990s and 2020, (ii) contamination levels are similar in organic and conventional cereals used for human consumption, and (iii) maintaining OTA concentrations below the maximum contamination levels ( $3.0 \mu\text{g}/\text{kg}$ ) set by the EU remains a major challenge.

#### KEYWORDS

aflatoxins, agronomic practices, beauvericin, deoxynivalenol, enniatin, fumonisin, ochratoxin A, post-harvest management, quality assurance, T-2/HT-2, zearalenone

## 1 | INTRODUCTION

Mycotoxins contamination of cereals and other crops has only recently been recognized as an important food safety and public health issue (Bryła et al., 2016; Deligeorgakis et al., 2023; Johns et al., 2022; Khaneghah et al., 2018). Concern is based on epidemiological evidence for harmful effects of mycotoxins on both human and animal health (Nleya et al., 2018; Schaarschmidt & Fauhl-Hassek, 2018; Streit et al., 2012; Wild & Gong, 2009). This has resulted in the European Commission setting maximum contamination levels (MCL) for a range of mycotoxins in foods (European Commission, 2006, 2021). However, consumer awareness about the health risks from mycotoxins is still relatively low, and insufficient consumer education has been described as the main reason (Mukhtar et al., 2023; Sanders et al., 2015).

Mycotoxins are secondary metabolites produced by certain fungal species in cereals and other crop plants during growth in the field and/or post-harvest (AHDB, 2016; Carvajal-Moreno, 2022; Dall'Asta & Berthiller, 2015; Gonçalves et al., 2019; Yu & Pedroso, 2023). Cereals are the main source of mycotoxins intakes by humans in both high- and low-income countries (Ayalew et al., 2006; Leblanc et al., 2005; Nleya et al., 2018; Wang, Hasanalieva, Wood, Markellou et al., 2020; Yu & Pedroso, 2023). Mycotoxins are a diverse group of chemicals with contrasting toxicological classifications and health impacts which are reflected in the MCLs set by the EU for the main mycotoxins (Table 1). The references cited in Table 1 provide more detailed information on the chemistry, toxicology and health impacts of the main mycotoxins. Further information is available in a recent review by Yu and Pedroso (2023), which describes the proportions of cereal sample found to be above the EU MCLs in different regions of the world. Information on the biosynthesis of mycotoxins and their metabolites (free, emerging, and masked) can also be found in recent reviews by Lu et al. (2020) and Ekwomadu et al. (2021).

In the majority of published mycotoxin surveys, and many quality assurance protocols used by food processors only deoxynivalenol (DON) and ochratoxin A (OTA) were assessed, and the results obtained for DON and OTA are then used to estimate total contamination levels of (i) *Fusarium* spp. and (ii) mycotoxins produced by *Aspergillus* and *Penicillium* spp., respectively. However, this practice is considered to be inaccurate, especially with respect to *Fusarium* mycotoxins (Bernhoft et al., 2022; Wang, Hasanalieva, Wood, Markellou et al., 2020), since (i) correlations between DON and other trichothecenes, zearalenones, and fumonisins were often found to be weak (Aureli et al., 2015; Borutova et al., 2012; Van Der Fels-Klerx et al., 2012) and (ii) *Fusarium* species that dominate in different climatic zones have contrasting mycotoxin profiles (Hope et al., 2005; Kelly et al., 2015; Lu et al., 2020; Medina & Magan, 2011; Popovski & Celar, 2013; Solarska et al., 2012; Van Der Fels-Klerx et al., 2012). Detailed information on the biosynthetic pathways for the main mycotoxins and their metabolites (also described as free, emerging, masked, modified, or conjugated mycotoxins) produced by *Fusarium* and mold (*Aspergillus* and *Penicillium*) species and their expressions under the influence of climatic factors can be found in recent reviews (Ekwomadu et al., 2021; Kolawole et al., 2021; Lu et al., 2020) and are therefore not detailed. However, it is now well established that infection by these fungal species tends to result in “multi-mycotoxin” contamination and that testing for DON, OTA, and selected aflatoxin (AFL; e.g., B<sub>1</sub>) only, may not always provide accurate estimates of total mycotoxin loads (Bernhoft et al., 2022).

The level of mycotoxin contamination in cereals is known to be affected by a range of factors including (i) climatic conditions during the growing season (especially after tillering) and at harvest (Bernhoft et al., 2012, 2022), (ii) agronomic management factors including crop protection, tillage, fertilization, rotation design/pre-crop, and variety choice (Bernhoft et al., 2022; Buerstmayr et al., 2021; Mielniczuk & Skwaryło-Bednarz, 2020; Powell &

**TABLE 1** Main toxicological classifications/effects and maximum contamination levels (MCL) set by the EU (EC, 2006, 2021) for mycotoxins produced by different groups of fungi and references that provide further information on the toxicology and health impacts.

Mycotoxin	Chemical characterization and confirmed or reported toxic effects	Food Type	EU-MCL ( $\mu\text{g}/\text{kg}$ )	For detailed information on the chemistry, toxicology health impact of mycotoxins see:
<b>Fusarium mycotoxins</b>				Ferrigo et al. (2016); Ji et al. (2019); Rocha et al. (2005); Reddy et al. (2010); Pitt et al. (2012); AHDB (2016)
DON	<b>Trichothene Type B</b> non-carcinogenic (causes diarrhea, vomiting, liver damage, anorexia, nausea, abdominal pain, headache and dizziness)	Grain <sup>1</sup>	1250 <sup>1</sup> or 1750 <sup>2</sup>	Foroud & Eudes (2009)
		CP	500 <sup>3</sup> or 750 <sup>4</sup>	Chen et al. (2019); EFSA et al. (2017)
		CP-BF	200	Mielniczuk & Skwaryło-Bednarz (2020)
HT-2/T-2	<b>Trichothene Type A</b> cytotoxic, hematopoietic	Grain	50 <sup>5</sup>	EFSA et al. (2017); Adhikari et al. (2017); EFSA, Arcella et al. (2017); EFSA (2013); Ji et al. (2019)
ZEA	<b>Resorcylic acid lactone</b> carcinogenic, estrogenic, teratogenic	Grain	100	EFSA et al. (2017); Zhang et al. (2018)
		CP	50 <sup>3</sup> or 75 <sup>4</sup>	
		CP-BF	20	
FUM	<b>Tricarboxylic acid</b> carcinogenic, neurotoxic	Grain	2000 <sup>6</sup>	Kamle et al. (2019)
		CP	400 <sup>6</sup>	
		CP-BF	200 <sup>6</sup>	
ENN	<b>Cyclic hexadepsipeptides</b> cytotoxic, antibacterial, anthelmintic, antifungal, herbicidal, insecticidal		No MCL	Prosperini et al. (2017)
BEA	<b>Cyclic hexadepsipeptide</b> cytotoxic, antibacterial (potential anticancer agent)		No MCL	Wu et al. (2018 & 2019)
<b>Claviceps mycotoxins<sup>7</sup></b>	<b>Clavines, lysergic acid amides, and peptides</b> (causes gangrene, spasms, diarrhea, vomiting, nausea, paresthesia, headaches, mania and psychosis)			Peraica et al. (1999); Reddy et al. (2010); Pitt et al. (2012)
ERG		CP	50-150 <sup>7,8</sup> (500 <sup>7,9</sup> )	AHDB (2018); Agriopoulou (2021)
		CP-BF	20	
<b>Mold mycotoxins</b>				Peraica et al. (1999); Pitt et al. (2012)
OTA	<b>Dihydroisocoumarin and L-<math>\beta</math>-phenylalanine component</b> carcinogenic, nephrotoxic, teratogenic, immunosuppressive	Grain <sup>1</sup>	5.0	Walker (2002); Jorgensen & Jacobsen (2002)
		CP	3.0	Bui-Klimke & Wu (2015)
		CP-BF	0.5	
AFL	<b>Coumarin derivatives</b> carcinogenic, hepatotoxic, teratogenic	Grain <sup>1</sup> , CP	2,0 <sup>10</sup> (4.0 <sup>11</sup> )	Barug et al. (2006); Dall'Aster & Berthiller (2015)
		CP	2,0 <sup>10</sup> (4.0 <sup>11</sup> )	Ferrigo et al. (2016); Khaneghah et al. (2018)
		CP-BF	0.1 <sup>10</sup>	Kumar et al. (2022)

Abbreviations: AFL, aflatoxins; BEA, beauvericin; CP, processed cereal products intended for human consumption; CP-BF, processed cereal-based foods and baby foods for infants and young children; DON, deoxynivalenol; ENN, enniatins; FUM, fumonisins; OTA, ochratoxin A; ZEA, zearalenone.

<sup>1</sup>Unprocessed cereal grain destined for human consumption other than durum wheat and oats; <sup>2</sup>Durum wheat and oats only; <sup>3</sup>Bread, pastries, biscuits, cereal snacks, and breakfast cereals; <sup>4</sup>Cereals products intended for direct human consumption including cereal flour, dried pasta, bran and germ, except for bread, pastries, biscuits, cereal snacks, and breakfast cereals; <sup>5</sup>Recommendation only, there is currently no legal EU-MCL; <sup>6</sup>Sum of fumosins B1 and B2 (limits set for maize only); <sup>7</sup>The MCL for ergot alkaloids refers to the lower bound sum of the following 12 ergot alkaloids: ergocornine/ergocorninine; ergocristine/ergocristinine; ergocryptine/ergocryptinine ( $\alpha$ - and  $\beta$ -form); ergometrine/ergometrinine; ergosine/ergosinine; ergotamine/ergotaminine; <sup>8</sup>Barley, wheat, spelt, and oats; <sup>10</sup>Aflatoxin B<sub>1</sub>; <sup>11</sup>Sum of aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>.

Vujanovic, 2021; Supronienė et al., 2012), and (iii) post-harvest management practices including drying and cleaning of harvested grain, and storage conditions (Alizadeh et al., 2021; Ayalew et al., 2006; Chandravarman et al., 2022; Gonçalves et al., 2019; Magan & Aldred, 2007; Mielniczuk & Skwaryło-Bednarz, 2020).

Since the 1990s there has been a rapid expansion of organic food production and reportedly, organic land area has grown from 11 million hectares (0.2 million farms) in 1999 to 76 million ha (3.7 million farms) in 2021 and is estimated to account for 1.6% of agricultural land area globally and nearly 10% in Australia and the EU (Willer et al., 2023). Demand for organic food is primarily driven by consumer perceptions that, compared with conventional intensive farming, organic farming delivers biodiversity, environmental, and food quality, safety, and security gains (Joshi & Rahman, 2015; Stolz et al., 2011).

In most countries organic farming standards are defined by government laws and regulations (European Commission, 2016; USDA, 2024). These regulations prohibit or restrict the use of many external inputs that are commonly used in conventional farming, primarily because they are (i) non-renewable resources (e.g., mineral P, K, and micronutrient fertilizers), (ii) energy intensive to produce (e.g., mineral N fertilizers and pesticides), and/or (iii) potentially deleterious to the environment and human health (e.g., mineral N and P fertilizers, synthetic chemical pesticides, antibiotics, and food additives) (Baker et al., 2002; Cooper et al., 2007; Rempelos et al., 2021, 2023).

Specifically, organic crop production prohibits the use of all synthetic chemical crop-protection products (including insecticides, acaricides, fungicides, herbicides, plant growth regulators, and soil disinfection chemicals) and mineral N, KCl, and superphosphate fertilizers (European Commission, 2016; USDA, 2024). Instead, weed, pest, and disease control in organic farming is based on preventative and non-chemical crop-protection methods, such as the use of (i) diverse crop rotations, (ii) more resistant/tolerant varieties, (iii) mechanical weeding, and (iv) biological disease and pest control products (Hansen, 2010; Rempelos et al., 2021, 2023). Organic crop production standards do, however, permit the use of certain plant (e.g., pyrethrum) or microbial (e.g., spinosad) extracts and/or mineral (e.g., Cu and S)-based crop-protection products, but it is recommended that these are only used as a last resort (European Commission, 2016; USDA, 2024). Organic farming standards prescribe regular inputs of organic fertilizers (e.g., manure and composts) and the use of legume crops in rotation (to increase N levels and balance N:P ratios in the soil). They also allow the restricted use of raw phosphate, potassium sulfate, and mineral micronutrient fertilizers if shown to be necessary by soil or plant analyses (European Commission, 2016; Rempelos et al., 2021; USDA, 2024). As

a result, organic and conventional cereal production protocols differ substantially in (i) the type of crop-protection protocols used and (ii) the types and quantities of organic and mineral fertilizers applied (Rempelos et al., 2020, 2021, 2023).

There is increasing evidence that the contrasting crop rotation designs, protection protocols, fertilization regimes, and varieties/genetics used in organic and conventional cereal production affects the concentrations of nutritionally relevant compounds in cereal grains. Specifically, the most recent systematic literature reviews and meta-analyses of composition differences between organic and conventional cereals/cereal products found higher antioxidant activity and phenolic concentrations, but lower cadmium, nitrate and nitrite, and pesticide concentrations in organic cereal grains/products (Baranski et al., 2014; Mie et al., 2016; Rempelos et al., 2020, 2023). However, there are, to our knowledge, no published systematic reviews/meta-analyses that compared mycotoxin incidence or concentrations in organic and conventional cereal grains/products.

As a result, there is still considerable scientific controversy and uncertainty about whether, and to what extent, the contrasting agronomic protocols used in organic and conventional cereal production systems affect mycotoxin levels and potential health risks for consumers and livestock (Benbrook, 2005; Bernhoft et al., 2012, 2022; Brodal et al., 2016; Gourama, 2015; Lairon, 2010; Magkos et al., 2006; Smith-Spangler et al., 2012; Trewavas, 2001, 2004). It is important to address this, because organic food consumption and production have rapidly increased globally over the last 30 years (Baudry et al., 2015; Nandi et al., 2016; Rempelos et al., 2021; Wier & Calverley, 2002; Wier et al., 2008; Yadav & Pathak, 2016).

Here we report the findings of a systematic literature review and meta-analysis of all accessible comparative (organic vs. conventional) mycotoxin contamination data for cereal grains and products, since cereals are the main dietary source for mycotoxins in humans and livestock (Bernhoft et al., 2022; Wang, Hasanalieva, Wood, Markelou et al., 2020). We tested the hypotheses that (i) the contrasting primary production methods used in conventional and organic cereal production systems affect the concentrations and profiles of different mycotoxins in cereal grains and products made from cereal grains, (ii) the climatic conditions during the cereal growing season (and associated infection pressure by mycotoxin-producing fungi) affect mycotoxin concentrations and profiles and the relative difference in mycotoxin concentrations between conventional and organic cereal grains, and (iii) post-harvest storage, processing, and quality assurance protocols applied to cereal grains destined for human consumption affect concentrations and profiles of mycotoxins,

and the relative difference in mycotoxin concentrations between organic and conventional cereals products that enter the human food chain.

To test these hypotheses our main objectives were to identify and quantify (i) differences in mycotoxin prevalence and concentrations between cereals and cereal-based products produced in conventional and organic farming systems and (ii) potential “confounding” effects of (a) cereal species, (b) country/climatic zone, (c) study type (farm and retail surveys, and experimental studies), (d) time period (years in which studies were carried out), (e) funding sources, and (f) mycotoxin analysis method by multilevel model meta-analyses. Funding source (whether studies obtained industry co-funding or not) was included as a parameter in the multilevel meta-analyses to test for potential bias. Mycotoxin analysis method was included in the multilevel meta-analyses to investigate whether the use of more sensitive HPLC methods affected study outcomes.

To achieve these objectives, we followed the methodology recommended and previously used for systematic reviews/meta-analyses that compared the incidence or concentrations of nutritionally relevant compounds in organic and conventional crops/plant foods (Baranski et al., 2014; Brandt et al., 2013; Smith-Spangler et al., 2012). However, as in previous reviews, some potential sources of bias and/or variation could not be excluded, because they were not described or could not be avoided/excluded in the studies that were used as data sources.

Most importantly, the three types of studies (retail surveys, farm surveys, and experimental studies) used in the meta-analysis are known to all have deficiencies. These have recently been described in detail by Baranski et al. (2014) and therefore only briefly summarized here.

Retail surveys, which compare organic and conventional cereal product samples (e.g., flour, breakfast cereals, bread, and pasta) purchased from supermarkets and other retail outlets over a specific time period in a specific location/region/country are thought to provide the best estimate of the differences in product quality as experienced by the consumers in each country. However, millers and cereal processors often blend grains/flour from different farms, regions within a country, or different countries and only a very small proportion of cereal product brands provide information on the farms, regions, or countries where grain was produced. In retail surveys it is therefore not possible to estimate the relative contribution of contrasting (i) production system (organic vs. conventional), (ii) environmental conditions during primary production, and (iii) post-harvest grain storage, blending, and processing on the difference in food composition (Baranski et al., 2014; Wang, Chatzidimitriou et al., 2020).

Farm surveys, which compare organic and conventional cereal grains collected after harvest from matched pairs of organic and conventional farms or groups of organic and conventional farms in the same location region or country, provide a greater level of control of confounding environmental factors and the results are not confounded by differences in post-harvest storage, blending, and processing of organic and conventional cereal grain samples. However, differences in agronomic protocols and environmental background conditions in both the organic and conventional farms included in the survey, may introduce bias/variation, especially if surveys are based on a small number of farms and/or growing seasons (Baranski et al., 2014). Meta-analyses of data from farm surveys carried out in different countries provide the most reliable estimate for effect of primary production protocols used on commercial organic and conventional farms on crop composition (Baranski et al., 2014).

Replicated field experiments are considered the best approach to identify and quantify differences in crop composition resulting from contrasting management protocols used in organic and conventional farming systems because they allow the impact of agronomic practices to be assessed under the same pedo-climatic background conditions. Also, the use of factorial field trial designs allows the effect of specific management practices (e.g., rotation design, tillage, fertilization, crop protection, and variety choice) and interactions between them on food composition to be identified (Rempelos et al., 2020, 2023). However, unless field trials are replicated in different seasons and in a range of different locations/environments, it remains unclear to what extent results can be extrapolated to other locations/environments (Baranski et al., 2014). A specific challenge in field trials that focus on assessing the effects of agronomic parameters (e.g., preceding crop, tillage, fungicide applications, and variety resistance) on mycotoxins incidence/concentration in field experiments, is the often very large spatial variation of fungal infection levels and/or mycotoxin incidence/concentration across fields and landscapes (Oerke et al., 2010).

It is important to note that more than 90% of the data used in our meta-analyses were from studies carried out in Europe, and meta-analyses results may therefore not accurately reflect mycotoxin contamination levels in other regions, especially those regions which have different climatic, agronomic, and food safety regulatory background conditions. The large contribution of European studies to the evidence base is due to (i) countries in Europe and North America accounting for the largest proportion of organic food consumption globally (Willer et al., 2023) and (ii) financial support for organic farming and food research from both the private and public sector being substantially

larger in Europe compared with North America (Baker, 2015).

## 2 | MATERIAL AND METHODS

The protocol used for the systematic literature review and meta-analyses were based on methodologies developed by Brandt et al. (2013) and Baranski et al. (2014) and was published online in 2018 (Wang et al., 2018).

### 2.1 | Literature search strategy

Relevant papers for the review were identified in the databases Web of Science, Scopus, and EBSCO. The research phrases contained four groups of terms combined with Boolean logic (“OR” and “AND”) and with asterisk truncation (\*) in order to find all contrasting interventions and participants for selected outcome:

- (Organic\* OR ecologic\* OR biodynamic\*) AND
- (Conventional\* OR integrated) AND
- (wheat OR barley OR oat OR spelt OR rye OR rice OR emmer OR buckwheat OR sorghum OR millet OR triticale OR fonio OR quinoa OR cereal\*) AND
- (deoxynivalenol OR aflatoxin OR beauvericin OR diacetoxyscirpenol OR enniatins OR fumonisin OR fusarenon X OR HT-2 OR T-2 OR monoacetoxyscirpenol OR moniliformin OR neosolaniol OR nivalenol OR ochratoxin OR zearalenone OR mycotoxin\*).

The online search was restricted to the period between January 1992 and December 2020. In addition, we (a) screened the list of references of all publications/articles from which suitable data could be extracted for additional articles and (b) contacted the authors of all papers from which suitable data could be extracted (see later) with requests for information on other publications or unpublished results. Studies published before 1992 were obtained via screening the list of references of articles published after January 1992 only. All articles from which data were extracted are listed in the reference list of the Supporting Information.

Papers in all languages were included and the translations of papers, that were published in languages other than English, were carried out by authors or external scientific collaborators. Summaries the literature search, and the number of studies and comparative datasets included in different analyses are provided in Figure S1 and Table S1. Lists of all articles used for extraction of data for (i) the standard weighted odds ratio meta-analyses, (ii) the standard weighted mean difference meta-analyses, and (iii) the unweighted mean difference meta-analyses (= sensi-

tivity analysis 1) are provided in Tables S2, S3, and S4, respectively.

### 2.2 | Criteria for including and excluding studies

#### 2.2.1 | Types of study designs

We included data that compared the incidence (proportions of samples testing positive) or concentrations of mycotoxin in cereal grains, flour, and/or processed cereal-based food samples from organic or conventional productions systems. Comparative data were from three types of studies and are described as (i) farm surveys (FS), (ii) retail surveys (RS), and (iii) controlled field experiment (EX).

Comparative data from farm surveys were from analyses of grain samples collected from organic and conventional farms in the same country or regions, and the number of farms included in surveys was considered as the sample size in the meta-analyses.

Comparisons in retail surveys (which were also described as basket studies in the articles that data were extracted from) were from analyses of processed cereal products labeled as organic or not-labeled as organic (= conventional) that were collected from the same retail outlets or retail outlets in the same area; the sample size was the number of samples collected and analyzed from retail outlets. Organic labels were compliant with European Union (EU), United States Department of Agriculture (USDA), or other national government agency certification schemes. Retail surveys carried out prior to the introduction of EU, USDA, or other national government standards could not be identified.

Comparisons in controlled experiments were based on analyses of grains from crops, which were grown in field experiments with a randomized block design, and the sample size was the number of replicate plots used in experiments.

For each type of study, the country where studies were carried out was recorded to allow results obtained in different climatic zones, organic certification, and other agricultural (e.g., environmental, pesticide and mycotoxin residue) regulatory backgrounds to be compared using multilevel meta-analyses.

#### 2.2.2 | Types of product (grain crops and processed foods)

The study population was comparative data obtained for (i) small-grain cereals and pseudocereal grains and (ii) processed food products made from small-grain cereals

and pseudocereals from organic and conventional production systems. Comparative datasets were found for common wheat (*Triticum aestivum* L., hexaploid); spelt wheat (*Triticum spelta*, L., hexaploid); durum wheat (*Triticum durum* Desf., tetraploid), emmer wheat (*Triticum dicoccum* Schrank ex Schübl, tetraploid), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* Moench), triticale (*Triticosecale* Wittm. ex A. Camus; tetraploid, hexaploidy, or octaploid *Triticum* x *Secale* hybrids), pearl millet (*Cenchrus americanus* Morrone), fonio (*Digitaria exilis* Stapf), buckwheat (*Fagopyrum esculentum* Moench), and quinoa (*Chenopodium quinoa* Willd.). However, since the literature review identified less than three comparative datasets for emmer wheat, triticale, buckwheat, sorghum, millets, fonio, and quinoa, only data for common wheat, durum wheat, spelt wheat, barley, oat, spelt, rye, and rice were included in meta-analyses. Data for common, spelt, and durum wheat were pooled for meta-analyses. Maize (corn, *Zea mays* L.) was not included because it differs from other cereals included in the meta-analyses in (i) physiology (e.g., by using C4 carbon fixation) and morphology, (ii) main breeding/selection method used to develop modern maize hybrids, and (iii) the agronomic methods used to produce maize crops (Carvajal-Moreno, 2022).

### 2.2.3 | Types of comparisons

Only studies directly comparing mycotoxin contamination between grain, flour, and other cereal-based processed foods made from grain produced in organic or conventional production methods were included. Conventional comparators were mycotoxin contamination data from cereal grains that were produced in farming systems that commonly use mineral fertilizers and/or pesticides, and processed, cereal-based food products made from grain produced in conventional/non-organic farming systems. Organic comparators were mycotoxin contamination data from cereal grains produced in farming systems that were described as (i) certified to organic farming standards, (ii) using organic production methods, or (iii) experimental plots under organic management, and processed, cereal/pseudocereal-based foods labeled as organic.

In several studies terms other than “conventional” or “organic” were used to describe non-organic and/or organic management practices/protocols. Therefore, management systems named “integrated,” “low input,” “extensive,” and protocols which, according to the authors’ description, involved the use of mineral fertilizers and/or pesticides, were treated as conventional. Farming systems

described or certified as “biodynamic,” “biological,” or “ecological,” that followed the “organic” principles and omitted the use of synthetic chemical mineral N and P fertilizers and pesticides in the production protocol, and/or described that organic fertilization and crop-protection regimes were used, were treated as organic.

Some studies compared more than two production systems which could be treated as organic and/or conventional. This could include different crop rotations, fertilizer types and input levels, crop-protection protocols, or tillage methods. However, only the organic and/or conventional (non-organic) systems identified in the study as being closest to the typical, contemporary organic and/or conventional farming systems were included in the meta-analysis (Baranski et al., 2014; Brandt et al., 2013).

### 2.2.4 | Types of outcome measures

All identified mycotoxin data were included. In addition to measures of concentration, data on the frequency of detection (proportion of samples testing positive for the presence of a given mycotoxin) were used.

## 2.3 | Data management, coding categories, and meta-analyses

### 2.3.1 | Screening and data extraction

A summary of the data search and selection process is presented in Figure S1.

The first screening stage of papers involved the evaluation of titles and abstracts. All papers that mention comparisons of mycotoxin levels in cereals and cereal-based foods in organic and conventional foods or farming systems in the title or abstract were recorded after any duplicates were removed. In the second screening stage, the full text of the papers was read to identify suitable datasets. All available publications were independently evaluated by two co-authors, to minimize the chances of suitable data being missed and to confirm the eligibility of data included in analyses. A wide range of background data (e.g., years, country/regions and/or climatic zones in which studies were carried out, sponsors of studies, details of agronomic practice, and assessment and analytical methods used) were recorded, and used in multilevel model-based meta-analyses.

Data reported as numerical values were copied directly into the database. Data published in graphical form was enlarged, printed, measured (using a ruler), and then entered into the database, when exact data could not be obtained from authors. All discrepancies and

disagreements were discussed and resolved by the whole reviewer group. The final list of publications and information about each paper used for data extraction are provided in Tables S2–S4 and S6.

### 2.3.2 | Climate classification

Climate is a key factor affecting the infection, growth, and metabolism of mycotoxin-producing fungi on cereal grains and mycotoxin contamination levels (Bernhoft et al., 2022). We therefore used the unified Koppen–Geiger climate classification scheme (Peel et al., 2007) to define climates for the geographic location of cultivation reported in FS and EX studies and the country of origin in RS studies. We also compared the following summary climate types: (i) temperate (temperate, not dry season, hot summer [Cfa]; temperate, dry summer, hot summer [Csa]; temperate, dry winter, hot summer [Cwa]; and temperate, no dry season, warm summer [Cfb] climates in the Koppen–Geiger classification scheme), (ii) continental (continental, no dry season, warm summer [Dfb]; continental, no dry season, cold summer [Dfc]; and continental, dry winter, hot summer [Dwa] climates in the Koppen–Geiger classification scheme), (iii) polar/alpine (polar, tundra [ET] climates in the Koppen–Geiger classification scheme), and (iv) dry (arid, steppe, cold [BSk] climates in the Koppen–Geiger classification scheme).

### 2.3.3 | Dealing with missing data, “not detected” and “zero” values

In the original publications, mycotoxin concentrations that were below the limit of detection (LOD) or limit of quantification (LOQ) were reported as 0, <LOD, or <LOQ. Also, mean mycotoxin concentrations were calculated in different ways by either (i) averaging concentrations measured in samples that tested positive only, (ii) averaging concentrations from all samples and using 0 as the value for all samples testing negative for the presence of mycotoxin (= concentrations < LOD or < LOQ), (iii) averaging concentration from all samples and using the LOD or LOQ as the value for all samples testing negative for the presence of mycotoxin or (iv) using  $\frac{1}{2}$  LOD or  $\frac{1}{2}$  LOQ as the value for all samples testing negative.

In the standard meta-analysis and sensitivity analyses we used  $\frac{1}{2}$  LOD or  $\frac{1}{2}$  the LOQ (if the LOD was not reported) as the value for all samples testing negative (Bernhoft et al., 2022).

When studies only reported data on the incidence (% of samples testing positive), but not concentrations of mycotoxins in positive samples, the authors were contacted

to obtain missing concentration values. If this was not successful, we used the method described by Lajeunesse (2013) to estimate concentrations, if this was possible. When both mean and median value were missing in studies, but the proportion of positive samples was reported and the number of samples testing positive was less than 50%, half of the LOD/LOQ was used as the median concentrations for calculation in the meta-analysis. If more than 50% of samples tested positive, no median concentration value was generated and used in meta-analyses. Information from two studies was used to estimate mycotoxin concentrations and estimated concentration data were only included in the unweighted meta-analyses (see sensitivity analyses 1 later).

For means reported without a measure of variation (SE or SD), we estimated the SE if the minimum and maximum values were reported using the rpois function in R (R Core Team, 2017). When only the maximum value was reported we used half of the LOD (or LOQ if the LOD was not reported) as the minimum value, if the number of samples testing positive was less than 100%. Studies for which calculations of SEs were not possible were excluded from the weighted meta-analysis.

If articles only reported mean concentrations of positive samples, but in addition the (a) of total number of samples and (b) the number of positive samples and (c) the LOD or LOQ, the following calculation was used to estimate mean concentrations in all samples:

$$\text{mean}_{\text{all}} = \frac{\text{mean}_{\text{pos}} \times n_{\text{pos}} + 0.5 \times \text{LOD} \times (n_{\text{tl}} - n_{\text{pos}})}{n_{\text{tl}}}$$

where,  $\text{mean}_{\text{all}}$  is concentration mean of all samples assessed;  $\text{mean}_{\text{pos}}$  is concentration mean of samples testing positive;  $n_{\text{tl}}$  is total number of all samples assessed;  $n_{\text{s}}$  is number of samples testing positive.

### 2.3.4 | Dealing with other unclear information

For studies which did not report the year in which experiments of surveys were carried out (Blajet-Kosicka et al., 2014; Champeil et al., 2004; Twaruzek et al., 2013) we assumed that the study was carried out 2 years prior to the year of publication.

When studies reported data for replicate samples or raw data for the same system, crop, year, and country, replicate data for each combination of crop, year, and country of origin were averaged and SEs calculated. For replicate samples in which no mycotoxins were detected (concentrations < LOD), half the LOD was used in the calculation of means.



### 2.3.5 | Assessment of risk of bias

Data suitable for weighted meta-analyses were critically appraised and evaluated for potential sources of bias associated with the study design, analytical methods, selective outcome reporting, and conflicts of interest. This assessment had a form of statements with three optional answers: (1) “YES” when the statement reflected the content of the paper; (2) “NO” when there was no information in the paper described by the statement; or (3) “Unclear” when information provided did not reflect the statement. The last point on the checklist was the final rating of the overall methodological quality of the study. None of the studies was excluded from the standard weighted meta-analysis based on these quality assessments; however, results of the publication quality assessments were taken into account during the evidence synthesis as part of the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system report (Table S5), and were used to identify poor quality studies that were excluded from sensitivity analysis 2; only data from acceptable and high quality articles were included in sensitivity analysis 2 (see Table S6).

## 2.4 | Data synthesis

Characteristics and findings of each study included in the literature review were presented as descriptive results. Both weighted and unweighted meta-analytical protocols were carried out using the “metafor” package in the R statistical environment (R Core Team, 2017) as previously described (Baranski et al., 2014). Data on concentrations and the proportion of positive samples were analyzed separately for each mycotoxin.

### 2.4.1 | Weighted meta-analysis

The mostly frequently reported values for mycotoxins contamination in cereals were (i) the mean concentration in all samples (estimated by using the measured values for positive samples and half the detection limit as the value for negative samples) and (ii) the incidence (= proportion of samples testing positive) of mycotoxin contamination.

The two “standard” weighted meta-analyses that were carried out, were therefore based on two effect sizes: the mean differences for comparison of mycotoxins concentrations and the odds ratio for comparison of the incidence of mycotoxins in organic and conventional comparators (= cereal grains and/or products). For both the mean difference (MD) and odds-ratio (OR)-based weighted

meta-analysis methods the corresponding sampling variance (95% confidence intervals) were calculated in R using the “Metafor” package (R Core Team, 2017).

The outcomes reported in studies were on the same meaningful scale, thus the meta-analysis could be performed directly on the crude difference of means (MD) (Borenstein et al., 2009).

The MD was used to compare mycotoxin concentrations between organic and conventional cereals grains and/or products and calculated as

$$MD = \bar{X}_o - \bar{X}_c$$

where  $\bar{X}_o$  was the mean concentration of organic samples, and  $\bar{X}_c$  the mean concentration of conventional cereals.

The variance of MD ( $V_{MD}$ ) was calculated as follows, with the  $S_1$  and  $S_2$  being the sample standard deviations of the two groups ( $S_1$ , organic;  $S_2$ , conventional), and  $n_1$  and  $n_2$  being the sample sizes in the two groups ( $n_1$ , organic;  $n_2$ , conventional):

$$V_{MD} = \frac{n_1 + n_2}{n_1 n_2} S_{pooled}^2$$

The pooled standard deviation ( $S_{pooled}$ ) was calculated as:

$$S_{pooled} = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

A positive MD value indicated that mean mycotoxin concentration was higher in organic samples, while a negative MD indicated that the mean concentrations was higher in conventional cereals.

The odds ratio (OR) was used to compare data on the proportion of samples testing positive for the presence of mycotoxins in organic and conventional cereal grains and/or products. It is an effect size based on binary data (Borenstein et al., 2009). The OR was calculated on the logarithmic scale as:

$$\ln(\text{odds ratio}) = \ln\left(\frac{a_i \times d_i}{b_i \times c_i}\right)$$

where  $a_i$  is the number of organic samples testing positive,  $b_i$  is the number of organic samples testing negative,  $c_i$  is the conventional samples testing positive, and  $d_i$  is a number of conventional samples testing negative for mycotoxin contamination.

The approximate variance of the OR was established as:

$$V_{\text{LogOddsRatio}} = \frac{1}{a_i} + \frac{1}{b_i} + \frac{1}{c_i} + \frac{1}{d_i}$$

A positive odds ratio means that the frequency of detection of a mycotoxin was higher in organic samples, and a negative odds ratio means that the frequency of detection was lower in organic samples.

## 2.4.2 | Tests of homogeneity

Tests of homogeneity (Q statistics and  $I^2$  statistics) were carried out on all the summary effect sizes (Higgins & Thompson, 2002; Higgins et al., 2003). Homogeneity was indicated if  $I^2$  was less than 25% and the  $p$ -value for the Q statistics was greater than 0.01.

Potential effects of moderators on mycotoxin contamination in cereals from conventional and organic production systems, such as cereal species, climate, country, and study type were explored using mixed-effect models and subgroup analyses in R (R Core Team, 2017).

## 2.4.3 | Identifying changes in mycotoxin concentration over time

For DON and OTA large comparative datasets were available. We therefore also carried out separate weighted meta-analyses for data from studies carried out (a) before 2004 (the year in which legal organic crop production standards were introduced in the EU), (b) between 2004 and 2009, (c) between 2010 and 2015, and (d) between 2016 and 2020 to examine whether contamination levels in organic and conventional cereal changed over time.

For DON, ZEA T-2/HT-2 and OTA we produced Forrest plots to investigate between study variation (Figures S2 to S9).

In addition, we carried out regression analysis to study trends for changes in DON and OTA mycotoxin contamination levels over time. This was based on regressions of mean incidence and concentrations of DON and OTA in organic and conventional cereals/cereal reported (a) before 2004, (b) between 2004 and 2009, (c) between 2010 and 2015, and (d) between 2016 and 2020.

## 2.4.4 | Assessment of publication bias and strength of evidence

Potential **publication bias** was assessed by inspection of funnel plots and using the Egger's regression test for funnel plot asymmetry (Baranski et al., 2014).

The overall strength of evidence derived from the meta-analysis was explored using the adaptation of the GRADE framework (Baranski et al., 2014; Guyatt et al., 2011), which included information about risk of bias for each study, as

well as inconsistency, indirectness and imprecision of the results, and publication bias (Table S5).

## 2.4.5 | Sensitivity analyses

Two sensitivity analyses were conducted to explore how data management and inclusion criteria affected the overall results of the meta-analyses.

*Sensitivity analysis 1 (unweighted meta-analyses).* In order to include published mean values for which measures of variability and/or sample size were not provided by the authors and/or could not be estimated, unweighted meta-analyses were carried using an established method (Baranski et al., 2014). The effect size was calculated as an In-transformed ratio of the concentration of mycotoxin in organic sample to the concentration of mycotoxin in conventional sample ( $\bar{X}_o/\bar{X}_c$ ), and was expressed as a percentage.

$$\text{Ln Ratio} = \text{Ln} \left( \frac{\bar{X}_o}{\bar{X}_c} \times 100\% \right)$$

The significance of difference between samples was evaluated comparing the arithmetic average of the result with  $\text{Ln}(100)$  using a resampling method.  $p$ -values were derived from Fisher's one-sample randomization test and a  $p < 0.05$  was considered statistically significant (Baranski et al., 2014).

*Sensitivity analysis 2.* This analysis explored the effects of excluding data from studies deemed to be poor quality in the GRADE assessment from weighted meta-analyses of concentrations data for DON and OTA (Table S5). Due to the small number of studies available for *Fusarium* mycotoxins other than DON and AFL, sensitivity analysis 2 was only carried out for DON and OTA.

## 3 | RESULTS

### 3.1 | Literature search

A total of 474 publications were identified in the initial literature search, 325 of which were excluded after reviewing the title and abstract. The full texts of the remaining 149 papers were read, and those which did not report suitable data were rejected. Overall, 85 publications (all of which were peer-reviewed articles) fulfilled the criteria of the meta-analysis as defined in the published protocol (Wang et al., 2018). A flow diagram of the search and selection process and the numbers of articles and datasets suitable for the (i) standard weighted mean difference (MD) data-based meta-analyses, (ii) standard weighted

**TABLE 2** Proportion of positive samples in organic and conventional cereal grains and cereal-based processed foods; results are from weighted odds ratio-based meta-analyses. Odds values <0 and >0 indicate a lower proportion of positive samples in organic and conventional cereal grains/products, respectively.

Mycotoxin	N	n	% Positive samples		Odds ratio	95% CI	p-value	Homogeneity tests		
			ORG	CON				I <sup>2</sup> -value	Q-statistics (p-value)	
<b>Fusarium mycotoxins</b>										
<b>DON (all years)</b>	36	93	47	58	-0.64	-0.85	-0.42	<0.0001	56	<0.0500
DON (before 2014)	15	37	56	68	-0.82	-1.13	-0.51	<0.0001	26	<0.0500
DON (2004–2009)	9	22	35	51	-0.68	-1.29	-0.08	<b>0.0272</b>	70	<b>0.0272</b>
DON (2010–2015)	9	21	50	58	-0.53	-1.04	-0.02	<b>0.0411</b>	72	<b>0.0411</b>
DON (2016–2021)	7	13	38	44	-0.34	-0.69	0.00	0.0520	5	<b>0.0520</b>
NIV	8	12	47	34	0.40	-0.61	1.41	NS	67	<0.0500
<b>HT-2/T-2</b>	12	32	44	64	-1.25	-1.65	-0.85	<0.0001	66	<0.0500
<b>ZEA (all years)</b>	16	41	27	34	-0.39	-0.77	-0.02	<b>0.0371</b>	34	<b>0.0659</b>
ZEA (before 2004)	6	11	19	29	-0.57	-1.36	0.23	NS	43	<b>0.0648</b>
ZEA (2004–2009)	1	6	5	15	-0.88	-1.96	0.19	NS	10	0.2708
ZEA (2010–2015)	7	14	26	33	-0.50	-1.00	0.00	0.0522	24	0.3368
ZEA (2016–2021)	5	10	50	50	0.22	-0.64	1.07	NS	34	0.2237
ENN	4	26	64	59	0.44	0.07	0.81	<b>0.0207</b>	37	<b>0.0080</b>
FUM	2	12	33	33	-0.02	-0.45	0.42	NS	4	
BEA	5	12	42	32	0.80	0.22	1.39	<b>0.0068</b>	0	0.7392
<b>Mould mycotoxins<sup>1</sup></b>										
<b>OTA (all years)</b>	18	67	52	41	0.52	0.22	0.82	<b>0.0008</b>	34	<b>0.0110</b>
OTA (before 2004)	7	39	64	54	0.59	0.14	1.04	<b>0.0099</b>	42	<b>0.0045</b>
OTA (2004–2009)	6	15	31	13	0.74	-0.04	1.51	0.0615	31	0.2799
OTA (2010–2015)	3	7	36	33	0.37	-0.41	1.15	NS	6	0.3811
OTA (2016–2020)	3	6	40	36	0.18	-0.35	0.70	NS	23	0.4468
<b>AFL</b>	5	13	9	23	-1.00	-1.97	-0.04	<b>0.0417</b>	57	<b>0.0016</b>

Abbreviations: AFL, aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2); BEA, beauvericin; CON, conventional; DON, deoxynivalenol; ENN, enniatin (ENA, ENA1, ENB, and ENB1); FUM, fumonisins (fumonisin B1, fumonisin B2, and fumonisin); N, number of publication used for extraction of data; n, number of comparisons/datasets extracted from publications; NIV, nivalenol; OTA, ochratoxin A; RG, organic; T-2/HT-2-toxins (T-2, T-2 tetraol, T-2 triol, and HT-2); ZEA, zearalenone.

<sup>1</sup>Produced by *Aspergillus* and/or *Penicillium* species.

odds ratio-based meta-analyses, and (iii) unweighted meta-analysis (= sensitivity analysis 1) are provided in Figure S1. The numbers of articles and datasets available for, and the reasons for articles being excluded from the two standard weighted meta-analyses and two sensitivity analyses carried, out are described in Table S1.

More than 90% of all studies that provided data used in meta-analysis were carried out in Europe, mostly in Germany, Poland, Italy, Spain, Switzerland, Turkey, and Denmark (Tables S7 and S8). Publications reported data on 28 different mycotoxins, 14 of which were included in the meta-analysis (Tables 2–6 and Tables S7–S10). For the other 14 mycotoxins less than three comparative data-points were available and they were therefore not included in the meta-analyses as recommended by Baranski et al. (2014).

### 3.2 | Effect of production system on mycotoxin incidence and concentrations

It is well documented that the relative effects of (i) agronomic practices and climatic conditions pre-harvest and (ii) post-harvest grain processing methods and storage conditions on fungal colonization/infection and mycotoxin production in cereal grains differ considerably between the main mycotoxin-producing fungal genera (*Claviceps*, *Fusarium*, and *Penicillium/Aspergillus*). Most importantly, climatic conditions during grain development and agronomic practices used pre-harvest are considered to be the most important drivers for *Claviceps* and *Fusarium* grain infections and mycotoxin levels (Bernhoft et al., 2022). Climatic conditions during harvest, and post-harvest grain processing (seed drying, clean-

**TABLE 3** Concentrations of mycotoxins in organic and conventional cereal grains and cereal-based processed foods; results are from weighted mean difference (MD) meta-analyses. MD values <0 and >0 indicate a lower concentration in organic and conventional cereal grains/products, respectively.

Mycotoxin	N	n	Concentration ( $\mu\text{g}/\text{kg}$ )			p-value	MD	95% CI	Homogeneity tests			
			Organic Mean	SE	Conventional Mean				SE	I <sup>2</sup> -value	Q-statistics (p-value)	
<b>Fusarium mycotoxins</b>												
DON (all years)	41	93	122.49	20.82	185.38	25.29	<0.0001	-37.99	-53.51	-22.47	97	<0.05
DON (before 2004)	19	41	90.08	15.09	168.24	31.63	0.0011	-42.35	-67.81	-16.89	76	<0.05
DON (2004–2009)	11	22	245.49	76.06	307.81	78.21	0.0026	-68.75	-113.56	-23.95	98	<0.05
DON (2010–2015)	10	19	98.21	23.77	156.53	36.07	0.0154	-26.66	-48.22	-5.10	86	<0.05
DON (2016–2021)	5	11	39.21	7.16	54.27	11.89	0.0706	-2.10	-4.37	0.18	0	0.57
NIV	9	13	25.68	8.55	31.73	11.80	NS	-3.28	-11.99	5.44	93	<0.05
HT-2/T-2	10	25	14.00	3.95	15.35	4.08	NS	0.00	-0.14	0.14	0	<0.05
ZEA (all years)	21	47	5.38	1.12	10.88	3.34	NS	-0.14	-0.49	0.21	46	<0.01
ZEA (before 2004)	10	15	8.76	3.01	21.10	9.76	NS	-0.37	-1.12	0.38	30	0.05
ZEA (2004–2009)	3	11	3.12	0.88	7.22	3.17	NS	0.15	-0.58	0.89	0	0.89
ZEA (2010–2015)	8	13	5.12	1.62	6.98	1.85	NS	-0.39	-1.09	0.31	37	0.20
ZEA (2016–2020)	3	8	2.59	0.46	3.08	0.51	NS	0.14	-0.57	0.85	69	<0.05
ENN	2	11	21.74	9.90	10.02	3.81	NS	0.12	-1.36	1.59	0	0.99
FUM	2	12	69.20	18.12	60.91	15.22	NS	20.81	-9.14	50.75	71	<0.05
BEA	3	7	1.83	0.86	1.78	0.87	NS	0.09	-0.04	0.22	97	<0.05
<b>Mold mycotoxins<sup>1</sup></b>												
OTA (all years)	19	70	1.55	0.36	0.90	0.16	NS	0.178	-0.04	0.39	99	<0.05
OTA (before 2004)	8	40	1.63	0.60	0.73	0.22	NS	-0.001	-0.15	0.15	95	<0.05
OTA (2004–2009)	7	19	1.26	0.34	0.85	0.30	NS	0.327	-0.30	0.96	99	<0.05
OTA (2010–2015)	3	6	1.04	0.15	0.94	0.29	0.0075	0.382	0.10	0.66	0	0.45
OTA (2016–2020)	2	5	2.61	0.47	2.42	0.42	NS	0.146	-0.53	0.82	79	<0.05
AFL	7	18	0.32	0.09	0.49	0.17	NS	-0.006	-0.04	0.03	94	<0.05

Abbreviations: 95% CI, 95% confidence interval; AFL, aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2); BEA, beauvericin; DON, deoxyvalenol; ENN, emmiatin (ENA, ENA1, ENB, and ENB1); FUM, fumonisins (fumonisin B1, fumonisin B2, and fumonisin); MD, mean difference; N, number of publications used for extraction of data; n, number of comparisons/datasets extracted from publications; NIV, nivalenol; T-2/HT-2-toxins (T-2, T-2 tetraol, and HT-2); OTA, ochratoxin A; ZEA, Zearealenone.

<sup>1</sup>Produced by *Aspergillus* and/or *Penicillium* species.

**TABLE 4** Concentrations of mycotoxins in organic and conventional cereal grains and cereal-based processed foods; results are from un-weighted meta-analyses (= sensitivity analysis 1). Mean difference values <0 and >0 indicate a lower concentration in organic and conventional cereal grains/products, respectively.

Mycotoxin	N	n	Concentration ( $\mu\text{g}/\text{kg}$ )				p-value	MD
			Organic mean	SE	Conventional Mean	SE		
<b>Fusarium mycotoxins</b>								
<b>DON (all years)</b>	64	175	165.20	38.08	204.37	26.14	<0.0001	-35.47
DON (before 2004)	29	86	99.16	12.76	167.75	22.38	<0.0001	-65.58
DON (2004–2009)	17	39	338.25	153.91	327.62	81.00	NS	10.62
DON (2010–2015)	16	34	130.59	57.18	170.29	64.47	<b>0.0025</b>	-39.71
DON (2016–2020)	8	16	155.39	55.31	151.53	50.53	NS	10.89
NIV	18	44	55.72	12.41	45.02	7.20	NS	12.87
<b>HT-2/T-2</b>	18	59	38.49	12.17	48.61	14.66	<b>0.0061</b>	-10.12
<b>ZEA (all years)</b>	28	64	11.28	4.79	19.79	6.31	<b>0.0001</b>	-8.51
ZEA (before 2004)	12	20	10.04	3.11	32.34	12.57	<b>0.0115</b>	-22.30
ZEA (2004–2009)	4	15	4.38	1.20	7.26	2.38	<b>0.0425</b>	-2.88
ZEA (2010–2015)	9	16	23.02	18.29	26.16	19.34	<b>0.0159</b>	-3.14
ZEA (2016–2020)	6	13	6.62	4.10	8.07	4.12	NS	-1.45
ENN	5	38	46.74	25.19	42.64	14.60	<b>0.0292</b>	4.98
FUM	2	12	69.20	18.12	60.91	15.22	NS	8.29
BEA	5	12	2.82	1.39	6.94	5.43	NS	-4.12
<b>Mold mycotoxins<sup>1</sup></b>								
<b>OTA (all years)</b>	22	80	1.50	0.32	1.13	0.28	0.0542	0.37
OTA (before 2004)	9	46	1.62	0.53	0.77	0.20	0.0898	0.85
OTA (2004–2009)	9	21	1.15	0.31	0.78	0.27	NS	0.36
OTA (2010–2015)	3	7	1.02	0.13	0.98	0.25	NS	0.04
OTA (2016–2020)	3	6	2.38	0.45	5.35	2.95	NS	-2.97
AFL	10	33	0.32	0.06	0.48	0.12	NS	-0.16

Abbreviations: AFL, aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2); BEA, beauvericin; DON, deoxynivalenol; ENN, enniatin (ENA, ENA1, ENB, and ENB1); FUM, fumonisins (fumonisin B1, fumonisin B2, and fumonisin); MD, mean difference; N, number of publications used for extraction of data; n, number of comparisons/datasets extracted from publications; NIV, nivalenol; OTA, ochratoxin A; T-2/HT-2-toxins (T-2, T-2 tetraol, T-2 triol, and HT-2); ZEA, zearalenone.

<sup>1</sup>Produced by *Aspergillus* and/or *Penicillium* species.

ing, and refining) and storage conditions are thought to be the main determinants for *Penicillium/Aspergillus* mold mycotoxin levels in cereals (Agriopoulou, 2021; Barug et al., 2006; Bernhoft et al., 2022; Bhat et al., 2010; Magan & Aldred, 2005; Wang, Chatzidimitriou et al., 2020). Results obtained for mycotoxins produced by *Claviceps*, *Fusarium*, and *Penicillium/Aspergillus* molds are therefore described in separate sections later.

### 3.2.1 | Mycotoxins produced by *Fusarium* spp

When data from all years were used in standard, random effect model, weighted odds ratio meta-analyses, the incidence (% of samples testing positive) of DON,

total T-2/HT-2 fusariotoxins (T-2/HT-2) and zearalenone (ZEA) was found to be slightly, but significantly, higher, while the incidence of contamination with enniatin (ENN) and beauvericin (BEA) was slightly, but significantly, lower in conventional compared with organic cereal grains/products (Table 2).

The standard, random effect model, weighted mean difference meta-analyses detected significant effects of production system only for DON ( $p < 0.0001$ ,  $n = 93$ ), and the estimated DON concentrations were 52% higher in conventional (185  $\mu\text{g}/\text{kg}$ ) compared with organic (122  $\mu\text{g}/\text{kg}$ ) cereal grains/products (Table 3).

However, it should be pointed out that heterogeneity was found to be high for both the odds ratio (OR;  $I^2 = 65\%$ ) and mean difference (MD  $I^2 = 97\%$ )-based weighted meta-analyses of DON data (Tables 1 and 2). The multilevel

**TABLE 5** Effects of study types, cereal species, product types, and climatic conditions on differences in deoxynivalenol (DON) concentrations and proportion of samples testing positive for DON found in organic and conventional cereal grain and processed cereal products; results are from multilevel, weighted meta-analyses models for mean differences (MD) and odds ratios (OR). MD and OR values <0 and >0 indicate a lower concentration or % positive samples in organic and conventional cereal grains/products, respectively.

Analyses Factor	Results of weighted meta-analyses to determine mean differences (MD)										Results of weighted meta-analyses to determine odds ratios (OR) for the proportions of contaminated samples									
	between mycotoxin concentrations					% positive samples					between mycotoxin concentrations					% positive samples				
	N	n	ORG	COM	CON	p-value	MD*	95% CI	N	n	ORG	CON	CON	p-value	OR*	95% CI				
<b>Random effect<sup>1</sup></b>	41	93	122	185	185	<0.0001	-38	-54	-22	36	93	47	58	<0.0001	-0.637	-0.854	-0.419			
<b>Multilevel</b>	41	93	122	185	185	0.0005	-39	-61	-17	36	93	47	58	0.0002	-0.567	-0.867	-0.268			
<b>Study type</b>																				
Retail surveys	21	47	89	160	160	0.0002	-40	-61	-20	23	59	55	63	0.0003	-0.498	-0.768	-0.229			
Farm surveys	15	40	164	227	227	0.0029	-40	-66	-14	12	33	31	49	<0.0001	-0.932	-1.315	-0.550			
Field experiments	5	6	109	111	111	NS	-13	-71	44	1	1	83	83	NS	0.000	-3.282	3.282			
<b>Cereal species</b>																				
Wheat	31	52	113	197	197	<0.0001	-49	-71	-28	26	47	49	63	<0.0001	-0.800	-1.105	-0.495			
Rye	7	15	45	69	69	NS	-17	-55	21	7	15	31	48	0.0003	-1.017	-1.565	-0.470			
Oat	4	7	142	150	150	NS	-14	-64	36	7	9	37	44	NS	-0.258	-0.945	0.429			
Barley	3	5	542	589	589	NS	-58	-134	17	4	4	59	68	NS	-0.287	-1.321	0.747			
Rice	3	3	26	71	71	NS	-21	-91	49	5	6	57	43	NS	0.569	-0.306	1.443			
Various <sup>2</sup>	7	11	96	158	158	NS	-38	-85	9	8	12	60	69	0.0780	-0.518	-1.093	0.058			
<b>Product type</b>																				
Grain	29	62	141	212	212	0.0005	-35	-55	-15	24	58	40	52	<0.0001	-0.571	-0.829	-0.313			
Flour	8	12	92	129	129	0.0897	-33	-70	5	9	13	62	70	0.0959	-0.514	-1.120	0.091			
Processed food	7	12	79	159	159	0.0003	-73	-112	-34	8	14	50	68	<0.0001	-1.286	-1.900	-0.672			
Baby food	1	1	49	29	29	NS	20	-89	129	1	1	4	26	NS	-2.050	-4.506	0.407			
Other <sup>3</sup>	3	6	89	102	102	NS	-11	-71	49	4	7	78	78	NS	-0.150	-0.955	0.655			
<b>Climate type</b>																				
Temperate	18	34	103	179	179	0.0002	-48	-74	-23	18	38	59	68	<0.0001	-0.553	-0.906	-0.201			
Continental	20	50	140	201	201	0.0027	-31	-52	-11	14	41	37	52	<0.0001	-0.877	-1.201	-0.553			
Polar/alpine	1	3	134	246	246	0.0001	-112	-169	-54	1	3	20	40	NS	-0.723	-2.505	1.059			
Dry	1	2	10	7	7	NS	3	-67	73	1	2	73	39	NS	1.218	-0.397	2.833			
Various <sup>4</sup>	3	4	121	95	95	NS	16	-40	72	4	9	49	56	NS	-0.327	-0.895	0.241			

Abbreviations: 95% CI, 95% confidence intervals; CON, conventional; N, number of publications used for extraction of data; n, number of comparisons/datasets extracted from publications; OR, organic.

\*MD and OR values <0 and >0 indicate a higher concentration or higher % positive samples in organic and conventional cereal grains/products, respectively.

<sup>1</sup>Results obtained with the random effects model used for the meta-analyses presented in Tables 1 and 3.

<sup>2</sup>Means reported were from two or more cereal species.

<sup>3</sup>Means reported were from two or more types of product types.

<sup>4</sup>Means reported were from samples collected in two or more climate zones.

**TABLE 6** Effects of study types, cereal species, product types, and climatic conditions on differences in ochratoxin A (OTA) concentrations and proportion of samples testing positive for OTA found in organic and conventional cereal grain and processed cereal products; results are from multilevel, weighted meta-analyses models for mean differences (MD), and odds ratios (OR); MD and OR values <0 and >0 indicate a lower concentration or % positive samples in organic and conventional cereal grains/products, respectively.

Analyses/Factors	N	n	Results of weighted meta-analyses to determine mean differences (MD) between mycotoxin concentrations					Results of weighted meta-analyses to determine odds ratios (OR) for the proportions of contaminated samples								
			Concentration ( $\mu\text{g}/\text{kg}$ )		p-value	MD	95%	CI	N	n	% Positive samples		p-value	OR	95%	CI
			ORG	CON							ORG	CON				
<b>Random effect<sup>1</sup></b>	19	70	1.55	0.90	NS	0.178	-0.039	0.395	18	67	52	41	<b>0.0008</b>	0.519	0.215	0.822
<b>Multilevel</b>	19	70	1.55	0.90	NS	0.312	-0.181	0.804	18	67	52	41	<b>0.0455</b>	0.452	0.009	0.895
<b>Study type</b>																
Retail survey	13	59	1.15	0.76	NS	0.194	-0.043	0.430	11	57	57	46	<b>0.0025</b>	0.520	0.183	0.857
Farm survey	6	11	3.69	1.69	NS	0.091	-0.477	0.658	7	10	21	17	NS	0.524	-0.218	1.266
<b>Cereal species</b>																
Wheat	14	34	0.83	0.80	NS	0.100	-0.162	0.361	12	30	56	45	<b>0.0236</b>	0.462	0.062	0.863
Rye	5	15	3.01	1.30	NS	0.146	-0.544	0.835	5	18	72	59	<b>0.0243</b>	0.696	0.090	1.302
Oat	4	4	1.33	1.48	NS	-0.040	-0.900	0.820	5	5	36	37	NS	-0.244	-1.263	0.774
Barley	4	6	3.62	1.35	NS	-0.150	-0.849	0.550	5	5	4	7	NS	0.138	-1.234	1.511
Rice	4	8	1.00	0.30	<b>0.0007</b>	1.036	0.439	1.634	3	7	36	2	<b>0.0001</b>	2.188	1.095	3.281
Various <sup>2</sup>	3	3	0.02	0.11	NS	-0.124	-0.991	0.744	2	2	12	50	NS	-0.846	-2.275	0.582
<b>Product type</b>																
Grain	10	32	2.02	1.07	NS	0.053	-0.315	0.420	11	32	43	31	<b>0.0035</b>	0.665	0.219	1.112
Flour	8	22	1.38	1.14	0.0919	0.302	-0.049	0.652	6	22	74	64	<b>0.0183</b>	0.543	0.092	0.994
Processed food	4	10	1.29	0.29	NS	0.429	-0.198	1.057	4	10	23	39	NS	0.512	-0.259	1.284
Baby food	2	6	0.10	0.14	NS	-0.046	-0.604	0.512	1	3	47	18	<b>0.0334</b>	-1.644	-3.158	-0.129
Not specified <sup>3</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Climate type</b>																
Temperate	8	20	0.92	1.01	NS	0.039	-0.297	0.375	7	15	31	34	NS	0.030	-0.581	0.641
Continental	6	33	2.05	1.00	NS	0.068	-0.271	0.406	6	35	67	55	<b>0.0040</b>	0.639	0.204	1.074
Arid	1	1	0.69	0.18	NS	0.514	-0.876	1.904	1	1	45	15	NS	1.534	-0.539	3.607
Various <sup>4</sup>	5	16	1.36	0.61	<b>0.0115</b>	0.606	0.136	1.076	5	16	39	20	<b>0.0291</b>	0.681	0.069	1.292

Abbreviations: 95% CI, 95% confidence intervals; CON, conventional; N, number of publications used for extraction of data; n, number of comparisons/datasets extracted from publications; ORG, organic;

<sup>1</sup>MD and OR values <0 and >0 indicate a higher concentration or higher % positive samples in organic and conventional cereal grains/products, respectively.

<sup>2</sup>Results obtained with the random effects model used for the meta-analyses presented in Tables 1 and 3.

<sup>3</sup>Means reported were from two or more cereal species.

<sup>4</sup>Means reported were from samples collected in two or more climate zones.

weighted meta-analyses and the two sensitivity analyses produced very similar results for DON (Tables 4 and 5 and Table S9).

The unweighted meta-analysis (= sensitivity analysis 1) detected significantly higher concentrations of DON ( $p = 0.0001$ ), HT-2/T2 ( $p = 0.0061$ ), and ZEA ( $p = 0.0001$ ) in conventional and significantly higher concentrations of ENN ( $p = 0.0292$ ) in organic cereal grain/products (Table 4). Unweighted meta-analyses were based on a larger number of comparative studies and datasets, primarily because a relatively large proportion of published articles did not include information on measures of variation and data from these studies could therefore not be used in weighted meta-analyses. Specifically, unweighted meta-analyses were based on 175, 44, 59, 64, 38, 12, and 12 comparative datasets, while weighted meta-analyses were based on only 91, 13, 25, 47, 11, 12, and 7 comparative datasets for DON, NIV, HT-2/T-2, ZEA, ENN, FUM, and BEA concentrations, respectively (Tables 3 and 4).

Sufficient data to analyze changes in *Fusarium* mycotoxin contamination levels over time were only available for DON and ZEA (Tables 3 and 4 and Figure S10). Results suggest that the mean concentrations of DON have decreased between the 2004–2009 and the 2016–2020 period (Table 2 and Figure S10). Also, when DON and ZEA incidence and concentrations in organic and conventional cereal grains/products recorded in the most recent time period (2016–2020) were compared, no significant effect of production systems was detected by both weighted and unweighted meta-analyses (Tables 2–4).

Sufficient data for the use of multilevel meta-analysis models to assess effects of study type, cereal species, product type, climatic zone, country, funding source, and mycotoxin analysis method on *Fusarium* mycotoxin levels were only available for DON (Table 5 and Tables S7 and S9). Multilevel, weighted meta-analyses detected significantly higher concentration and/or incidence of DON in conventional compared with organic cereal samples only when data from (i) retail and farm surveys (but not field experiment), (ii) wheat and rye (but not oats, barley, and rice) grain/products, (iii) cereal grain and processed cereal products other than flour and baby food, (but not flour and baby food products), (iv) temperate, continental, and polar/alpine (but not dry) climates, (v) Austria, Belgium, the Czech Republic, Germany, Italy, and Poland (but not Switzerland, Spain, Finland, France, Great Britain, Croatia, Ireland, Korea, Latvia, the Netherlands, Slovenia, and Slovakia), (vi) studies which received public funding only (but not industry co-sponsored studies), and (vii) studies that used liquid chromatography (but not ELISA or gas chromatography) were compared (Table 5 and Tables S7 and S9).

### 3.2.2 | Mycotoxins produced by *Aspergillus/Penicillium* molds

When data from all years were used for standard, random effect model odds ratio meta-analyses, the incidence (% of samples testing positive) of OTA contamination was found to be significantly higher, while the incidence of AFL contamination was significantly lower in organic compared with conventional cereal grains/products (Table 2). The standard multilevel model odds ratio meta-analysis also detected a higher incidence of OTA contamination in organic cereals (Table 6).

Both, the random effect and multilevel model weighted, and the unweighted mean difference meta-analyses detected no significant effects of production system on OTA and/or AFL concentrations (Tables 3, 4, and 6). However, heterogeneity was found to be high for both the odds ratio and mean difference-based weighted meta-analyses of OTA and AFL data (Tables 2 and 3).

Sufficient data to analyze changes in *Aspergillus/Penicillium* mycotoxin contamination over time were only available for OTA (Tables 2–4). When data from studies published before 2004 were compared, weighted OR meta-analyses detected a significantly higher OTA incidence in organic compared with conventional cereal grains/products (Table 2). However, no significant effect of production system on OTA incidence was detected in the three more recent time periods (2004–2009, 2010–2015, and 2016–2020) (Table 2). Weighted MD meta-analyses detected significantly higher estimated OTA concentrations in organic compared with conventional cereal grains/products in only one time period (2010 and 2015) (Table 3). Unweighted meta-analysis found no significant effect of production system on concentrations of OTA in all four time periods (Table 4). OTA concentrations slightly decreased in organic and slightly increased in conventional cereals/cereal products between the “before 2004” and the “2010–2015” time period, but more than doubled (to  $\sim 2.5 \mu\text{g}/\text{kg}$ ) between the “2010–2015” and the 2016–2020 time period in both organic and conventional cereal/cereal products (Tables 2 and 3 and Figure S12). The exact reasons for the relatively high mean OTA concentrations reported for both organic and conventional samples in the most recent time period (2016–2020) remain unclear and this warrants further study. However, it should be noted that all studies that reported very high mean OTA concentrations ( $>6 \mu\text{g}/\text{kg}$ ) were published before 2010.

Sufficient data for use of multilevel meta-analysis models to assess effects of study type, cereal species, product type, climatic zone, country, funding source, and mycotoxin analysis method on *Aspergillus/Penicillium* mold



mycotoxin contamination were only available for OTA (Table 6 and Tables S8 and S10).

Multilevel, weighted mean difference meta-analyses detected significantly higher concentration of OTA in organic compared with conventional cereal grain/products only when data from (i) rice (but not wheat, rye, oats, and barley), (ii) Spain (but not any of the other countries), and (iii) studies which received public funding only (but not industry co-sponsored studies) were compared (Table 6 and Tables S8 and S10).

When results from retail and farm surveys were compared no significant difference in OTA concentrations between organic and conventional samples were detected in both retail and farm surveys, but mean concentration reported in farm surveys were 2–3 times higher than those reported in retail surveys (Table 6).

Multilevel, weighted odds ratio meta-analyses detected a higher incidence of OTA in organic compared with conventional samples when data from (i) wheat, rye and rice (but not oat and barley), (ii) grain, flour, and baby food (but not other processed foods), (iv) continental (but not temperate and arid) climates, (v) samples analyzed by liquid chromatography (but not ELISA), and (vi) studies which received public funding only (but not industry co-sponsored studies) were compared (Table 6 and Tables S8 and S10).

### 3.2.3 | Ergot mycotoxins produced by *Claviceps purpurea* and other *Claviceps* spp

Only two publications (Lauber et al., 2005; Malysheva et al., 2014) reported comparative data on ergot mycotoxin concentrations in conventional and organic cereal grains/products and meta-analyses were therefore not carried out. However, both studies reported higher ergot alkaloids contamination levels in conventional compared with organic rye grain samples (Lauber et al., 2005; Malysheva et al., 2014). It should be noted that the literature review also identified a survey of rye-based cereal products which reported that “*there were no apparent differences in the ergot levels between the organic and non-organic products*” (Crews et al., 2009). However, the authors did not publish information on mycotoxin incidence and concentrations in organic and conventional samples and therefore did not provide data that were suitable for meta-analyses.

## 3.3 | Strength of evidence

The overall assessment of the strength of evidence using an adapted GRADE approach (Guyatt et al., 2011) identified uncertainties in the evidence base, but there was

little evidence for publication bias and the overall reliability/strength of evidence was moderate for the majority of parameters. For example, for DON and OTA, the two mycotoxins with the largest evidence base (number of publications with suitable comparative data) the overall reliability was found to be moderate because, although the precision was high and there was no publication bias, the effect magnitude was small and the inconsistency in the data was high (Table S5 and Figures S2 and S8).

## 4 | DISCUSSION

According to FAO statistics, cereals are the main dietary source of carbohydrates, protein, and energy in human diets worldwide, although consumption amounts and cereal species/types consumed differ significantly between regions (Food and Agriculture Organization of the United Nations (FAO) (2022)). For example, cereals account for ~50% of total dietary energy intake in Africa and Asia, ~30% in Europe and the Americas, and ~23% in Oceania (Food & Agriculture Organization of the United Nations (FAO), 2022). Maize (which is not covered by this review) accounted for 12% of global crop production (1.2 billion tons), with a large proportion (~65%) being used as livestock feed (FAO, 2022). However, maize is also an important staple food, in particular in South America and Africa, and in North America and Europe the increase in gluten free consumption has also resulted in an increased use of maize-based food products (Carvajal-Moreno, 2022). Rice and wheat, the main small-grain cereals used for human consumption, each accounted for 8% (0.8 billion tons) of global crop production in 2020 (FAO, 2022).

Small-grain cereals (including wheat, rice, barley, rye, and oats) are also known to be important dietary source for (i) protein with a good nutritional value and (ii) nutritionally desirable fiber, phytochemicals (e.g., phenolics) and mineral micronutrients (e.g., Cu, Fe, Se, and Zn) if consumed as wholegrain (Poutanen et al., 2022; Wang, Chatzidimitriou et al., 2020).

Substituting animal products/protein (especially red meat) with cereal and legume grain/protein consumption is increasingly recognized as an important component strategy toward more sustainable and healthy diets, because it has the potential to simultaneously (i) reduce greenhouse emissions and other negative environmental impacts from agriculture, (ii) address the negative health effects of Western diets, and (iii) improve global food security (Hasanaliyeva et al., 2023; Poutanen et al., 2022). For example, recent analyses estimated that the production of 100 g of cereal protein requires ~5 m<sup>2</sup> of land and is associated with greenhouse gas (GHG) emissions equivalent to

~3 kg CO<sub>2</sub>, while the production of 100 g of beef protein requires 164 m<sup>2</sup> of land and is associated with GHG emissions equivalent to 50 kg CO<sub>2</sub> (Ritchie & Roser, 2024 March 1). Adopting this approach is of particular importance in North America, Europe, and Oceania, where animal products (i) currently account for ~60% dietary protein intake, which is double the global average and a major contributing factors to the increase in obesity and associated chronic diseases (e.g., metabolic syndrome and coronary heart disease) in these regions (FAO, 2020).

It is interesting to note that the difference in land area required and GHG emissions between conventional and organic cereal production is negligible compared with the differences between cereal and livestock production described above. For example, recent systematic reviews and meta-analyses reported that organic cereal yields are ~25%–30% lower compared with intensive conventional systems (de Ponti et al., 2012; Seufert et al., 2012), which would mean that organic farming would require 25%–30% more land (6.25–6.5 m<sup>2</sup>/100 g cereal protein) compared with conventional production, if the global estimate of land requirement for cereal production (~5 m<sup>2</sup>/100 g) (Ritchie & Roser, 2024) is assumed to be the land requirement for conventional production. Similarly recent lifecycle analysis-based estimates of GHG emissions per unit production in UK organic and conventional cereal and livestock production systems, found no significant difference between production systems for GHG emission per unit production for all types of livestock and oat crops, but slightly lower GHG emissions for organic compared with conventional milling wheat, triticale, and rye production (Smith et al., 2019).

One of the main challenges of moving toward more sustainable and healthy diets based on replacing animal product/protein with small-grain cereal grain/protein consumption is the risk that this will also increase mycotoxin intakes (Hasanalieva et al., 2023; Thielecke & Nugent, 2018).

To ensure that mycotoxin levels used for human consumption are below the MCL set by regulatory agencies, it is now common practice to test all batches of cereals delivered by farmers and traders to commercial grain storage and processing facilities for a range of indicator mycotoxin compounds (e.g., DON and OTA) (Wang, Hasanalieva, Wood, Markellou et al., 2020). It is also common practice that cereal with mycotoxin levels that are above the MCL is used as animal feeds (Bernhoft et al., 2022). Cereal processors may also blend cereal batches with no detectable or very low mycotoxin concentrations with cereal batches that are above the MCL in order to increase the amount of grain below the MCL available for processing into food products, but there is no information on how common this practice is.

Globally, approximately 40% of total cereal production is currently processed into food products while ~35% of grain is used as animal feed (FAO, 2022). However, it is important to consider that (i) the majority of small-grain cereals used for human consumption is refined/milled to remove the outer germ and bran layers of the grain, (ii) the bran/germ fraction contains most of the nutritionally desirable fiber, phytochemicals, and minerals, and (iii) although protein is more uniformly distributed in cereal grains, the bran/germ fraction removed by refining accounts for 25%–30% of the grain volume and is primarily used as animal feed (McKevith, 2004; Poutanen et al., 2022; Shewry & Halford, 2002).

In order to facilitate the recommended change to more sustainable cereal grain-based diets at the global level, a larger proportion of cereal production would have to be used as food and therefore have mycotoxin concentrations below the MCLs set for food. It is difficult to assess to what extent this is feasible, because there are to our knowledge no reliable estimates of the proportion of harvested cereal crops that (i) are rejected for food use due to mycotoxin contamination above the MCLs and instead used as animal feed and that (ii) are used as animal feed, but could potentially be used in the human food supply chain because it has below MCL mycotoxin concentrations and fulfils other quality and safety norms set by cereal processors. However, reports that grain processors already resort to blending grain batches to generate enough cereal grain with mycotoxin levels below the MCL suggests that this may be a significant challenge at least in some regions.

The meta-analyses reported here found substantially higher mean DON and OTA concentrations in grain samples collected in farm surveys compared with cereal products samples collected in retail surveys and this also indicates that post-harvest quality assurance protocols remove a substantial proportion of harvested grain because mycotoxin levels are deemed too high, as previously suggested (Bernhoft et al., 2022; Wang, Hasanalieva, Wood, Markellou et al., 2020).

The main strategy to increase the proportion of cereals available for human consumption should therefore be to reduce the proportion of cereals that have mycotoxin concentrations above the MCL via (i) breeding/selection of cereal genotypes with increase resistance against mycotoxin-producing fungi, (ii) use of agronomic practices that reduce the risk of fungal infection and mycotoxin production, (iii) improvements of the facilities used for grain drying and storage (thought to be of particular importance in low-income countries), and (iv) development and use of physical, thermal, and chemical processing methods that break down or detoxify mycotoxins (Table 1).

There have been claims that organic food consumption will increase mycotoxin exposure, because organic farming

systems prohibit the use of fungicides for crop protection, although they provided no conclusive evidence to substantiate this theory (Gomiero, 2018; Trewavas, 2001, 2004). However, there is increasing evidence that following recommendations to increased whole-grain consumption will also increase dietary mycotoxin intake (Thielecke & Nugent, 2018; Wang, Hasanalieva, Wood, Markellou et al., 2020). This may indirectly lead to higher mycotoxin intakes by organic food consumers, who have been reported to have “healthier” diets, with higher consumption of fruit, vegetable, and whole-grain products (Baudry et al., 2015; Eisinger-Watzl et al., 2015; Rempelos et al., 2021, 2023; Wang, Hasanalieva, Wood, Markellou et al., 2020). Since there have been, to our knowledge, no systematic literature reviews and meta-analysis based on the whole available evidence, there is still considerable controversy and uncertainty about the effects of agricultural production methods and post-harvest quality assurance protocols used in organic and conventional production on mycotoxin contamination in cereal grains/products.

The systematic literature review and standard model-based meta-analyses reported here, were therefore designed to address the controversy/uncertainty about whether and to what extent the contrasting agricultural production protocols used in conventional and organic production systems affect the incidence and concentrations of mycotoxins in cereal grains/products (see discussion section entitled *Effects of cereal production system (organic vs. conventional)* later).

The multilevel model-based meta-analyses were designed to identify potential explanatory factors for the large variation in outcomes reported in the literature. This focused on identifying potential confounding effects of (i) cereal species/variety, (ii) climatic background conditions, and (iii) post-harvest grain processing and QA protocols (results are discussed in separate sections later). A discussion of (i) *Strategies to reduce mycotoxin contamination in organic and conventional cereals*, (ii) *Potential impacts of the meta-analyses results*, (iii) *Relevance for livestock health and product quality*, and (iv) *Study limitations* is also provided in separate sections later.

## 4.1 | Effects of cereal production system (organic vs. conventional)

### 4.1.1 | *Fusarium* mycotoxins

The findings of higher incidence and/or concentrations of DON and other *Fusarium* mycotoxins concentrations in conventional samples are broadly consistent with most previous qualitative literature reviews (Benbrook, 2005; Bernhoft et al., 2022; Brodal et al., 2016; Gottschalk et al.,

2007) and the only previous meta-analysis of comparative DON contamination data in wheat (Smith-Spangler et al., 2012), which reported significantly higher levels of DON in conventional compared with organic wheat samples (SMD  $-0.94$ ;  $p < 0.01$ ;  $I^2 = 63$ ;  $n = 7$ ).

However, it is important to consider that modified mycotoxins were not monitored in any of the comparative studies included in meta-analyses. Modified (also described as “masked”) mycotoxins have been defined as “*mycotoxin derivatives whose structure changes in plants and cannot be detected by conventional analytical techniques*” and their global occurrence, major transformation mechanisms and analysis methods have recently been reviewed (Lu et al., 2020). Modified forms are known for all major *Fusarium* mycotoxins including DON, T-2, HT-2, ZEN, and FUM, many modified mycotoxins (e.g., sulfate and glucoside conjugates of the parent compounds) are the result of plant detoxification processes and the majority of modified mycotoxins are considered less toxic than their parent forms (Lu et al., 2020). However, cereal processing (e.g., heat, enzyme, or acid/alkaline treatments) and/or hydrolysis in the digestive tract may result in modified forms being converted back into their parent form or compounds with similar toxicity to the parent compounds (Lu et al., 2020). Since the ratios of parent to modified mycotoxin concentrations can vary significantly, there is ongoing research effort to develop analytical methods/protocols for modified mycotoxins, which are not currently assessed as part of standard regulatory mycotoxin monitoring programmes and quality assurance protocols used by cereal storage, processing, and marketing companies (EFSA, Knutsen, Alexander, Barregård, Bignami, Brüschweiler, Ceccatelli, Cottrill, Dinovi, Edler et al., 2017; Lu et al., 2020), see also the discussion section on “Study limitation” later).

A unique, recent study compared concentrations of “free” (= parent) and modified *Fusarium* mycotoxins (DON, H-2, HT-2, and NIV) in oat grain from organic and conventional production systems (Daud et al., 2023)]. Results confirmed previous studies by showing that concentration of free mycotoxins were higher than the respective modified forms and that the ratio of free/modified mycotoxin concentrations differed between compounds (e.g., was greater for H-2 and HT-2 than DON). The study also reported for the first time that the ratios of free/modified mycotoxins were similar in organic and conventional cereals. If future studies confirm that production system does not significantly affect the proportions of free/modified mycotoxins being present in cereals, it would validate the results of the meta-analyses reported here with respect to the relative difference in *Fusarium* mycotoxin concentrations between organic and conventional cereals.

Results of the meta-analyses reported here are also consistent with the most recently published retail surveys of wheat flour (Wang, Hasanalieva, Wood, Markellou et al., 2020) carried out in Germany and the United Kingdom, which suggested that, although *Fusarium* mycotoxin levels are higher in conventional wheat flour, concentrations in both organic and conventional flour are ~10 times lower than the MCLs set by the EU and pose no health risks to consumers. Results from these retail surveys also suggest that consumption of wholegrain instead of refined cereal products would result in a significantly higher exposure to DON and H-2/HT-2 (a *Fusarium* mycotoxin with relatively high toxicity compared with DON) with conventional flour, but not with organic wheat flour (Figure 1) (Wang, Hasanalieva, Wood, Markellou et al., 2020).

Results of the meta-analyses also suggest that DON contamination has decreased over time and that DON concentrations were substantially lower in retail compared with farm surveys. This may be, at least partially, due to (i) the introduction of more stringent mycotoxin-testing-based QA systems (e.g., in response to growing concerns about negative health impacts and the introduction of MCLs by the EU) and/or (ii) grain processing (especially refining) methods used for cereals destined for human consumption.

These findings are consistent with previous studies which reported lower *Fusarium* mycotoxin contamination in refined compared with wholegrain flour (Thielecke & Nugent, 2018; Wang, Hasanalieva, Wood, Markellou et al., 2020) or grain samples collected in farm surveys compared with samples of processed cereal products collected in retail surveys (Bernhoft et al., 2022).

Information on the agronomic factors that may have contributed to the higher *Fusarium* mycotoxin contamination in conventional cereals has recently been reviewed (Bernhoft et al., 2022) and is therefore only summarized later (Table 7). Most studies into the effects of climatic and agronomic parameters on *Fusarium* mycotoxin contamination in cereal grain have focused on wheat, because modern common and durum wheat varieties are more susceptible to *Fusarium* head blight (FHB) and associated mycotoxin contamination, compared with other small-grain cereal species (e.g., rye, triticale, and oats) used for human consumption (Gaikpa et al., 2020; Wegulo et al., 2011). Briefly, the use of more diverse rotations, which is prescribed by organic farming standards, is known to reduce *Fusarium* disease pressure and mycotoxin risk by minimizing the build-up of soil/crop residues and breaking the life cycle of fungal pathogens (Table 7). In contrast, cereal monocultures and especially growing cereals such as wheat after maize or rice, practices which are widely used in conventional but not organic production systems, substantially increases the risk of *Fusar-*

*ium* head blight and mycotoxin contamination (Table 7). Other agronomic management practices that were shown to result in an increased risk of *Fusarium* grain infection and mycotoxin contamination and are exclusively or more widely used in conventional farming systems include (i) high inputs of mineral nitrogen fertilizer, (ii) use of minimum or no tillage systems, and (iii) the use of modern, short-straw varieties (Table 7). There is also evidence that the use of certain fungicides (e.g., strobilurins) may increase mycotoxin production by *Fusarium* spp. due to stress imposed on the fungal pathogen and/or by augmenting competition from commensal (e.g., *Cladosporium*, *Itersonilla*, and *Holtermanniella* spp.) (Rojas et al., 2020) or other plant pathogenic fungi (e.g., *Septoria* spp. and *Microdochium nivale*) (Duba et al., 2018; Simpson et al., 2001) on cereal leaves and/or grain (Table 7).

It is important to note that climatic parameters are known to interact with and augment the effects of agronomic factors (Bernhoft et al., 2022; Magan et al., 2002) and that this may explain the large variation observed between and within studies for the incidence and concentrations of *Fusarium* mycotoxin contamination in organic and conventional cereals/cereal products. For example, in seasons with climatic conditions that increase the risk of lodging (strong winds together with rain after tillering), it is likely that (i) the use of short-straw varieties and growth regulators in conventional production will reduce *Fusarium* infection by minimizing lodging, while (ii) the use of longer-straw varieties in organic systems will increase the risk of lodging and *Fusarium* grain infection. In contrast, in seasons with dry, calm conditions after tillering, it is likely that the use of short-straw varieties and growth regulators in conventional systems will increase, while the use of longer-straw varieties in organic systems will reduce the risk of *Fusarium* grain infection and mycotoxin contamination (Bernhoft et al., 2022; Buerstmayr et al., 2020, 2021; Supronienė et al., 2012). However, further research is required to gain a better understanding of the interactions between climatic conditions and contrasting agronomic practices used in organic and conventional systems and their impact on *Fusarium* mycotoxin contamination.

The finding of (i) a higher incidence of ENN and BEA (in the weighted OR meta-analysis) and (ii) higher concentrations of ENN (in the unweighted meta-analysis) in organic cereals should to be interpreted with caution, because these compounds were only assessed in a small number ( $\leq 5$ ) of studies (Table 2). Also, there are currently no MCLs set by regulators for both ENN and BEA (Table 1), because, compared with other *Fusarium* mycotoxins, there is very limited information about their toxicity and potential health impacts (Prosperini et al., 2017; Wu et al., 2018) (Table 1). For example, the EFSA CONTAM Panel reported

**TABLE 7** Reported effects of climatic, primary production/agronomic, and post-harvest/processing factors on mycotoxin contamination risk in wheat that may explain the variability observed between and within studies included in the meta-analyses.

Factors	Effect on mycotoxin contamination risk	Mechanism suggested by authors	Reference
<b>Seed used</b>			
Untreated, infected seed; farm-saved seed	↑ FM, ERG	Seed used introduces fungal inoculum that infects grain of the next crop	Gonçalves et al. (2019), AHDB, 2021
Certified seed	↓ ERG	Seed tested for seed-borne diseases	AHDB 2021
Fungicide seed treatment	↓ FM	Reduction of fungal inoculum present on seed	Gonçalves et al. (2019)
<b>Climate</b>			
Rainfall during flowering <sup>1</sup>	↑ FM	Increased <i>Fusarium</i> flower infection	AHDB (2021); Cheli et al. (2017)
Rainfall before harvest	↑ FM	Increased <i>Fusarium</i> grain colonization	AHDB (2021); Cheli et al. (2017)
<b>Rotation design</b>			
Wheat grown before wheat	↑ DON, NIV	High <i>Fusarium</i> inoculum on pre-crop residues	Krebs et al. (2000); Wenda-Piesik et al. (2017)
Maize grown before wheat	↑ DON, ZEA	High <i>Fusarium</i> inoculum on pre-crop residues	Krebs et al. (2000); Birzele et al. (2002); Pirgozliev et al. (2003); Edwards & Jennings (2018); Vogelgsang et al. (2019); AHDB (2021)
Rice grown before wheat	↑ DON, ZEA	High <i>Fusarium</i> inoculum on pre-crop residues	Qiu et al. (2016)
Broad leaf crops grown before wheat	↓ DON, NIV	No or low <i>Fusarium</i> inoculum on pre-crop residues	Wenda-Piesik et al. (2017); Vogelgsang et al. (2019);
Biofumigant break, cover and inter-crops	↓	Reduction of <i>Fusarium</i> inoculum after maize crops	Drakopoulos, Gimeno et al. (2021), Drakopoulos, Kägi, et al. (2021)
<b>Tillage</b>			
Minimum or no tillage <sup>2</sup>	↑ DON, ZEA	High proportion of crop residues and associated <i>Fusarium</i> inoculum remains on soil surface	Dill-Macky & Jones (2000); Krebs et al. (2000); Oldenburg et al. (2007); Edwards & Jennings (2018); Vogelgsang et al. (2019)
Minimum or no tillage	↓ DON	Lower tiller/stem density resulting in better aeration/lower humidity within the crop canopy	Supronienė et al. (2012)
<b>Lodging</b>	↑ DON, NIV	<ul style="list-style-type: none"> <li>Cereal heads get closer to fungal inocula on the soil surface</li> <li>Increased humidity/moisture in the crop canopy</li> </ul>	Nakajima et al. (2008); Bernhoft et al. (2012); Bernhoft et al. (2022)
<b>High mineral N fertilizer inputs</b>	↑ DON, ZEA	<ul style="list-style-type: none"> <li>Delayed flag leaf senescence/grain maturity</li> <li>Reduced foliar disease resistance</li> </ul>	Heier et al. (2005); Bernhoft et al. (2012); Supronienė et al. (2012); Scarpino et al. (2022) Rempelos et al. (2021); Rempelos et al., 2023)

(Continues)

TABLE 7 (Continued)

Factors	Effect on mycotoxin contamination risk	Mechanism suggested by authors	Reference
<b>Crop protection</b>			
<i>Fusarium</i> -specific fungicides <sup>3</sup>	↓ FM	Inhibition of <i>Fusarium</i> infection/growth	Wegulo et al. (2011), Poole & Arnaudin (2014); Shah et al. (2018); Wenda-Piesik et al. (2017); AHDB (2021); Moraes et al. (2022)
Herbicide use for weed control	↓ FM ↑ FM	Reduction of <i>Fusarium</i> inoculum <sup>4</sup> Increased <i>Fusarium graminearum</i> infection	Nugmanov et al. (2018); Bernhoft et al. (2012)
Strobilurin and other fungicides <sup>5</sup>	↑ DON, NIV	<ul style="list-style-type: none"> <li>Removal of competition from commensal or other plant pathogenic fungi on leaves/grain</li> <li>Stress responses by <i>Fusarium</i> species</li> </ul>	Felix D'Mello et al. (1998) Simpson et al. (2001) Köpke et al. (2007); Magan et al. (2002); Müllenborn et al. (2008)
<b>Growth regulators</b>			
Chlormequat <sup>6</sup>	↑ ZEA	<ul style="list-style-type: none"> <li>Delayed flag leaf senescence/grain maturity</li> <li>Reduced plant height</li> </ul>	Supronienė et al. (2012); Mankevičienė et al. (2008)
Trinexapac-ethyl, Etefon	↑ DON, ZEA, T-2	<ul style="list-style-type: none"> <li>Delayed flag leaf senescence/grain maturity</li> <li>Reduced plant height</li> </ul>	Suproniene (2006)
<b>Variety/species choice</b>			
Use of FHB-resistant modern varieties	↓ FM	Reduction in <i>Fusarium</i> infection/grain colonization <sup>6</sup>	Rudd et al. (2001); Rocha et al. (2005); Wegulo et al., 2011; Edwards & Jennings (2018); Gaikpa et al. 2020; AHDB (2021); Buerstmayr et al. (2020, 2021); Bernhoft et al. (2022); Moraes et al. (2022)
Use of longer-straw wheat varieties or species <sup>5</sup>	↓ FM	<ul style="list-style-type: none"> <li>Less grain infection via rain splash from crop residue inoculum on the soil surface</li> <li>Reduced leaf wetness periods and humidity in the crop canopy</li> </ul>	Oldenburg et al. (2007); Gaikpa et al. 2020; Buerstmayr et al. (2020, 2021); Rempelos et al. (2023); Rempelos et al. (2021); Bernhoft et al. (2022)
<b>Grain harvest/processing/storage</b>			
Delay of harvest or grain drying after harvest	↑ DON, ZEA	Longer time period for <i>Fusarium</i> infection/growth in grain tissues; increased risk of secondary infection by common mold fungi	Birzele et al. (2000); Magan et al. (2010); Cheli et al. (2017); Edwards & Jennings (2018)
Physical decontamination	↓ ERG	Removal of ergot sclerotia	Cheli et al. (2017);
Grain drying immediately after harvest	↓ DON, ZEA, OTA, AFL	Inhibition of fungal growth and mycotoxin synthesis in grain	Birzele et al. (2000); Magan & Aldred (2005, 2007); Cheli et al. (2017)
Poor on-farm storage facilities	↑ OTA, AFL	Increased fungal growth and mycotoxin production	Jorgensen & Jacobsen (2002); Food Standards Agency (2007); Magan & Aldred (2007)

(Continues)

TABLE 7 (Continued)

Factors	Effect on mycotoxin contamination risk	Mechanism suggested by authors	Reference
Optimized storage conditions (humidity, temperature, CO <sub>2</sub> )	↓ FM, OTA, AFL	Inhibition of fungal growth and mycotoxin production	Food Standards Agency (2007); Cheli et al. (2017); Magan et al. (2010); Gonçalves et al. (2019),
Pest infestation/damage <sup>9</sup>	↑ OTA, AFL	Increased fungal growth	Magan et al. (2010); Cheli et al. (2017);
Refining of grains	↓ FM, OTA, AFL	Removal of mycotoxins present on outer layers of the grain <sup>10</sup>	Magan et al. (2010); Cheli et al. (2017); Schaarschmidt & Faulstich (2018); Wang et al. (2020)

Abbreviations: ↑, described to increase in mycotoxin contamination; ↓, described to decrease in mycotoxin contamination; AFL, aflatoxins; DON, deoxynivalenol; FHB, *Fusarium* head blight; FM, *Fusarium* mycotoxins; NIV, nivalenol; OTA, ochratoxin A; T2, T2-toxin; ZEA, zearalenone.

<sup>1</sup> GS59-GS69.

<sup>2</sup> Especially when maize or other cereals were used as previous crops.

<sup>3</sup> Application of a fungicide with activity against mycotoxin-producing *Fusarium* spp., ideally during early flowering stages (GS63-GS65).

<sup>4</sup> Grass weeds are alternative hosts for *Fusarium* spp. and may allow overwintering of *Fusarium*.

<sup>5</sup> Other pesticides without activity against *Fusarium* spp. were also reported to increase *Fusarium* mycotoxin contamination (Felix D'Mello et al., 1998).

<sup>6</sup> Plant growth regulator used to reduce stem length and lodging risk.

<sup>7</sup> Fungicides treatments often provide only partial and or variable control of FHB (Bernhofs et al., 2022).

<sup>8</sup> For example, spelt wheat (*Triticum spelta*).

<sup>9</sup> Especially if combined with high humidity/moisture during grain storage.

<sup>10</sup> Fungal infestation levels and mycotoxin concentrations are usually higher near the grain surface and lower in the endosperm.

that “the most important contributors to the chronic dietary exposure to beauvericin and the sum of enniatins were grains and grain-based products” and the panel concluded that “acute exposure to beauvericin and enniatins do not indicate concern for human health” and “that there might be a concern with respect to chronic exposure but no firm conclusion could be drawn, thus relevant in vivo toxicity data are needed to perform a human risk assessment” (EFSA, 2014). It is interesting to note that BEA was recently shown to have an anticancer activity and is currently being investigated as a potential new cancer therapeutic (Wu et al., 2019).

#### 4.1.2 | Mycotoxins produced by *Aspergillus/Aspergillus* molds

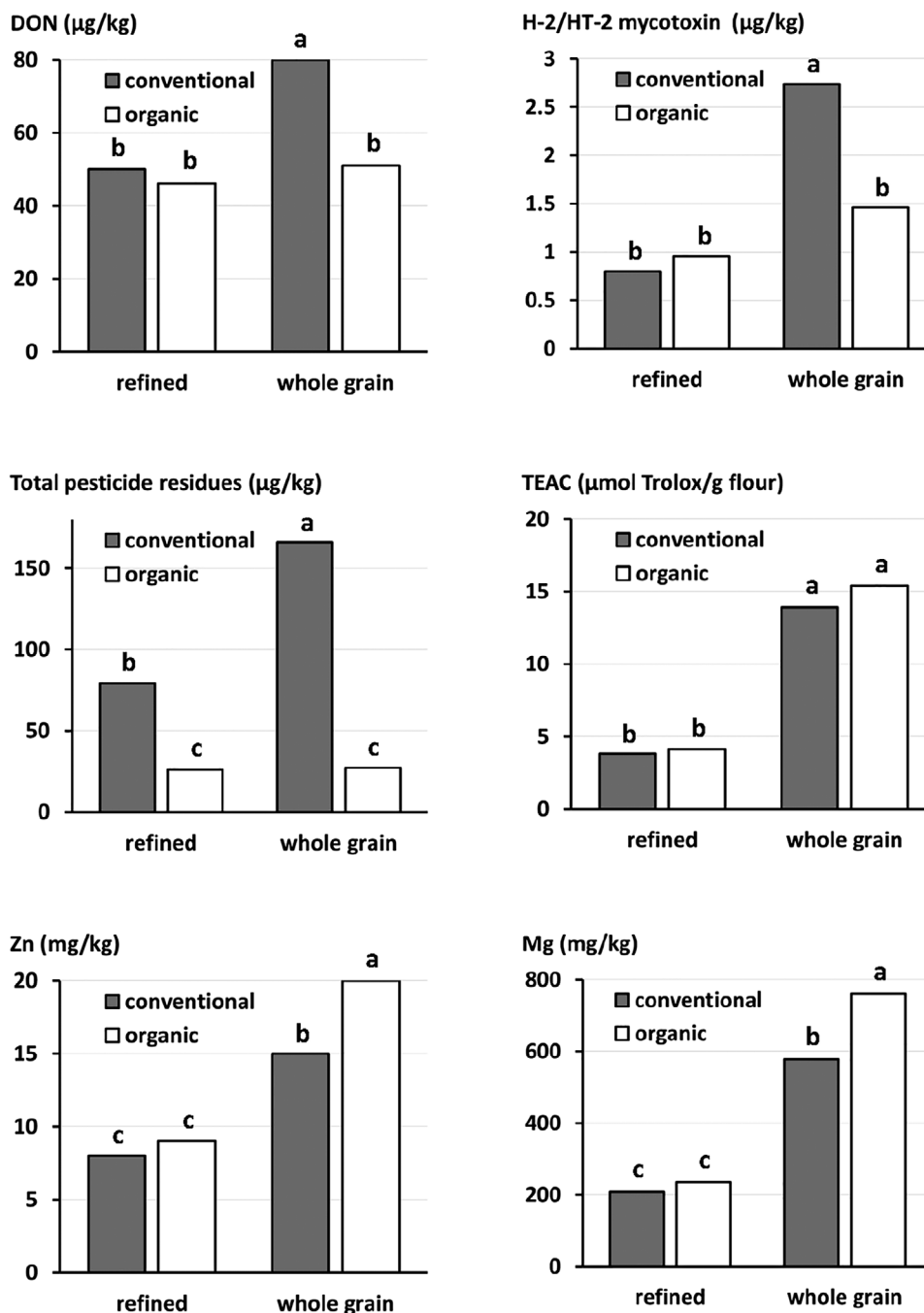
The overall trends found for *Aspergillus/Penicillium* mycotoxins (OTA and AFL) were different to those detected for *Fusarium* mycotoxins. When all available data were used for meta-analyses no significant effects of production systems on OTA and AFL concentrations were detected (Tables 3 and 4), although OTA was more frequently detected in organic, while AFLs were more frequently detected in conventional grain and cereal products (Table 2). However, a significantly higher incidence of OTA contamination in organic samples was detected when data collected before 2004 was compared and no significant difference in AFL concentrations between organic

and conventional comparators was detected in both the weighted and unweighted meta-analyses (Tables 3 and 4). It is important to note that modified forms of OTA and AFL were not monitored in any of the comparative studies included in meta-analyses (see also the discussion section on “Study limitations” later).

Agronomic practices (rotation, tillage, fertilization, and crop protection) used pre-harvest, are thought to have no or only small effects on mold mycotoxin contamination levels in cereals, but high OTA contamination has often been linked to delayed or inefficient drying of harvested grain and poor grain storage conditions (Magan & Aldred, 2005, 2007; Magan et al., 2010) (Table 7).

The importance of efficient post-harvest drying and storage of cereal grain for minimizing OTA contamination may also explain the finding that the incidence (% of samples testing positive) of OTA contamination was significantly higher in organic cereal grains/products only when comparative data published before 2004 were compared. This view is supported by several comparative studies published before 2004 (Jørgensen & Jacobsen, 2002; Jørgensen et al., 1996) and a review by Köpke et al. (2007), which concluded that the higher OTA contamination in organic grains was due to poor grain drying and/or storage facilities available on organic farms at that time.

Although analyses of more recent data did not detect significant effects of production system, it is important to highlight that meta-analyses of the most recent OTA data (collected between 2016 and 2020) showed that mean



**FIGURE 1** Effect of production system (organic vs. conventional) and processing (refined vs. whole grain) on concentrations of the *Fusarium* mycotoxins (deoxynivalenol and H-2/HT-2), pesticide residues, and the mineral micronutrients Mg and Zn, and antioxidant capacity/activity in wheat flour brands available in United Kingdom and German retail outlets. TEAC, antioxidant activity/capacity assessed using the TEAC-assay. Bars labeled with the same letter are not significantly different ( $p < 0.05$ ). Data are from Wang, Hasanalieva, Wood, Markellou et al. (2020), Wang, Chatzidimitriou, Wood, Hasanalieva et al. (2020), Wang, Hasanaliyeva, Wood, Anagnostopoulos et al. (2020).

OTA concentrations in both conventional and organic cereal grains were  $\sim 2.5 \mu\text{g kg}^{-1}$  and therefore close to the MCL set for older children and adults ( $3 \mu\text{g/kg}$ ) and 5 times higher than MCL for cereal-based foods and baby foods for infants and young children ( $0.5 \mu\text{g/kg}$ ) set by the EU for OTA (Table 1) (European Commission, 2006).

However, it is also important to consider that the comparative data available for baby food suggest that mean OTA concentrations were  $< 0.2 \mu\text{g/kg}$  in both organic and conventional samples and below the EU-MCL, which suggests that more stringent QA systems are applied to cereal-based baby foods. Also mean AFL concentrations in



both organic and conventional cereals were  $<0.5 \mu\text{g}/\text{kg}$  and substantially lower than the MCL of  $4 \mu\text{g}/\text{kg}$  set by the EU for total AFL contamination (Table 1).

The results reported for OTA in this study are consistent with the findings of the most recently published European retail survey, which concluded that maintaining OTA-contamination levels below the EU-MCL continues to be a challenge in Europe (Wang, Hasanalieva, Wood, Markellou et al., 2020). This should be of some concern to EU regulators, because OTA was shown to be nephrotoxic, immunosuppressive, carcinogenic, and teratogenic at relatively low concentrations in all animal species used for toxicity testing (Bui-Klimke & Wu, 2015; Walker, 2002). The relatively low thresholds set for OTA reflect the considerable health risks and especially cancer-promoting activity that were linked to even very small intakes of OTA (Bhat et al., 2010; Bui-Klimke & Wu, 2015; Peraica et al., 1999; Reddy et al., 2010; Walker, 2002).

A substantial proportion of cereals produced are currently rejected for use in human consumption due to high OTA levels and are instead used as livestock feed, which may have negative effects on animal health and product safety (Bui-Klimke & Wu, 2015; Imade et al., 2021; Kabak et al., 2006; Walker, 2002), see also discussions section on “*Relevance for livestock health and product quality*” later). It is therefore important to investigate how OTA levels can be further reduced by innovations in crop breeding, primary production protocols, and especially improvements in harvest and post-harvest grain drying protocols and storage technology to minimize carry-over effects into livestock production.

#### 4.1.3 | Ergot mycotoxins produced by *Claviceps purpurea* and other *Claviceps* spp

Ergotism (the oldest known mycotoxicosis) was a common acute mycotoxicosis in the middle ages (Agriopoulou, 2021). However, it is now very rare in Europe, primarily because a large proportion of ergot sclerotia (the source of ergot mycotoxins/alkaloids in cereal grain) can be removed from harvested cereals by seed cleaning machines based on photocells (Agriopoulou, 2021; Crews et al., 2009). These machines are now routinely used in most European countries for grain destined for both human consumption (Agriopoulou, 2021), but their use is not a standard practice in many low-income countries, and for cereals used as livestock feed (Agriopoulou, 2021; Imade et al., 2021). Cleaning and processing of cereal grain can only remove up to 82% of the sclerotia, and can therefore not completely eliminate contamination of cereals and high ergot alkaloid (EA) concentrations have occasionally been detected in Europe (Agriopoulou, 2021).

There was insufficient comparative data to compare ergot mycotoxin contamination in organic and conventional cereal grain/products by meta-analyses. This is a major gap in the evidence base, because EAs contamination in European cereals has recently increased and therefore remains a source of concern for human and animal health (Agriopoulou, 2021; AHDB, 2021).

#### 4.1.4 | Confounding effect of cereal species/variety

Due to the low number of studies and comparative datasets available for cereal species other than wheat and rye and the relatively large variation between studies results for DON and OTA contamination in oat, barley, and rice species need to be interpreted with caution and cannot be used to draw general conclusions on the effect of production system in these species.

However, it is interesting to note that differences in DON, and to a lesser extent, OTA concentrations between wheat and rye were greater than the differences found between organic and conventional grain of the same cereal species. This indicates that the contrasting numbers of studies/data being available for different cereal species (e.g., substantially more studies/data for wheat than minor cereal species) may explain some of the variations and may have confounded the overall differences found between organic and conventional cereals in the standard model-based meta-analyses.

Specifically, DON contamination was higher in wheat than rye and a significant effect of production system was only detected for wheat. This is consistent with the well-documented higher susceptibility of wheat to *Fusarium* infection compared with rye (Bernhoft et al., 2022; Mielniczuk & Skwaryło-Bednarz, 2020). The susceptibility to both *Fusarium* and *Claviceps purpurea* infection and associated mycotoxin risks differs considerably between cereal species. For example, maize and wheat are considered to be more at risk from *Fusarium* infection/mycotoxins than rye, barley, and oats (which have longer stems/straw than modern wheat varieties, and are produced with lower mineral N fertilizer inputs and without growth regulator applications in conventional production systems), while rye is most at risk from *C. purpurea* infection and ergot contamination (Agriopoulou, 2021; AHDB, 2002, 2016, 2018; Foroud & Eudes, 2009). There are also substantial differences in disease resistance between varieties of wheat, rye, and other cereals and varieties used in different countries/region of Europe (Magistrali et al., 2020; Rempelos et al., 2020; Tupits et al., 2022). For example, longer-straw wheat varieties are thought to be less susceptible to *Fusarium* infection/mycotoxin contamination (Foroud & Eudes,

2009; Köpke et al., 2007), unless lodging occurs, which increases the risk of (i) colonization of grains by *Fusarium*, *Aspergillus*, and *Penicillium* species and (ii) both DON and OTA contamination (Konvalina et al., 2016; Nakajima et al., 2008). The higher density of tillers/stems within the crop canopy of modern short-straw varieties generates a more humid microclimate, which is known to enhance *Fusarium* infection. Also, in short-straw varieties the ears and grains are closer to the soil surface and this is thought to facilitate a more rapid progress of infection from primary *Fusarium* inoculum on the soil surface to lower leaves, from leaf to leaf, and eventually from the flag leaf to the grain (Köpke et al., 2007). The use of plant growth regulators (e.g., chlormequat) is thought to exacerbate infection by further shortening the stem length (Köpke et al., 2007).

In contrast, OTA concentrations were higher in rye compared with wheat, but the reasons for this remain unclear. However, differences in agronomic methods and/or post-harvest processing and quality assurance protocols used for wheat and rye may have been at least partially responsible for the contrasting trends for DON and OTA contamination observed (Bernhoft et al., 2022; Mielniczuk & Skwaryło-Bednarz, 2020). It is also important to consider that data available for wheat and rye were from studies in a different group of countries/climatic zones; this may have confounded the differences in mycotoxin levels between cereal species, since climatic background conditions during the growing season and harvest are known to substantially affect mycotoxin levels (see section on “Confounding effects of climatic conditions” later).

#### 4.1.5 | Confounding effects of climatic conditions

Wet/humid and warm conditions during later growth stages, harvest, and storage are known to increase the risk of mycotoxin contamination of cereal grains. For example, several studies reported that higher temperatures, relative humidity, and rainfall during cultivation (and especially during flowering/anthesis) may significantly increase *Fusarium* mycotoxin contamination (AHDB, 2021; Cheli et al., 2017; Van Der Fels-Klerx et al., 2012). Specifically, temperatures between 15 and 30°C and relative humidity  $\geq 90\%$  between 10 days before and after anthesis are considered as high-risk conditions for FHB and *Fusarium* mycotoxin contamination in cereals and climatic conditions during this period are used in FHB prediction models (Matengu et al., 2023). In contrast, wet/humid conditions during grain harvest and storage are known to increase the risk of OTA and AFL contamination of cereal grains (Köpke et al., 2007).

The finding of substantially higher overall DON and OTA contamination in cereals grown in temperate and continental climates with higher rainfall/humidity levels compared with cereals grown in dry climates was therefore not surprising. However, it is interesting to note that significant higher DON concentrations in conventional compared with organic cereal samples were detected in studies from temperate, continental, and polar/alpine, but not dry climates, while no significant effect of production system on OTA concentrations was detected in studies from all climatic regions. Previous studies suggest that this may be explained by high rainfall and humidity during flowering being a major driver for *Fusarium* infection and DON contamination, but not *Aspergillus/Penicillium* infection and OTA contamination in cereals (Aldred & Magan, 2004; Barug et al., 2006; Bhat et al., 2010; Magan et al., 2010; Mielniczuk & Skwaryło-Bednarz, 2020).

Climate change may affect the overall risk of mycotoxin contamination, the profile of mycotoxins found in cereals and the relative difference in contamination between cereals from organic and conventional production systems; see reviews by Paterson and Lima (2010) and Perrone et al. (2020) for detailed descriptions of the potential effects of climate change on mycotoxin contamination risks. Overall, they predict that in areas which become dryer and hotter during the growing season mycotoxin contamination may decrease, while colder regions which become temperate and wet will experience greater problems associated with both *Fusarium* and *Aspergillus/Penicillium* mycotoxins. For example, an increase of temperatures in cold countries like Norway may cause an increase in both *Fusarium graminearum* and *Fusarium langsethiae* infections resulting in higher levels of DON and HT-2/T-2 contamination, respectively (Bernhoft et al., 2012).

#### 4.1.6 | Confounding effects of post-harvest grain processing and QA protocols

The setting of MCLs by the EU in 2006, has resulted in the development and widespread use of mycotoxin testing-based quality assurance protocols, which involves the analysis of all cereal batches that are delivered by farmers to grain storage/processing companies for *Fusarium* and *Aspergillus/Penicillium* mycotoxin contamination (Bernhoft et al., 2022; Binder et al., 2007; Marin et al., 2013; Rodrigues & Naehrer, 2012; Van Der Fels-Klerx et al., 2012; Wang, Hasanalieva, Wood, Markellou et al., 2020).

This may, at least partially, explain why *Fusarium* mycotoxins concentrations in small-grain cereals have (i) decreased over time since the early 2000s and (ii) are now very similar in both organic and conventional production (Table 4 and Figure S1), since most

comparative studies were carried out in Europe. Trends toward a decrease in the concentrations of some mycotoxins in Europe have been reported previously and linked to the development of (a) regulatory systems and testing regimes for mycotoxins and/or (b) improved agricultural and post-harvest processing/storage practices and/or (c) application of improved HACCP-systems throughout the grain supply chain (Aldred & Magan, 2004; Kabak et al., 2006; van Egmond et al., 2007). For DON and other *Fusarium* mycotoxins, changes in primary production protocols (use of more resistant varieties, FHB forecasting, improved timing of harvests and in conventional systems also improved fungicide treatments) may have also contributed to reducing contamination (Powell & Vujanovic, 2021). However, some agronomic practices that increase mycotoxin risk (e.g., minimum tillage in conventional farming and growing wheat after wheat or maize pre-crops in organic production) are thought to be used more widely now than 20 years ago (Qiu et al., 2016; Rempelos et al., 2020, 2021, 2023; Riley et al., 1994; Townsend et al., 2016).

The finding that mean concentrations of mycotoxins in cereal grains assessed in farm surveys were 2–3 times higher than those reported in cereal products in retail surveys is also thought to be mainly due to QA protocols preventing a proportion of harvested grain with too high mycotoxin levels from entering the human food chain, although milling/refining can also result in a reduction of mycotoxin concentrations in processed cereal products (Bernhoft et al., 2022; Wang, Hasanalieva, Wood, Markelou et al., 2020). Since most of the grain rejected for human consumption and milling waste is used as animal feed, future studies should investigate whether the improvement in QA-procedures for cereals entering the human food chain, may have, inadvertently, increased the proportion of harvested cereal crops that is (i) deemed unsuitable for human consumption and used as animal feed or (ii) discarded because it is unsuitable for both human and livestock consumption.

Different to the *Fusarium* mycotoxins, results obtained in different time periods indicate that concentrations of the *Aspergillus/Penicillium* mold mycotoxins OTA have slightly increased recently in both organic and conventional production, but are also now very similar in organic and conventional cereals/cereal products (Table 4 and Table S12). While improvements in post-harvest storage conditions and quality assurance in the organic sector after 2004 may explain that there is now no significant difference in OTA concentrations between organic and conventional cereal grains/products, the reasons for the increase in the latest time period (2016–2020) in both systems are unknown.

It is important to note, that OTA incidence (% positive samples) was higher in organic, while AFL was

higher in conventional samples when data from all years were included in the weighted odds ratio meta-analyses (Table 2). This may be explained by differences in the pre- and especially post-harvest environmental conditions in the 18 studies which provided OTA data compared with the 5 studies which provided AFL data; only 2 studies assessed both OTA and AFL in the same sets of organic and conventional cereal samples. However, contrasting trends for OTA and AFL were also found in the two studies which assessed both OTA and AFL incidence in the same sets of (i) oat (Solarska et al., 2012) or (ii) barley and wheat (Bakutis et al., 2006) grain samples. It is therefore more likely that overall the pre- and especially post-harvest protocols used for organic cereals favored the development of mold species linked to OTA production (*Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Penicillium verrucosum*, *Penicillium cyclopium*, *Penicillium nordicum*, *Penicillium viridicatum*) while protocols used for conventional cereals favored species (*Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus*) linked to AFL production (Deligeorgakis et al., 2023; Mukhtar et al., 2023; Perrone et al., 2020). However, the exact reasons for the different trends observed for OTA and AFL remain unclear. Given the relatively high toxicity of both OTA and AFL (Table 1), this should be further investigated in the future.

It is also important to consider that consumption of fruit (especially dried fruit), vegetables, and nuts (especially peanuts), can also significantly contribute to dietary mycotoxin intakes, and that post-harvest quality assurance for some of these crops may not be as efficient as those used in cereals. For example, there are reports that inefficient out-grading of apples with visible lesions/symptoms of fungal infections (which is a good indicator for mycotoxin contamination risk)(AHDB, 2002) has resulted in unacceptably high levels of patulin (a mycotoxin produced by *Aspergillus*, *Penicillium*, and *Byssoschlamys* spp.) in organic apple juice (Piemontese et al., 2005; Piqué et al., 2013; Piqué et al., 2013).

## 4.2 | Strategies to reduce mycotoxin contamination in organic and conventional cereals

The methodologies and strategies currently available to minimize the risk of mycotoxin contamination in primary production, and during harvest and subsequent grain storage and processing are summarized in Table 7 (for detailed information see Agriopoulou, 2021; Aldred & Magan, 2004; Barug et al., 2006; Bhat et al., 2010; Chandravarman et al., 2022; Cheli et al., 2017; Fumagalli et al., 2021; Gonçalves et al., 2019; Hamad et al., 2023; Kabak et al., 2006; Köpke

et al., 2007; Liu et al., 2021; Magan & Aldred, 2005, 2007; Mielniczuk & Skwaryło-Bednarz, 2020; Mohammadi Shad et al., 2022; Rudd et al., 2001; Siddiq & Vemireddy, 2021).

In this section we therefore focus on summarizing (i) to what extent existing mycotoxin risk mitigation strategies are already implemented in the organic and conventional sector and (ii) the methodologies/technologies that have the greatest potential for further reducing mycotoxin contamination in the organic and conventional food supply chains. Strategies for primary production and post-harvest storage/processing are described in separate subsections.

#### 4.2.1 | Strategies for primary production

Organic farming standards prohibit the use of inputs (e.g., mineral N fertilizers and stem shortening plant growth regulators) that were linked to an increased risk of mycotoxin contamination in conventional cereal production (Table 7). They also prescribe or recommend a range of practices are known to reduce the risk of mycotoxin contamination (e.g., use of diverse crop rotation with legume as fertility building break crops, varieties with high levels of disease resistance and competitiveness against weeds) (Table 7). Since the use of herbicides is also not permitted, the use of inversion ploughing for tillage/weed control (which efficiently incorporates fungal inoculum present on plant residues from preceding crop into the soil and thereby reduces mycotoxin risk) is also more widely used in organic farming, compared with intensive conventional production, where the use of minimum and no tillage (practices which are known to increase the risk of mycotoxin contamination) has increased substantially (Table 7; (Benbrook, 2005; Bernhoft et al., 2012, 2022; Hasanaliyeva et al., 2023; Rempelos et al., 2020, 2021, 2023).

In contrast, the use of fungicides with activity against mycotoxin-producing fungi is only permitted in conventional farming systems, where it can significantly reduce mycotoxin grain contamination in wheat, rice, and other cereals (AHDB, 2016, 2018; Gonçalves et al., 2019; Kumar et al., 2021; Poole & Arnaudin, 2014; Shah et al., 2018; Wenda-Piesik et al., 2017). However, the correct timing of fungicide application is known to be important for achieving satisfactory levels of control in the field. For example, for optimum control of FHB in wheat it is essential that all heads are emerged, especially when fungicides that are not capable of translocating quickly are used. However, wet weather conditions that provide favorable conditions for infection by mycotoxin production fungi, also create soil conditions unfavorable for ground spraying. Farmers therefore often have trouble to achieve an optimum timing of fungicide application under field con-

ditions. This is a main reason why fungicide efficiency was often found to be extremely variable and often insufficient for controlling FHB in infected wheat (Bernhoft et al., 2022; Shah et al., 2018). However, fungicide resistance in important mycotoxin-producing fungi is also a growing concern and may further reduce the efficacy of the currently used chemical treatments in the future (de Chaves et al., 2022).

Multilevel meta-analyses in the study detected significantly higher concentrations of DON in conventional grain in farm surveys and cereal products in retail surveys (Table 5). In farm surveys only the climatic conditions during the growing season (which can be assumed to have been similar for both production systems in farm surveys) and agronomic protocols (which differed between organic and conventional production) used were explanatory factors/drivers for DON and other *Fusarium* mycotoxins. This suggests that the (i) preventative management-based crop-protection regimes used in organic farming provide better control of *Fusarium* infection and mycotoxin production compared with the (ii) fungicide and/or plant growth regulator intervention-based protocols used in conventional farming and confirms the conclusions of a recent qualitative literature reviews of farm survey-based studies (Bernhoft et al., 2022).

There is currently substantial research focused on further reducing mycotoxin contamination in primary production and this includes efforts to (i) breed and/or select resistant/tolerant cereal varieties/cultivars, (ii) improve crop management practices, and (iii) alternative treatment methods that may further reduce mycotoxin contamination in primary production. The suitability of these methods/strategies for organic and conventional production systems is summarized later.

#### *Breeding for resistance/tolerance against mycotoxin production fungi*

The development of cereal varieties/cultivars with improved resistance against mycotoxin-producing fungi is widely recognized to be one of most promising strategies for (i) reducing mycotoxin contamination in primary cereal production globally and (ii) increasing the amount of cereal grain fit for human consumption (Buerstmayr et al., 2020; Ghimire et al., 2020; Jéon & Ordon, 2024; Rempelos et al., 2023; Rudd et al., 2001; Siddiq & Vemireddy, 2021; Wolfe et al., 2008).

Until recently all conventional and organic small-grain cereal production was based on varieties/cultivars from conventional farming breeding programs which primarily focused on improving grain yields in conventional high input production systems (Petitti et al., 2023; Rempelos et al., 2023). In rice and wheat (and to a lesser extent in minor cereal species) this involved the introduction of

semi-dwarfing genes to reduce stem/straw length which (i) increased harvest index (grain/straw ration) and (ii) reduced the risk of lodging. However, the modern, short-straw wheat and rice varieties require high mineral NPK fertilizer and pesticide inputs to deliver their genetic yield potential and are considered to not have important traits (e.g., suppressiveness against weeds, high nutrient use efficiency from organic fertilizers, and resistance against foliar diseases including FHB) required in organic farming systems (Petitti et al., 2023; Rempelos et al., 2023).

The profile of wheat varieties used in organic and conventional farming has therefore diverged, with conventional farmer using the most recently developed modern cultivars from conventional breeding programs, while organic farmers increasing use longer-straw varieties released (i) by conventional wheat breeders between the 1970s and 1990s or (ii) from organic farming-focused breeding/selection programs (Murphy et al., 2007; Rempelos et al., 2023). A trend toward diverging variety use is also now emerging for rice (Petitti et al., 2023). For wheat there is now increasing evidence that in organic production longer-straw varieties deliver (i) similar or higher grain yields/yield stability, (ii) greater competitiveness against weeds, (iii) higher grain protein contents and baking quality, (iv) reduced FHB susceptibility and mycotoxin contamination risk, and (v) without substantially increasing the risk of lodging when compared with modern short-straw varieties (Hasanaliyeva et al., 2023; Rempelos et al., 2020, 2023; Wilkinson et al., 2022).

In contrast, the use of straw length as a *Fusarium* mycotoxin mitigation trait in conventional breeding programs is not currently recommended (Buerstmayr et al., 2020) because longer-straw varieties were shown to substantially increase the risk of lodging in intensive conventional production systems (due mainly to the high mineral N inputs used) which leads to not only (i) lower yields and yield stability but also (ii) an increased risk of mycotoxin contamination (Nakajima et al., 2008; Rempelos et al., 2023). However, there has been considerable progress in the identification of FHB-resistance QTLs and the understanding of the role of other morphological and phenological traits on FHB resistance that can be exploited in both conventional and organic farming-focused breeding programs. For a recent review of the “*Efficiency of indirect selection for fusarium head blight resistance and mycotoxin accumulation in winter wheat*” see Michel et al. (2024). The most promising strategy to reduce mycotoxin contamination risks without reducing yields in conventional farming is therefore the use of crop breeding/selection (including molecular-assisted breeding and selection tools) to introduce fungal resistance traits other than increased stem length (Buerstmayr et al., 2020; Ghimire et al., 2020; Liu

et al., 2021; Rempelos et al., 2023; Siddiq & Vemireddy, 2021).

In this context it should be noted that the breeding/selection methods used in organic and conventional crop breeding have also diverged. Specifically, the use of marker-assisted and/or genomic selection methods to target the introduction of specific traits/genes is becoming increasingly important in conventional cereal breeding programs (Buerstmayr et al., 2020). Also, selection is usually done using standardized conventional agronomic protocols which includes high mineral NPK fertilizer inputs and on farms/fields owned or managed by breeding companies. In contrast, in organic wheat breeding programs, farmer participatory and/or evolutionary plant breeding approaches are increasingly used to develop/select locally adapted cultivars, variety mixtures, and/or “heterogeneous populations” that combine productivity, robustness, resource use efficiency, and grain quality traits desired by farmers and consumers (Rempelos et al., 2023). There have now been several studies which reported that variety mixtures or populations resulting from evolutionary plant breeding programs have lower disease and pest incidence/severity compared with varieties from conventional breeding programs in organic, “low-input” and/or regenerative farming backgrounds (Döring et al., 2015, 2011; Knapp et al., 2020; Merrick et al., 2020; Vidal et al., 2020; Wuest et al., 2021). However, there is to our knowledge no information on the FHB and mycotoxin contamination levels in wheat populations.

#### *Crop management innovations to minimize mycotoxin risk*

Recent reviews and studies (e.g., AHDB, 2021; Bernhoft et al., 2022; Edwards & Jennings, 2018; Gonçalves et al., 2019) concluded that there is considerable scope to reduce mycotoxin contamination by avoiding practices known to increase risk and/or adopting practices known to mitigate risk (see Table 7). For example, growing maize before wheat (which was reported to more than double mycotoxin concentrations in wheat grain), cereal monoculture or consecutive crops of the same cereal species have all been described as significant risk factors for mycotoxin contamination especially if combined with minimum tillage systems (AHDB, 2021; Birzele et al., 2002; Edwards & Jennings, 2018; Krebs et al., 2000; Pirgozliev et al., 2003; Vogelgsang et al., 2019). Although the density of maize and small-grain cereals is thought to be substantially higher in conventional arable rotations, the need (and possibly growing need) to further diversify organic arable rotations has also been recognized for some time (Robson et al., 2002). For example, growing (i) two consecutive wheat crops (e.g., in years with high organic wheat prices) and (ii) growing wheat after maize (e.g., on farms which grow maize for

feed or use in biogas units) is thought to have become more common in organic farming (Rempelos et al., 2021).

The biggest challenge for the intensive conventional cereal sector is that many of the agronomic practices (e.g., cereal dense rotations and maize–wheat/rice double cropping rotations) and inputs (high mineral N fertilizer inputs and use of plant growth regulators) that increase mycotoxin risk, are also thought to be essential to maintaining the current (i) grain yields per unit area and (ii) cereal production volume from intensive conventional systems (Rempelos et al., 2021). However, a recent series of field experiments by Drakopoulos, Gimeno et al. (2021), Drakopoulos, Kägi, et al. (2021) demonstrated that innovative use of break-, cover-, and intercrops can substantially reduce mycotoxin contamination in intensive, cereal dense and maize/wheat double cropping rotations. Specifically, they showed that the use of (i) Indian mustard, white mustard, or Berseem clover as “cut-and-carry” biofumigant break crops in maize/wheat rotations, (ii) Indian mustard, white mustard or winter pea as an interval cover-crop after silage maize, and (iii) Indian mustard or white mustard as intercrops in grain maize significantly reduced (by up to 87%) mycotoxin contamination in subsequent wheat crops. Cut-and-carry biofumigation break crops and cover crops also increased winter and spring wheat yields by 15% and 25%, respectively.

It is important to note that the use of minimum tillage, which increases the risk of FHB and mycotoxin contamination, is recommended and increasingly used in both conventional and organic farming, due to its proven economic, agronomic, and environmental benefits which include reduced (i) labor and fuel costs, (ii) run-off and nutrient/fertilizer losses from soil, (iii) damage to soil structure and the soil biota, and (iv) soil carbon/organic matter losses (Arshad et al., 2023; Khangura et al., 2023; Krauss et al., 2020).

#### *Novel treatment options for the control of toxigenic fungi and mycotoxin biosynthesis*

Fungicides are likely to remain an important component of mycotoxin control in conventional small-grain cereal production especially in regions with high *Fusarium* disease pressure (Cuperlovic-Culf et al., 2017). However, the need to reduce the current reliance on azole fungicides, due to the risk of resistance development, is also widely recognized, and there is continuing research focused on the development of new active compounds and formulations for synthetic fungicides (e.g., pydiflumetofen, novel triazoles, pydiflumetofen coformulated with prothioconazole, and prothioconazole formulated with essential oil) (Edwards, 2022; He et al., 2023; Wu et al., 2023; Xia et al., 2021). More recently there has also been considerable research effort on identifying antifungal microbial metabo-

lites that can be produced by fermentation technology and some of these microbial fungicides (e.g., Frenolicin B produced by *Streptomyces* strain NEAU-H<sub>3</sub>) have shown activity against FHB similar to synthetic fungicides in field trials (Han et al., 2021).

A range of alternative treatments inhibit infection by mycotoxin-producing fungi (including *Fusarium*, *Aspergillus*, *Alternaria*, and *Penicillium* spp.) and/or mycotoxin biosynthesis in cereal crops and this includes that use of (i) antifungal peptides and proteins (Martinez-Culebras et al., 2021), (ii) biological control based on bacterial, yeast, and fungal antagonists (Habschied et al., 2021; Powell & Vujanovic, 2021), and nanomaterials (Abd-Elsalam et al., 2017). Most of these treatments are in early stage of development, currently not commercially available, and/or licensed for use as crop-protection products.

The development of crop-protection products for mycotoxin-producing fungi based on microbial metabolite-based fungicides and biological control agents may also provide an additional strategy for the organic farming sector, because different to synthetic fungicides they can be permitted/licensed for use under organic farming standards (European Commission 2016). Integrated disease management based on (i) FHB-resistant/tolerant varieties/populations with (ii) microbial fermentation-derived fungicide, and/or (iii) biological control treatments is thought to be a particularly promising strategy that should be explored in the future (Powell & Vujanovic, 2021)

#### 4.2.2 | Post-harvest control strategies

The most important methods to for avoiding increases in mycotoxin contamination post-harvest are (i) immediate drying of grain (especially when harvest is delayed and/or has to occur under wet/humid conditions), (ii) physical decontamination/cleaning of grain to remove ergot sclerotia and weed seed, (iii) the use of modern climate-controlled grain storage facilities, and (iv) efficient control of post-harvest storage pests in grain stores (see Table 7 for references that provide further details of the main currently used post-harvest control methods).

In low-income countries the lack of appropriate grain drying and storage facilities are contributing considerably to high mycotoxin loads and this is thought to also have been the case in the 1990s in the emerging and rapidly growing organic cereal sector in Europe (Jørgensen & Jacobsen, 2002). In the 1990s a large proportion of organic wheat grain was produced by small-scale farmers which had no immediate access to grain drying and this was described as the main reason for higher OTA concentrations found in organic compared with conventional grain

at that time (Jørgensen & Jacobsen, 2002). This may also explain the higher concentrations of OTA in organic compared with conventional rice identified in the multilevel model meta-analysis (Table 6). OTA is produced by *A. ochraceus* in rice and generally considered to be a post-harvest problem caused primarily by inadequate drying and unsuitable storage facilities (Gonçalves et al., 2019).

A range of new emerging methods and technologies are currently being investigated/developed for post-harvest management of mycotoxins. This includes the (i) use of ionizing and non-ionizing radiation, cold (atmospheric) plasm, pulse light, ultrasound, pulse electric field and high-pressure processing and (ii) treatment with polyphenols/flavonoids, nanoparticles or natural essential oils to remove fungal inoculum, and/or prevent fungal growth and mycotoxin production and/or to degrade/detoxify mycotoxins. Reviews by Abd-Elsalam et al. (2017), Alizadeh et al. (2021), Hamad et al. (2023), and Sipos et al. (2021) describe the relative effectiveness these technologies and suggest that most can partially (i) reduce the growth of mycotoxigenic fungi, and/or (ii) modify the structure of mycotoxins, while maintaining nutritional value of cereal grain, although none can achieve complete decontamination. The authors also suggest that considerable additional R&D (including post-treatment toxicological and food quality assessments) and industrial upscaling will be necessary before these technologies can be safely and cost-effectively used in commercial grain storage and processing.

Microbe-based (biological) strategies to control grain infecting fungi and mycotoxins during storage have also been investigated (e.g., for the control of AFL during rice storage) and results of these studies were recently reviewed by Manna and Kim (2016) and Nešić et al. (2021). They describe that, in laboratory studies, antagonistic bacteria isolated from stored rice can inhibit the growth or mycotoxin production by fungi under storage conditions, or detoxify (or biodegrade) mycotoxins, but that considerable additional research is required before commercial bio-control products post-harvest use will be available.

### 4.3 | Potential impacts of the meta-analyses results

The meta-analysis confirmed the main conclusion of most previous qualitative literature reviews (e.g., Benbrook, 2005; Bernhoft et al., 2022) that (i) organic primary production protocols result in lower *Fusarium* mycotoxin concentrations in cereal grain and (ii) mycotoxin concentrations in organic and conventional cereal products entering the human food chain are not significantly different and below the EU-MRLs.

These findings are likely to further increase the demand for organic foods, because (i) this study demonstrates that concerns raised about higher mycotoxin loads in organic cereals consumed in Europe and North America (e.g., Avery, 2001; Trewavas, 2001, 2004) are unfounded, while (ii) other literature reviews and meta-analysis found that organic cereal production protocols results in higher concentrations of nutritionally desirable phenolics and antioxidants and, but lower concentrations of nutritionally undesirable pesticides and Cd in cereals (in particular wheat) (Baranski et al., 2014; Mie et al., 2016).

Also, a recent retail survey of wheat flour, found that mineral micronutrient concentrations (Cu, Mg, and Zn) are also significantly higher in organic compared with conventional wheat flour (Wang, Chatzidimitriou et al., 2020). This survey, uniquely, monitored simultaneously (i) mycotoxin concentrations, (ii) pesticide residues, (iii) phenolic concentrations and profiles, (iv) antioxidant activity, and (v) mineral micronutrient concentrations in all brands of wholegrain and refined flour made from common and spelt wheat (*T. aestivum* and *T. spelta*) available in the United Kingdom and German supermarkets. Results suggest that following dietary recommendations to increase wholegrain consumption, results in significantly higher intakes of some *Fusarium* mycotoxins (DON, H-2/HT-2) and pesticides with conventional, but not organic cereal products, when compared with products made from refined (white) cereal flour (Figure 1; Wang, Chatzidimitriou et al., 2020, 2020, 2020). This study also confirmed the results of the meta-analysis by Baranski et al. (2014) which found significantly higher concentrations of phenolic and antioxidant concentrations in organic cereals, but also demonstrated, for the first time, that differences between organic and conventional cereal products were larger in wholegrain compared with refined flour for many nutritionally relevant parameters (Figure 1; Wang, Chatzidimitriou et al., 2020, 2020, 2020).

Both the meta-analyses results reported here and the recent retail survey by Wang, Hasanalieva, Wood, Markelou et al. (2020) suggest that *Fusarium* and mold mycotoxin levels are well below the MCLs set by European regulators and this may help to increase whole-grain consumption by consumers currently concerned about higher mycotoxin concentrations in wholegrain products (Mukhtar et al., 2023; Ragona, 2016). Also, results from Wang, Chatzidimitriou et al. (2020) suggest that switching from conventional to organic whole-grain consumption allows consumers to increase antioxidant/phenolic and mineral micronutrient intake without a significant increase in dietary intake of both mycotoxins and pesticides (Figure 1).

In this context it should be considered that (i) refining not only removes most micronutrients but also 20%–30%

**TABLE 8** Maximum contamination levels (MCLs) allowed in cereal-based human foods and livestock feeds in the EU, United States, and China.

	Maximum contamination levels permitted in:					
	Human food products			Livestock feed		
	EU <sup>1</sup>	US <sup>2</sup>	China <sup>2</sup>	EU <sup>1</sup>	US <sup>2</sup>	China <sup>2</sup>
<b><i>Fusarium</i> mycotoxins</b>						
Deoxynivalenol (DON)	1250–1750*	1000	1000	8000	5000–10,000 <sup>#</sup>	1000–5000 <sup>#</sup>
Zearalenone (ZEA)	100–350*	NR	60	2000–3000 <sup>#</sup>	NR	100–500 <sup>#</sup>
Fumonisin B <sub>1</sub> + B <sub>2</sub> (FUM)	1000–4000*	2000–4000*	NR	60,000	5000–10,000 <sup>#</sup>	5000–50,000 <sup>#</sup>
<b>Mould mycotoxins</b>						
Ochratoxin A (OTA)	0.5–5*	NR	5	250	NR	100
Aflatoxin B <sub>1</sub>	2–5*	20	5–20*	20		10–50 <sup>#</sup>
Total aflatoxins (AFL)	4–10*	20	20	50	20–300 <sup>#</sup>	

\*Range of MCLs set for grain and different food types (excluding baby food).

<sup>#</sup>Range of MCLs set for feeds used for different livestock species.

<sup>1</sup>European Commission (2006).

<sup>2</sup>Yu & Petrosa (2023).

of energy and protein from grains and that (ii) organic food consumers were reported to consume not only more wholegrain but also less meat (Baudry et al., 2015; Eisinger-Watzl et al., 2015). Both increased whole-grain and organic food consumption may therefore, indirectly reduce the pressure on crop production to increase grain yields, by (i) utilizing more the harvest energy and protein in grain in human diets and (ii) reducing the amount of cereal required for livestock production.

#### 4.4 | Relevance for livestock health and product quality

Consumption of cereals with high mycotoxin concentrations can have significant negative effects on the health and performance of livestock and product quality and the effects of mycotoxins found in cereals on different livestock species has been reviewed extensively (Felix D’Mello et al., 1999; Iheshiulor et al., 2011; Xu et al., 2022; Yu & Pedrosa, 2023). Briefly, mycotoxins were shown to impair the function of kidneys, liver and/or the reproductive, digestive, neurological, and/or immune systems and that this leads to reduced productivity and reproductive capacity and increased incidence of infectious diseases and/or animal death (Yu & Pedrosa, 2023). It is important to note that the sensitivity to specific mycotoxins differs between livestock species (Yu & Pedrosa, 2023), and this is reflected in the MCL set for different livestock species by regulatory authorities (Table 8). However, MCLs set for livestock feed are between 2 and 50 times higher than those set for food (Table 8).

Since cereals that are rejected for human consumption due to high mycotoxin loads, are widely used as live-

stock feed, the higher *Fusarium* mycotoxin concentrations found in conventional compared with organic cereal grains may indicate that the risk of negative health impacts from exposure to *Fusarium* mycotoxins in concentrate feeds is greater in conventional livestock production systems. In ruminant livestock production the difference in mycotoxin exposure may be particularly large, since the use of cereal-based concentrate feeds is substantially higher in conventional compared with organic ruminant meat and dairy production systems in Europe (Średnicka-Tober et al., 2016).

Multi-mycotoxin occurrence in animal feeds (Daud et al., 2023) can also result in carry-over to animal-derived food products and thereby contribute to dietary mycotoxin intakes. This issue has recently been reviewed by Tolosa et al. (2022) who describe that mycotoxin concentrations found in animal-derived foods can sometimes exceed the MCL set for food and that there is particular concern about AFL in milk and dairy products, and OTA in meat byproducts.

#### 4.5 | Study limitations

An important limitation of this study was that data from a large proportion of published articles could not be included in the weighted meta-analysis because (a) they did not report measures of variation and (b) very few authors replied and provided this information after they were contacted to request a “measure of variation” for the means they published. Also, many of these articles (e.g., Bernhoft et al., 2012) were large, well-designed studies and would have been graded as having high or satisfactory quality if measures of variation could have



been obtained. This significantly reduced the statistical power of the weighted meta-analyses, and may explain why the unweighted meta-analyses (sensitivity analysis 1) detected significant effects of production system for a wider range of *Fusarium* mycotoxins.

The small number of studies reporting comparative datasets for mycotoxins other than DON and OTA and the fact that modified mycotoxins were not monitored in any of the studies are other important limitations. This was emphasized by a recently published farm survey-based study (Daud et al., 2023), which for the first time, measured both the free DON, NIV, and H-2/HT-2 and plant sugar-conjugated glucoside forms of the parent mycotoxins in both organic and conventional oat samples. Specifically, they reported that (i) H-2/HT-2 (not DON) was the most frequently detected mycotoxin and present in the highest concentrations in both organic and conventional samples, (ii) concentrations of the glucoside forms were substantially lower than those recorded for the parent compounds, but accounted for a larger proportion of total concentrations (free + conjugated) for DON compared with NIV and H-2/HT-2, and (iii) the proportion of positive samples and concentrations for DON, NIV, and H-2/HT-2 were higher in conventional oat samples, although the difference in concentrations between organic and conventional samples was only significant for H-2, HT-2, and T-2 glycoside (Daud et al., 2023).

Organic food fraud, which is defined as the fraudulent mislabeling and/or selling of or conventional foods as “certified organic” is also now recognized as a significant problem (Manning & Kowalska, 2021; van Ruth & de Pagter-de Witte, 2020) and should be considered as a potential study limitation because it may have confounded the mean incidence and concentration values obtained for organic, but not conventional, samples in some of the retail and farm survey-based studies included in the meta-analyses. This is because, it can be assumed that conventional samples collected in farm and retail surveys were produced with conventional farming protocols, because it makes no commercial sense for conventional cereal producers to use organic production protocols or for certified organic cereals to be sold into the conventional food sector. In contrast, there have been well-documented cases of organic food fraud which involved either (i) cereals produced on conventional farms being sold as labeled and sold as “organic” or (ii) organic farmers using prohibited inputs (e.g., mineral NPK fertilizers or synthetic chemical pesticides) for cereal production (Berezow, 2018; Manning & Kowalska, 2021; Roseboro, 2023; van Ruth & de Pagter-de Witte, 2020). However, since there are no reliable estimates of the extent of fraud it is not possible to estimate to what extent it was a confounding factor in the farm

and retail survey-based studies that provided data for the meta-analyses in this study.

A limitation of the replicated field experiment-based studies that provided data for the meta-analyses was that the organically managed plots were not certified to organic farming standards. This was primarily because, the land, labor, and financial resources available to researchers make it virtually impossible to comply with the (i) large ( $\geq 6$  m) separation distance between certified organic and conventionally managed agricultural land and (ii) a 2–3 year conversion period (during which land has to be managed to organic farming standards, but foods produced cannot be marketed as organic) prescribed under organic farming legislation (European Commission, 2016; USDA, 2024). It is therefore possible that greater interference between organically and conventionally managed experimental crops (e.g., cross contamination of organic crops with fungicides applied in conventional plots and cross contamination of conventional crops with fungal inoculum produced in organic plots) was a confounding factor in field experiments. Similarly, the relatively short length of time that soils were managed to organic farming standards in field experiments (compared with soils on commercial organic farms that had to go through a 2–3 year conversion period) may potentially have had a confounding effect on the relative difference in mycotoxin incidence/concentrations between organic and conventional crops (e.g., by affecting crop physiological parameters linked to foliar disease resistance).

## 5 | CONCLUSION

Overall, the results of the meta-analysis reported here suggest that historically conventional cereal grains/products used for human consumption had higher concentrations of *Fusarium* mycotoxin, while the incidence of OTA contamination was higher in organic cereals. Our findings also confirm previous studies which reported that *Fusarium* mycotoxin loads have decreased over time and that both *Fusarium* and *Aspergillus/Fusarium* mycotoxins are now broadly similar in organic and conventional cereals and lower than the MCL levels set by the EC for grains/products destined for human consumption, although maintaining OTA levels below the MCL remains a challenge.

The decrease in *Fusarium* contamination since the well-documented FHB epidemics in the late 1990s/early 2000s can be attributed to improvements in both (i) primary cereal production and (ii) post-harvest drying, and quality assurance protocols (e.g., introduction of detailed mycotoxin testing) since the early 2000s.

However, it remains unclear whether this also applies to cereals used as livestock feeds, because the improvements in QA systems may have resulted in a larger proportion of cereal batches with high mycotoxin loads being rejected for human consumption and used as livestock feed. Mycotoxin contamination therefore remains a serious challenge in livestock production.

Results from this and previous systematic literature reviews/meta-analyses that compared nutritionally relevant compounds in organic and conventional foods suggest that organic cereal product consumption may allow an increased intake of nutritionally desirable compounds (e.g., phenolics, antioxidants, and mineral micronutrients) without an increase in dietary mycotoxin and pesticide exposure.

Future research should therefore focus on improving agronomic protocols and genetic resistance against mycotoxin-producing fungi, especially in regions for which climate change is predicted to increase mycotoxin pressure.

One particular area of concern is that, average concentrations of OTA in both organic and conventional cereal grains/products in the last time period examined (2010 to 2015) were still higher than the MCL set by the EC for cereal-based foods and baby foods for infants and young children.

Also, the risk of exposure to mixtures of mycotoxins has rarely been investigated and exposure to mixtures of mycotoxins may still affect human and animal health, even if concentrations for each individual mycotoxin are below the EC threshold.

## AUTHOR CONTRIBUTIONS

**Juan Wang:** Conceptualization; methodology; data curation; investigation; validation; formal analysis; writing—original draft; visualization. **Enas Khalid Sufar:** Investigation; writing—review and editing. **Aksel Bernhoft:** Validation; investigation; formal analysis; writing—review and editing. **Chris Seal:** Validation; supervision; writing—review and editing. **Leonidas Rempelos:** Methodology; data curation; software; investigation; validation; formal analysis; writing—review and editing. **Gultekin Hasanaliyeva:** Investigation; formal analysis; writing—review and editing. **Bingqiang Zhao:** Conceptualization; investigation; writing—review and editing; validation. **Per Ole Iversen:** Validation; writing—review and editing. **Marcin Baranski:** Conceptualization; software; methodology; data curation; investigation; formal analysis; supervision; writing—review and editing; visualization. **Nikolaos Volakakis:** Investigation; validation; writing—review and editing. **Carlo Leifert:** Conceptualization; methodology; data curation; investigation; validation; formal analysis; supervision; funding acquisition;

project administration; resources; writing—original draft; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

Carlo Leifert owns conventionally managed farm land in Germany and organically managed farm land in Greece. Nikolaos Volakakis owns and managed organic farmland in Greece. All other authors declare no conflicts of interest. The funders of the research had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request by the first author Juan Wang.

## ORCID

Carlo Leifert  <https://orcid.org/0000-0002-2974-3612>

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## SUPPORTING INFORMATION

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