Europ. J. Agronomy 85 (2017) 12–24

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Effect of agronomic programmes with different susceptibility to deoxynivalenol risk on emerging contamination in winter wheat

Massimo Blandino[a,*], Valentina Scarpino[a], Michael Sulyok[b], Rudolf Krska[b], Amedeo Reyneri[a]

[a] University of Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo P. Braccini 2, I-10095 Grugliasco (TO), Italy
[b] Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, Tulln 3430, Austria

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 13 December 2016
Accepted 3 January 2017

Keywords:
Common wheat
Durum wheat
Fungicide
Fusarium Head Blight
Emerging mycotoxins

ABSTRACT

Deoxynivalenol (DON) is the most prevalent mycotoxin in small cereal crops throughout the world, and its occurrence is closely linked to the presence of Fusarium Head Blight (FHB) disease.

In order to minimize the sanitary risk, wheat cropping systems are commonly designed to control DON contamination, as this represents the main target contaminant. However, several other mycotoxins and secondary metabolites produced by *Fusarium* and other fungal species have been detected in wheat. The objective of this study was to evaluate whether the application of agronomic programmes with different susceptibility to DON contamination could also affect the occurrence of emerging mycotoxins in wheat kernels.

Field experiments have been conducted in North Italy, under naturally-infected conditions, over a period of 3 growing seasons, by comparing 4 field programmes, which were constituted by the combination of wheat cultivars (a durum wheat variety that is susceptible to DON contamination and a common moderately resistant one) and 2 fungicide applications at heading (untreated control compared to an azole application at heading).

Grain samples have been analyzed by means of a dilute-and-shoot multi-mycotoxin LC–MS/MS method, and 43 fungal metabolites were detected. In addition to DON, the most abundant compounds were aurofusarin, culmorin and deoxynivalenol-3-glucoside, which were detected in all the growing seasons and agronomic strategies. Other trichothecenes and zearalenone derivatives were also found, but in clearly lower concentrations.

Contamination by enniatins and moniliformin, produced by other *Fusarium* species e.g. *Fusarium avenaceum*, alternariol, alternariol methyl ether and tentoxin, produced by *Alternaria* species, has been observed for all the compared growing seasons. The presence of other mycotoxins and secondary metabolites was clearly affected by the climatic conditions: fumonisins, beauvaricin, bikaverin, fusaric acid and butenolid were detected in the warmer growing seasons, while chrysogine, infectopyrone, secalonic acid and ergot alkaloids (sum of 13 toxins) were only found in the more rainy and cool seasons. Equisetin, decalonectrin, toxin T-2 and HT-2 were only found in traces.

Abbreviations: 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; AME, alternariol methyl ether; AOH, alternariol; ANOVA, analysis of variance; AUR, aurofusarin; BEA, beauvericin; BIK, bikaverin; BUT, butenolide; CHRY, chrysogine; CULM, culmorin; DEC, decalonectrin; DON, deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside; EC, European Commission; EFSA, European Food Safety Authority; ENNs, enniatins A, A_1, B, B_1, B_2; EQU, equisetin; EAs, ergot alkaloids; FA, fusaric acid; FB, fumonisins; FUS, fusaproliferin; GDD, accumulated growing degree days; GS, growth stage; INF, infectopyrone; LC–MS/MS, liquid chromatography coupled with tandem mass spectrometry detection; LOD, limit of detection; LOQ, limit of quantification; MON, moniliformin; MR, moderately resistant; MS, mass spectrometry detection; NIV, nivalenol; S, susceptible; TENT, tentoxin; TW, test weight; SAD, secalonic acid; ZEA, zearalenone; ZEA-4-S, zearalenone-4-sulphate.

* Corresponding author.
E-mail address: massimo.blandino@unito.it (M. Blandino).

http://dx.doi.org/10.1016/j.eja.2017.01.001
1161-0301/

The application of the field programmes clearly affected DON contamination in each growing season: a significant increase in this toxin has been observed moving from the lowest risk agronomic strategy to the highest one. The application of the most favourable DON control field programme (a moderately resistant variety combined with fungicide application at heading) reduced the content of this mycotoxin by 89%, compared to the worst programme (untreated susceptible variety).
The application of the less risky agronomic strategy for DON contamination led to a significant reduction (>84%) of all the other mycotoxins produced by the DON producing fungal species. Moreover, although the considered agronomic factors (variety susceptibility and fungicide application) resulted in a control efficacy that varied in function of the environmental conditions and the type of mycotoxin, the results show a clear reduction trend, after the application of agronomic strategies that are able to minimize the DON content, for almost all the other *Fusarium*, *Alternaria*, *Claviceps* and *Penicillium* metabolites.
The results summarized in this work, which have been obtained under different environmental and agronomical conditions, allow a first assessment to be made of the agronomic strategies that could be applied to control emerging mycotoxins in wheat.

1. Introduction

Mycotoxins are secondary metabolites that are produced by several fungal species, which could have a range of toxic properties, including carcinogenicity and neurotoxicity, as well as developmental and reproductive toxicity for humans and reared animals and could result in illnesses and economic losses (Pestka and Smolinski, 2005).

Among the various agricultural commodities, cereals are most likely to be contaminated, and deoxynivalenol (DON), a type-B trichothecene produced by *Fusarium* spp., is the most prevalent toxin in small cereal crops throughout the world (Larsen et al., 2004). Regulatory limits have been set by the European Commission (EC) to protect humans from this mycotoxin exposure through cereal consumption (EC No. 1881/2006 and EC No. 1126/2007, with a limit of 1250 and 1750 μg kg^{-1} in unprocessed common and durum wheat, respectively). The occurrence of DON in wheat and other small cereals is closely linked to the presence of Fusarium Head Blight (FHB) disease, which causes total or partial premature ear senescence and consequent negative impacts on both crop yields and grain quality. Different *Fusarium* species are involved in promoting this disease, although *F. graminearum sensu stricto* and *F. culmorum*, are the most important FHB agents and the main causes of DON accumulation in grains in temperate areas (Yli-Mattila, 2010; Somma et al., 2014).

Although DON contamination in wheat grains depends on the meteorological conditions, particularly at flowering (van der Fels-Klerx et al., 2013), an important role is played by agronomic factors, such as crop rotation, debris management, variety susceptibility and fungicide applications (Pirgozliev et al., 2003; Koch et al., 2006). At present, the most effective approaches adopted to minimize DON occurrence in wheat are the use of preventive agronomic practices to reduce the pathogen inocula in the field, the cultivation of varieties that are less susceptible to FHB and the application of fungicides that are effective in controlling *Fusarium* spp, according to an integrated approach that addresses all of the possible risk factors (Blandino et al., 2012). Thus, in order to ensure low sanitary risks, wheat cropping systems in temperate areas are generally designed to control DON contamination, as this is the main target contaminant. However, to date, about 400 different mycotoxins have been identified in different commodities, and several of these have been found in cereals (Berthiller et al., 2013). These other fungal metabolites, some of which have been referred to as "emerging" (Streit et al., 2013), have not yet received detailed scientific attention. The European Food Safety Authority (EFSA) is currently working on establishing a scientific opinion on the risks to public health related to the presence of emerging mycotoxins in feeds and foods (EFSA, 2010, 2014). Nowadays, it is necessary to collect occurrence data on these mycotoxins in the most important cereal areas in the EU, in order to correctly consider the risk of exposure and to make risk assessments. In addition, there is also a greater interest in verifying the effect of the Good Agricultural Practices (GAP) that are normally applied to control FHB and DON, which is the reference mycotoxin for wheat in temperate areas, on the content of emerging mycotoxins in this crop. Since these compounds could be produced by other *Fusarium* species that are not directly involved in FHB disease, as well as by other fungal species belonging to the *Alternaria*, *Penicillium* and *Claviceps* families, more detailed knowledge on the environmental and agronomic conditions that promote their occurrence is essential in order to set up field programmes that will be able to minimize the overall sanitary risk for grain.

The aim of this study was to investigate the effect of agronomic strategies, with different susceptibility to DON, on the occurrence of emerging mycotoxins and fungal metabolites in wheat in different production situations.

2. Materials and methods

2.1. Chemicals

Methanol and acetonitrile (both LC gradient grade) were purchased from J.T. Baker (Deventer, The Netherlands); ammonium acetate (MS grade) and glacial acetic acid (p.a.) were obtained from Sigma–Aldrich (Vienna, Austria). Water was purified successively by means of reverse osmosis and a Milli-Q plus system from Millipore (Molsheim, France).

All the fungal metabolite standard solutions were stored at −20 °C and were brought to room temperature before use.

2.2. Field experimental design and samples

The effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on emerging mycotoxin occurrence in wheat was studied in North-West Italy in the 2010–11 growing season at Cigliano (45°18′N, 8°01′E; altitude 237 m), in a sandy-loam soil (Typic Hapludalfs) and in the 2011–12 and 2012–13 growing seasons at Carmagnola (44°50′N, 7°40′E; altitude 245 m) in a loam soil (Typic Udifluvents).

In each growing season, 4 field programmes, resulting from a factorial combination of 2 wheat cultivars (cv), with different susceptibility to DON contamination, and 2 fungicide applications, were compared in naturally-infected field conditions: