DOI: 10.5965/223811712122022176

Revista de Ciências Agroveterinárias 21 (2): 2022
Universidade do Estado de Santa Catarina

Deoxynivalenol biosynthesis and accumulation in wheat under application of carrageenan

Biossíntese e acúmulo de desoxinivalenol em trigo submetido à aplicação de carragena

Rafael Dal Bosco Ducatti[1*] (ORCID 0000-0001-8916-6557), João Americo Wordell Filho[2] (ORCID 0000-0003-4091-1290), Sergio Miguel Mazaro[1] (ORCID 0000-0003-2787-9409)

[1]Federal University of Technology, Pato Branco, PR, Brazil. *Author for correspondence: rducatti@alunos.utfpr.edu.br; rafaelducatti1007@gmail.com
[2]Research Center for Family Agriculture, Research and Rural Extension of Santa Catarina, Chapecó, SC, Brazil.

Submission: 03/11/2021 | Acceptance: 15/12/2021

ABSTRACT

Mycotoxins pose a large threat to human and livestock besides reducing the quality of food/feed. It is believed that these toxins are biosynthesized as a mechanism of defense (stress-response) of fungi. The most important mycotoxin for winter-cereal crops is known by deoxynivalenol (DON), a trichothecene biosynthesized mainly by *Fusarium graminearum*. This manuscript brings data of three years (2018, 2019 and 2020) of field research (24 field experiments) in Western Santa Catarina and North-Western Rio Grande do Sul, Brazil using a sulphated polysaccharide exclusive from red seaweed, carrageenan, associated with fungicides to help suppressing deoxynivalenol (DON) biosynthesis and accumulation in wheat kernels. A reduction of 36.4% in DON contamination was observed. Weather conditions have influenced the accumulation of DON in wheat kernels. Carrageenan has shown to be a biological compound capable of helping on the suppression of DON biosynthesis and accumulation in wheat kernels regardless of weather conditions.
KEYWORDS: DON; mycotoxins; *Solieria chordalis*;*Triticum aestivum* L.

RESUMO

As micotoxinas representam uma grande ameaça para humanos e animais, além de reduzirem a qualidade de alimentos/rações. Acredita-se que essas toxinas sejam biossintetizadas como mecanismo de defesa (resposta ao estresse) de fungos. A micotoxina de maior interesse para cereais de inverno é conhecida por desoxinivalenol (DON), um tricoteceno biossintetizado principalmente por *Fusarium graminearum*. Esse estudo traz dados de três anos (2018, 2019 e 2020) de pesquisa de campo (24 experimentos) conduzidos no Oeste de Santa Catarina e no Nordeste do Rio Grande do Sul, Brasil, utilizando um polissacarídeo sulfatado exclusivo de algas vermelhas, carragena, associado a fungicidas para ajudar na supressão da biossíntese e acúmulo de desoxinivalenol (DON) em grãos de trigo. Foi observada uma redução de 36,4% na contaminação por DON. As condições climáticas influenciaram no acúmulo de DON nos grãos de trigo. A carragena se demostrou como um composto biológico capaz de atenuar a biossíntese e acúmulo de DON em grãos de trigo independente das condições climáticas.
PALAVRAS-CHAVE: DON; micotoxinas, *Solieria chordalis*, *Triticum aestivum* L.

Evidences have shown that the biosynthesis of mycotoxins, invisible secondary metabolites biosynthesized by filamentous fungi, might be linked to stress-response mechanisms triggered by abiotic and/or biotic stresses such as high/cold temperature, humidity, growth media pH variations, competition against other biotic organisms, application of fungicides, among others (WEGULO 2012, MARIN et al. 2013, PONTS 2015). The most problematic mycotoxin for wheat is called deoxynivalenol (DON), a trichothecene B mainly produced by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schweinitz) Petch) (WEGULO 2012, PONTS 2015).

Deoxynivalenol, also known as vomitoxin, has raised the attention of all governmental agencies all around the world in the last few decades (BOUTRIF & CANET 1998, FAO 2004) because of the high levels of toxicity it causes when consumed over threshold levels (MARIN et al. 2013, PINTON & OSWALD 2014). Besides its toxicity, DON causes large economic losses to farmers and industries (MARIN et al. 2013, MIEDANER et al. 2017). The incidence of *F. graminearum* in wheat fields is also linked to large decreases in crop yields (REIS et al. 2016), mainly associated to the incidence of the *Fusarium* Head Blight disease (FHB) (MIEDANER et al. 2017).

The search to understand what drives different species of *Fusarium* to produce specific mycotoxins and, the same species of *Fusarium* to produce different classes of mycotoxins is still not completely understood (WEGULO 2012, PONTS 2015). However, the need to find biological/inorganic compounds with the potential to diminish the contamination of wheat kernels by these toxins is essential to provide food and feed with raised quality to humans and livestock (KRSKA et al. 2016).

In this context, plant elicitation might play a big role to help solving this problem. Plant elicitation drives plants to, through their co-evolution, find ways to produce their own defense mechanisms against biological organisms such as fungi (DALIO et al. 2014, SHUKLA et al. 2016). These mechanisms, triggered by pathogen-associated molecular patter-recognition receptors (PRR - *PAMP-Recognition Receptor*) and thereafter by *Effector-Triggered Immunity* (ETI) can considerably diminish the incidence of biotic and abiotic stresses on plants (DALIO et al. 2014) by the production of many secondary metabolites, which might affect fungi growth and survival. Carrageenan, a sulphated marine polysaccharide found exclusively on red seaweed can elicit plants to produce their own mechanisms and compounds of defense (SHUKLA et al. 2016). It's been shown that carragennan helps on the suppression of mycotoxins caused by *Fusarium* in maize, wheat and barley (DUCATTI et al. 2021a,b).

Therefore, this research was developed in order to try to stablish: 1) if weather conditions for Southern Brazil have influenced the incidence of DON in wheat kernels during three wheat harvest seasons (2018, 2019 and 2020); and 2) if the bio-compound exclusively found on red seaweed, carrageenan, could help on the reduction of the amount of DON biosynthesized by *F. graminearum* over time.

Data from 24 field experiments performed during the wheat harvest seasons of 2018, 2019 and 2020 in Western Santa Catarina and North-Western Rio Grande do Sul, Brazil, were compiled and analyzed. These field experiments used different wheat cultivars with different levels of tolerance to FHB, different sowing densities, and made use of different amounts of fertilizers and chemical controls (fungicides, insecticides and herbicides). The only variable that varied within each of these field trials was the use, or not, of carrageenan for the treated plots. Carrageenan was supplied with a product entitled Algomel PUSH®, produced with a red seaweed called *Solieria chordalis* (C. Agardh) J. Agardh 1842. Algomel PUSH®, for all trials, was applied through a unique foliar application at the dose of 1.0 L ha^{-1} in the beginning of the tillering stage of plants (applied either in mixture with other products or alone). The area harvested and used for yield assessment in each field experiment was of 1000 m^{-2}. DON analyses were performed sampling 500 g of wheat grains from each treatment (Algomel PUSH and Control) in each field trial. Data for climatic conditions from 20 weather stations located in these regions were collected from the Brazilian Meteorologic Database of the National Institute of Meteorology (BDMEP/INMET 2021).

DON quantification was performed through Near-Infrared Spectrometry (NIRs) following the methodology of GARCIA et al. (2017). All data were analyzed by ANOVA (parametric data) or by Kruskal-Wallis (non-parametric data) and means were compared using either Tukey's HSD or Mann-Whitney statistical methods. All statistical analyses were performed with the software JASP 0.13.1 (RRID:SCR_015823).

The data gathered from the BDMEP/INMET for Western Santa Catarina state (SC) and North-Western Rio Grande do Sul state (RS), Brazil, shows that the rainfall between the months of August to November, corresponding to the periods of flowering, kernel filling, and kernel maturation dropped considerably from the harvest seasons 2018 to 2020 (Table 1). For temperature, no correlation with either mycotoxin contamination or yield could be found, therefore this dataset is not presented.

Weather conditions influence the incidence/growth of fungi and DON biosynthesis in the fields (WEGULO 2012). This could be observed during these three years of field trials. Nevertheless, for these three years, carrageenan was able to decrease the amount of DON accumulation in kernels when compared to parcels that have not received Algomel PUSH® (Figure 1).

The reduction of DON contamination was, on average, 68.2%, 34.4%, and 19.5% for the harvest seasons of 2018, 2019, and 2020, respectively. Furthermore, by plotting the correlation of DON incidence and the mean precipitation for the evaluated years (Figure 1), a positive effect on the overall incidence of DON in wheat fields without carrageenan was observed (R^2=0,91). However, a weak correlation was seen (R^2=0,38) when carrageenan was applied, meaning the results were stable over time regardless of weather conditions.

Table 1. Monthly mean precipitation records for North-Western Rio Grande do Sul (RS) and Western Santa Catarina (SC), Brazil, for the harvest seasons of 2018, 2019, and 2020. The gray-shaded columns indicate the critical period for FHB incidence and DON biosynthesis in wheat ears/kernels for these regions.

State	Harvest Season	May	June	July	August	September	October	November
SC	2018	61.14	92.22	26.96	77.6	164.96	220.08	140.96
	2019	282.38	34.72	65.68	21.44	65.26	178.96	207.04
	2020	105.62	172.98	120.92	103.38	40.18	32.88	103.16
RS	2018	113.06	138.68	82.74	69.12	162.18	205.18	192.20
	2019	239.02	27.92	131.82	63.74	51.26	203.68	141.22
	2020	141.52	187.12	189.56	102.08	69.32	34.18	81.70

RS: weather stations of Cruz Alta, Erechim, Frederico Westphalen, Ibirubá, Palmeira das Missões, Passo Fundo, Santa Rosa, Santo Augusto, São Luiz Gonzaga and Soledade. SC: weather stations of Caçador, Campos Novos, Chapecó, Curitibanos, Dionísio Cerqueira, Joaçaba, Lages, Novo Horizonte, São Miguel do Oeste and Xanxerê.

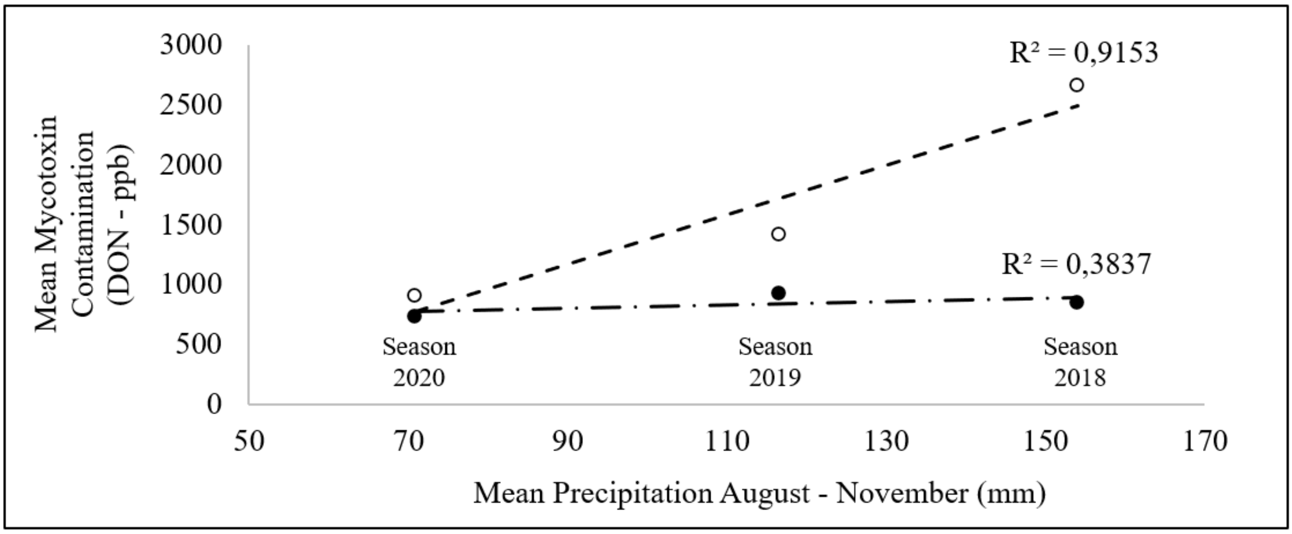

Figure 1. Pearson' correlation between the mean mycotoxin contamination (DON - ppb) and the mean precipitation (mm) of each harvest season. (○) Control (only fungicides) and (●) Treatment (Fungicides + Carrageenan (Algomel PUSH® 1.0 L ha^{-1})).

WEGULO (2012) performed a review showing the factors influencing DON accumulation in small grain cereals. In this investigation, the author has demonstrated that weather conditions, including rainfalls, temperature and relativity humidity, are the most important factors contributing to the incidence of FHB and the accumulation of DON in wheat kernels.

According to this author, the accumulation of DON in small grain crops is weekly positive correlated to the fungus biomass and the FHB disease (R^2=0.50). This is important because having large amounts of fungi biomass in a field does not mean the biosynthesis and accumulation of DON will be greater than a field with lower amounts of fungi biomass. This assumption holds true because it is believed that mycotoxin production is associated with a stress-response mechanism for the protection of fungi (PONTS 2015), i.e. more mycotoxin is synthesized and accumulated with increasing stressful conditions. Acidic pH, for example, promoted higher biosynthesis of DON by *Fusarium* (MERHEJ et al. 2010).

Because fungi are sessile organisms, the production of secondary metabolites (which involves metabolic costs), such as mycotoxins, are mainly carried out to defend and guarantee a long-term survival to the fungi (PONTS 2015). For a study performed by PONTE et al. (2012) during three harvest seasons (2006 - 2008) in commercial wheat fields of 38 municipalities in Rio Grande do Sul, Brazil, the correlation found between DON accumulation and *Fusarium* Damaged Kernels was of R^2=0.27. It again showsthat the presence of *Fusarium* in the kernels does not necessarily mean that DON biosynthesis and accumulation will always follow the fungi biomass.

There cannot be disagreed that the use of fungicides, mainly triazoles, exert a good result in reducing fungi biomass in the fields (MCMULLEN et al. 2012, BONFADA et al. 2020). However, fungi that can survive the application of these chemicals face a large period of stress, which in turns might contribute to increase the biosynthesis of mycotoxins as a defense response from these organisms (PONTS 2015). In a meta-analysis of more than 100 field experiments using different fungicides to control FHB and DON biosynthesis in wheat, MCMULLEN et al. (2012) showed that the most efficient fungicide to reduce *Fusarium* biomass in wheat fields was a combination of prothioconazole + tebuconazole, with a reduction of 52%. However, this same combination was able to reduce DON biosynthesis by only 42%.

These results suggest that fungicides have a positive effect on reducing DON biosynthesis by *Fusarium*. However, this reduction is believed to be linked to a reduction in fungi biomass in the fields much more than to DON biosynthesis itself. This is because FHB and DON are weakly correlated (PONTE et al. 2012, WEGULO 2012). It means that, for DON biosynthesis itself, one would expect to see higher biosynthesis of mycotoxin by the *Fusarium* that survived the application of fungicides, in other words, fungi that thrive the application of fungicide will tend to synthetize much more DON as a stress defense mechanism.

To better understand these assumptions, in the harvest season of 2020 an experiment in the region of Campo Novo, RS, Brazil, was developed. This experiment (4 replicates) was composed of four treatments that consisted in: 1) negative control (nothing being applied), 2) positive control (only fungicides), 3) positive control + carrageenan, and 4) only carrageenan. For DON biosynthesis and accumulation, the results for this specific experiment can be found in Figure 2.

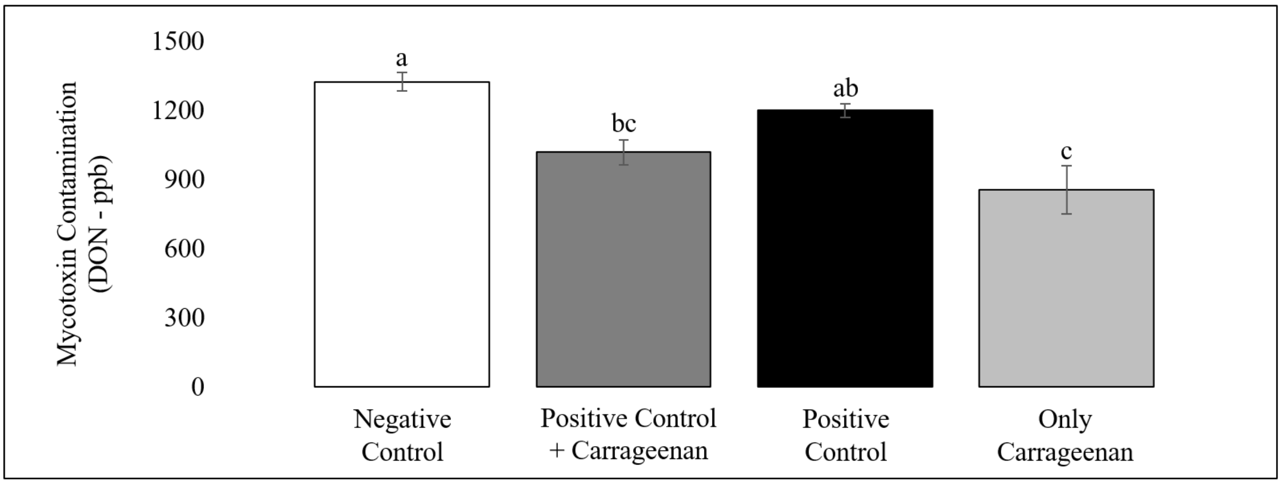

Figure 2. Mycotoxin contamination of wheat kernels influenced by fungicides and carrageenan. Fungicide applications happened at the tillering stage (trifloxystrobin + tebuconazole - 750 mL ha^{-1}), elongation stage (pyraclostrobin + epoxiconazole - 330 mL ha^{-1}), and flowering stage (pyraclostrobin + metconazole - 750 mL ha^{-1}). Carrageenan application happened only at the tillering stage (applied in combination with fungicide) at the dose of 1.0 L ha^{-1} of Algomel PUSH®. This experiment was conducted as a completely randomized block design with 4 replicates. Columns with different letters are statistically different according to Tukey's HSD statistical test (confidence level of 5%).

By analyzing Figure 2, one could see, regarding DON contamination, that parcels that received only fungicides (positive control) showed lower contamination than the negative control. The FHB disease incidence was not assessed in these parcels; nevertheless, this decrease in DON contamination is possibly more linked to the reduction on fungi biomass than directly linked to DON biosynthesis by each point of *Fusarium* found in the field (new studies to prove this ought to be performed). When only carrageenan or when carrageenan + fungicides was applied, a greater decrease in DON contamination was observed, probably because of the effects that this biological compound produces directly on plants and indirectly on fungi (DUCATTI et al. 2021b).

Carrageenan can elicit the primary and secondary mechanisms of growth and defense of plants. Not only mimicking the attack of pathogens/microbes on plants, allowing them to produce ahead of time, secondary metabolites of defense, these compounds can stimulate the mechanisms of growth such as photosynthesis, activity of the Krebs cycle, hormone regulation, changes in redox status, among others (SHUKLA et al. 2016, DUCATTI et al. 2021a). It is believed that, yet to be investigated, the power of elicitation linked to carrageenan produces an effect of long duration within plants due to its solubility and degree of sulphation. This hypothesis has been raised by the authors due to the time of application of carrageenan in all the experiments (beginning of the tillering stage) and the results seen after kernel harvest.

Among all the secondary metabolites produced by plants after elicitation with carrageenan, salicylic acid (SA) exerts a fundamental role in signaling pathways within plants (SHUKLA et al. 2016, HAO et al. 2019). Yet HAO et al. (2019) have shown the ability of some strains of *F. graminearum* to degrade plants' SA, it is believed that the high amounts of SA produced over time by plants after elicitation with carrageenan may have a strong effect on fungi, reducing fungi metabolism, making fungi less prone to suffer and get stressed by unfavorable conditions, reducing the amount of carbon consumed from the plant, thus, reducing mycotoxin biosynthesis and helping to increase the final yield and quality of kernels. Overall, for these three years of field research, a decrease in DON contamination of 36.4% was observed (Figure 3).

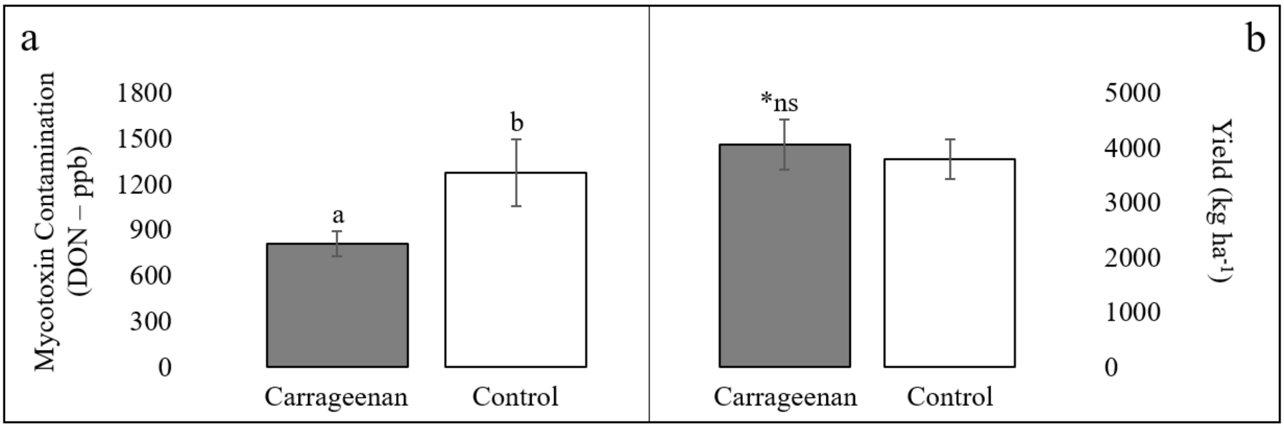

Figure 3. Mean overall mycotoxin contamination (DON - ppb) (a) and yield (b) for the three harvest seasons evaluated. (a) Columns with different letters are statistically different according to Mann-Whitney statistical test (confidence level of 5%). (b) Columns with different letters are statistically different according to Tukey´s HSD statistical test (confidence level of 5%). *ns: not significant.

Carrageenan has shown to be a biological compound capable of helping on the suppression of DON biosynthesis and accumulation in wheat kernels over time regardless of weather conditions. To farmers and industries, Algomel PUSH® may be used as an exllent tool for the suppression of DON and the acquisition of better quality kernels to be used for feed and food. Nevertheless, further research must be performed to better understand the direct and indirect effects of carrageenan over plants and *Fusarium*.

ACKNOWLEDGEMENTS

We would like to thank Doctor Siumar Pedro Tironi from the Federal University of Fronteira Sul – Campus Chapecó, for the help and advises in the development of this study.

REFERENCES

BDMEP/INMET. 2021. Banco de Dados Meteorológicos – Instituto Nacional de Meteorologia. Meteorologic Database. Brazil. Disponível em: https://bdmep.inmet.gov.br. Acesso: 30 jul. 2021.

BONFADA EB et al. 2020. Performance of fungicides on the control of fusarium head blight (*Triticum aestivum* L.) and deoxynivalenol contamination in wheat grains. Summa Phytopathologica 4: 374-380.

BOUTRIF E & CANET C. 1998. Mycotoxin prevention and control: FAO programmes. Revue de Médecine Vétérinaire 149: 681-694.

DALIO RJD et al. 2014. Efetores nas interações Planta-Patógeno. Revisão Anual de Patologia de Plantas 22: 25-68.

DUCATTI RDB et al. 2021a. Use of carrageenan for the reduction of deoxynivalenol in wheat and barley kernels. Journal of Biotechnology and Biodiversity 9: 40-47.

DUCATTI RDB et al. 2021b. An algal sulphated polysaccharide capable of reducing mycotoxin biosynthesis by *Fusarium*. Communications in Plant Science 11: 57-59.

FAO. 2004. Food and Agriculture Organization of the United Nations. Worldwide regulations for mycotoxins in food and feed in 2003. Rome: FAO.

GARCIA J et al. 2017. Deoxynivalenol screening in wheat kernels using hyperspectral imaging. Biosystems Engineering 155: 24-32.

HAO G et al. 2019. Characterization of a *Fusarium graminearum* salicylate hydroxylase. Frontiers in Microbiology 9: 3219.

KRSKA R et al. 2016. Safe food and feed through an integrated toolbox for mycotoxin management: the MyToolBox approach. World Mycotoxin Journal 9: 487-495.

MARIN S et al. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology 60: 218-237.

MCMULLEN M et al. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight disease. Plant Disease 96: 1712-1728.

MERHEJ J et al. 2010. Acidic pH as a determinant of TRI gene expression and trichothecene B biosynthesis in *Fusarium graminearum*. Food Additives & Contaminants 27: 710-717.

MIEDANER T et al. 2017. Editorial: Management of *Fusarium* species and their mycotoxins in cereal food and feed. Frontiers in Microbiology 8: 1543.

PINTON P & OSWALD IP. 2014. Effect of deoxynivalenol and other type B trichothecenes on the intestine: a review. Toxins 6: 1615-1643.

PONTE EMD et al. 2012. Deoxynivalenol and nivalenol in commercial wheat grain related to Fusarium Head Blight epidemics in southern Brazil. Food Chemistry 132: 1087-1091.

PONTS N. 2015. Mycotoxins are a component of *Fusarium graminearum* stress-response system. Frontiers in Microbiology 6: 1234.

REIS EM et al. 2016. Anthesis, the infectious process and disease progress curves for fusarium head blight in wheat. Summa Phytopathologica 42: 134-139.

SHUKLA PS et al. 2016. Carrageenans from red seaweeds as promoters of growth and elicitors of defense response in plants. Frontiers in Marine Science 3: 81.

WEGULO S. 2012. Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins 4: 1157-1180.