TRI Genotyping and Chemotyping: A Balance of Power

Ria T. Villafana, Amanda C. Ramdass and Sephra N. Rampersad *

Department of Life Sciences, Faculty of Science and Technology, The University of the West Indies, St. Augustine, Trinidad and Tobago; therese.villafana@my.uwi.edu (R.T.V.); amanda.ramdass@my.uwi.edu (A.C.R.)

* Correspondence: sephra.rampersad@sta.uwi.edu

Received: 29 October 2019; Accepted: 16 January 2020; Published: 21 January 2020

Abstract: Fusarium is among the top 10 most economically important plant pathogens in the world. Trichothecenes are the principal mycotoxins produced as secondary metabolites by select species of Fusarium and cause acute and chronic toxicity in animals and humans upon exposure either through consumption and/or contact. There are over 100 trichothecene metabolites and they can occur in a wide range of commodities that form food and feed products. This review discusses strategies to mitigate the risk of mycotoxin production and exposure by examining the Fusarium-trichothecene model. Fundamental to mitigation of risk is knowing the identity of the pathogen. As such, a comparison of current, recommended molecular approaches for sequence-based identification of Fusaria is presented, followed by an analysis of the rationale and methods of trichothecene (TRI) genotyping and chemotyping. This type of information confirms the source and nature of risk. While both are powerful tools for informing regulatory decisions, an assessment of the causes of incongruence between TRI genotyping and chemotyping data must be made. Reconciliation of this discordance will map the way forward in terms of optimization of molecular approaches, which includes data validation and sharing in the form of accessible repositories of genomic data and browsers for querying such data.

Keywords: genotyping; chemotyping; Fusarium; molecular detection; trichothecenes

Key Contribution: The reasons underlying discordance between TRI genotyping and chemotyping data are presented. Fundamental to the success and reliability of one approach is the induction of trichothecene production in vitro, which is complicated and as yet wholly undefined. The other approach is hinged on high level maintenance of the TRI gene cluster in the genomes of Fusarium species.

1. Introduction

Mycotoxins are produced by some fungi as toxic secondary metabolites and impose a serious economic impact at all levels of food and feed production, including crop and animal health and production, processing, and human health [1,2]. These toxic effects can be reversible and irreversible depending on a number of factors [3]. Mycotoxin-producing fungi can be broadly classified into two groups: field fungi (e.g., Fusarium species) infect seeds before harvest and produce mycotoxins in the field (pre-harvest infection in seeds with a high moisture content (22 to 25%); and storage fungi (e.g., Aspergillus, Fusarium and Penicillium species) infect stored seeds/grain and produce mycotoxins on stored produce (post-harvest in seeds/grain with 12 to 18% moisture content) (http://www.fao.org). Mycotoxins remain as residues in stored produce within 24 h after fungal infestation [4]. There is, however, overlap between the two designations [3,5].
An effective mycotoxin management program should address prevention of mycotoxin production, detoxification and decontamination, strategies for routine surveillance, implementation of mycotoxin thresholds in contaminated food and feed, and measures to regulate the movement of mycotoxin-contaminated material in national and international trade. In Europe, increasing levels of T-2 and HT-2 Type A trichothecenes in small grain cereals (e.g., wheat, barley, oat, rye, and triticale) is an emerging issue of food safety as these mycotoxins are considered to be high risk due to their common occurrence and high acute toxicity [6–8]. Deoxynivalenol (DON) and its acetylated derivatives (3-ADON and 15-ADON) as Type B trichothecenes occur as the predominant mycotoxin in the northern hemisphere and its toxigenic impact is significant to animal health and causes acute human toxicosis [9].

The Food and Agriculture Organization (FAO) and the Codex Alimentarius Commission have adopted a “Hazard Analysis and Critical Control Point (HACCP)” approach, which is a coordinated system that identifies, evaluates, and defines the means by which to control hazards with the potential to cause adverse health effects (http://www.fao.org). Factors that impact upon such regulatory decisions towards mitigating the risk of mycotoxin exposure in human food/feed include but are not limited to:

- Identifying the source of mycotoxin contamination, i.e., fungus and toxin identification;
- Toxicological profiling of mycotoxin residues in stored food/feed;
- Assessing the current analytical methods to identify and quantify such residues;
- Defining the relationship between mycotoxin levels and different types of food/feed;
- Effects of mycotoxins on human and animal health.

Using the *Fusarium*-trichothecene (TRI) mycotoxin model, at the core of this HACCP system, central to the mitigation of risk of mycotoxin exposure, the following aspects form the basis of this review:

1. Rapid and accurate detection and identification of the *Fusarium* species infecting plant material as an indication of the source of mycotoxin contamination;
2. Identification and quantification of mycotoxins;
3. Toxicological profiling of mycotoxin residues and their metabolites through testing for the presence of the end-products of trichothecene (TRI) biosynthesis, which includes utilization of chemotyping techniques according to their advantages and disadvantages;
4. Potential for prediction of mycotoxin contamination through TRI genotyping, which involves PCR-based methods to detect target genes within the trichothecene biosynthesis gene cluster, and in some cases, assessing the expression levels of TRI genes at the level of the transcript;
5. Characterization of the causes associated with discordance between genotyping and chemotyping data and factors affecting reliability of both approaches to mycotoxin detection.

2. **Molecular Identification of *Fusarium* Species**

Correct identification of *Fusarium* species is fundamental to determining the potential for trichothecene production. The *Fusarium* genus has a membership of 300 phylogenetically distinct species, 20 species complexes and nine monotypic lineages [10,11]. Identification of *Fusarium* to the species level based on morphological characteristics of colony and micro- and macroconidia is prone to error due to the plasticity of morphological traits. Furthermore, not all features needed for identification are well-developed in culture (e.g., the inability of isolates to produce macroconidia after subculture). As such, morphology frequently fails to distinguish among Fusaria at the species level [12].
Multi-locus sequence data comparison is the foundation of current *Fusarium* species identification strategies [11,13,14]. Sequence repositories that house validated protein-coding gene sequences are accessible at *Fusarium* MLST at the CBS-KNAW Fungal Biodiversity Centre (http://www.cbs.knaw.nl/Fusarium/). This database only banks carefully curated sequences of isolates that are available from the CBS-KNAW, *Fusarium* Research Center (FRC, http://plantpath.psu.edu/directory/specialties/Fusarium-research-center) or ARS Culture Collection (NRRL, http://nrrl.ncaur.usda.gov/). Additionally, the accession records that identify a sequence to the phylogenetic species by EF-1α haplotype can be retrieved, e.g., FIESC 25-a, where “25” is the species and “a” is the haplotype within species [15].

The recommended markers for identification of *Fusarium* species are, minimally, the protein-coding genes of the translation elongation factor 1 (EF-1α/TEF-1/TEF1), and the RNA Polymerase II largest and/or second largest subunit (RPB1 and/or RPB2, respectively). The rationale is: (i) sequence comparisons of two independent loci improves the accuracy of identification, (ii) these gene targets are faithfully amplified by PCR and sequenced using primers that are can be successfully used for most members of this genus, (iii) these markers distinguish among sequences at or near species-level, and (iv) these gene sequences are well-represented in the *Fusarium* MLST database [11,16]. Multi-locus sequence typing schemes may include other genetic markers (e.g., Calmodulin—CAM, beta- tubulin—βTUB) specifically developed for identification of members of defined species complexes. There are also specific primers for the detection of *F. culmorum*, *F. poae*, *F. sporotrichioides*, *F. cerealis*, *F. pseudograminearum*, *F. graminearum sensu lato*, and *F. graminearum sensu stricto* (Table 1).
Table 1. – PCR primers for the detection of *Fusarium* species as known trichothecene mycotoxin producers.

| Fusarium Species | Primer Name | Target Gene | Primer Sequence 5' to 3' | Amplicon/bp | Reference |
|------------------|-------------|-------------|--------------------------|-------------|-----------|
| *F. culmorum*    | FC01F (fwd) | SCAR specific | ATGGTGAAACTGTCGCGGGC   | 570         | [17,18]   |
|                  | FC01R (rev) | SCAR        | CTCCTTATACGCCAACATCTCG  |             |           |
|                  | Fcg17F (fwd) | SCAR         | TCGATATACCCGATTCC      | 340         |           |
|                  | Fcg17R (rev) | SCAR        | TACGACACCGTACGGG     |             |           |
|                  | Fcu-F (fwd) | IGS         | GACTATATTATGCTCGAGAG   | 200         |           |
|                  | Fgc-R (rev) | IGS         | CTCTCATATACCCCTCGG    |             |           |
| *F. graminearum* + fungi belonging to FGSC - *F. asiaticum; F. meridionale* | Fg16F (fwd) | SCAR FGSC members | CTCCGGATATGGTCGCAA | 400–500 | [18–20] |
|                  | Fg16R (rev) | SCAR        | GTTAGTGTACAAAAGGTG   |             |           |
|                  | Fgr-F (fwd) | IGS specific | GTTATGGGTAACAAGTTG   | 500         | [21]      |
|                  | Fgc-R (rev) | IGS         | CTCTCATATACCCCTCGG   |             |           |
|                  | GOPW (fwd)  | gaoA gene   | ACCTCTGTGTTCTCCAGACGG | 472         | [22]      |
|                  | GORV (rev)  | gaoA gene   | CTGTCAGATATACCGGTGTG  |             |           |
| *F. poae*        | FPs2F (fwd) | SCAR specific | CAAGCAAAACGCTCTACC   | 220         | [23]      |
|                  | FPs2R (rev) | SCAR        | TTTCCACCTCAGGACAGTT  |             |           |
|                  | PoaeIGS-R (fwd) | IGS         | FAAGCTCCTCCTGGAGACTCGA | 306         | [24]      |
|                  | CNL12 (rev) | IGS         | CTGAAAGCCTTCAAGTCAG   |             |           |
|                  | Fps-F (fwd) | IGS specific | CGCAAGTATAGTGGACAG    |             |           |
|                  | Fpo-R (rev) | IGS         | CGCGCCACCCCTGAGC     | 400         |           |
| *F. sporotrichioides* | AF330109CF (fwd) | TRI13 specific | AAAAGCCCAAATTGCTGATG | 332         | [25]      |
|                  | AF330109CR (rev) | TRI13 | TGCGATGTTCATTACCCTCAGC |             |           |
|                  | FspTTS2K (fwd) | ITS specific | CTGGTATGCTTGATCTGCACTA | 288         | [26]      |
|                  | P208L (rev) | ITS         | ACAATATACACTCGGCGCAGA |             |           |
|                  | Fps-F (fwd) | IGS specific | CGCAAGTATAGTGGACAG    | 400         | [21]      |
|                  | Fsp-R (rev) | IGS         | GTGACAGGACGGCATTCCGC  |             |           |
| *F. pseudograminearum* | FP1-1 (fwd) | degenerate | CCGGTTAGTTCACACTTTCYG | 523         | [27]      |
|                  | FP1-2 (rev) |             | GGAATATGTAGAGACATAT  |             |           |
| *F. cerealis*    | CRO-AF (fwd) | specific | CTCACTGTCACCGGGTTCTCAG | 842         | [28]      |
|                  | CRO-AR (rev) |             | CTCACTGTCACCGGGTTCTCAG |             |           |
Assignment of correct identities to multi-locus sequences involves careful editing of nucleotide sequences to ensure primer sequences are removed, there are no ambiguities in the sequences and the top BLASTn hits match the same species names. Additionally, BLASTx can first be used to verify the identity of the protein-coding regions of the sequences. Where there are several top BLASTn hits with different Fusarium species names with similar scores, it is usually necessary to sequence additional loci; however, selection of additional markers excludes the use of ITS and D1/D2 sequences, which cannot resolve sequences to the species level due to low degree of sequence variation [11]. It was also reported that up to 50% of ITS and D1/D2 Fusarium sequences curated in GenBank are misidentified, and hypervariable ITS2 sequences of the ITS1-5.8S-ITS2 rDNA array can exist as paralogues or orthologues in several species’ complexes [29,30].

3. Fusarium Species Known to Produce Trichothecenes

Type A trichothecene (e.g., T-2, HT-2, NEO, DAS) producers, either singly or in co-mixtures, include F. langsethiae, F. poae, F. polyphialidicum, and F. sporotrichioides (Biomin http://www.mycotoxins.info/mycotoxins/common-mycotoxins/t-2-toxin/) [31–34]. Type B trichothecene (e.g., NIV, DON) producers are F. culmorum, F. graminearum, F. poae, F. meridionale, F. sambucinum, and F. solani, some of which are capable of producing one or more trichothecenes of either Type A and/or Type B, e.g., DON in the US [35], DAS, T-2, NEO in Egypt, and NIV, 15-ADON in Argentina [36].

4. Chemotyping

The chemotype is defined as the chemical phenotype of a given fungal strain including a profile of the organisms’ secondary metabolites [37]. Chemotyping trichothecene-producing Fusaria is important to determining the potential risk of toxin production in contaminated food and feed, and to devising preventative measures to mitigate this risk. Trichothecenes have been classified into four groups (Types A, B, C, and D) based on the substitution pattern of 12, 13-epoxytrichothec-9-ene [38]. Pasquali et al. [39] described two chemotypes within Type B trichothecenes (Chemotype I and II). Chemotype I which pertains to DON and its acetylated derivative producers, is subdivided into Chemotype IA for 3-ADON producers, and Chemotype IB for 15-ADON producers, while Chemotype II are the NIV and/or 4-ANIV producers.

Analytical Techniques for Chemotyping

Ideally, an orthogonal approach to chemotyping is preferred by which the toxin is both quantified and mass-verified. In some cases, a chemical phenotype may relate to a continuum of chemotypes, rather than discrete groupings. The goal is to derive objective classification systems to identify Fusarium species based on their chemical profiles. The choice of screening and quantitative methods for the most common Type A and B trichothecenes will depend on the instrumentation available, detection limit required, matrix composition, and the properties of the analyte (Table 2).

5. Genotyping

Genotyping generates TRI gene information pertaining to which taxa are potentially toxic and aids in assigning the toxic share of genotypes in the environment and/or in a given pathogen population. A TRI genotype refers to a specific nucleotide sequence of one or more of the TRI genes found in the genomes of some Fusaria that encodes an enzyme which enables production of a specific trichothecene(s) and, therefore, detection targets the TRI gene sequences. A chemotype is the secondary metabolite profile of an organism as determined by chemical analysis and, therefore, detection targets the end products of trichothecene biosynthesis. A genotype is not a chemotype or vice versa and should not be used interchangeably. As such, on the basis of the proportion of potentially ‘toxic’ genotypes, it should be possible to predict whether there is a risk of toxin production and subsequent exposure [40]. A number of studies have utilized genotyping as a proxy to determining chemotype [41–49]. Table 3 outlines the various research efforts in applying TRI genotyping to Fusarium species over the last 20 years.
Table 2. Current analytical techniques for TRI chemotyping.

| Technique                          | Advantages                                                                 | Disadvantages                                                                 | TRI Toxin                      | References |
|------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------|------------|
| Enzyme-Linked Immunosorbent Assay  | (1) several ELISA-kits capable of detecting DON in the relevant concentration range set by the FDA and EU are commercially available; analysis of several samples in a single test - high throughput and portability for on-site application; simple sample processing; high sensitivity and specificity; does not require toxic reagents; can detect the presence of fungi in food even after heat treatment which enables the evaluation of contamination in processed foods; rapid screening (<0.5-2h); in situ use; test kits available for use with low sample volume requirements and less clean-up steps compared to methods like TLC and HPLC; simultaneous analysis of multiple samples. | (1) cross-reactivity and dependence on a specific matrix - cross-reactivity data for 3-ADON, 15-ADON and/or DON-3G were reported in some studies using different commercially available ELISA-kits; (2) matrix interference (presence of other substances lead to alteration of results); (3) semi-quantitative method and confirmatory reference method is required; (4) narrow operating range; (5) false positive/negative results possible; (6) each kit detects only a single mycotoxin and is designed for one-time use; thus, it can be costly if one needs to test samples contaminated with multiple mycotoxins; (7) each test kit is specified by the manufacturer and while some third-party validations, e.g., by AOAC, have been done for some mycotoxin ELISA kits, the validation and marketing are for use with specific toxins under specific contamination levels within specified matrixes and, therefore, the kit cannot be used for all food matrices and all contamination levels. | DON, T-2, T-2/HT-2 | [50–57] |
| Technique                                                                 | Advantages                                                                 | Disadvantages                                                                                     | TRI Toxin                                                                 | References |
|--------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|------------|
| Liquid chromatography-mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS); LC-MS/MS followed by structure confirmation via Q-TOF | (1) selective detection; (2) low detection limits; (3) qualitative and quantitative results; (4) generation of structural information of analyte; (5) little sample treatment required; (6) applicable to complex matrices; (7) multi-analyte analysis; (8) no derivatization required | (1) expensive technology—high-end instrumentation to achieve suitable detection limits; (2) specialist expertise required to perform analysis; (3) time consuming when compared to rapid test; (4) sensitivity is dependent on ionization technique—challenge to achieve tight chromatographic conditions (especially pH and additives to the mobile phase) for optimal ionization; (5) optimal ionization only achievable in modern instruments with rapid switching between negative and positive modes as mycotoxins vary, e.g., polarity, molecular mass, and heavy reliance on correct sample preparation and purification; (6) reliable quantification achievable only by matrix-matched calibration and internal standards; (7) matrix-matched calibration to improve performances; (8) may require different extraction solvents, types of clean-up (solid phase extraction (SPE), QuEChERS, and immunooaffinity column (IAC)) as well as calibration approaches (external or matrix matched) | NIV, DON, 3-Ac-DON, 15-Ac-DON, HT-2, T-2 toxin (maize); LC-ESI-MS/MS: NIV, DON, 3-Ac-DON, 15-Ac-DON, HT-2 toxin, T-2 toxin, DAS, neosolaniol, monoacetoxyscirpenol, T-2 triol, and T-2 tetraol (wheat and oat); LC-APCI-MS- DOM-1, HT-2 toxin, T-2 toxin, acetyl T-2 toxin, DAS, monoacetoxyscirpenol, neosolaniol (oats, maize, barley and wheat); T-2 and HT-2 and their glucosylated and acetylated derivatives (T2 toxin-3-glucoside, 3-acetyl-T2 toxin and 3-acetyl-HT-2 toxin) in staple flours, barley, maize, oats, rye, and wheat | [58–63]    |
| Technique                                                                 | Advantages                                                                                      | Disadvantages                                                                                                     | TRI Toxin                                | References |
|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------|
| High Performance Liquid Chromatography (HPLC); Columns: T-2/HT-2™ HPLC columns - T-2 and HT-2 toxins | (1) high sensitivity and selectivity; (2) applicable to complex matrices; (3) high reliability and accuracy; (4) short analysis time; (5) automated (auto-sampler) | (1) expensive technology; (2) laborious; (3) require the use toxic chemicals and there is a cost attributed to waste storage and disposal; (4) specialist expertise required to perform analysis; (5) time consuming when compared to rapid test; (6) compounds must possess UV absorption or fluorescence properties; (7) derivatization may be required | HT-2 toxin, T-2 toxin, DON (cereals and grains) | [58–60,64] |
| HPLC with a specific detector—fluorescence (FL), ultraviolet (UV), diode array (DAD), or MS; Ultra HPLC-MS/MS (UHPLC-MS/MS) | HPLC-FL- highly specific and sensitive, lower cost than LC-MS methods; method validation performed according to Commission Decision 2002/657/EC (EC, 2002, 2014, 2017), revealed precision | HPLC-FL—specificity for fluorescing compounds which must be well separated on column for reliable quantification | HPLC-FL- DON, NIV, T-2 toxin, HT-2 toxin, NEO, DAS, 3-Ac-DON, 15-Ac-DON (wheat and corn); HPLC-MS- DON; DON and its acetylated and glucosylated metabolites, HT-2 and T-2 toxins in maize | [62,65–67] |
| Combination of immunological capture and HPLC-MS/MS | monoclonal antibody developed against DON for purification of cereal extract, before the follow-up HPLC-MS/MS analysis | N/A                                                                                                             | DON, 3-ADON, and 15-ADON from wheat, oatmeal, and maize | [68] |
| Competitive immunochromatographic assay or lateral flow immunoassay | N/A                                                                                                                                                     | N/A                                                                                                             | DON in maize extracts                  | [69] |
5.1. Genotyping Platforms

The development and optimization of molecular tools for detecting genes involved in trichothecene biosynthesis are hinged on an understanding of the arrangement, diversity and evolutionary maintenance of TRI genes as part of a biosynthetic gene cluster [70]. Furthermore, TRI biosynthesis should be characterized as an interconnected network in which various intermediates and a range of end-products are generated; there are alternate routes of synthesis and it is very unlikely the reactions occur as a linear pathway [71].

The commonly used genotyping platforms to investigate the extent and pattern of genetic variation (genotype) that contributes to production of a specific toxin or class of toxin (chemotype) include genotyping-by-sequencing, nucleotide polymorphism detection, quantitative detection of toxins and quantitative detection of toxin-producing *Fusarium* by expression analysis. Detecting and characterizing unique genetic features highlighted by genotyping assays require that the template DNA and RNA be of high integrity regardless of the assay. A genotype-phenotype correlation is not always evident or straightforward due to interactions between different genes, the underlying and often complex biochemical mechanisms of gene expression and the interplay of environmental factors. According to Houle et al. [72], genotype data supplements rather than supplants phenotypic information.

### Table 3. TRI genotyping of *Fusarium* species over the last 20 years.

| *Fusarium* Species | Host Species | Country      | TRI Gene Target | Chemotype    | Reference |
|--------------------|--------------|--------------|-----------------|--------------|-----------|
| *F. asiaticum*     | Triticum sp. (wheat) | China         | TRI3, TRI12     | 3-ADON       | [73]      |
| *F. asiaticum*     | Hordeum vulgare (barley) | Japan        | TRI3, TRI12     | NIV          | [73]      |
| *F. asiaticum*     | Triticum sp. (wheat) | Taiwan       | TRI13           | 15-ADON and NIV | [74] |
| *F. avenaceum*     | Zea mays (maize) | Brazil       | TRI3, TRI12     | 3-ADON       | [73]      |
| *F. avenaceum*     | herbageous vine | Venezuela     | TRI3, TRI12     | NIV          | [73]      |
| *F. cerealis*      | potato tuber | Netherlands  | TRI3, TRI12     | NIV          | [75]      |
| *F. cerealis*      | Azalea        | New Zealand  | TRI3, TRI12     | NIV          | [75]      |
| *F. culmorum*      | Ammophila arenaria (European beachgrass) | Netherlands | TRI3, TRI12     | NIV          | [75]      |
| *F. culmorum*      | Triticum sp. (wheat) | France       | TRI3, TRI12     | 3-ADON       | [75]      |
| *F. culmorum*      | Populus nigra (European black poplar) | Portugal    | TRI3, TRI12     | 3-ADON       | [75]      |
| *F. culmorum*      | Ammophila arenaria (European beachgrass) | Netherlands | TRI3, TRI12     | NIV          | [75]      |
| *F. culmorum*      | Hordeum vulgare (barley) | Denmark     | TRI3, TRI12     | 3-ADON       | [73]      |
| *F. culmorum*      | Avena sativa (oat) | Canada       | TRI3, TRI12     | 3-ADON       | [76]      |
| *F. culmorum*      | Hyacinthus orientalis (Hyacinth) | Netherlands | TRI3, TRI12     | NIV          | [75]      |
| *F. culmorum*      | Triticum sp. (wheat) | Poland       | TRI3, TRI12     | NIV and 3-ADON | [77] |
| *F. culmorum*      | Triticum sp. (wheat) | UK           | TRI3, TRI7, TRI13 | DON and NIV | [78] |
| *F. culmorum*      | Triticum sp. (wheat) | Tunisia      | TRI3, TRI5, TRI7, | DON, NIV    | [79]      |
| *F. graminearum*   | Zea mays (maize) | Iran         | TRI3, TRI12     | NIV          | [73]      |
| *F. graminearum*   | Triticum sp. (wheat) | South Africa | TRI3, TRI12     | 15-ADON      | [75]      |
| *F. graminearum*   | Rumohra adiantiformis (leatherleaf fern) | Netherlands | TRI3, TRI12     | NIV          | [73]      |
| *F. graminearum*   | Triticum sp. (Louisiana, wheat) | USA          | TRI3, TRI12     | 15-ADON      | [80]      |
| *F. graminearum*   | Triticum sp. (Ohio, wheat) | USA          | TRI3, TRI12     | 3-ADON       | [75]      |
| *F. graminearum*   | Zea mays (Michigan, maize) | USA          | TRI3, TRI12     | 15-ADON      | [73]      |
| *F. graminearum*   | Zea mays (Ohio, maize) | USA          | TRI3, TRI12     | 15-ADON      | [73]      |
| *F. graminearum*   | Triticum sp. (Kansas, wheat) | USA          | TRI3, TRI12     | 15-ADON      | [73]      |
| *F. graminearum*   | Sorghum bicolor (sorghum) | Ethiopia    | TRI3, TRI12     | 15-ADON      | [78]      |
| *F. graminearum*   | Zea mays (maize) | Nepal        | TRI3, TRI12     | NIV          | [73]      |
| *F. graminearum*   | Avena sativa (oat) | Sweden       | TRI3, TRI12     | 3-ADON       | [75]      |
5.1.1. Targeted Detection of TRI Genes by Conventional PCR: Single, Duplex, and Multiplex PCR Assays

One molecular approach to trichothecene genotyping is based on PCR detection and identification of specific TRI genes involved in the trichothecene biosynthesis pathway. For an historical account of the development of primers and PCR conditions for *Fusarium* genotyping see review by Pasquali and Migheli [96]. Specific TRI genes enabled differentiation between different trichothecenes: TRI3 and TRI12, for the differentiation of genotypes into 3-ADON, 15-ADON, or NIV [73,74,78,81,83,91,97] TRI7 and TRI13, for the differentiation of genotypes into DON and NIV [74,81,83,91]; and TRII along with

| Fusarium Species | Host Species | Country | TRI Gene Target | Chemotype | Reference |
|------------------|--------------|---------|-----------------|-----------|-----------|
| *F. graminearum* | Zea mays (maize) | South Africa | TRI3, TRI12 | 15-ADON | [73] |
| *F. graminearum* | Triticum sp. (wheat) | England | TRI3, TRI12 | 15-ADON | [73] |
| *F. graminearum* | Triticum sp. (wheat) | Poland | TRI3, TRI12 | 3-ADON, 15-ADON and NIV | [73] |
| *F. graminearum* | Zea mays (maize) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON and NIV | [81] |
| *F. graminearum* | Hordeum vulgare (barley) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON and NIV | [81] |
| *F. graminearum* | Triticum sp. (wheat) | Taiwan | TRI13 | 15-ADON and NIV | [74] |
| *F. graminearum* | Triticum sp. (wheat) | Canada | TRI3 | 3-ADON, 15-ADON and 3-ANX | [82] |
| *F. graminearum* | Zea mays (maize) | Canada | TRI1, TRI8, TRI12, TRI3 | 3-ADON, 15-ADON and 3-ANX | [82] |
| *F. graminearum* | Zea mays (maize) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON and NIV | [81] |
| *F. graminearum* | Hordeum vulgare (barley) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON and NIV | [81] |
| *F. graminearum* | Triticum sp. (wheat) | Taiwan | TRI13 | 15-ADON and NIV and 3-ADON | [83] |
| *F. graminearum* | Triticum sp. (wheat) | Canada | TRI3 | DON, NIV | [35] |
| *F. graminearum* | Zea mays (maize) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON, NIV, NX-2 | [84] |
| *F. graminearum* | Hordeum vulgare (barley) | Korea | TRI3, TRI4, TRI5, TRI7, TRI8, TRI11 | DON, NIV, NX-2 | [84] |
| *F. graminearum* | Triticum sp. (wheat), wild grass | USA (New York) | TRI1 | DON, NIV, NX-2 | [85] |
| *F. graminearum* | Triticum sp. (wheat) | Multi | USA | TRI1 | DON, NIV, NX-2 | [84] |
| *F. graminearum* | Galium aparine, Triticum sp. | Germany, France | TRI7, TRI13 | 3-ADON, 15-ADON, DON, NIV | [86] |
| *F. graminearum* | Hordeum vulgare (barley) | USA (north) + Canada (south) | TRI1 | NX-2 | [40] |
| *F. graminearum* | Hordeum vulgare (barley) | Argentina | TRI7, TRI13 | DON, NIV | [87] |
| *F. graminearum* | Zea mays (maize) | USA (New York) | TRI1 | DON, NIV | [87] |
| *F. graminearum* | Hordeum vulgare (barley) | Nepal | TRI13 | DON, NIV | [89] |
| *F. graminearum* | Hordeum vulgare (barley) | Uruguay | TRI1 | 15-ADON, NX-2 | [17] |
| *F. graminearum* | Hordeum vulgare (barley) | Argentina | TRI3, TRI7, TRI13 | 15-ADON, DON, NIV | [90] |
| *F. graminearum* | Hordeum vulgare (barley) | Argentina | TRI3, TRI7, TRI13 | 3-ADON, 15-ADON, DON, NIV | [19] |
| *F. graminearum* | Hordeum vulgare (barley) | Italy | TRI5, TRI7, TRI13 | DON, NIV | [91] |
| *F. graminearum* | Hordeum vulgare (barley) | Uruguay | TRI3, TRI5, TRI7, TRI13 | 15-ADON, DON | [92] |
| *F. graminearum* | Hordeum vulgare (barley) | Italy | TRI5, TRI7, TRI12 | 3-ADON, 15-ADON, NIV | [20] |
| *F. graminearum* | Glycine max (soybean) | Argentina | TRI3, TRI5, TRI7 | 15-ADON, DON, NIV | [93] |
| *F. graminearum* | Triticum sp. (wheat) | Brazil | TRI3, TRI12 | 3-ADON, 15-ADON, NIV | [94] |
| *F. graminearum* | Triticum sp. (wheat) | Brazil | TRI3, TRI12, TRI13 | 15-ADON, DON | [95] |
| *F. graminearum* | orange twig | New Caledonia | TRI3, TRI12 | NIV | [73] |
| *F. graminearum* | Zea mays (maize) | Nepal | TRI3, TRI12 | NIV | [73] |
| *F. graminearum* | Triticum sp. (wheat) | Taiwan | TRI13 | NIV | [74] |
| *F. graminearum* | Musa sp. (banana) | Honduras | TRI3, TRI12 | NIV | [73] |
| *F. graminearum* | Acacia mearnsii (black wattle) | South Africa | TRI3, TRI12 | NIV | [73] |
| *F. graminearum* | Hordeum vulgare (barley) | Australia | TRI3, TRI12 | 3-ADON | [73] |
| *F. graminearum* | Zea mays (maize) | Korea | TRI3, TRI4, TRI5, TRI8, TRI11 | NIV | [81] |
| *F. graminearum* | Hordeum vulgare (barley) | Korea | TRI3, TRI4, TRI5, TRI8, TRI11 | NIV | [81] |
| *F. graminearum* | Zea mays (maize) | Nepal | TRI3, TRI4, TRI5, TRI8, TRI11 | NIV | [81] |
| *F. graminearum* | Hordeum vulgare (barley) | Uruguay | TRI3, TRI4, TRI5, TRI8, TRI11 | NIV | [81] |
its regulatory genes TRI6 and TRI10 [39] and TRI3 and TRI12 [83,98], for the differentiation of 3ANX (NX-2) and NX (NX-3) from 3-ADON, 15-ADON, and DON [82].

5.1.2. Detection of TRI1 Gene Sequence Polymorphisms by PCR-RFLP

Polymorphisms in the target sequence to be amplified by PCR have been used to differentiate between 3-ADON and NX-2-producers [98]. TRI1 specific PCR assays are based on amplification of TRI1 gene sequence using a reverse primer (TRI1-R; 5’-TTCCTGCAGGGGCTTGATG-3’) and one of two forward primers for the detection of 3-ADON (5’-AATGCTCGCGAACTAATCAG-3’), and for the detection of 3-ANX (5’AATGCTAGCGAAATGATCAA-3’) genotypes. Polymorphisms in the amplified sequence allows cleavage by Apol restriction enzyme into a specific banding pattern (PCR fingerprinting) that is characteristic of NX-2-producing F. graminearum strains, and hence, the NX-2 genotype can be distinguished from Type B genotypes (i.e., 15-ADON, 3-ADON and NIV) of F. graminearum [40]. Polymorphisms in the TRI13 gene sequence is used to distinguish between the DON and the NIV genotypes based on differential size of the amplicon produced: ~227 bp is produced for a DON genotype when primers Tri13F and Tri13DONR are used, whereas, ~312 bp is produced for a NIV genotype by primers Tri13NIVF and Tri13R [99–101].

5.1.3. TRI5-TRI6 Intergenic Region Sequencing

Bakan et al. [102] developed a PCR-based approach for discriminating between high-producing and low-producing F. culmorum strains based on amplification with specific primers that target the intergenic region between TRI5 and TRI6 genes (TRI6-54, N1-2, N1-2R, 4056, 3551). PCR amplification with primer pair, N1-2 and N1-2R, resulted in a 200 bp amplicon for the high-producing strains, whereas no amplification was obtained for low-producing strains. PCR amplification with the 4056 and 3551 primers, resulted in an amplicon of 650 bp for the low-producing strains, and no amplification was obtained for high-producing strains. A duplex PCR was carried out with N1-2/N1-2R and the 4056/3551 primer pairs; this enabled differentiation of the high-producing from the low-producing F. culmorum strains.

5.1.4. Multi-locus Genotyping Assay (MLGT)

A multi-locus genotyping assay (MLGT) was developed to allow simultaneous determination of species identity and trichothecene genotype [103]. Six gene targets, species identification genes (RED, MAT, and TEF-1) and TRI genes (TRI101, TRI12, TRI3), were amplified in a multiplex PCR. PCR products were subjected to allele-specific primer extension (ASPE) reactions in multiplex reactions containing 48 ASPE probes consisting of each species and a type B trichothecene genotype targeted [103–106]. The resulting biotinylated extension products from ASPE reactions were then hybridized to polystyrene microsphere sets and detection were performed using a Luminex 100 flow cytometer (Luminex Corporation) [103,107].

5.1.5. Quantitation of TRI Gene Products by Real-time qPCR

qPCR single and multiplex PCR detection can be used to determine genotype profile by directly using the fungal substrate or in food [75,83,108–110]. Data from qPCR analysis can be used for qualitative and quantitative analyses in addition to generating gene expression profiles of specific TRI genes involved in DON biosynthesis during (i) infection, (ii) colonization, and (iii) according to substrate composition [111,112].

6. Advantages of TRI Genotyping

The genome sequences of several F. graminearum species complex (FGSC) strains have been published. Additionally, the nucleotide sequences of the core trichothecene biosynthetic gene cluster of many representative strains that produce 3-ADON, 15-ADON, and NIV have been deposited in
GenBank. This availability of sequence information has enabled the design and selection of several primer sets for molecular characterization of various Fusarium strains and species. There are currently 14 complete nucleotide sequences of the trichothecene biosynthetic gene cluster of F. graminearum and F. culmorum in GenBank. Genotyping requires the design and optimization of primer pairs that target one or more gene of the trichothecene biosynthetic pathway. Availability of several species’ genome sequences have allowed development of the primers. Genotyping also allows screening a large number of isolates in a given fungal population and, therefore, provides the option for high-throughput analysis sample sizes far in excess of what is possible in an analytical chemotype determination in terms of speed and number of samples to process.

The practicality of genotyping is highlighted in the identification of novel chemical groups and Fusarium species. Ward et al. [103], through genotyping, confirmed the replacement of a once dominant FHB strain in the US by a more highly toxigenic F. graminearum population, which explained the shift in the chemotype composition of F. graminearum. A shift in genotype profile may hint at a shift in species [94,99,113–119]. Newly encountered sequence variation in the specific TRI genes can lead to the production of different chemical end-products which could escape detection by chemical analysis as in the case of NX-2. Monitoring changes in amino acid sequence of TRI genes would enable prediction of a shift in toxin production. The NIV-producing population in Louisiana, USA [106,120], and similarly, a new species in Ethiopia were identified through genotyping [104]. The discovery of novel trichothecene metabolites indicates suggests that the TRI gene markers used for genotyping are integral to genotyping and there may be a need to develop novel and more universal markers for toxin detection [121].

In breeding against Fusarium head blight (FHB) susceptibility, it is important to understand the chemotype diversity of the pathogen [122–126]. In fact, assessing the sequence (nucleotide and amino acid) diversity of TRI genes of Fusarium strains and their toxin-producing capabilities in breeding programs are considered to be crucial for developing varieties that are more tolerant or resistant to infection [127].

Introduction of novel genotypes into new agroecosystems would challenge Fusarium disease management schemes because selection drives the establishment of the more pathogenic fungus. Therefore, for rapid and accurate detection of TRI, genotypes should be considered as an important aspect of quarantine and biosecurity mechanisms [128]. In terms of transnational and international trade, grains produced and exported from NIV-producing populations in different countries should be more closely monitored for toxin contamination.

7. Incongruence between Chemotype and Genotype

There is a need for continuous monitoring of Fusarium populations at two main tiers: (i) to determine changes in Fusarium species, e.g., new introductions, in a given environment [117,129,130] and (ii) to detect shifts in toxin production where a more potent toxin is being produced, e.g., NIV is more toxic than DON, and/or where a toxin is being produced at higher concentrations than previously recorded as a result of species introduction and/or host-environmental adaptation [103,108,114,122,126,131–133].

Monitoring requires rapid, accurate, and affordable tools to predict toxin production in the field and molecular diagnostic methods can be used as an interim proxy for determining levels of risk. Trichothecene genotyping is a fast and reliable method for the prediction of trichothecene production in various Fusarium species based on the identification of specific TRI genes involved in the trichothecene biosynthesis pathway [83]. Some studies reported that genotyping was highly correlated to chemotyping and that trichothecene genotyping enabled rapid prediction of production of different toxins [74,83,90,95,108,134,135]. Other studies report incongruence between chemotype and genotype data and warn against using genotyping to solely identify the risk of mycotoxin production [19,37,82,87,136,137].
There can be incongruence between the TRI genotype and chemotype, which underscores the need for optimized molecular and chemical analytics for the characterization of different toxigenic *Fusarium* species. Additional research into methods to support chemotype determination is required as the currently available chemotyping techniques are dependent on induction of trichothecene production by isolates in vitro. The chemotype profile in the field and the profile determined in the laboratory are at the very least partially discordant, and for which, for various reasons (some of them still undefined), chemotype data may fail to predict the “real” risk of toxin production by select *Fusarium* strains. Genotyping provides baseline data for evaluating mycotoxin risk, as this approach confirms the presence of TRI genes in a given genome [138]. Kelly et al. [84] stated that it is possible to accurately infer chemotype from trichothecene genotype based on the NX-2 Type B trichothecene case study. The challenge lies in the induction of mycotoxins in vitro for subsequent chemical detection and quantification.

8. Factors Affecting the Reliability of Genotype-chemotype Association

In vitro toxin induction, production and concentration are strain-, substrate-, temperature-, pH-, water activity-/relative humidity-, and time-dependent for many *Fusarium* species [139]. In addition, the expression of TRI genes that lead to synthesis of trichothecenes and their metabolites or acetylated derivatives is not a linear pathway and should be considered to be a network with multiple routes to a given toxin [71]. This expression is subject to a number of factors that are as yet undefined [37,140].

In vitro induction of trichothecenes for subsequent chemical determination of chemotype minimally necessitates that media (composition of substrate) and incubation conditions are considered for maximum mycotoxin induction. There are no defined conditions for toxin induction for several *Fusarium* species, excluding *F. graminearum*; however, research has shown that even within a given species, different strains within the same geographical location can behave differently under laboratory conditions [39,96,141–144]. Furthermore, although there are reports of protocols for the induction of trichothecenes by *F. graminearum* in vitro based on liquid cultures [145–150], induction of trichothecene production under laboratory conditions remains the limiting factor in detection. Genotyping of *F. graminearum* sensu stricto strains infecting wheat in Minnesota resulted in 3-ADON genotype, but chemical analysis indicated that neither DON nor NIV nor its acetylated derivatives were produced in vitro [151]. The danger in this case was these ‘no trichothecene’ producers were then studied as potential biocontrol agents and the failure to induce trichothecine in vitro and the resultant failure of the chemical analytical method to detect any toxin were not considered [152]. Sugiara et al. [153] also pointed out that NIV-producing strains can also produce low levels of DON, but DON-producers cannot produce NIV. Chemical analysis has to cater to the different options to produce trichothecenes by these strains.

One possible explanation for the similar accumulation of both acetyl derivatives by strains of different chemotype and genotypes could be that the acetyl derivatives biosynthesis (DON) is regulated by temperature [39,154,155]. Temperature influences trichothecene chemotypes [39,154,155]. At high temperatures (above 30 °C), 3-ADON production is favored, with minimum production of 15-ADON between 30–35 °C, while at low temperatures (below 10 °C) 15-ADON production is favored, with minimum production of 3-ADON between 5–10 °C [39,154,156,157]. Variations in the respective concentrations can also be expected for each chemotype with trace amounts of 15-ADON being produced for Chemotype 1A (between 30–35 °C) and vice versa for Chemotype 1B between 5–10 °C. In the case of Chemotype B (NIV and its derivatives) producers, changes in climate had no effect on the production of this mycotoxin [156,158].

Most studies that focused on the effects of abiotic factors on mycotoxin production examined the effects of temperature and incubation time, but few have looked at the effects of water activity *aw* on mycotoxin production [144,159]. Hope et al. [160] determined that the minimum *aw* required for DON production to occur in *F. graminearum* and *F. culmorum* is > 0.93 *aw* at optimum temperature; however, below 0.90 *aw*, no DON is produced. In another study, maximum DON production was observed at
0.995 \textit{aw} at 25 °C, over a 40 days period, on wheat-based media, but as the water availability decreased, DON production also decreased at a significant rate. At lower temperatures (15 °C), maximum DON production was only observed at > 0.981 \textit{aw}. The study also looked at the effects of water availability and NIV production under the same conditions, specifically at 0.995 \textit{aw} and 25 °C, NIV production was 10 times less than DON over a 40-day period but was greater at 15 °C, with maximum NIV production occurring at 0.981 \textit{aw} [161].

Simultaneous production of different toxins can often be missed by analytical chemotyping methods where testing is for one target toxin. Different toxins (e.g., NIV and DON) can be simultaneously produced by the same isolate depending on induction conditions [86,162]. Chemical analysis would also have to cover detection of acetylated and non-acetylated forms of DON as several studies reported the co-production of acetylated forms of trichothecenes by certain strains (3-ADON and 15-ADON), albeit in different relative amounts [86,163–165]. New toxins would escape detection by chemotyping methods due to a lack of standards for a new toxin [166].

The quantitative toxin production capacity of individual \textit{Fusarium} strains can vary significantly, indicating that strains isolated from the same geographical region may have different abilities to produce toxins and produce them in differing levels in vitro [167]. Toxin production is often variable among isolates and some strains simply do not produce any toxins under laboratory conditions [136,168,169].

Conditions that regulate toxin production in the field are impacted by complex environment-plant-pathogen interactions and toxin production in vitro may not reveal the toxigenic potential of a given strain, which can be used to devise strategies to mitigate risk of exposure [170–172]. In planta field inoculation may be able to demonstrate the toxigenic capability of \textit{Fusarium} strain, but even so, there is a myriad of factors that influence toxin production under field conditions [173,174]. The interrelated conditions that simulate the toxin-producing behavior of a strain in the field are not defined. For instance, fungicide use in a given agroecosystem may have an effect on the chemotype of the \textit{Fusarium} population in that environment [175]. Specifically, carbenazim (MBC) resistance can be associated with higher toxin production [176]. The 3-ADON chemotype in Asia is predominant in \textit{F. graminearum} and \textit{F. asiaticum} populations [101]. According to Zhang et al. [177], the 3-ADON chemotype had an adaptive advantage over NIV-producing \textit{F. asiaticum} strains, in that they were more resistant to benzimidazoles. However, these data were relevant to a specific \textit{Fusarium} population and the history and conditions under which MBC was utilized must be considered. Carbenazim-resistant isolates produced higher concentrations of trichothecenes (DON+3-ADON or DON+15-ADON) than carbenazim-S isolates in vitro and in field inoculated wheat heads [178]. Kulik et al. [77] carried out mycotoxin analysis of three \textit{F. graminearum} isolates of 3-ADON, 15-ADON, and NIV chemotypes using RT-qPCR and the results indicated an increase in trichothecene accumulation in most of the tebuconazole-treated samples.

The host plant also plays a defining role in determining the toxicity of a toxin and specific examples are: (i) the ability of potato host to transform DON into NIV [33], (ii) NX-2 is detoxified to non-toxic rearrangement products (NX-3 and NX-3-M1) in planta [166], and (iii) the ability of resistant wheat genotypes to metabolize DON [174,179].

Trichothecenes also function as virulence factors for different \textit{Fusarium} head blight (FHB) pathogens [39,180–182]. As such, different \textit{Fusarium} species have been shown to exhibit host preference and different levels of competitiveness in cases of co-infection [183]. In China, maize is more susceptible to infection by \textit{F. asiaticum} (Chemotype II) and is generally more aggressive than other Chemotype I-producing \textit{Fusarium} sp. [39,99,154,184]. On the other hand, in wheat and rice, Chemotype IA-producers, e.g., \textit{F. asiaticum} are more aggressive than Chemotype II producers and produce higher concentrations of DON [101,155].

9. Future Prospects—Data Sharing and Quality Control

Concerns have been raised about sequence identities in GenBank as the largest and most widely used database. Apart from the discrepancy in using ITS sequences alone for sequence identification,
some entries lack descriptive and up-to-date annotations due to the rapid pace of fungal taxonomy revision, type strains may not be clearly indicated, sequences may be unnamed or only partially named, and there are unpublished sequences in GenBank, which is an indication of authenticity to many researchers [185].

O’Donnell et al. [11] published clear guidelines and a 10-step primer for obtaining accurate species-level identification based on BLASTn queries. The study described sequence-based identification of Fusaria using databases that contain a library of sequences from type strains and whose sequence identity has been verified, i.e., the CBS-KNAW Biodiversity Centre (http://www.cbs.knaw.nl/Fusarium/). The reporting standards in Fusarium MLST ensure that (i) submitted data are sufficient for clear interpretation and querying by other researchers, (ii) data formats are standardized, (iii) terminology used is consistent, (iv) TEF1, RPB2, and RPB1 sequences specific for Fusarium identification are verified, (v) haplotype data based on TEF1 analysis is consistent, and finally, (vi) there is clear and accessible information for contacting the specific curators dealing with Fusarium-related matters and software/website-related matters.

9.1. Data Repositories for Fusarium Genome Sequences

One of the challenges of developing a genotype database is the availability of molecular as well as other metadata in relation to identification of Fusarium strains. There are several repositories, outside of GenBank, that house searchable genomic data for a range of Fusarium species. Screenshots of the home page of each of these databases and/or searchable browsers are presented as figures (Figures 1–5) under each repository as proof of recent activity and on-going curation. All websites were accessed on 25th October, 2019.

Figure 1. Fusarium MLST (http://www.wi.knaw.nl/Fusarium/).

Figure 2. Luxemberg Fusarium collection and the European Fusarium collection (https://catalogue.luxmcc.lu/).
9.5. **FungiDB**

FungiDB belongs to the EuPathDB suite of databases and offers an integrated genomic and functional genomic database for fungi. FungiDB also includes experimental and environmental isolate sequence data, comparative genomics, analysis of gene expression, supplemental bioinformatics analyses, and a web interface for data mining.

9.6. **MycoBank**

This database offers up-to-date taxonomic features and nomenclature, species descriptions, and illustrations in addition to metrics that track these changes. Pairwise sequence alignments of fungi and yeasts against curated references databases are enabled.

---

**Figure 3.** EnsemblFungi ([https://fungi.ensembl.org/index.html](https://fungi.ensembl.org/index.html)).

**Figure 4.** FungiDB ([https://fungidb.org/fungidb/](https://fungidb.org/fungidb/)).

**Figure 5.** MycoBank ([http://www.mycobank.org/](http://www.mycobank.org/)).

9.2. **Fusarium MLST**

This database can be queried against for DNA sequence-based identification of single and multiple sequences. The single sequence alignment algorithm compares the sequence of an unknown against sequences present in the *Fusarium* MLST reference database. The multiple sequences option, sequences from two or more loci from the unknown are queried against the *Fusarium* MLST database using tools within the BioloMICS software.
9.3. **A European Database of *F. graminearum* and *F. culmorum* Trichothecene Genotypes**

This is a freely accessible and updatable database of trichothecene genotypes of strains from three *Fusarium* species, collected over the period 2000–2013: 1147 *F. graminearum*, 479 *F. culmorum*, and 3 *F. cortaderiae* strains from 17 European countries in addition to mapping of trichothecene type B genotype with respect to distribution according to species [39]. Information on host plant, country of origin, sampling location, year of sampling, and previous crop are available. This information is important to epidemiological analysis of potential spatial and temporal trichothecene genotype shifts in Europe.

9.4. **Ensembl Fungi**

Ensembl Fungi is a browser used for exploring fungal genomes. The genome sequences are accessed from the databases of the International Nucleotide Sequence Database Collaboration (the European Nucleotide Archive at the EBI, GenBank at the NCBI, and the DNA Database of Japan).

9.5. **FungiDB**

FungiDB belongs to the EuPathDB suite of databases and offers an integrated genomic and functional genomic database for fungi. FungiDB also includes experimental and environmental isolate sequence data, comparative genomics, analysis of gene expression, supplemental bioinformatics analyses, and a web interface for data-mining.

9.6. **MycoBank**

This database offers up-to-date taxonomic features and nomenclature, species descriptions, and illustrations in addition to metrics that track these changes. Pairwise sequence alignments of fungi and yeasts against curated references databases are enabled [186,187].

**Author Contributions:** Conceptualization, S.N.R.; methodology, S.N.R.; formal analysis, A.C.R., S.N.R.; data curation, A.C.R., R.T.V., S.N.R.; writing—original draft preparation, S.N.R.; writing—review and editing, A.C.R., R.T.V., S.N.R.; funding acquisition, S.N.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** The University of the West Indies, St. Augustine, Campus Research and Publication Grant: CRP.3.MAR16.12.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Wu, F. Measuring the economic impacts of *Fusarium* toxins in animal feeds. *Anim. Feed Sci. Technol.* 2007, 137, 363–374. [CrossRef]
2. Bryden, W.L. Food and feed, mycotoxins and the perpetual pentagram in a changing animal production environment. *Anim. Prod. Sci.* 2012, 52, 383–397. [CrossRef]
3. Introduction to the Mycotoxin Issue. Available online: [https://www.youtube.com/watch?v=GMEMiH-Njmc&list=PLUsKEB0unbGiH96SILXJ_3U37AcS_rvI9&index=2&t=0s](https://www.youtube.com/watch?v=GMEMiH-Njmc&list=PLUsKEB0unbGiH96SILXJ_3U37AcS_rvI9&index=2&t=0s) (accessed on 20 October 2019).
4. Gewinner, J.; Harnisch, R.; Mück, O. *Manual of the Prevention of Post-Harvest Grain Losses*, 2nd ed.; Deutsche Gesellschaft für Technische Zusammenarbeit: Eschborn, Germany, 1996.
5. Weidenbörner, M. *Mycotoxins in Feedstuffs*, 1st ed.; Springer: New York, NY, USA, 2007; p. 308.
6. Edwards, S.; Barrier-Guillot, B.; Clasen, P.E.; Hietaniemi, V.; Pettersson, H. Emerging issues of HT-2 and T-2 toxins in European cereal production. *World Mycotoxin J.* 2009, 2, 173–179. [CrossRef]
7. Schuhmacher-Wolz, U.; Heine, K.; Schneider, K. Report on toxicity data on trichothecene mycotoxins HT-2 and T-2 toxins. *EFSA Support. Publ.* 2010, 7, 65E. [CrossRef]
8. van der Fels-Klerx, H.J. Occurrence data of trichothecene mycotoxins T-2 toxin and HT-2 toxin in food and feed. *EFSA Support. Publ.* 2010, 7, 66E. [CrossRef]
9. Sobrova, P.; Adam, V.; Vasatková, A.; Beklova, M.; Zeman, L.; Kizek, R. Deoxynivalenol and its toxicity. *Interdiscip. Toxicol.* 2010, 3, 94–99. [CrossRef]
10. Balajee, S.A.; Borman, A.M.; Brandt, M.E.; Cano, J.; Cuenca-Estrella, M.; Dannaoui, E.; Guarro, J.; Haase, G.; Kibbler, C.C.; Meyer, W.; et al. Sequence-based identification of Aspergillus, Fusarium, and Mucorales species in the clinical mycology laboratory: Where are we and where should we go from here? J. Clin. Microbiol. 2009, 47, 877–884. [CrossRef]

11. O’Donnell, K.; Ward, T.J.; Robert, V.A.; Crous, P.W.; Geiser, D.M.; Kang, S. DNA sequence-based identification of Fusarium: Current status and future directions. Phytoparasitica 2015, 43, 583–595. [CrossRef]

12. Taylor, J.W.; Jacobson, D.J.; Kroken, S.; Kasuga, T.; Geiser, D.M.; Hibbett, D.S.; Fisher, M.C. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 2000, 31, 21–32. [CrossRef]

13. Guarro, J. Fusariosis, a complex infection caused by a high diversity of fungal species refractory to treatment. Eur. J. Clin. Microbiol. Infect. Dis. 2013, 32, 1491–1500. [CrossRef]

14. van Diepeningen, A.D.; Feng, P.; Ahmed, S.; Sudhadham, M.; Bunyaratavej, S.; de Hoog, G.S. Spectrum of Fusarium infections in tropical dermatology evidenced by multilocus sequencing typing diagnostics. Mycoses 2015, 58, 48–57. [CrossRef] [PubMed]

15. O’Donnell, K.; Gueidan, C.; Sink, S.; Johnston, P.R.; Crous, P.W.; Glen, A.; Riley, R.; Zitomer, N.C.; Colyer, P.; Waalwijk, C.; et al. A two-locus DNA sequence database for typing plant and human pathogens within the Fusarium oxysporum species complex. Fungal Genet. Biol. 2009, 46, 936–948. [CrossRef] [PubMed]

16. O’Donnell, K.; Sutton, D.A.; Rinaldi, M.G.; Sarver, B.A.; Balajee, S.A.; Schroers, H.J.; Summerbell, R.C.; Robert, V.A.; Crous, P.W.; Zhang, N.; et al. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J. Clin. Microbiol. 2010, 48, 3708–3718. [CrossRef] [PubMed]

17. Garmendia, G.; Pattarino, L.; Negrín, C.; Martínez-Silveira, A.; Pereyra, S.; Ward, T.J.; Vero, S. Species composition, toxigenic potential and aggressiveness of Fusarium isolates causing head blight of barley in Uruguay. Food Microbiol. 2018, 76, 426–433. [CrossRef] [PubMed]

18. Nicholson, P.; Simpson, D.R.; Weston, G.; Rezanaoor, H.N.; Lees, A.K.; Parry, D.W.; Joyce, D. Detection and quantification of Fusarium culmorum and Fusarium graminearum genotypes and production profiles of Fusarium poae isolates causing head blight of barley in Uruguay. Food Microbiol. 2014, 76, 426–433. [CrossRef] [PubMed]

19. Covarelli, L.; Beccari, G.; Salvi, S. Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. Food Chem. Toxicol. 2011, 49, 2365–2369. [CrossRef]

20. Jurado, M.; Vázquez, C.; Patiño, B.; González-Jaén, M.T. PCR detection assays for the trichothecene-producing species Fusarium graminearum, Fusarium langsethiae, Fusarium poae, Fusarium equiseti and Fusarium sporotrichioides. Syst. Appl. Microbiol. 2005, 28, 562–568. [CrossRef]

21. Biazio, G.R.D.; Leite, G.G.S.; Tessmann, D.J.; Barbosa-Tessmann, I.P. A new PCR approach for the identification of Fusarium graminearum. Braz. J. Microbiol. 2008, 39, 554–560. [CrossRef]

22. Parry, D.W.; Nicholson, P. Development of a PCR assay to detect Fusarium poae in wheat. Plant Pathol. 1996, 45, 383–391. [CrossRef]

23. Konstantinova, P.; Yli-Mattila, T. IGS-RFLP analysis and development of molecular markers for identification of Fusarium poae, Fusarium langsethiae, Fusarium sporotrichioides and Fusarium kyushuense. Int. J. Food Microbiol. 2004, 95, 321–331. [CrossRef]

24. Demeke, T.; Clear, R.M.; Patrick, S.K.; Gaba, D. Species-specific PCR-based assays for the detection of Fusarium species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. Food Microbiol. 2005, 103, 271–284. [CrossRef] [PubMed]

25. Kulik, T.; Fordosi, G.; Pszczółkowska, A.; Podzieder, K.; Lapinski, M. Development of PCR assay based on ITS2 rDNA polymorphism for the detection and differentiation of Fusarium sporotrichioides. FEMS Microbiol. Lett. 2004, 239, 181–186. [CrossRef] [PubMed]

26. Aoki, T.; O’Donnell, K. Morphological and molecular characterization of Fusarium pseudograminearum sp. nov., formerly recognized as the group 1 population of F. graminearum. Mycologia 1999, 91, 597–609. [CrossRef]

27. Yoder, W.T.; Christianson, L.M. Species-specific primers resolve members of Fusarium section Fusarium: Taxonomic status of the edible “quorn” fungus reevaluated. Fungal Genet. Biol. 1998, 23, 68–80. [CrossRef] [PubMed]

28. O’Donnell, K.; Cigelnik, E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol. Phylogenet. Evol. 1997, 7, 103–116. [CrossRef]
30. O’Donnell, K.; Cigelnik, E.; Nirenberg, H.I. Molecular systematics and phylogeography of the Giberella fujikuroi species complex. *Mycologia* 1998, 90, 465–493. [CrossRef]
31. Li, Y.; Wang, Z.; Beier, R.C.; Shen, J.; Smet, D.D.; De Saeger, S.; Zhang, S. T-2 toxin, a trichothecene mycotoxin: Review of toxicity, metabolism, and analytical methods. *J. Agric. Food Chem.* 2011, 59, 3441–3453. [CrossRef]
32. Kraska, R.; Nährer, K.; Richard, J.L.; Rodrigues, I.; Schuhmacher, R.; Slate, A.B.; Whitaker, T.B. Guide to mycotoxins featuring mycotoxin risk management in animal production. In *Special Edition World Nutrition Forum*; Binder, E.M., Ed.; Anytime Publishing Services: Leicestershire, UK, 2012; p. 165.
33. Marin, S.; Ramos, A.J.; Cano-Sancho, G.; Sanchis, V. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol.* 2013, 60, 218–237. [CrossRef]
34. Shi, W.; Tan, Y.; Wang, S.; Gardiner, D.; De Saeger, S.; Liao, Y.; Wang, C.; Fan, Y.; Wang, Z.; Wu, A. Mycotoxicogenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling. *Toxins* 2017, 9, 6. [CrossRef]
35. Delgado, J.A.; Schwarz, P.B.; Gillespie, J.; Rivera-Varas, V.V.; Secor, G.A. Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. *Phytopathology* 2010, 100, 290–296. [CrossRef] [PubMed]
36. Castillo, M.; Samar, M.M.; Moltó, G.; Resnik, S.; Pacion, A. Trichothecenes and zearalenone production by *Fusarium* species isolated from Argentinean black beans. *Mycotaxon* 2002, 8, 31–36. [CrossRef] [PubMed]
37. Desjardins, A.E. Natural product chemistry meets genetics: When is a genotype a chemotype? *J. Agric. Food Chem.* 2008, 56, 7587–7592. [CrossRef] [PubMed]
38. Desjardins, A.E. Natural product chemistry meets genetics: When is a genotype a chemotype? *J. Agric. Food Chem.* 2008, 56, 7587–7592. [CrossRef] [PubMed]
39. McCormick, S.P.; Stanley, A.M.; Stover, N.A.; Alexander, N.J. Trichothecenes: From simple to complex mycotoxins. *Toxins* 2011, 3, 802–814. [CrossRef]
40. Pasquali, M.; Beyer, M.; Logrieco, A.; Audenaert, K.; Balmas, V.; Basler, R.; Boutigny, A.L.; Chrpova, J.; Czembor, E.; Gagkaeva, T.; et al. A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. *Front. Microbiol.* 2016, 7, 406. [CrossRef]
41. Kelly, A.; Proctor, R.H.; Belzile, F.; Chulze, S.N.; Clear, R.M.; Cowger, C.; Elmer, W.; Lee, T.; Obanor, F.; Waalwijk, C.; et al. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. *Fungal Genet. Biol.* 2016, 95, 39–48. [CrossRef]
42. Bukowska, A.; Kalin’ski, T.; Koper, M.; Kostrzewska-Szalowska, I.; Kwiatowski, J.; Mazur-Marzec, H.; Jasser, I. Predicting blooms of toxic cyanobacteria in eutrophic lakes with diverse cyanobacterial communities. *Sci. Rep.* 2017, 7, 8342. [CrossRef] [PubMed]
43. Ooko, S.; Rose, L.; Okuo, A.; Nsirhifelhe, N.; Sila, H.; Viljoen, A. Assessing genotype-by-environment interactions in aspergillus car rot and pre-harvest aflatoxin accumulation in maize inbred lines. *Agronomy* 2017, 7, 86. [CrossRef]
44. Kelly, L.T.; Wood, S.A.; McAllister, T.G.; Ryan, K.G. Development and application of a quantitative PCR assay to assess genotype dynamics and anatoxin content in *Microcoleus autumnalis*-dominated mats. *Toxins* 2018, 10, 431. [CrossRef]
45. Yu, B.; Huai, D.; Huang, L.; Kang, Y.; Ren, X.; Chen, Y.; Zhou, X.; Luo, H.; Liu, N.; Chen, W.; et al. Identification of genomic regions and diagnostic markers for resistance to aflatoxin contamination in peanut (*Arachis hypogaea* L.). *BMC Genet.* 2019, 20, 32. [CrossRef]
46. Laabei, M.; Recker, M.K.; Aldeljawi, M.; Gulay, Z.; Sloan, T.J.; Williams, P.; Jeffries, E.; Endres, J.L.; Bayles, K.W.; Fey, P.D.; et al. Predicting the virulence of MRSA from its genome sequence. *Genome Res.* 2014, 24, 839–849. [CrossRef] [PubMed]
47. Ooko, S.; Rose, L.; Ouko, A.; Netshifelhe, N.; Sila, H.; Viljoen, A. Assessing genotype-by-environment interactions in aspergillus car rot and pre-harvest aflatoxin accumulation in maize inbred lines. *Agronomy* 2017, 7, 86. [CrossRef]
48. Kelly, L.T.; Wood, S.A.; McAllister, T.G.; Ryan, K.G. Development and application of a quantitative PCR assay to assess genotype dynamics and anatoxin content in *Microcoleus autumnalis*-dominated mats. *Toxins* 2018, 10, 431. [CrossRef]
49. Yu, B.; Huai, D.; Huang, L.; Kang, Y.; Ren, X.; Chen, Y.; Zhou, X.; Luo, H.; Liu, N.; Chen, W.; et al. Identification of genomic regions and diagnostic markers for resistance to aflatoxin contamination in peanut (*Arachis hypogaea* L.). *BMC Genet.* 2019, 20, 32. [CrossRef]
51. Park, J.W.; Shon, D.H.; Kim, Y.B. Application of an enzyme-linked immunosorbent assay for detecting mold contamination in agricultural commodities and comparison with conventional assays. Food Agric. Immunol. 2003, 15, 159–166. [CrossRef]

52. Meirelles, P.G.; Ono, M.A.; Ohe, M.C.T.; Maroneze, D.M.; Itano, E.N.; Garcia, G.T.; Sugiyara, Y.; Ueno, Y.; Hirooka, E.Y.; Ono, E.Y. Detection of Fusarium sp. contamination in corn by enzyme-linked immunosorbent assay. Food Agric. Immunol. 2006, 17, 79–89. [CrossRef]

53. Dzuman, Z.; Vaclavikova, M.; Polisenska, I.; Vepríková, Z.; Fenclova, M.; Zachariasova, M.; Hajslova, J. Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: Investigation of the impact of sample matrix on results accuracy. Anal. Bioanal. Chem. 2014, 406, 505–514. [CrossRef]

54. Righetti, L.; Galaverna, G.; Dall’Asta, C. Group detection of DON and its modified forms by an ELISA kit. Food Addit. Contam. Part A 2017, 34, 248–254. [CrossRef]

55. Ruprich, J.; Ostrý, V. Immunochromatographic methods in health risk assessment: Cross reactivity of antibodies against mycotoxin deoxynivalenol with deoxynivalenol-3-glucoside. Cent. Eur. J. Public Health 2008, 16, 34–37. [CrossRef] [PubMed]

56. Tangni, E.K.; Motte, J.C.; Callebaut, A.; Pussemier, L. Cross-reactivity of antibodies in some commercial deoxynivalenol test kits against some fusariotoxins. J. Agric. Food Chem. 2010, 58, 12625–12633. [CrossRef] [PubMed]

57. Zachariasova, M.; Hajslova, J.; Kostelanska, M.; Poustka, J.; Krplova, A.; Cuhra, P.; Hoehel, I. Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry. Anal. Chim. Acta 2008, 625, 77–86. [CrossRef] [PubMed]

58. Shephard, G.S. Current status of mycotoxin analysis: A critical review. J. AOAC Int. 2016, 99, 842–848. [CrossRef]

59. Alshannaq, A.; Yu, J.H. Occurrence, toxicity, and analysis of major mycotoxins in food. Int. J. Environ. Res. Public Health 2017, 14, 632. [CrossRef]

60. Pereira, V.L.; Fernandes, J.O.; Cunha, S.C. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. Trends Food Sci. Technol. 2014, 36, 96–136. [CrossRef]

61. Nakagawa, H.; Matsuo, Y.; McCormick, S.; Lim, C.W. Development of an LC-MS/MS determination method for T-2 toxin and its glucoside and acetyl derivatives for estimating the contamination of total T-2 toxins in staple flours. J. AOAC Int. 2018, 101, 658–666. [CrossRef]

62. Miro-Abella, E.; Herrero, P.; Canela, N.; Arola, L.; Ras, R.; Fontanals, N.; Borrull, F. Determination of trichothecenes in cereal matrices using subcritical water extraction followed by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Food Anal. Methods 2018, 11, 1113–1121. [CrossRef]

63. Yoshinari, T.; Takeda, N.; Watanabe, M.; Sugita-Konishi, Y. Development of an analytical method for simultaneous determination of the modified forms of 4, 15-diacetoxyscirpenol and their occurrence in Japanese retail food. Toxins 2018, 10, 178. [CrossRef]

64. Ok, H.E.; Lee, S.Y.; Chun, H.S. Occurrence and simultaneous determination of nivalenol and deoxynivalenol in rice and bran by HPLC-UV detection and immunoaffinity cleanup. Food Control 2018, 87, 53–59. [CrossRef]

65. Cigić, I.K.; Prosen, H. An overview of conventional and emerging analytical methods for the determination of mycotoxins. Int. J. Mol. Sci. 2009, 10, 62–115. [CrossRef]

66. Buttinger, G.; Krksa, R. Determination of B-trichothecenes in wheat by post column derivatisation liquid chromatography with fluorescence detection (PCC-HPLC-FLD). Mycotoxin Res. 2003, 19, 139–143. [CrossRef]

67. Arroyo-Manzanares, N.; De Ruyck, K.; Uka, V.; Gámiz-Gracia, L.; García-Campana, A.M.; De Saeger, S.; Di Mavungu, J.D. In-house validation of a rapid and efficient procedure for simultaneous determination of ergot alkaloids and other mycotoxins in wheat and maize. Anal. Bioanal. Chem. 2018, 410, 5567–5581. [CrossRef] [PubMed]

68. Zuo, H.G.; Zhu, J.X.; Shi, L.; Zhan, C.R.; Guo, P.; Wang, Y.; Zhang, Y.; Liu, J. Development of a novel immunoaffinity column for the determination of deoxynivalenol and its acetylated derivatives in cereals. Food Anal. Methods 2018, 11, 2252–2260. [CrossRef]

69. Urusov, A.E.; Gubaidullina, M.K.; Petrakova, A.V.; Zherdev, A.V.; Dzantiev, B.B. A new kind of highly sensitive competitive lateral flow immunoassay displaying direct analyte-signal dependence: Application to the determination of the mycotoxin deoxynivalenol. Mikrochim. Acta 2018, 185, 29. [CrossRef]
70. Villafana, R.T.; Ramdass, A.C.; Rampersad, S.N. Selection of *Fusarium* trichothecene toxin genes for molecular detection depends on TRI gene cluster organization and gene function. *Toxins* 2019, 11, 36. [CrossRef]
71. Kimura, M.; Tokai, T.; Takahashi-Ando, N.; Ohsato, S.; Fujimura, M. Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: Pathways, genes, and evolution. *Biosci. Biotechnol. Biochem.* 2007, 71, 070310525. [CrossRef]
72. Houle, D.; Govindaraju, D.R.; Omholt, S. Phenomics: The next challenge. *Nat. Rev. Genet.* 2010, 11, 855. [CrossRef]
73. Ward, T.J.; Bielawski, J.P.; Kistler, H.C.; Sullivan, E.; O’Donnell, K. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. *Proc. Natl. Acad. Sci. USA* 2002, 99, 9278–9283. [CrossRef]
74. Wang, C.L.; Cheng, Y.H. Identification and trichothecene genotypes of *Fusarium graminearum* species complex from wheat in Taiwan. *Bot. Stud.* 2011, 58, 4. [CrossRef]
75. Kulik, T. Development of TaqMan assays for 3ADON, 15ADON and NIV *Fusarium* genotypes based on TRI12 gene. *Cereal Res. Commun.* 2011, 39, 200–214. [CrossRef]
76. Tóth, B.; Mesterházy, Á.; Nicholson, P.; Téren, J.; Varga, J. Mycotoxin production and molecular variability of European and American isolates of *Fusarium culmorum*. In *Molecular Diversity and PCR-Detection of Toxigenic Fusarium Species and Ochratoxigenic Fungi*, 1st ed.; Mulé, G., Bailey, J.A., Cooke, B.M., Logrieco, A., Eds.; Springer: Dordrecht, The Netherlands, 2004; pp. 587–599.
77. Kulik, T.; Lojko, M.; Jestoi, M.; Perkowski, J. Sublethal concentrations of azoles induce tri transcript levels and trichothecene production in *Fusarium graminearum*. *FEMS Microbiol. Lett.* 2012, 335, 58–67. [CrossRef] [PubMed]
78. Jennings, P.; Coates, M.E.; Turner, J.A.; Chandler, E.A.; Nicholson, P. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. *Plant Pathol.* 2004, 53, 182–190. [CrossRef]
79. Kammoun, L.G.; Gargouri, S.; Barreau, C.; Richard-Forget, F.; Hajaoui, M.R. Trichothecene chemotypes of *Fusarium culmorum* infecting wheat in Tunisia. *Int. J. Food Microbiol.* 2010, 140, 84–89. [CrossRef] [PubMed]
80. Starkey, D.E.; Ward, T.J.; Anki, T.; Gale, L.R.; Kistler, H.C.; Geiser, D.M.; Suga, H.; Toth, B.; Varga, J.; O’Donnell, K. Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genet. Biol.* 2007, 44, 1191–1204. [CrossRef] [PubMed]
81. Lee, T.; Oh, D.W.; Kim, H.S.; Lee, J.; Kim, Y.H.; Yun, S.H.; Lee, Y.W. Identification of deoxynivalenol and nivalenol-producing chemotypes of Gibberella zeae by using PCR. *Appl. Environ. Microbiol.* 2001, 67, 2966–2972. [CrossRef]
82. Crippin, T.; Renaud, J.B.; Sumarah, M.W.; Miller, J.D. Comparing genotype and chemotype of *Fusarium graminearum* from cereals in Ontario, Canada. *PLoS ONE* 2019, 14, e0216735. [CrossRef]
83. Tralamazza, S.M.; Braghini, R.; Corrêa, B. Trichothecene genotypes of the *Fusarium graminearum* species complex isolated from Brazilian wheat grains by conventional and quantitative PCR. *Front. Microbiol.* 2016, 7, 246. [CrossRef]
84. Kelly, A.C.; Clear, R.M.; O’Donnell, K.; McCormick, S.; Turkington, T.K.; Tekauz, A.; Gilbert, J.; Kistler, H.C.; Busman, M.; Ward, T.J. Diversity of *Fusarium* head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. *Fungal Genet. Biol.* 2015, 82, 22–31. [CrossRef]
85. Lofgren, L.; Riddle, J.; Dong, Y.; Kuhnem, P.R.; Cummings, J.A.; Del Ponte, E.M.; Bergstrom, G.C.; Kistler, H.C. A high proportion of NX-2 genotype strains are found among *Fusarium graminearum* isolates from north-eastern New York state. *Eur. J. Plant Pathol.* 2018, 150, 791–796. [CrossRef]
86. Mugrabi de Kuppler, A.M.; Steiner, U.; Sulyok, M.; Kraska, R.; Orkée, E.C. Genotyping and phenotyping of *Fusarium graminearum* isolates from Germany related to their mycotoxin biosynthesis. *Int. J. Food Microbiol.* 2011, 151, 78–86. [CrossRef] [PubMed]
87. Sampietro, D.A.; Díaz, C.G.; González, V.; Vattuone, M.A.; Ploper, L.D.; Catalán, C.A.N.; Ward, T.J. Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. *Int. J. Food Microbiol.* 2011, 145, 359–364. [CrossRef] [PubMed]
88. Fulcher, M.R.; Winans, J.B.; Quan, M.; Oladipo, E.D.; Bergstrom, G.C. Population genetics of *Fusarium graminearum* at the interface of wheat and wild grass communities in New York. *Phytopathology* 2019, 109, 2124–2131. [CrossRef] [PubMed]
Desjardins, A.E.; Proctor, R.H. Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a putative new lineage. *Fungal Biol.* 2010, 115, 38–48. [CrossRef] [PubMed]

Reynos, M.M.; Ramirez, M.L.; Torres, A.M.; Chulze, S.N. Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. *Int. J. Food Microbiol.* 2011, 145, 444–448. [CrossRef]

Quarta, A.; Mita, G.; Haidukowski, M.; Logrieco, A.; Mule, G.; Visconti, A. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. *FEBS Microbiol. Lett.* 2006, 259, 7–13. [CrossRef]

Pan, D.; Mionetto, A.; Calero, N.; Reynoso, M.M.; Torres, A.; Bettucci, L. Population genetic analysis and trichothecene profiling of *Fusarium graminearum* from wheat in Uruguay. *Genet. Mol. Res.* 2016, 15, 1–11. [CrossRef]

Barros, G.G.; Oviedo, M.S.; Ramirez, M.L.; Chulze, S.N. Safety aspects in soybean food and feed chains: Fungal and mycotoxins contamination. In *Soybean-Biochemistry, Chemistry and Physiology*, 1st ed.; Ng, T.B., Ed.; IntechOpen: London, UK, 2011; pp. 1–17.

Astolfi, P.; Dos Santos, J.; Schneider, L.; Gomes, L.B.; Silva, C.N.; Tessmann, D.J.; Del Ponte, E.M. Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in southern Brazil. *Int. J. Food Microbiol.* 2011, 148, 197–201. [CrossRef]

Scoz, L.B.; Astolfi, P.; Reartes, D.S.; Schmale, D.G., III; Moraes, M.G.; Del Ponte, E.M. Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. *Plant Pathol.* 2009, 58, 344–351. [CrossRef]

Pasquali, M.; Migheli, Q. Genetic approaches to chemotype determination in type B-trichothecene producing Fusaria. *Int. J. Food Microbiol.* 2014, 189, 164–182. [CrossRef]

Astolfi, P.; Reynoso, M.M.; Ramirez, M.L.; Alves, T.C.A.; Tessmann, D.J.; Del Ponte, E.M. Genetic population structure and trichothecene genotypes of *Fusarium graminearum* isolated from wheat in southern Brazil. *Plant Pathol.* 2012, 61, 289–295. [CrossRef]

Liang, J.M.; Xayamongkhon, H.; Broz, K.; Dong, Y.; McCormick, S.P.; Abramova, S.; Ward, T.J.; Ma, Z.H.; Kistler, H.C. Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper midwestern United States. *Fungal Genet. Biol.* 2014, 73, 83–92. [CrossRef] [PubMed]

Pasquali, M.; Giraud, F.; Brochot, C.; Cocco, E.; Hoffmann, L.; Bohn, T. Genetic *Fusarium* chemotyping as a useful tool for predicting nivalenol contamination in winter wheat. *Int. J. Food Microbiol.* 2010, 137, 246–253. [CrossRef] [PubMed]

Puri, K.D.; Zhong, S. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology* 2010, 100, 1007–1014. [CrossRef] [PubMed]

Zhang, H.; Van der Lee, T.; Waalwijk, C.; Chen, W.; Xu, J.; Xu, J.; Zhang, Y.; Feng, J. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS ONE* 2012, 7, e31722. [CrossRef] [PubMed]

Bakan, B.; Giraud-Delville, C.; Pinson, L.; Richard-Molard, D.; Fournier, E.; Brygoo, Y. Identification by PCR of *Fusarium culmorum* strains producing large and small amounts of deoxynivalenol. *Appl. Environ. Microbiol.* 2002, 68, 5472–5479. [CrossRef]

Taw, T.J.; Clear, R.M.; Rooney, A.P.; O’Donnell, K.; Gaba, D.; Patrick, S.; Starkey, D.E.; Gilbert, J.; Geiser, D.M.; Nowicki, T.W. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genet. Biol.* 2008, 45, 473–484. [CrossRef]

O’Donnell, K.; Ward, T.J.; Aberra, D.; Kistler, H.C.; Aoki, T.; Orwig, N.; Kimura, M.; Bjørnstad, Å.; Klemsdal, S.S. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. *Fungal Genet. Biol.* 2008, 45, 1514–1522. [CrossRef]

Yli-Mattila, T.; Gagkaeva, T.; Ward, T.J.; Aoki, T.; Kistler, H.C.; O’Donnell, K. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian far East. *Mycologia* 2009, 101, 841–852. [CrossRef]
106. Sarver, B.A.J.; Ward, T.J.; Gale, L.R.; Broz, K.; Kistler, H.C.; Aoki, T.; Nicholson, P.; Carter, J.; O’Donnell, K. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 2011, 48, 1096–1107. [CrossRef]

107. Boutigny, A.L.; Ward, T.J.; Ballois, N.; Iancu, G.; Ioos, R. Diversity of the Fusarium graminearum species complex on French cereals. Eur. J. Plant Pathol. 2014, 138, 133–148. [CrossRef]

108. Nielsen, L.K.; Jensen, J.D.; Rodriguez, A.; Jørgensen, L.N.; Justesen, A.F. TRI12 based quantitative real-time PCR assays reveal the distribution of trichothecene genotypes of F. graminearum and F. culmorum isolates in Danish small grain cereals. Int. J. Food Microbiol. 2012, 157, 384–392. [CrossRef] [PubMed]

109. Nielsen, L.K.; Jensen, J.D.; Nielsen, G.C.; Jensen, J.E.; Spliid, N.H.; Thomsen, I.K.; Justesen, A.F.; Collinge, D.B.; Jørgensen, L.N. Fusarium head blight of cereals in Denmark: Species complex and related mycotoxins. Phytopathology 2011, 101, 960–969. [CrossRef] [PubMed]

110. Yli-Mattila, T. Ecology and evolution of toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 2002, 108, 611–624. [CrossRef]

111. Okorski, A.; Polak-Śliwińska, M.; Karpiesiuk, K.; Pszczółkowska, A.; Kozera, W. Real time PCR: A good tool to estimate mycotoxin contamination in pig diets. World Mycotoxin J. 2017, 10, 219–228. [CrossRef]

112. Preiser, V.; Goetsch, D.; Sulyok, M.; Kraska, R.; Mach, R.L.; Farnleitner, A.; Brunner, K. The development of a multiplex real-time PCR to quantify Fusarium DNA of trichothecene and fumonisin producing strains in maize. Anal. Methods 2015, 7, 1358–1365. [CrossRef]

113. Audenaert, K.; Van Broeck, R.; Bekaert, B.; De Witte, F.; Heremans, B.; Messens, K.; Höfte, M.; Haesaert, G. Fusarium head blight (FHB) in Flanders: Population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. Eur. J. Plant Pathol. 2009, 125, 445–458. [CrossRef]

114. Beyer, M.; Pogoda, F.; Pallez, M.; Lazic, J.; Hoffmann, L.; Pasquali, M. Evidence for a reversible drought induced shift in the species composition of mycotoxin producing Fusarium head blight pathogens isolated from symptomatic wheat heads. Int. J. Food Microbiol. 2014, 182–183, 51–56. [CrossRef]

115. Bottalico, A.; Perrone, G. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 2002, 108, 611–624. [CrossRef]

116. Malikhipour, A.; Gilbert, J.; Piercey-Normore, M.; Cloutier, S. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of Fusarium isolates causing head blight in wheat. Plant Dis. 2012, 96, 1016–1025. [CrossRef]

117. Nielsen, L.K.; Jensen, J.D.; Nielsen, G.C.; Jensen, J.E.; Spliid, N.H.; Thomsen, I.K.; Justesen, A.F.; Collinge, D.B.; Jørgensen, L.N. Fusarium head blight of cereals in Denmark: Species complex and related mycotoxins. Phytopathology 2011, 101, 960–969. [CrossRef] [PubMed]

118. Yang, L.; Van der Lee, T.; Yang, X.; Yu, D.; Waalwijk, C. Fusarium populations on Chinese barley show a dramatic gradient in mycotoxin profiles. Phytopathology 2008, 98, 719–727. [CrossRef] [PubMed]

119. Baltova, T.; Poulova, I. Structure and expression of the 5S rRNA gene region in the genomes of the virulent and avirulent populations of Fusarium head blight pathogens isolated from symptomatic wheat heads. Fungal Genet. Biol. 2009, 46, 337–347. [CrossRef] [PubMed]

120. Gale, L.R.; Harrison, S.A.; Ward, T.J.; O’Donnell, K.; Milus, E.A.; Gale, S.W.; Kistler, H.C. Relative aggressiveness and production of 3-or 15-acetyl deoxynivalenol and deoxynivalenol by Fusarium graminearum in spring wheat. Can. J. Plant Pathol. 2010, 32, 146–152. [CrossRef] [PubMed]

121. Frühmann, P.; Weigl-Pollack, T.; Mikula, H.; Wiesenberger, G.; Adam, G.; Varga, E.; Berthiller, F.; Kraska, R.; Hametner, C.; Fröhlich, J. Methylthiodeoxynivalenol (MTD): Insight into the chemistry, structure and toxicity of thia-Michael adducts of trichothecenes. Org. Biomol. Chem. 2014, 12, 5144–5150. [CrossRef]

122. Gilbert, J.; Clear, R.M.; Ward, T.J.; Gaba, D.; Tekauz, A.; Turkington, T.K.; Woods, S.M.; Nowicki, T.; O’Donnell, K. Relative aggressiveness and production of 3-or 15-acetyl deoxynivalenol and deoxynivalenol by Fusarium graminearum in spring wheat. Can. J. Plant Pathol. 2010, 32, 146–152. [CrossRef] [PubMed]

123. Gosman, N.; Steed, A.; Chandler, E.; Thomsett, M.; Nicholson, P. Evaluation of type I Fusarium head blight resistance of wheat using non-deoxynivalenol-producing fungi. Plant Pathol. 2010, 59, 147–157. [CrossRef]

124. He, X.; Singh, P.K.; Duveiller, E.; Schleng, N.; Dreisigacker, S.; Singh, R.P. Identification and characterization of international Fusarium head blight screening nurseries of wheat at CIMMYT, Mexico. Eur. J. Plant Pathol. 2013, 136, 123–134. [CrossRef]

125. Spolit, P.; Barros, N.C.; Gomes, L.B.; Dos Santos, J.; Del Ponte, E.M. Phenotypic and pathogenic traits of two species of the Fusarium graminearum complex possessing either 15-ADON or NIV genotype. Eur. J. Plant Pathol. 2012, 133, 621–629. [CrossRef]
126. von der Ohe, C.; Gauthier, V.; Tamburic-Ilincic, L.; Brule-Babel, A.; Fernando, W.D.; Clear, R.; Ward, T.J.; Miedaner, T. A comparison of aggressiveness and deoxynivalenol production between Canadian Fusarium graminearum isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur. J. Plant Pathol. 2010, 127, 407–417. [CrossRef]

127. Horevaj, P.; Gale, L.R.; Milus, E.A. Resistance in winter wheat lines to initial infection and spread within spikes by deoxynivalenol and nivalenol chemotypes of Fusarium graminearum. Plant Dis. 2011, 95, 31–37. [CrossRef] [PubMed]

128. Bilska, K.; Jurczak, S.; Kulik, T.; Ropelewksa, E.; Olszewski, J.; Zelechowski, M.; Zapotoczny, P. Species composition and trichothecene genotype profiling of Fusarium field isolates recovered from wheat in Poland. Toxins 2018, 10, 325. [CrossRef] [PubMed]

129. Fredlund, E.; Gidlund, A.; Sulyok, M.; Börjesson, T.; Kraska, R.; Olsen, M.; Lindblad, M. Deoxynivalenol and other selected Fusarium toxins in Swedish oats—Occurrence and correlation to specific Fusarium species. Int. J. Food Microbiol. 2013, 167, 276–283. [CrossRef] [PubMed]

130. Xu, X.M.; Parry, D.W.; Nicholson, P.; Thomsett, M.A.; Simpson, D.; Edwards, S.G.; Cooke, B.M.; Doohan, F.M.; Brennan, J.M.; Moretti, A.; et al. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. Eur. J. Plant Pathol. 2005, 112, 143–154. [CrossRef]

131. Guo, X.W.; Fernando, W.D.; Seow-Brock, H.Y. Population structure, chemotype diversity, and potential chemotype shifting of Fusarium graminearum in wheat fields of Manitoba. Plant Dis. 2008, 92, 756–762. [CrossRef]

132. Waalwijk, C. Fusarium species on wheat in the Netherlands: Inventory and molecular identification. In European Seminar on Fusarium—Mycotoxins, Taxonomy and Pathogenicity. Proceedings of the Seventh International Symposium; Chelkowski, P., Golinski, P., Kozlowska, M., Kwaska, H., Perkowski, J., Eds.; Warsaw Agricultural University: Poznan, Poland, 2002; Volume 43, pp. 125–130.

133. Foroud, N.A.; Ouellet, T.; Laroche, A.; Oosterveen, B.; Jordan, M.C.; Ellis, B.E.; Eudes, F. Differential transcriptome analyses of three wheat genotypes reveal different host response pathways associated with Fusarium head blight and trichothecene resistance. Plant Pathol. 2012, 61, 296–314. [CrossRef]

134. Demeke, T.; Gräfenhan, T.; Clear, R.M.; Phan, A.; Ratmayaka, I.; Chapados, J.; Patrick, S.K.; Gaba, D.; Lévesque, C.A.; Seifert, K.A. Development of a specific TaqMan® real-time PCR assay for quantification of Fusarium graminearum clade 7 and comparison of fungal biomass determined by PCR with deoxynivalenol content in wheat and barley. Int. J. Food Microbiol. 2010, 141, 45–50. [CrossRef]

135. Dinolfo, M.I.; Barros, G.G.; Stenglein, S.A. Development of a PCR assay to detect the potential production of nivalenol in Fusarium poae. FEMS Microbiol. Lett. 2012, 323, 99–104. [CrossRef]

136. Tan, D.C.; Flematti, G.R.; Ghisalberti, E.L.; Sivasithamparam, K.; Chakraborty, S.; Obanor, F.; Jayasena, K.; Barbetti, M.J. Mycotoxins produced by Fusarium spp. associated with Fusarium head blight of wheat in western Australia. Mycotoxin Res. 2012, 28, 89–96. [CrossRef]

137. Kulik, T.; Busko, M.; Bilska, K.; Ostrowska-Kołodziejczak, A.; van Diepeningen, A.; Perkowski, J.; Stenglein, S. Depicting the discrepancy between tri genotype and chemotype on the basis of strain CBS 139514 from a field population of F. graminearum ssp. associated with Fusarium head blight of wheat in Ontario, central Canada. Plant Pathol. 2016, 66, 14–27. [CrossRef]

138. Vogelgsang, S.; Sulyok, M.; Bänziger, I.; Kraska, R.; Schuhmacher, R.; Forrer, H.R. Effect of fungal strain and cereal substrate on in vitro mycotoxin production by Fusarium poae and Fusarium avenaceum. Food Addit. Contam. 2008, 25, 745–757. [CrossRef] [PubMed]

139. Miller, J.D.; Greenhalgh, R.; Wang, Y.; Lu, M. Trichothecene chemotypes of three Fusarium species. Mycologia 1991, 83, 121–130. [CrossRef]

140. Frisvad, J.C. Media and growth conditions for induction of secondary metabolite production. In Fungal Secondary Metabolism: Methods and Protocols, 1st ed.; Keller, N.P., Turner, G., Eds.; Humana Press: Totowa, NJ, USA, 2012; Volume 944, pp. 47–58.

141. Kulik, T.; Abarenkov, K.; Busko, M.; Bilska, K.; van Diepeningen, A.D.; Ostrowska-Kołodziejczak, A.; Krawczyk, K.; Brankovics, B.; Stenglein, S.; Sawicki, J.; et al. ToxGen: An improved reference database for the identification of type B-trichothecene genotypes in Fusarium. PeerJ 2017, 5, e2992. [CrossRef]
Toxins 2020, 12, 64

143. Buško, M.; Kulik, T.; Ostrowska, A.; Góral, T.; Perkowski, J. Quantitative volatile compound profiles in fungal cultures of three different *Fusarium graminearum* chemotypes. *FEBS Microbiol. Lett.* 2014, 359, 85–93. [CrossRef] [PubMed]

144. Kokkonen, M.; Jestoi, M.; Laitila, A. Mycotoxin production of *Fusarium langsethiae* and *Fusarium sporotrichioides* on cereal-based substrates. *Mycotaxon Res.* 2012, 28, 25–35. [CrossRef]

145. Harris, L.J.; Alexander, N.J.; Saparno, A.; Blackwell, B.; McCormick, S.P.; Desjardins, A.E.; Robert, L.S.; Tinker, N.; Hattori, J.; Piche, C.; et al. A novel gene cluster in *Fusarium graminearum* contains a gene that contributes to butenolide synthesis. *Fungal Genet. Biol.* 2007, 44, 293–306. [CrossRef] [PubMed]

146. Jiao, F.; Kawakami, A.; Nakajima, T. Effects of different carbon sources on trichothecene production and Tri gene expression by *Fusarium graminearum* in liquid culture. *FEBS Microbiol. Lett.* 2008, 285, 212–219. [CrossRef]

147. Gardiner, D.M.; Kazan, K.; Manners, J.M. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. *Fungal Genet. Biol.* 2009, 46, 604–613. [CrossRef]

148. Gardiner, D.M.; Osborne, S.; Kazan, K.; Manners, J.M. Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. *Microbiology* 2009, 155, 3149–3156. [CrossRef]

149. Lowe, R.G.; Jubault, M.; Canning, G.; Urban, M.; Hammond-Kosack, K.E. The induction of mycotoxins by trichothecene producing *Fusarium species*. In *Plant Fungal Pathogens: Methods and Protocols*, 1st ed.; Bolton, M.D., Thomma, B.P.H.J., Eds.; Humana Press: Totowa, NJ, USA, 2012; Volume 835, pp. 439–455.

150. Lowe, R.G.; McCorkelle, O.; Bleackley, M.; Collins, C.; Faou, P.; Mathivahan, S.; Anderson, M. Extracellular peptidases of the cereal pathogen *Fusarium graminearum*. *Front. Plant Sci.* 2015, 6, 962. [CrossRef] [PubMed]

151. Gale, L.R.; Ward, T.J.; Kistler, H.K. A subset of the newly discovered Northland population of *Fusarium graminearum* from the U.S. does not produce the B-type trichothecenes DON, 15ADON, 3ADON or NIV. In Proceedings of the National Fusarium Head Blight Forum, Milwaukee, WI, USA, 7–9 December 2010; Canty, S., Clark, A., Anderson-Scully, A., Ellis, D., Van Sanford, D., Eds.; University of Kentucky: Lexington, KY, USA, 2010; pp. 48–49.

152. Yuen, G.Y.; Jochum, C.C.; Du, L.; Arreguin, I.; Gale, L.R. Pre-inoculation of wheat heads with a nontoxigenic *Fusarium* isolate inhibits deoxynivalenol production by a toxigenic pathogen. In *Pathogen Biology and Genetics, Proceedings of the National Fusarium Head Blight Forum, Milwaukee, WI, USA, 7–9 December 2010*; Canty, S., Clark, A., Anderson-Scully, A., Ellis, D., Van Sanford, D., Eds.; University of Kentucky: Lexington, KY, USA, 2010; pp. 57–60.

153. Sugiuwa, Y.; Watanabe, Y.; Tanaka, T.; Yamamoto, S.; Ueno, Y. Occurrence of Gibberella zeae strains that produce both nivalenol and deoxynivalenol. *Appl. Environ. Microbiol.* 1990, 56, 3047–3051. [CrossRef] [PubMed]

154. Schmidt-Heydt, M.; Parra, R.; Geisen, R.; Magan, N. Modelling the relationship between environmental factors, transcriptional genes and deoxynivalenol mycotoxin production by strains of two *Fusarium* species. *J. R. Soc. Interface* 2010, 8, 117–126. [CrossRef] [PubMed]

155. Wang, J.; Zhao, Z.; Yang, X.; Yang, J.; Gong, A.; Zhang, J.; Chen, L.; Zhou, C. Fusarium graminearum species complex and trichothecene genotype. In *Mycotoxins and Food Safety*, 1st ed.; DeVries, J.W., Trucksess, M.W., Jackson, L.S., Eds.; IntechOpen: London, UK, 2019; pp. 1–19.

156. Covarelli, L.; Beccari, G.; Prodi, A.; Generotti, S.; Etruschi, F.; Juan, C.; Ferrer, E.; Mañes, J. *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *J. Sci. Food Agric.* 2015, 95, 540–551. [CrossRef] [PubMed]

157. Magan, N.; Hope, R.; Aldred, D. Ecophysiology of *Fusarium culmorum* and mycotoxin production. In *Advances in Food Mycology: Advances in Experimental Medicine and Biology*, 1st ed.; Hocking, A.D., Pitt, J.I., Samson, R.A., Thrane, U., Eds.; Springer: Boston, MA, USA, 2006; Volume 571, pp. 123–136.

158. Albuquerque, D.R.; Patriarca, A.; Pinto, V.F. Can discrepancies between *Fusarium graminearum* and *Fusarium sporotrichioides* affect rice grain quality? *Int. J. Food Microbiol.* 2005, 106, 291–296. [CrossRef]

159. Hope, R.; Aldred, D.; Magan, N. Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. *Int. J. Food Microbiol.* 2006, 106, 291–296. [CrossRef]

160. Ramirez, M.L.; Chulze, S.; Magan, N. Temperature and water activity effects on growth and temporal deoxynivalenol production by *Fusarium culmorum* and *Fusarium graminearum* on wheat grain. *Lett. Appl. Microbiol.* 2005, 40, 295–300. [CrossRef]
161. Hope, R.; Magan, N. Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by Fusarium culmorum on a wheat-based substrate. Lett. Appl. Microbiol. 2003, 37, 70–74. [CrossRef]
162. Gilbert, J.; Breteron, P.; MacDonald, S. Assessment of dietary exposure to ochratoxin A in the UK using a duplicate diet approach and analysis of urine and plasma samples. Food Addit. Contam. 2001, 18, 1088–1093. [CrossRef]
163. Christ, D.S.; Märländer, B.; Varrelmann, M. Characterization and mycotoxigenic potential of Fusarium species in freshly harvested and stored sugar beet in Europe. Phytopathology 2011, 101, 1330–1337. [CrossRef]
164. Korn, U.; Müller, T.; Ulrich, A.; Müller, M.E.H. Impact of aggressiveness of Fusarium graminearum and F. culmorum isolates on yield parameters and mycotoxin production in wheat. Mycotoxin Res. 2011, 27, 195–206. [CrossRef] [PubMed]
165. Kawakami, A.; Nakajima, T.; Hirayae, K. Effect of carbon sources and amines on induction of trichothecene production by Fusarium asiaticum in liquid culture. FEMS Microbiol. Lett. 2014, 352, 204–212. [CrossRef] [PubMed]
166. Llorens, A.; Mateo, R.; Hinojo, M.J.; Valle-Algarra, F.M.; Jiménez, M. Influence of environmental factors on the biosynthesis of type B trichothecenes by isolates of Fusarium spp. from Spanish crops. Int. J. Food Microbiol. 2004, 94, 43–54. [CrossRef] [PubMed]
167. Spolti, P.; Del Ponte, E.M.; Cummings, J.A.; Dong, Y.; Bergstrom, G.C. Fitness attributes of Fusarium graminearum isolates from wheat in New York possessing a 3-ADON or 15-ADON trichothecene genotype. Phytopathology 2014, 104, 513–519. [CrossRef] [PubMed]
168. Proctor, R.H.; McCormick, S.P.; Kim, H.S.; Cardoza, R.E.; Stanley, A.M.; Lindo, L.; Kelly, A.; Brown, D.W.; Lee, T.; Vaughan, M.M.; et al. Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. PLoS Pathog. 2018, 14, e1006946. [CrossRef] [PubMed]
169. Mirocha, C.J.; Abbas, H.K.; Windels, C.E.; Xie, W. Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by Fusarium graminearum isolates. Appl. Environ. Microbiol. 1989, 55, 1315–1316. [CrossRef]
170. Müller, H.M.; Schwadorf, K. A survey of the natural occurrence of Fusarium toxins in wheat grown in a southwestern area of Germany. Mycopathologia 1993, 121, 115–121. [CrossRef]
171. Malbrán, I.; Mourelos, C.A.; Giotto, J.R.; Balatti, P.A.; Lori, G.A. Toxigenic capacity and trichothecene production by Fusarium graminearum isolates from Argentina and their relationship with aggressiveness and fungal expansion in the wheat spike. Phytopathology 2014, 104, 357–364. [CrossRef] [PubMed]
172. Gang, G.; Miedaner, T.; Schuhmacher, U.; Schollenberger, M.; Geiger, H.H. Deoxynivalenol and nivalenol production by Fusarium culmorum isolates differing in aggressiveness toward winter rye. Phytopathology 1998, 88, 879–884. [CrossRef]
173. Audenaert, K.; De Boevre, M.; Vanheule, A.; Callewaert, J.; Bekaut, B.; Höfte, M.; De Saeger, S.; Haesaert, G. Mycotoxin glucosylation in commercial wheat varieties: Impact on resistance to Fusarium graminearum under laboratory and field conditions. Food Control 2013, 34, 756–762. [CrossRef]
174. Gale, L.R.; Ward, T.J.; Balmas, V.; Kistler, H.C. Population subdivision of Fusarium graminearum sensu stricto in the upper midwestern United States. Phytopathology 2007, 97, 1434–1439. [CrossRef] [PubMed]
175. Zhang, C.Q.; Hu, J.L.; Wei, E.L.; Zhu, G.N. Evolution of resistance to different classes of fungicides in Botrytis cinerea from greenhouse vegetables in eastern China. Phytoparasitica 2009, 37, 351–359. [CrossRef]
176. Zhang, L.; Jia, X.; Chen, C.; Zhou, M. Characterization of carbendazim sensitivity and trichothecene chemotypes of Fusarium graminearum in Jiangsu Province of China. Physiol. Mol. Plant Pathol. 2013, 84, 53–60. [CrossRef] [PubMed]
177. Becher, R.; Weihmann, F.; Deising, H.B.; Wirsel, S.G. Development of a novel multiplex DNA microarray for Fusarium graminearum and analysis ofazole fungicide responses. BMC Genomics 2011, 12, 52. [CrossRef] [PubMed]
178. Miedaner, T.; Korzun, V. Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology 2012, 102, 560–566. [CrossRef]
180. Proctor, R.H.; Hohn, T.M.; McCormick, S.P. Reduced virulence of Gibberella zeae caused by disruption of a trichothecene toxin biosynthetic gene. *MPMI Mol. Plant Microbe Interact.* 1995, 8, 593–601. [CrossRef]

181. Poppenberger, B.; Berthiller, F.; Lucyshyn, D.; Sieberer, T.; Schuhmacher, R.; Krska, R.; Kuchler, K.; Glössl, J.; Luschnig, C.; Adam, G. Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. *J. Biol. Chem.* 2003, 278, 47905–47914. [CrossRef]

182. Spanic, V.; Zdunic, Z.; Drezner, G.; Sarkanj, B. The pressure of Fusarium disease and its relation with mycotoxins in the wheat grain and malt. *Toxins* 2019, 11, 198. [CrossRef]

183. Yli-Mattila, T.; Rämö, S.; Hietaniemi, V.; Hussien, T.; Carlobos-Lopez, A.L.; Cumagun, C.J.R. Molecular quantification and genetic diversity of toxigenic Fusarium species in northern Europe as compared to those in southern Europe. *Microorganisms* 2013, 1, 162–174. [CrossRef]

184. Carter, J.P.; Rezanoor, H.N.; Holden, D.; Desjardins, A.E.; Plattner, R.D.; Nicholson, P. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. *Eur. J. Plant Pathol.* 2002, 108, 573–583. [CrossRef]

185. Nilsson, R.H.; Ryberg, M.; Kristiansson, E.; Abarenkov, K.; Larsson, K.H.; Kõljalg, U. Taxonomic reliability of DNA sequences in public sequence databases: A fungal perspective. *PLoS ONE* 2006, 1, e59. [CrossRef] [PubMed]

186. Crous, P.W.; Gams, W.; Stalpers, J.A.; Robert, V.; Stegehuis, G. MycoBank: An online initiative to launch mycology into the 21st century. *Stud. Mycol.* 2004, 50, 19–22.

187. Robert, V.; Vu, D.; Amor, A.B.H.; van de Wiele, N.; Brouwer, C.; Jabas, B.; Szoke, S.; Dridi, A.; Triki, M.; Daoud, S.B.; et al. MycoBank gearing up for new horizons. *IMA Fungus* 2013, 4, 371. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).