

Review

Deoxynivalenol: A Major Player in the Multifaceted Response of *Fusarium* to Its Environment

Kris Audenaert ^{1,*}, Adriaan Vanheule ¹, Monica Höfte ² and Geert Haesaert ¹

¹ Department of Applied BioSciences, Faculty Bioscience Engineering, Ghent University, Valentin Vaerwyckweg, 1, Ghent 9000, Belgium; E-Mails: adriaan.vanheule@ugent.be (A.V.); geert.haesaert@ugent.be (G.H.)

² Department of Crop Protection, Laboratory of Phytopathology, Faculty Bioscience Engineering, Ghent University, Coupure links 653, Ghent 9000, Belgium; E-Mail: monica.hofte@ugent.be

* Author to whom correspondence should be addressed. E-Mail: kris.audenaert@ugent.be; Tel.: +32-477-97-00-75; Fax: +32-9-242-42-93.

Received: 24 October 2013; in revised form: 16 December 2013 / Accepted: 16 December 2013 / Published: 19 December 2013

Abstract: The mycotoxin deoxynivalenol (DON), produced by several *Fusarium* spp., acts as a virulence factor and is essential for symptom development after initial wheat infection. Accumulating evidence shows that the production of this secondary metabolite can be triggered by diverse environmental and cellular signals, implying that it might have additional roles during the life cycle of the fungus. Here, we review data that position DON in the saprophytic fitness of *Fusarium*, in defense and in the primary C and N metabolism of the plant and the fungus. We combine the available information in speculative models on the role of DON throughout the interaction with the host, providing working hypotheses that await experimental validation. We also highlight the possible impact of control measures in the field on DON production and summarize the influence of abiotic factors during processing and storage of food and feed matrices. Altogether, we can conclude that DON is a very important compound for *Fusarium* to cope with a changing environment and to assure its growth, survival, and production of toxic metabolites in diverse situations.

Keywords: trichothecene; oxidative stress; virulence factor; fungicides; primary metabolism

1. Introduction

Fusarium head blight (FHB) is an important disease of small-grain cereals that is caused by a diverse set of *Fusarium* species. Although yield reduction is a serious consequence of *Fusarium* infection in the field, the primary interest in FHB research is driven mainly by the ability of *Fusarium* to produce mycotoxins that have toxic effects on plants, animals and humans [1,2]. Deoxynivalenol (DON) is one of the most prevalent mycotoxins encountered in grain fields. Consequently, although it is not the most toxic one, DON is considered to be the most economically important mycotoxin. DON belongs to the structural group of trichothecenes all bearing a common tricyclic 12,13-epoxytrichothec-9-ene core structure. Type A, B, C and D trichothecenes can be distinguished based on substitutions at position C-4, C-7, C-8 and/or C15 [3]. DON belongs to the type B trichothecenes and is mainly produced by *Fusarium graminearum* and *F. culmorum*, two important members of the FHB-causing species complex [4]. Historically, DON, also called vomitoxin, has been notorious because it provokes acute and chronic disease symptoms in humans and animals that consume contaminated grains [5]. Its toxic effects range from diarrhea, vomiting, gastro-intestinal inflammation, necrosis of the intestinal tract, the bone marrow and the lymphoid tissues. It causes inhibition of protein, DNA and RNA synthesis and inhibition of mitochondrial function. In addition, it has effects on cell division and membrane integrity and induces apoptosis [6]. Only after its toxicity for mammals had been established, were dedicated efforts initiated to unravel the conditions under which *Fusarium* species produce DON.

Many environmental factors are reported to affect DON levels during the infection process [7,8]. For instance, humidity and intensive rainfall during and after anthesis result in increased DON production and proliferated FHB symptoms [9–16]. Moreover, the weather conditions during the vegetative growth of wheat are important parameters determining *Fusarium* and DON load, reflecting the importance of survival of the primary inoculum present in soil and on crop debris during winter [14]. Furthermore, FHB and DON are influenced by many agronomic and other anthropogenic factors: no-, minimal-, or non-inversive tillage systems are beneficial for *Fusarium* [17]. Crop rotation, nitrogen fertilization, and weed management shape the structure of the soil biota and influence *Fusarium* survival [14,18,19]. Finally, the germplasm of the host has been shown to influence FHB and DON synthesis for example by the ability of resistant genotypes to metabolize DON [20,21].

Although this information is very valuable, in most studies no mechanistic clues are provided on how these factors affect the toxigenic machinery of the fungus. In addition, there are many other abiotic factors affecting DON of which the physiological relevance is not always clear. Obviously, a thorough insight into the functional rationale of DON production may provide hints towards an adjustment of control measures in order to avoid DON presence in the field. Therefore, we have placed the factors known to induce DON production in a relevant physiological frame, namely the different phases in the life cycle of *Fusarium* during the growing season of wheat (*Triticum* sp.) as a model host. Where possible we combine this information into working models that should be experimentally validated to obtain a holistic view on DON production by *Fusarium*.

2. The Saprophytic Phase

2.1. Survival of the Fittest

During the saprophytic phase, *F. graminearum* can survive on dead organic matter to persist in the absence of a living host, which is an important asset during the active invasion of hosts later on in the season. Therefore, saprophytic fitness is a significant component of the overall pathogen vigor [22]. Strikingly, information on the role of DON during this saprophytic period is scarce, although it covers a major part in the pathogen's life cycle and determines the primary inoculum load. Indeed, recently, DON production during the saprophytic survival on wheat stubble has been shown to be correlated with the aggressiveness of the isolates during their pathogenic phase [22].

The ability of most *F. graminearum* isolates to produce DON provides a dual advantage at the saprophytic state in the competition for niches on crop residues and organic matter. Firstly, DON is an antimicrobial metabolite that is effective against other eukaryotic soil organisms because of its interference with protein biosynthesis [5]. Secondly, DON can affect the metabolite production of other soil-residing fungi, such as *Trichoderma* sp., that are known for their strong outcompeting capacity by mycoparasitism, orchestrated by chitinases and other degrading enzymes [23]. In co-inoculation experiments, DON proved to repress the chitinase activity in *T. atroviride* [24], although a reduction in the *Trichoderma* biomass due to DON production by *F. graminearum* could not be observed [25].

Despite the very limited amount of information on the role of DON during the saprophytic phase, indirect evidence may come from comparative studies on the saprophytic survival of different *Fusarium* species. Apparently, *F. poae* which is considered a weak pathogen, is a better saprophytic survivor that outcompetes *F. graminearum* from soil and crop debris samples [26,27]. Since *F. poae* produces a more toxic blend of mycotoxins than *F. graminearum*, comprised of both type A and type B trichothecenes, it is tempting to speculate that this feature accounts for its better saprophytic survival capacity. The remarkable omnipresence of *F. poae* in the subsequent growth phase on living plant tissue, may thus originate from a “strength in numbers” strategy, originating from an inoculum build-up during the saprophytic phase.

2.2. Linkage between DON Production and Formation of Conidia and Ascospores

As the infection of *F. graminearum* is realized via production of conidia and ascospores, the formation of these reproductive structures is a very important phase in the pathogen's life cycle. Recent research has shown that both DON production and conidia/ascospore formation are under tight regulation by overlapping cellular factors [28], some of which are mentioned below. APSES proteins are a conserved class of transcription factors regulating development, secondary metabolism and pathogenicity [29,30]. Recently, *FgStuA*, a *F. graminearum* gene encoding a protein with high homology to APSES transcription factors has been characterized. Using a knock-out approach, *FgStuA* was shown to influence spore development and DON biosynthesis amongst other processes [31]. Several other regulatory cellular proteins such as the C-type cyclin like protein CID1, the ZIF1 b-zip transcription factor and the Wor-1 like nuclear protein *Fgp1* are all involved in sexual reproduction

and influence DON production [32–35]. These results highlight a tight link between reproductive fungal development and secondary metabolite production.

3. DON in the Pathogenic Phase: A Lethal Weapon of a Hemibiotrophic Cereal Killer

3.1. Plant Defense: A Matter of Making the Good Choices at the Right Time

Plants are endowed with a sophisticated set of plant defense mechanisms that can be activated upon pathogen infection. These defense responses can be divided into two main signaling pathways. One pathway involves a prompt induction of reactive oxygen species (ROS) followed by the accumulation of salicylic acid (SA), activating the plant's defense machinery. This type of defense often coincides with a programmed cell death (PCD)-type response and a hypersensitive response (HR) that isolate the pathogen and deprive it from nutrients. This SA-type defense is generally accepted to be efficient against biotrophic pathogens that need viable cells for survival. The other pathway involves jasmonic acid (JA). This type of response is especially activated during the plant defense against necrotrophic pathogens [36,37].

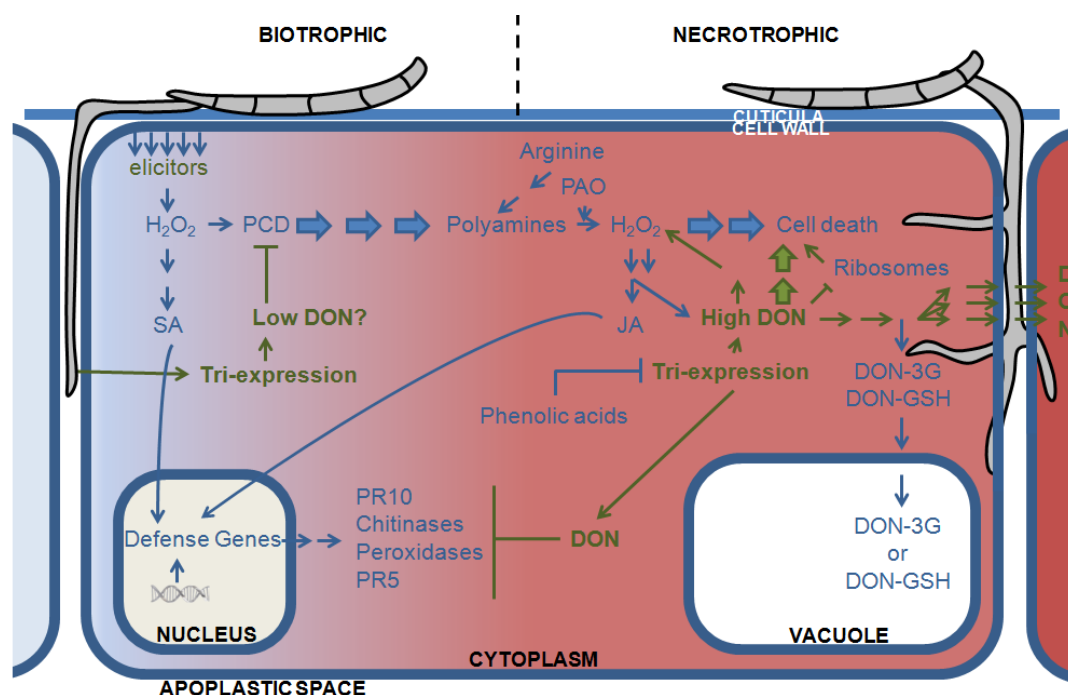
However, some pathogens, such as DON-producing *Fusarium* spp, are hemibiotrophic and have both a biotrophic and a necrotrophic phase during the colonization of their host. Hence, in such interactions, a coordinated and ordered expression of SA- and JA-dependent defense responses in the plant is crucial to halt the fungus [38], but at the same time, it provides multiple opportunities for interference by the pathogen.

3.2. DON and the Plant Defense Response: Hijacking the Plants Oxidative Armor

There is ample evidence suggesting that DON production during infection is a sophisticated strategy of the fungus to circumvent and hijack the plant's defense system. When a rain-splashed conidium or wind-dispersed ascospore lands on the exposed vulnerable parts of a crop plant (glumae, floral cavity, lemma, palea, or anthers) during or just after anthesis, it can germinate and penetrate the plant [39]. An initial superficial and intercellular growth of the fungus is eventually followed by the actual penetration of the plant, which involves the formation of infection cushions and foot-like structures invaginating the host tissue [40,41]. In this first phase, the fungus grows biotrophically into the intercellular spaces and the role of DON is assumed to be unimportant. Still, during this biotrophic phase several reports describe *Tri* gene expression at the hyphal tip [42–44]. Recently, the ability of very low DON concentrations to inhibit PCD has been illustrated [45] which could interfere with PCD, thus disrupting the biotroph-type defense (Figure 1).

Afterward, the fungus switches to a more invasive intracellular growth, including necrosis and cell death [40]. During this second necrotrophic infection phase, the production of the mycotoxin DON becomes apparent and is necessary for the spread of the fungus in the rachis of wheat [46]. Previously, studies have demonstrated that *tri5* knockout mutants, which cannot produce DON because the inactive *Tri5* gene does not convert farnesyl pyrophosphate to trichodiene, are less virulent due to the lack of spread in the rachis, implying that DON is crucial in ear colonization [42,47,48].

Figure 1. Hypothetical model of the effect of DON during the biotrophic and necrotrophic phases of *F. graminearum* infection of wheat, based on defense-related responses in wheat. The left part depicts the biotrophic phase and the right and red parts indicate the necrotrophic phase of the fungus. Green lines and arrows mark pathways of the fungus, whereas the blue lines reflect pathways of the plant. DON: deoxynivalenol; DON-3G: DON-glucoside; DON-GSH: DON-glutathione; JA: jasmonic acid; PAO: polyamine oxidases; PCD: programmed cell death; PR: pathogenesis related; SA: salicylic acid; Tri: trichothecenes.



The induction of cell death is a well-known defense strategy of plants against biotrophic but not against necrotrophic fungi [49]. In this context, it is interesting that high DON concentrations were shown to trigger H_2O_2 synthesis and subsequent cell death (Figure 1). Moreover, using an *in vitro* approach, several research groups demonstrated that H_2O_2 is an efficient inducer of DON production, especially when applied at early stages of spore germination [50–52]. Physiologically, these observations indicate that if H_2O_2 is one of the first defense molecules encountered by the invading *Fusarium* hyphae, it also establishes a positive feedback loop leading both to increased DON and H_2O_2 levels. Consequently, DON production by *Fusarium* in the necrotrophic infection phase may interfere with the two-step defense response against hemibiotrophs, because it directs the plant towards an oxidative burst which is not effective against necrotrophs. The eventual activation of H_2O_2 -mediated defense responses comprising phenolic acids, chitinases, glucanases and peroxidases [46], might come too late or at the wrong time point for the plant to defend itself against the invasive necrotrophic growth of *F. graminearum*. Indeed, it is generally recognized that both timing and localization of defense or signaling compounds determine the outcome of a plant-pathogen interaction. The importance of H_2O_2 in the induction of DON was confirmed by the effectiveness of anti-oxidative phenolic acids, such as ferulic acid, to inhibit trichothecene accumulation at a transcriptional level

in vitro [53–55]. In addition, *in planta*, the presence of ferulic acid in wheat cultivars correlated negatively with the accumulation of DON during *F. graminearum* infection [56].

Finally, it seems that DON-producing *Fusarium* species also interfere with the plant defense pathway further downstream of the oxidative burst. Indeed, SA can be used by *F. graminearum* as a carbon source [36], which may result in reduced expression of the typical SA-dependent defense genes such as pathogenesis-related protein 1 (PR1), nonexpressor of PR genes 1 (NPR1), and PR4, possibly impeding the control of symptoms development [36]. Moreover, the production of other defense-related compounds, such as PR10, chitinases, peroxidases, PR5, and PR10, is inhibited by DON at later time points during infection [49].

Nevertheless, DON is not essential in all *F. graminearum* plant interactions. For instance, although eventually a high DON load is measured as well, the infection of barley and rice with *F. graminearum* strains does not involve this mycotoxin [47,57,58].

3.3. Directing DON to the Vacuoles: Deprivation of the Pathogen of Its Virulence Factor

From the above, it is clear that DON is a powerful tool of *F. graminearum* to grow within the wheat host. Nevertheless, the plant is endowed with detoxification mechanisms to dampen the detrimental effects of the mycotoxin (for review [21]). Most important is the covalent binding of DON to hydrophilic molecules, such as glucose and glutathione (γ -glutamyl-cysteinyl-glycine, GSH). Conjugated DON is then transported via membrane-bound transporters to the vacuoles or apoplastic space [59,60]. The detoxifying effect of the conjugation is beyond dispute, but intriguingly, glutathione, a product derived from glyoxylate in the Calvin cycle, also plays an important role in modulating the redox status of the host cell, which determines the outcome of plant-pathogen interactions. Hence, it is tempting to speculate that through conjugation to DON, the fungus sequesters glutathione that affects the antioxidative status and, consequently, the defense machinery of the host cell. Still, it is important to notice that the oxidative status of plant cells is very complex with amongst others catalases, ascorbate peroxidases, superoxide dismutases and NADPH oxidases establishing the oxidative equilibrium.

4. The Plant's Primary Carbohydrate and Nitrogen Metabolism Feed into DON Production and Fungal Growth

Although current research particularly focuses on downstream defense signaling, the energy and carbon skeletons used in the defense reactions activated in wheat upon infection with *Fusarium* require the redistribution of energy from the primary metabolism of the plant. Interestingly, pathogens themselves seemingly also drain energy from the primary metabolism of the host to the advantage of their own pathogenic growth and production of their virulence factors [61,62].

When a plant is attacked by a pathogen, the availability of ready-to-use energy, reducing agents, and carbon skeletons is a prerequisite for optimal activation of defense. In many plant pathosystems, photosynthesis, which generates ATP and NADPH, decreases at the site of infection, establishing novel sink tissues [61]. Carbohydrate partitioning between source and sink tissues is a highly dynamic process during the plant's life cycle and the physiological balance can easily be disrupted. Because of reduced photosynthesis, the plant will mobilize monosaccharides to the infection site by activating

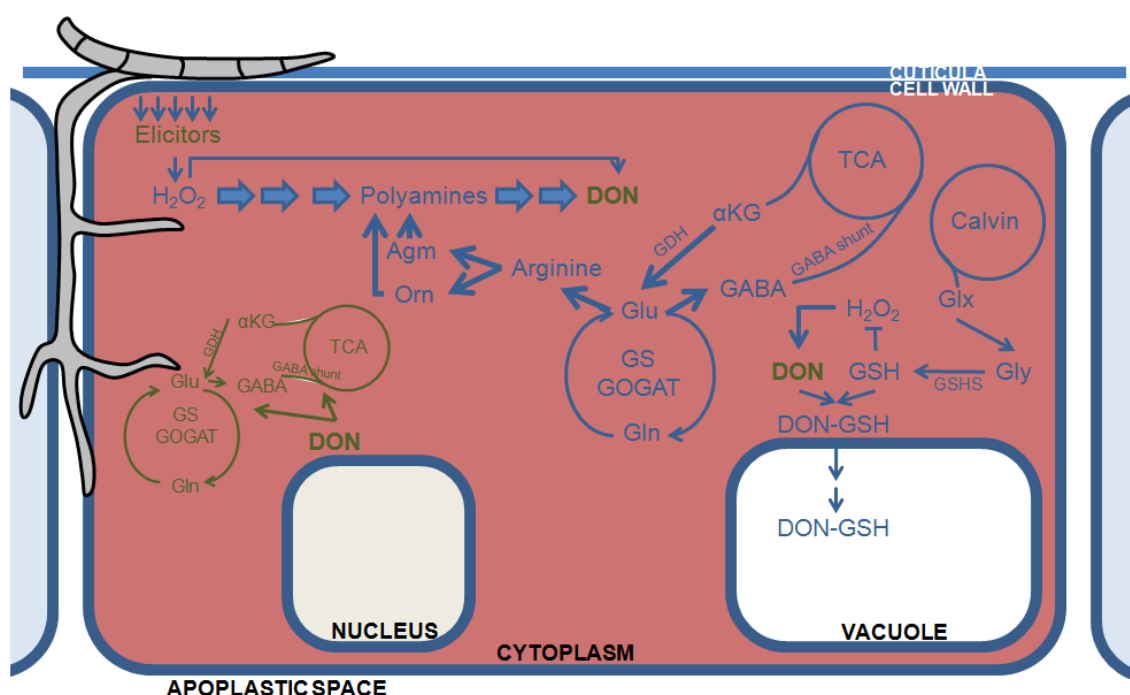
membrane-bound invertases that cleave apoplastic sucrose, thus generating energy and carbohydrate skeletons for diverse metabolic processes, including defense. However, sucrose is also an important inducer of the *Tri* gene machinery. Especially *Tri5* and *Tri4*, which are both involved in the initial steps of trichothecene biosynthesis by converting farnesylpyrophosphate to trichodiene and the latter to 15-decalonectrin, respectively, are strongly upregulated by sucrose, resulting in increased DON biosynthesis [63].

In the *F. graminearum*-wheat interaction, several plant invertases are upregulated, indicating that the fungus exploits sucrose not only as a trigger for DON biosynthesis, but also as a monosaccharide source that can be used for its own growth [64]. However, the contributions to the metabolism of the plant and of the fungus are difficult to distinguish. Indeed, pathogenic fungi also produce invertases that can potentially disturb the source-sink balance and the repartitioning of the carbon sources in the plant and, hence, affect the infection process.

The importance of nitrogen in plant defense is mainly situated at three levels. Firstly, nitrogen is indirectly involved as an energy source. Inorganic nitrogen is usually taken up as NH_4 or NO_3 after which it is incorporated into amino acids, such as glutamate, glutamine, asparagine, and aspartate via glutamine synthase. Subsequently, these amino acids are transported or stored in the plant by the glutamine-oxoglutarate aminotransferase (GOGAT) cycle. When the energy demand of the plant cells increases, for example upon pathogen infection, these amino acids are diverted to the energy-generating tricarboxylic acid (TCA) cycle, in part via the γ -aminobutyrate (GABA) shunt, leading to reducing equivalents and ultimately ATP [61,62]. Secondly, nitrogen is a main compound in the regulation of the redox status of plant cells. Reactive nitrogen species, such as nitric oxide (NO), but also polyamines, produced from the precursor L-arginine, can be directly involved in plant defense through HR induction [65]. Moreover, N-containing glutathione is an important antioxidant alleviating oxidative damage during an HR [66]. Thirdly, the plant's nitrogen metabolism has been suggested to be involved in the defense response through a pivotal mechanism of evasion or endurance [62]. During the evasion process, implicated in a successful defense response against biotrophic pathogens, nitrogen is uploaded in the phloem as asparagine or glutamine and transported away from the invaded area to deprive the pathogen from the necessary nitrogen sources. During the endurance process nitrogen is remobilized from noninfected tissues providing infected cells with sufficient nitrogen to keep them alive; a strategy that is very efficient against necrotrophic pathogens [62].

Just as with the carbohydrate metabolism, pathogens, including DON-producing *Fusarium* species, appear to hijack the primary nitrogen metabolism of the plant for their own benefit. For instance, several pathogens can use the plant's amino acids as N-sources. Moreover, upon infection with *F. graminearum*, the primary GOGAT cycle appears to be redirected toward the production of ornithine and arginine, resulting in the formation of polyamines [67] (Figure 2). Indeed, a metabolo-proteomics approach revealed the induction of the agmatin-to-polyamine conversion [68]. As described above, the accumulation of polyamines can lead to ROS through the formation of NO and the action of polyamine oxidases [38,69], which could hypothetically contribute positively to the necrotrophic phase of *F. graminearum*. Finally, in an *in vitro* study, polyamines have been shown to induce DON production as well, further contributing to the fungus pathogenicity [70].

Figure 2. Hypothetical model of the interaction of DON with the primary metabolism of the host and the pathogen. Green lines and arrows indicate pathways of the fungus, blue lines reflect pathways of the plant. Bullet lines represent inhibitory actions. Agm: agmatine; α KG: α -ketoglutarate; DON: deoxynivalenol; GABA: γ -aminobutyric acid; GDH: glutamate dehydrogenase; Gln: glutamine; Glu: glutamate; Glx: glyoxylate; Gly: glycine; GOGAT: glutamine oxoglutarate aminotransferase; Orn: ornithine; TCA: tricarboxylic acid.



Although evidence is scarce, DON may interfere with aspects of the primary metabolism of the fungus itself. Although DON is considered to be a secondary metabolite, knocking out of the *Tri5* gene has a very profound impact on the primary metabolism of the fungus leading to decreased levels of glutamate and GABA and reduced glutamine synthase and GABA transferase activities [71]. Consequently the complete GABA shunt, TCA cycle, and polyamine metabolism are negatively affected (Figure 2). Conversely, upon infection, the GABA shunt becomes activated in DON-producing *F. graminearum* strains, suggesting a replenishment of the TCA cycle during the interaction with a host [72]. Moreover, metabolomic studies of wheat ears have revealed that the TCA cycle of the host is disturbed as well upon infection with *F. graminearum*, resulting in an increased activity of glutamate dehydrogenase that converts α -ketoglutarate to glutamate although a direct link with DON production was not investigated. Interestingly, in other pathosystems involving necrotrophic and/or toxin-producing plant pathogens, a similar exhaustion of the TCA cycle of the host takes place, suggesting this might be a conserved and effective virulence strategy [73,74].

5. Of Crops and Men: The DON Molecule and Man's Chemical Warfare

Because *Fusarium* infects an important economic crop cultivated within an agro-ecosystem, the plant-fungus interaction is more complex than in a natural ecosystem. Indeed, farmers interfere to minimize the presence of DON and other mycotoxins in the crop. Whereas the effect of chemical

fungicides on fungal outgrowth is quite straightforward and generally results in reduced fungal load, reports on the impact of fungicides on the production of fungal secondary metabolites, especially mycotoxins, are rather inconsistent and fragmentary. Still, careful analysis of the information reveals important insights into the function of DON in the reaction of fungi to fungicide applications.

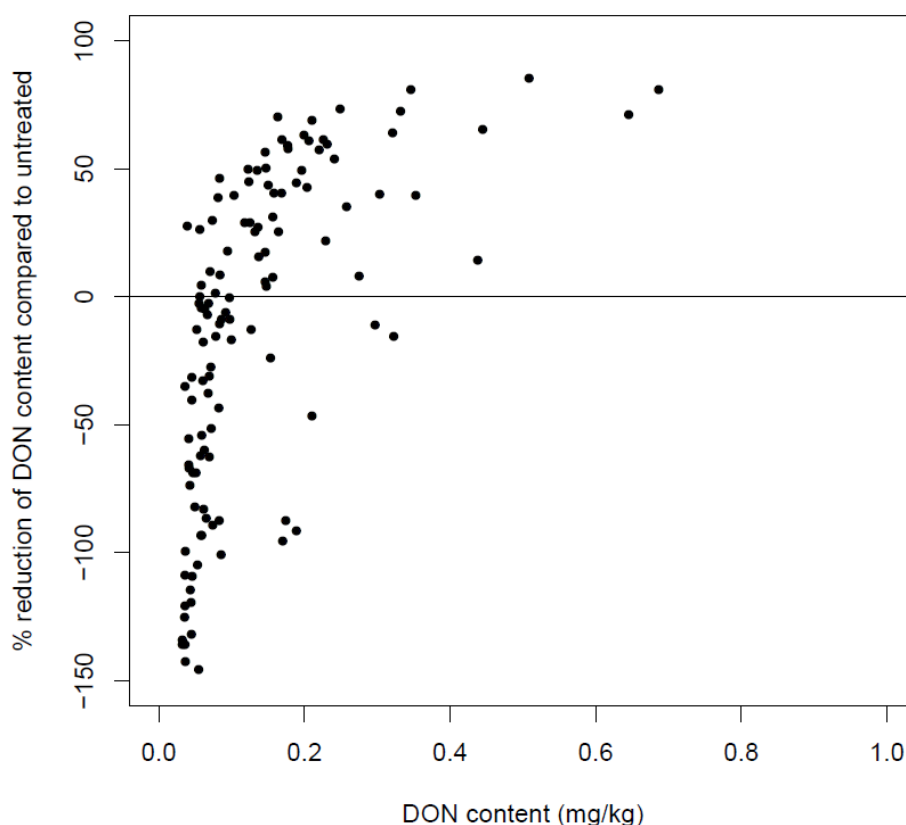
The effect of the strobilurin fungicide azoxystrobin on DON production varies from an increase [75–78] to a reduction [79] depending on environmental factors. Some other fungicides, such as carbendazim and thiram, have been tested for their efficiency to reduce DON in grain samples, but no clear effect was observed [80]. Nevertheless, the mycotoxin chemotype and the sensitivity toward carbendazim fungicides correlated well. As such, most strains producing nivalenol (NIV) or 15-acetyl-deoxynivalenol (15ADON) were susceptible, whereas all carbendazim-resistant isolates were 3-acetyl-deoxynivalenol (3ADON) producers [81].

The most important fungicides currently used to control *Fusarium* are the azoles. A multi-year and multi-location experiment carried out in Belgium illustrated that the effect of azole fungicides with respect to DON depended on the DON concentration in the wheat host. In plants containing low and high DON amounts, fungicide applications often resulted in an increase and a reduction of DON load, respectively. These field trials also demonstrated that it was impossible to decrease the DON levels by more than 75% of the control fields (Figure 3). This observation may imply that highly contaminated fields, in which DON levels exceed the legislative values multiple fold, cannot be rescued by fungicide applications.

Within the group of azole fungicides, field doses of tebuconazole [75,77,82–85], metconazole [79,82,85], and prothioconazole [85] consistently reduced DON biosynthesis or content. In contrast, application of another azole fungicide, propiconazole, either decreased or increased DON levels [76,85]. Intriguingly, DON amounts are increased by application of a sublethal dose of prothioconazole, which is meticulously regulated through the production of H₂O₂ as an oxidative stress response of the fungus. Indeed, oxidative stress as a booster of toxigenic pathways is now considered a trait common to various toxigenic fungi from different genera of the fungal kingdom [86]. Moreover, qRT-PCR analyses have revealed that the expression of *Tri4*, *Tri5*, and *Tri11* was higher in cultures of *F. graminearum* isolates supplemented with sublethal concentrations of tebuconazole and propiconazole than that in nontreated controls, although the fold change in the *Tri* transcript levels differed according to the type of azole used [87].

Typically, azole sensitivity in fungi is modified by either point mutations in the cytochrome P450 monooxygenase-encoding target gene *CYP51* [88,89], overexpression of *CYP51* [90], presence of paralogous *CYP51* genes [91], the presence of fungal drug transporters, belonging to the ABC or MDR classes [92], or an altered composition of the sterol content [93]. However, considering the effect of low fungicide levels on DON production, the question arises whether DON interferes with the fungicide effectiveness. Indirect proof comes from *in vitro* fungicide assays with a *tri5* knockout mutant of *F. graminearum*. The overall fitness and fecundity of the mutant was comparable to that of the parent strain; but, when homeopathic levels of azole fungicides were applied, only the mutant fungus promptly stopped growing [94]. Apparently, when a strain cannot produce its toxic secondary metabolite DON, it becomes hypersensitive to azole fungicides. Additionally, when *F. graminearum* strains were allowed to adapt to azole fungicides, they showed an increased production of the B-type trichothecene NIV [95].

Figure 3. Percentage of reduced DON content after application of triazole fungicides at GS 39 and GS 55 on different wheat varieties in function of the DON content present in the untreated experimental field trials. All data points are the result of four independent replications and experiments were carried out at several locations in a three-year-experiment.



Although direct evidence on the role of DON in fungicide resistance is currently lacking, *Sacharomyces cerevisiae* is known to be very resistant against DON because of the presence of multiple ABC transporters that pump the mycotoxin out of the cell [96]. In addition, expression of ABC transporters of the plant pathogen *Mycosphaerella graminicola* in an ABC transporter-lacking mutant of *S. cerevisiae* clearly indicated a wide functional overlap between the ABC transporters induced by azole fungicides and those by the A-type trichothecene diacetoxyscirpenol [97]. Finally, transcriptional profiling of ABC transporters upon fungicide application points toward a mechanism alleviating the impact of the fungicide [95]. Together, this fragmentary information seems to imply that the mycotoxin production capacity and resistance against fungicides converge at the ABC efflux level or other MDR pumps. It is not unlikely that (some) efflux pumps activated upon mycotoxin biosynthesis are also activated during exposure to fungicides. Interestingly, at least one ABC transporter has been shown to be important in the virulence of *F. graminearum*, but an effect on the mycotoxin efflux from the deletion mutant was not reported [98].

6. Abiotic Factors Influencing DON Biosynthesis in the Field and during Storage

The impact of abiotic factors on mycotoxin production has recently been reviewed [7,8]. Therefore, we highlight only new research findings that deal with environmental effects on DON biosynthesis.

6.1. pH

Although it is currently unknown whether and how the pH fluctuates during a wheat infection with *Fusarium*, it is well established that a low extracellular pH results in an increased trichothecene production [99]. *Tri* gene expression is regulated by a zinc finger transcription factor *FgPac1* at acidic pH values [100], but the regulation at neutral or basic pH remains unclear. As information on the extracellular pH during wheat infection by *F. graminearum* and during grain storage remains scarce, it is very difficult to place the results on the pH effects in a physiological context. Probably, a dynamic window of pH fluxes influences DON production during the infection process.

6.2. a_w and Temperature

The availability of free water (a_w) and the incubation temperature will determine whether there will be an outgrowth of *F. graminearum*, especially during storage of wheat grains after harvest. In addition, the toxigenic outcome of fungal growth also depends on the a_w value and the temperature. Indeed, high a_w values increase DON production in contaminated wheat grain batches [101] as well as elevating the incubation temperature from 15 °C to 30 °C [102]. Several reports also describe a clear interaction between temperature and a_w value [103].

6.3. Light

In plants, several important pathways follow a diurnal regulation based on the day/night regime. Although fungi do not depend on photosynthesis for their energy supply, their secondary metabolism is often fine-tuned by light. One of the most important light-regulatory protein complexes is the velvet complex, comprising at least *FgVe1* (VeA) and *FgVeB*. Although the velvet complex has been elaborately investigated with regard to the switch between asexual and sexual phases of the fungus, recent research highlights its significance in the regulation of the *Tri* gene machinery. By means of a gene replacement strategy, VeA has been demonstrated to regulate trichothecene production at the level of the biosynthetic genes *Tri4* and *Tri5* and the transcriptional regulator genes *Tri6* and *Tri10* [104,105]. Results with knockout mutants have revealed that *FgVeB* plays a role in the regulation of *Tri5* and *Tri6* as well [106].

6.4. Post-Harvest Anthropogenic Factors Influencing the DON Content

After harvest, grains are often stored for some time in silos before final use as animal feed or human food. Although DON production during storage is, exceptions notwithstanding, rather rare, effects of changed storage conditions on fungal outgrowth and DON production have been reported. Modified storage atmosphere, chemical preservation systems, and biocontrol with lactic acid bacteria have been proposed as antifungal measures [102]. Detailed insights into the effect of these measures on DON production are still lacking. Chemical compounds, such as antioxidants and essential oils applied during storage of wheat grains clearly have a very variable impact on the DON levels. In an experiment in which wheat grains were inoculated with *F. graminearum* and subsequently treated with neutralized electrolyzed water, the ROS present in the electrolyzed water reduced the fungal load in the wheat commodities. However, at sublethal levels, this decrease in biomass coincided with an

increase in DON level. The ROS liberated from the electrolyzed water oxidatively stimulated the *Tri* gene machinery to produce DON [94].

7. Conclusions and Challenges for the Future

In the present review, we gathered available data on diverse factors known to affect DON production by *Fusarium*. We combined this information into hypothetical models on the effect of DON on defense-related processes and the primary metabolism of wheat as a model host. Altogether, based on the present literature, we claim that DON is a molecule that is crucial throughout the fungal life cycle. During saprophytic survival, DON might be involved in competition for niche. Furthermore, DON production and conidia- and/or ascospore formation are tightly linked processes.

During the interaction with its host, it seems that *Fusarium* uses DON to disturb the defense system at several critical time points of infection assuring successful colonization and symptom development (Figure 1). Moreover, DON appears to be deployed to hijack the primary C and N metabolism of the plant to improve fungal growth and production of virulence factors (Figure 2). Although parts of the proposed models are still highly speculative and not supported by direct experimental evidence, we hope they provide valuable working hypotheses for future research.

An additional challenge is to decipher the function of other type A and type B trichothecenes produced by other members of the FHB disease complex. Is the importance of DON in the life cycle of *Fusarium* spp. unique or can the functions be extrapolated to other mycotoxins? More generally, searching for parallels between *Fusarium* and other toxin-producing plant pathogens might reveal conserved infection strategies typical for this type of phytopathogens.

Acknowledgments

We greatly acknowledge Maarten Ameye, Martine De Cock, and Danny Vereecke for critical reading of the manuscript. Kris Audenaert and Adriaan Vanheule are indebted to the Research Fund of University College Ghent for a post-doctoral and PhD grant, respectively.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Arunachalam, C.; Doohan, F.M. Trichothecene toxicity in eukaryotes: Cellular and molecular mechanisms in plants and animals. *Toxicol. Lett.* **2013**, *27*, 149–158.
2. Maresca, M. From the gut to the brain: Journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. *Toxins* **2013**, *23*, 784–820.
3. McCormick, S.P.; Stanley, A.M.; Stover, N.A.; Alexander, N.J. Trichothecenes: From simple to complex mycotoxins. *Toxins* **2011**, *3*, 802–814.
4. Goswami, R.S.; Kistler, H.C. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* **2004**, *5*, 515–525.
5. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516.

6. Pestka, J.J. Toxicological mechanisms and potential health effects of deoxynivalenol and nivalenol. *World Mycotoxin J.* **2010**, *3*, 323–347.
7. Wegulo, S.N. Factors influencing deoxynivalenol accumulation in small grain cereals. *Toxins* **2012**, *4*, 1157–1180.
8. Merhej, J.; Richard-Forget, F.; Barreau, C. Regulation of trichothecene biosynthesis in *Fusarium*: Recent advances and new insights. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 519–528.
9. Hooker, D.C.; Schaafsma, A.W.; Tamburic-Ilinic, L. Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. *Plant Dis.* **2002**, *86*, 611–619.
10. Schaafsma, A.W.; Tamburic-Ilinic, L.; Miller, J.D.; Hooker, D.C. Agronomic considerations for reducing deoxynivalenol in wheat grain. *Can. J. Plant Pathol. Rev. Can. Phytopathol.* **2001**, *23*, 279–285.
11. Moschini, R.C.; Fortugno, C. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. *Eur. J. Plant Pathol.* **1996**, *102*, 211–218.
12. Klem, K.; Vanova, M.; Hajslova, J.; Lancova, K.; Sehnalova, M. A neural network model for prediction of deoxynivalenol content in wheat grain based on weather data and preceding crop. *Plant Soil Environ.* **2007**, *53*, 421–429.
13. Kriss, A.B.; Paul, P.A.; Xu, X.M.; Nicholson, P.; Doohan, F.M.; Hornok, L.; Rietini, A.; Edwards, S.G.; Madden, L.V. Quantification of the relationship between the environment and *Fusarium* head blight, *Fusarium* pathogen density, and mycotoxins in winter wheat in Europe. *Eur. J. Plant Pathol.* **2012**, *133*, 975–993.
14. Landschoot, S.; Waegeman, W.; Audenaert, K.; Vandepitte, J.; Baetens, J.M.; De Baets, B.; Haesaert, G. An empirical analysis of explanatory variables affecting *Fusarium* head blight infection and deoxynivalenol content in wheat. *J. Plant Pathol.* **2012**, *94*, 135–147.
15. Lindblad, M.; Borjesson, T.; Hietaniemi, V.; Elen, O. Statistical analysis of agronomical factors and weather conditions influencing deoxynivalenol levels in oats in Scandinavia. *Food Add. Contam. Part A Chem.* **2012**, *29*, 1566–1571.
16. Gourdain, E.; Piraux, F.; Barrier-Guillot, B. A model combining agronomic and weather factors to predict occurrence of deoxynivalenol in durum wheat kernels. *World Mycotoxin J.* **2011**, *4*, 129–139.
17. Leplat, J.; Friberg, H.; Abid, M.; Steinberg, C. Survival of *Fusarium graminearum*, the causal agent of *Fusarium* head blight. A review. *Agron. Sustain. Dev.* **2013**, *33*, 97–111.
18. Bernhoft, A.; Torp, M.; Clasen, P.E.; Loes, A.K.; Kristoffersen, A.B. Influence of agronomic and climatic factors on *Fusarium* infestation and mycotoxin contamination of cereals in Norway. *Food Add. Contam. Part A Chem.* **2012**, *29*, 1129–1140.
19. Lemmens, M.; Haim, K.; Lew, H.; Ruckenbauer, P. The effect of nitrogen fertilization on *Fusarium* head blight development and deoxynivalenol contamination in wheat. *J. Phytopathol.* **2004**, *152*, 1–8.
20. Miedaner, T.; Korzun, V. Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology* **2012**, *102*, 560–566.
21. Berthiller, F.; Crews, C.; Dall'Asta, C.; De Saeger, S.; Haesaert, G.; Karlovsky, P.; Oswald, I.P.; Seefelder, W.; Speijers, G.; Stroka, J. Masked mycotoxins: A review. *Mol. Nutr. Food Res.* **2013**, *57*, 165–186.

22. Tunali, B.; Obanor, F.; Erginbas, G.; Westecott, R.A.; Nicol, J.; Chakraborty, S. Fitness of three *Fusarium* pathogens of wheat. *FEMS Microbiol. Ecol.* **2012**, *81*, 596–609.
23. Lorito, M.; Farkas, V.; Rebuffat, S.; Bodo, B.; Kubicek, C.P. Cell wall synthesis is a major target of mycoparasitic antagonism by *Trichoderma harzianum*. *J. Bacteriol.* **1996**, *178*, 6382–6385.
24. Lutz, M.P.; Feichtinger, G.; Defago, G.; Duffy, B. Mycotoxigenic *Fusarium* and deoxynivalenol production repress chitinase gene expression in the biocontrol agent *Trichoderma atroviride* P1. *Appl. Environ. Microbiol.* **2003**, *69*, 3077–3084.
25. Naef, A.; Senatore, M.; Defago, G. A microsatellite based method for quantification of fungi in decomposing plant material elucidates the role of *Fusarium graminearum* DON production in the saprophytic competition with *Trichoderma atroviride* in maize tissue microcosms. *FEMS Microbiol. Ecol.* **2006**, *55*, 211–220.
26. Pereyra, S.A.; Dill-Macky, R. Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to *Fusarium* head blight inoculum. *Plant Dis.* **2008**, *92*, 800–807.
27. Landschoot, S.; Audenaert, K.; Waegeman, W.; Pycke, B.; Bekaert, B.; De Baets, B.; Haesaert, G. Connection between primary *Fusarium* inoculum on gramineous weeds, crop residues and soil samples and the final population on wheat ears in Flanders, Belgium. *Crop Protect.* **2011**, *30*, 1297–1305.
28. Calvo, A.M.; Wilson, R.A.; Bok, J.W.; Keller, N.P. Relationship between secondary metabolism and fungal development. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 447–459.
29. Twumasi-Boateng, K.; Yu, Y.; Chen, D.; Gravelat, F.N.; Nierman, W.C.; Sheppard, D.C. Transcriptional profiling identifies a role for BrlA in the response to nitrogen depletion and for StuA in the regulation of secondary metabolite clusters in *Aspergillus fumigatus*. *Eukaryot. Cell* **2009**, *8*, 104–115.
30. Tong, X.Z.; Zhang, X.W.; Plummer, K.M.; Stowell, K.M.; Sullivan, P.A.; Farley, P.C. GcSTUA, an APSES transcription factor, is required for generation of appressorial turgor pressure and full pathogenicity of *Glomerella cingulata*. *Mol. Plant Microbe Interact.* **2007**, *20*, 1102–1111.
31. Lysoe, E.; Pasquali, M.; Breakspear, A.; Kistler, H.C. The transcription factor FgStuAp influences spore development, pathogenicity, and secondary metabolism in *Fusarium graminearum*. *Mol. Plant Microbe Interact.* **2011**, *24*, 54–67.
32. Pasquali, M.; Spanu, F.; Scherm, B.; Balmas, V.; Hoffmann, L.; Hammond-Kosack, K.E.; Beyer, M.; Migheli, Q. FcStuA from *Fusarium culmorum* controls wheat foot and root rot in a toxin dispensable manner. *PLoS ONE* **2013**, *8*, 1–15.
33. Zhou, X.Y.; Heyer, C.; Choi, Y.E.; Mehrabi, R.; Xu, J.R. The CID1 cyclin C-like gene is important for plant infection in *Fusarium graminearum*. *Fungal Genet. Biol.* **2010**, *47*, 143–151.
34. Wang, Y.; Liu, W.D.; Hou, Z.M.; Wang, C.F.; Zhou, X.Y.; Jonkers, W.; Ding, S.L.; Kistler, H.C.; Xu, J.R. A novel transcriptional factor important for pathogenesis and ascosporeogenesis in *Fusarium graminearum*. *Mol. Plant Microbe Interact.* **2011**, *24*, 118–128.
35. Jonkers, W.; Dong, Y.H.; Broz, K.; Kistler, H.C. The Wor1-like protein Fgp1 regulates pathogenicity, toxin synthesis and reproduction in the phytopathogenic fungus *Fusarium graminearum*. *PLoS Pathog.* **2012**, *8*, 1–18.

36. Qi, P.F.; Johnston, A.; Balcerzak, M.; Rocheleau, H.; Harris, L.J.; Long, X.Y.; Wei, Y.M.; Zheng, Y.L.; Ouellet, T. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. *Fungal Biol.* **2012**, *116*, 413–426.
37. Robert-Seilanianantz, A.; Grant, M.; Jones, J.D.G. Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. *Annu. Rev. Phytopathol.* **2011**, *49*, 317–343.
38. Ding, L.N.; Xu, H.B.; Yi, H.Y.; Yang, L.M.; Kong, Z.X.; Zhang, L.X.; Xue, S.L.; Jia, H.Y.; Ma, Z.Q. Resistance to hemi-biotrophic *F-graminearum* infection is associated with coordinated and ordered expression of diverse defense signaling pathways. *PloS ONE* **2011**, *6*, 1–17.
39. Parry, D.W.; Jenkinson, P.; McLeod, L. *Fusarium* ear blight (Scab) in small grain cereals—A review. *Plant Pathol.* **1995**, *44*, 207–238.
40. Kazan, K.; Gardiner, D.M.; Manners, J.M. On the trail of a cereal killer: Recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Mol. Plant Pathol.* **2012**, *13*, 399–413.
41. Boenisch, M.J.; Schafer, W. *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. *BMC Plant Biol.* **2011**, *11*, 1–13.
42. Desjardins, A.E.; Proctor, R.H.; Bai, G.H.; McCormick, S.P.; Shaner, G.; Buechley, G.; Hohn, T.M. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Mol. Plant Microbe Interact.* **1996**, *9*, 775–781.
43. Cowger, C.; Arellano, C. *Fusarium graminearum* infection and deoxynivalenol concentrations during development of wheat spikes. *Phytopathology* **2013**, *103*, 460–471.
44. Hallen-Adams, H.E.; Wenner, N.; Kuldau, G.A.; Trail, F. Deoxynivalenol biosynthesis-related gene expression during wheat kernel colonization by *Fusarium graminearum*. *Phytopathology* **2011**, *101*, 1091–1096.
45. Diamond, M.; Reape, T.J.; Rocha, O.; Doyle, S.M.; Kacprzyk, J.; Doohan, F.M.; McCabe, P.F. The *Fusarium* mycotoxin deoxynivalenol can inhibit plant apoptosis-like programmed cell death. *PloS ONE* **2013**, *8*, 1–8.
46. Walter, S.; Nicholson, P.; Doohan, F.M. Action and reaction of host and pathogen during *Fusarium* head blight disease. *New Phytol.* **2010**, *185*, 54–66.
47. Langevin, F.; Eudes, F.; Comeau, A. Effect of trichothecenes produced by *Fusarium graminearum* during *Fusarium* head blight development in six cereal species. *Eur. J. Plant Pathol.* **2004**, *110*, 735–746.
48. Jansen, C.; Von Wettstein, D.; Schafer, W.; Kogel, K.H.; Felk, A.; Maier, F.J. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16892–16897.
49. Desmond, O.J.; Manners, J.M.; Stephens, A.E.; MacClean, D.J.; Schenk, P.M.; Gardiner, D.M.; Munn, A.L.; Kazan, K. The *Fusarium* mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defence responses in wheat. *Mol. Plant Pathol.* **2008**, *9*, 435–445.
50. Audenaert, K.; Callewaert, E.; Hofte, M.; De Saeger, S.; Haesaert, G. Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. *BMC Microbiol.* **2010**, *10*, 1–14.

51. Ponts, N.; Pinson-Gadais, L.; Barreau, C.; Richard-Forget, F.; Ouellet, T. Exogenous H₂O₂ and catalase treatments interfere with Tri genes expression in liquid cultures of *Fusarium graminearum*. *FEBS Lett.* **2007**, *581*, 443–447.
52. Ponts, N.; Pinson-Gadais, L.; Verdal-Bonnin, M.N.; Barreau, C.; Richard-Forget, F. Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*. *FEMS Microbiol. Lett.* **2006**, *258*, 102–107.
53. Boutigny, A.L.; Atanasova-Penichon, V.; Benet, M.; Barreau, C.; Richard-Forget, F. Natural phenolic acids from wheat bran inhibit *Fusarium culmorum* trichothecene biosynthesis *in vitro* by repressing Tri gene expression. *Eur. J. Plant Pathol.* **2010**, *127*, 275–286.
54. Boutigny, A.L.; Barreau, C.; Atanasova-Penichon, V.; Verdal-Bonnin, M.N.; Pinson-Gadais, L.; Richard-Forget, F. Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and Tri gene expression in *Fusarium* liquid cultures. *Mycol. Res.* **2009**, *113*, 746–753.
55. Atanasova-Penichon, V.; Pons, S.; Pinson-Gadais, L.; Picot, A.; Marchegay, G.; Bonnin-Verdal, M.N.; Ducos, C.; Barreau, C.; Roucolle, J.; Sehabiague, P.; *et al.* Chlorogenic acid and maize ear rot resistance: A dynamic study investigating *Fusarium graminearum* development, deoxynivalenol production, and phenolic acid accumulation. *Mol. Plant Microbe Interact.* **2012**, *25*, 1605–1616.
56. Engelhardt, G.; Koeniger, M.; Preiss, U. Influence of wheat phenolic acids on *Fusarium* head blight resistance and deoxynivalenol concentration. *Mycotoxin Res.* **2002**, *18*, 100–103.
57. Goswami, R.S.; Kistler, H.C. Pathogenicity and *in planta* mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. *Phytopathology* **2005**, *95*, 1397–1404.
58. Boddu, J.; Cho, S.; Kruger, W.M.; Muehlbauer, G.J. Transcriptome analysis of the barley-*Fusarium graminearum* interaction. *Mol. Plant Microbe Interact.* **2006**, *19*, 407–417.
59. Bowles, D.; Lim, E.K.; Poppenberger, B.; Vaistij, F.E. Glycosyltransferases of lipophilic small molecules. *Annu. Rev. Plant Biol.* **2006**, *57*, 567–597.
60. Coleman, J.O.D.; BlakeKalff, M.M.A.; Davies, T.G.E. Detoxification of xenobiotics by plants: Chemical modification and vacuolar compartmentation. *Trends Plant Sci.* **1997**, *2*, 144–151.
61. Bolton, M.D. Primary metabolism and plant defense: Fuel for the fire. *Mol. Plant Microbe Interact.* **2009**, *22*, 487–497.
62. Seifi, H.S.; Van Bockhaven, J.; Angenon, G.; Hofte, M. Glutamate metabolism in plant Disease and defense: Friend or foe? *Mol. Plant Microbe Interact.* **2013**, *26*, 475–485.
63. Jiao, F.; Kawakami, A.; Nakajima, T. Effects of different carbon sources on trichothecene production and Tri gene expression by *Fusarium graminearum* in liquid culture. *FEMS Microbiol. Lett.* **2008**, *285*, 212–219.
64. Guenther, J.C.; Hallen-Adams, H.E.; Bucking, H.; Shachar-Hill, Y.; Trail, F. Triacylglyceride metabolism by *Fusarium graminearum* during colonization and sexual development on wheat. *Mol. Plant Microbe Interact.* **2009**, *22*, 1492–1503.
65. Romero-Puertas, M.C.; Perazzolli, M.; Zago, E.D.; Delledonne, M. Nitric oxide signalling functions in plant-pathogen interactions. *Cell. Microbiol.* **2004**, *6*, 795–803.

66. Elzahaby, H.M.; Gullner, G.; Kiraly, Z. Effects of powdery mildew infection of barley on the ascorbate-glutathione cycle and other antioxidants in different host-pathogen interactions. *Phytopathology* **1995**, *85*, 1225–1230.
67. Gardiner, D.M.; Kazan, K.; Praud, S.; Torney, F.J.; Rusu, A.; Manners, J.M. Early activation of wheat polyamine biosynthesis during *Fusarium* head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *BMC Plant Biol.* **2010**, *10*, doi:10.1186/1471-2229-10-289.
68. Gunnaiah, R.; Kushalappa, A.C.; Duggavathi, R.; Fox, S.; Somers, D.J. Integrated metabolo-proteomic approach to decipher the mechanisms by which wheat QTL (Fhb1) contributes to resistance against *Fusarium graminearum*. *PloS ONE* **2012**, *7*, 1–15.
69. Lysoe, E.; Seong, K.Y.; Kistler, H.C. The transcriptome of *Fusarium graminearum* during the infection of wheat. *Mol. Plant Microbe Interact.* **2011**, *24*, 995–1000.
70. 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.
71. Chen, F.F.; Zhang, J.T.; Song, X.S.; Yang, J.; Li, H.P.; Tang, H.R.; Liao, Y.C. Combined metabonomic and quantitative real-time PCR analyses reveal systems metabolic changes of *Fusarium graminearum* induced by Tri5 gene deletion. *J. Prot. Res.* **2011**, *10*, 2273–2285.
72. Carapito, R.; Hatsch, D.; Vorwerk, S.; Petkovski, E.; Jeltsch, J.M.; Phalip, V. Gene expression in *Fusarium graminearum* grown on plant cell wall. *Fungal Genet. Biol.* **2008**, *45*, 738–748.
73. Tsuge, T.; Harimoto, Y.; Akimitsu, K.; Ohtani, K.; Kodama, M.; Akagi, Y.; Egusa, M.; Yamamoto, M.; Otani, H. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. *FEMS Microbiol. Rev.* **2013**, *37*, 44–66.
74. Brauc, S.; De Vooght, E.; Claeys, M.; Geuns, J.M.C.; Hofte, M.; Angenon, G. Overexpression of arginase in *Arabidopsis thaliana* influences defence responses against *Botrytis cinerea*. *Plant Biol.* **2012**, *14*, 39–45.
75. Zhang, Y.J.; Fan, P.S.; Zhang, X.; Chen, C.J.; Zhou, M.G. Quantification of *Fusarium graminearum* in harvested grain by real-time polymerase chain reaction to assess efficacies of fungicides on *Fusarium* head blight, deoxynivalenol contamination, and yield of winter wheat. *Phytopathology* **2009**, *99*, 95–100.
76. Magan, N.; Hope, R.; Colleate, A.; Baxter, E.S. Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. *Eur. J. Plant Pathol.* **2002**, *108*, 685–690.
77. Simpson, D.R.; Weston, G.E.; Turner, J.A.; Jennings, P.; Nicholson, P. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *Eur. J. Plant Pathol.* **2001**, *107*, 421–431.
78. Gaurilcikiene, I.; Mankeviciene, A.; Suproniene, S. The effect of fungicides on rye and triticale grain contamination with *Fusarium* fungi and mycotoxins. *Zemdirbyste* **2011**, *98*, 19–26.
79. Pirgozliev, S.R.; Edwards, S.G.; Hare, M.C.; Jenkinson, P. Effect of dose rate of azoxystrobin and metconazole on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in wheat grain. *Eur. J. Plant Pathol.* **2002**, *108*, 469–478.
80. Zhang, Y.J.; Yu, J.J.; Zhang, Y.N.; Zhang, X.; Cheng, C.J.; Wang, J.X.; Hollomon, D.W.; Fan, P.S.; Zhou, M.G. Effect of carbendazim resistance on trichothecene production and aggressiveness of *Fusarium graminearum*. *Mol. Plant Microbe Interact.* **2009**, *22*, 1143–1150.

81. 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.
82. Edwards, S.G.; Pirgozliev, S.R.; Hare, M.C.; Jenkinson, P. Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head blight of winter wheat. *Appl. Environ. Microbiol.* **2001**, *67*, 1575–1580.
83. Haidukowski, M.; Pascale, M.; Perrone, G.; Pancaldi, D.; Campagna, C.; Visconti, A. Effect of fungicides on the development of *Fusarium* head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. *J. Sci. Food Agric.* **2005**, *85*, 191–198.
84. Ioos, R.; Belhadj, A.; Menez, M.; Faure, A. The effects of fungicides on *Fusarium* spp. and *Microdochium nivale* and their associated trichothecene mycotoxins in French naturally-infected cereal grains. *Crop Prot.* **2005**, *24*, 894–902.
85. Paul, P.A.; Lipps, P.E.; Hershman, D.E.; McMullen, M.P.; Draper, M.A.; Madden, L.V. Efficacy of triazole-based fungicides for *Fusarium* head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. *Phytopathology* **2008**, *98*, 999–1011.
86. Reverberi, M.; Ricelli, A.; Zjalic, S.; Fabbri, A.A.; Fanelli, C. Natural functions of mycotoxins and control of their biosynthesis in fungi. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 899–911.
87. 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.
88. Wyand, R.A.; Brown, J.K.M. Sequence variation in the CYP51 gene of *Blumeria graminis* associated with resistance to sterol demethylase inhibiting fungicides. *Fungal Genet. Biol.* **2005**, *42*, 726–735.
89. Leroux, P.; Walker, A.S. Multiple mechanisms account for resistance to sterol 14 alpha-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **2011**, *67*, 44–59.
90. Hamamoto, H.; Hasegawa, K.; Nakaune, R.; Lee, Y.J.; Makizumi, Y.; Akutsu, K.; Hibi, T. Tandem repeat of a transcriptional enhancer upstream of the sterol 14 alpha-demethylase gene (CYP51) in *Penicillium digitatum*. *Appl. Environ. Microbiol.* **2000**, *66*, 3421–3426.
91. Liu, X.; Yu, F.; Schnabel, G.; Wu, J.B.; Wang, Z.Y.; Ma, Z.H. Paralogous cyp51 genes in *Fusarium graminearum* mediate differential sensitivity to sterol demethylation inhibitors. *Fungal Genet. Biol.* **2011**, *48*, 113–123.
92. De Waard, M.A.; Andrade, A.C.; Hayashi, K.; Schoonbeek, H.J.; Stergiopoulos, I.; Zwiers, L.H. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. *Pest Manag. Sci.* **2006**, *62*, 195–207.
93. Löffler, J.; Einsele, H.; Hebart, H.; Schumacher, U.; Hrastnik, C.; Daum, G. Phospholipid and sterol analysis of plasma membranes of azole-resistant *Candida albicans* strains. *FEMS Microbiol. Lett.* **2000**, *185*, 59–63.

94. Audenaert, K.; Monbaliu, S.; Deschuyffeleer, N.; Maene, P.; Vekeman, F.; Haesaert, G.; De Saeger, S.; Eeckhout, M. Neutralized electrolyzed water efficiently reduces *Fusarium* spp. *in vitro* and on wheat kernels but can trigger deoxynivalenol (DON) biosynthesis. *Food Control* **2012**, *23*, 515–521.
95. Becher, R.; Weihmann, F.; Deising, H.B.; Wirsal, S.G.R. Development of a novel multiplex DNA microarray for *Fusarium graminearum* and analysis of azole fungicide responses. *BMC Genomics* **2011**, *12*, doi:10.1186/1471-2164-12-52.
96. Poppenberger, B.; Berthiller, F.; Lucyshyn, D.; Sieberer, T.; Schuhmacher, R.; Krska, R.; Kuchler, K.; Glossl, 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.
97. Zwiers, L.H.; Stergiopoulos, I.; Gielkens, M.M.C.; Goodall, S.D.; De Waard, M.A. ABC transporters of the wheat pathogen *Mycosphaerella graminicola* function as protectants against biotic and xenobiotic toxic compounds. *Mol. Genet. Genomics* **2003**, *269*, 499–507.
98. Gardiner, D.M.; Stephens, A.E.; Munn, A.L.; Manners, J.M. An ABC pleiotropic drug resistance transporter of *Fusarium graminearum* with a role in crown and root diseases of wheat. *FEMS Microbiol. Lett.* **2013**, *348*, 36–45.
99. Gardiner, D.M.; Osborne, S.; Kazan, K.; Manners, J.M. Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. *Microbiol. Sgm* **2009**, *155*, 3149–3156.
100. Merhej, J.; Richard-Forget, F.; Barreau, C. The pH regulatory factor Pad1 regulates Tri gene expression and trichothecene production in *Fusarium graminearum*. *Fungal Genet. Biol.* **2011**, *48*, 275–284.
101. Ramirez, M.L.; Chulze, S.; 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.
102. Magan, N.; Aldred, D.; Mylona, K.; Lambert, R.J.W. Limiting mycotoxins in stored wheat. *Food Add. Contam. Part A Chem.* **2010**, *27*, 644–650.
103. Kokkonen, M.; Ojala, L.; Parikka, P.; Jestoi, M. Mycotoxin production of selected *Fusarium* species at different culture conditions. *Int. J. Food Microbiol.* **2010**, *143*, 17–25.
104. Jiang, J.H.; Liu, X.; Yin, Y.N.; Ma, Z.H. Involvement of a velvet protein FgVeA in the regulation of asexual development, lipid and secondary metabolisms and virulence in *Fusarium graminearum*. *PloS ONE* **2011**, *6*, e28291.
105. Merhej, J.; Urban, M.; Dufresne, M.; Hammond-Kosack, K.E.; Richard-Forget, F.; Barreau, C. The velvet gene, FgVe1, affects fungal development and positively regulates trichothecene biosynthesis and pathogenicity in *Fusarium graminearum*. *Mol. Plant Pathol.* **2012**, *13*, 363–374.
106. Jiang, J.H.; Yun, Y.Z.; Liu, Y.; Ma, Z.H. FgVELB is associated with vegetative differentiation, secondary metabolism and virulence in *Fusarium graminearum*. *Fungal Genet. Biol.* **2012**, *49*, 653–662.