Cereal Research Communications 43(3), pp. 481–491 (2015)
DOI: 10.1556/0806.43.2015.010
First published online 18 June 2015

Preliminary Survey on the Co-occurrence of DON and T2+HT2 Toxins in Durum Wheat in Italy

G. Aureli[1]*, T. Amoriello[2], A. Belocchi[1], M.G. D'Egidio[1], M. Fornara[1], S. Melloni[1] and F. Quaranta[1]

[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di ricerca per la valorizzazione qualitativa dei cereali (CRA-QCE), Via Cassia n. 176, 00191 Rome, Italy
[2]Consiglio per la Ricerca e la sperimentazione in Agricoltura, Centro di ricerca per gli alimenti e la nutrizione (CRA-NUT), Via Ardeatina 546, 00178 Rome, Italy

(Received 27 June 2014; Accepted 24 November 2014)

This study was carried out to determine the co-occurrence of deoxynivalenol (DON) and the sum of T2 and HT2 toxins in durum wheat samples belonging to eight cultivars grown in a national network experimental trials over a three-year period (2011–2013). The effect of several factors (cultivar, year and cultivation area) affecting the occurrence of the two types of mycotoxins and their relationship with several agronomic and grain quality parameters were assayed by statistical analysis (GLZ). The results highlighted the different trend of incidence and contamination rate of the two types of mycotoxins in relation to the cropping year and to the growing examined areas. Year and its interaction with the cultivation area was the most important factor affecting the DON contamination, whereas genotype and its interaction with the year mainly influenced T2+HT2 toxins contamination rate. DON and T2+HT2 contamination levels were not significantly correlated with each other. The evidence that the two types of mycotoxins were differently related with several agronomic and grain quality parameters could be connected to the effects of the respective fungal disease on wheat plant.

Keywords: deoxynivalenol, T2, HT2, *Fusarium*, durum wheat

Introduction

Trichothecenes are a large group of chemically related toxic fungal compounds mainly produced by *Fusarium* spp. in cereal grains. These mycotoxins have multiple toxic effects on eukaryotic cells including inhibition of protein, DNA and RNA synthesis (Lemmens et al. 2005; Rocha et al. 2005; Lancova et al. 2008; Yazar and Omurtag 2008; Gauthier et al. 2013). Deoxynivalenol (DON) is the most common mycotoxin among the type B group of trichothecenes found at high concentration in cereals and mainly produced by *F. graminearum* and *F. culmorum* (Terzi et al. 2007; Häller et al. 2008; Miller 2008; Edwards 2009). The main features of DON on animals are constituted by an emetic effect (DON or "vomitoxin") and immunosuppressive effects also representing a risk for human health (SCF 2002; He et al. 2010; Pestka and Smolinski 2005; Maresca 2013). T2 and

* Corresponding author; E-mail: gabriella.aureli@entecra.it; Fax: +39 0636306022

HT2 toxins belong to the type A group of trichothecenes known as the most toxic members of this group of mycotoxins. The T2 toxin is rapidly transformed to a large group of products, HT2 toxin resulting as the major metabolite with equivalent toxicity, and the sum of T2 and HT2 was considered in the determination (Edwards et al. 2009; EFSA 2011). T2 and HT2 toxins are reported to be implicated in human Alimentary Toxic Aleukia (ATA) due to the consumption of contaminated grain (Wu et al. 2010). The main source of trichothecenes in the food chain is *Fusarium*-contaminated grain and for this reason Fusarium Head Blight (FHB or "scab") outbreak of cereal crops is of great interest. *F. graminearum* Schwabe and *F. culmorum* (WG Smith) are the most relevant species of fungi causing the FHB disease often determining both severe reduction of crop yield and production of fungal secondary metabolites (e.g. DON) in grains which are mainly influenced by environment, genotype and management practices (Pestka and Smolinski 2005; Rocha et al. 2005; Shephard 2008; Váňová et al. 2008; Berthiller et al. 2009; Russel et al. 2010; Zain 2011). The susceptibility shown by durum wheat (*Triticum durum* Desf.) to the FHB disease is of particular concern in Italy due to the important role of this species in the "pasta" production. As regards fungi capable of producing T2 and HT2 toxins *F. langsethiae* is considered the main producer (Edwards et al. 2012). This species results closely related to *F. sporotrichioides* Scherb. and *F. poae* (Schmidt et al. 2004) and the same is known in Europe as grain contaminant of small grain cereals such as oats (the most susceptible type of cereal), barley and wheat. Moreover, the influence of temperature on infection, growth, and mycotoxin production by *F. langsethiae* and *F. sporotrichioides* (Nazari et al. 2014) and the distribution of T2 and HT2 in milling fractions (Pascale et al. 2011) were assayed on durum wheat. *F. langsethiae* was also identified on wheat in Italy (Infantino et al. 2007). Nowadays the approach to the problem mycotoxins in foods is mainly oriented on one side to improve prevention in field, and on the other one to assay the occurrence of simultaneous presence of different mycotoxins (e.g.: deoxynivalenol, T2 and HT2 toxins, nivalenol, etc.). The attention of the European Community is focusing more and more on that direction through the recently adopted Recommendation (2013/165/UE; EFSA 2011) concerning the "indicative" levels for the sum of T2 and HT2 in different matrices: for unprocessed durum wheat the maximum level is 100 μg/kg. The same Recommendation reports also an invitation to the European countries to ascertain the co-occurrence of T2 and HT2 and other toxins from *Fusarium* spp. assessing the degree of concentration. Therefore, today from the *Fusarium* toxins belonging to the group of trichothecenes, only for deoxynivalenol maximum limits were set in cereals and cereal products (EC Regulation 1881/2006, EC Regulation 1126/2007) which are of 1750 μg/kg in unprocessed durum wheat. The aim of this study was to investigate the co-occurrence of DON and the sum of T2 and HT2 (T2+HT2) toxins in durum wheat crops in Italy with regard to the following topics: 1) the levels of DON and T2+HT2 contamination in unprocessed kernels of durum wheat also considering the influence of several important factors (cultivar, year and cultivation area); 2) the relationships between the two aforesaid A and B types of trichothecenes detected and their correlation with several agronomic/grain quality parameters.

Materials and Methods

The overall 240 durum wheat kernel samples were collected during the 2011–2013 three-year period within the Italian durum wheat network experimental trials (Quaranta et al. 2013). A total of 10 locations were included in this study, grouped in five macro-areas (Fig. S1*): North, Centre-East, Centre-West, South and Sicily (only one location). A number of 8 cultivars were evaluated every year (Claudio, Duilio, Dylan, Iride, Kanakis, Ramirez, Sculptur and Simeto). Each experimental trial was carried out according to a randomized complete block experimental design (RCBD) with three replications (10 m^2 plots); this experimental protocol was the same both for year and location. Agronomic traits were determined at plot level: after harvesting, grain was air-cleaned and weighed to determine grain yield (t/ha), corrected to 13% of moisture content (GY). On the samples test-weight (TW)(kg/hL) (FOSS Infratec™1241 Grain Analyzer, Sweden) and thousand-kernels weight (TKW) (g) (Pfeuffer Contador GmbH, Germany) were determined. Subsequently grains from the three replication plots were bulked and a representative whole grain subsample was milled (particle size ≤1 mm) using Cyclotec 1093 (FOSS, Sweden). The whole meal was used for protein content determination (% d.m.) by Dumas method (Leco FP 428, Germany). The same whole milled grain subsamples were submitted to the mycotoxins analyses by Enzyme-Linked Immuno-Sorbent Assay (ELISA). DON determination was made on acqueous (distilled water) extract of matrix according to the Ridascreen® DON method with LOD of 18.5 µg/kg (R-Biopharm AG, Germany). The analyses were performed using the Basic Robotic Immunoassay Operator (BRIO, SEAC, Radim Group, Italy). The T2+HT2 detection was carried out on methanolic extracts of matrix according to the instructions of Veratox® T2/HT2 toxin method (Neogen, USA), with LOD of 25.0 µg/kg. The absorbance values were read using Sirio-S Microplate Reader (SEAC, Radim Group, Italy) and concentration data were obtained by the use of RIDA® Soft Win software (R-Biopharm AG, Germany). Distilled water was obtained from Water Purification System Zeener Power I (Human Corporation, Korea) and methanol (analytical grade) was supplied by Sigma (Sigma-Aldrich, UK) (Fig. S1).

In order to statistically analyze the data, samples with mycotoxins value below LOD were equalized to zero (Tittlemier et al. 2013). Descriptive statistics, including mean, standard deviation (SD), minimum (Min) and maximum (Max) value, 25th percentile (Q1), median (Q2), 75th percentile (Q3) and 95th percentile (P95) were calculated for DON and T2+HT2. The data were graphically reported by box plots concerning 25–75% range, median, not outliers range, both suspected outlier and outlier data. Generalized linear model (GLZ) was applied to assess the influence of three factors (cultivar, year and cultivation area) and all their two-way interactions on DON and T2+HT2 contamination. We assumed the response variable $Y_{A,B}$ (DON or T2+HT2) had a Poisson distribution and the logarithm of its expected value E(Y) could be modelled by a linear combination of unknown parameters. Poisson regression models were GLZ with the logarithm as the canonical link function and the Poisson distribution function. The statistical model was:

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

$$\log (Y_{A,B}) = \beta_0 + \tau_i + \delta_l + \zeta_k + \tau_i \cdot \delta_l + \tau_i \cdot \zeta_k + \delta_l \cdot \zeta_k + \varepsilon_{ilk}$$

where $Y_{A,B}$ = DON (A) or T2+HT2 (B) mycotoxins; β_0 = average effect common to all observations; τ_i = cultivar (CV); i = 1, …, 8 (Claudio, Duilio, Dylan, Iride, Kanakis, Ramirez, Sculptur, Simeto); δ_l = year; l = 1, …, 3; (1 = 2011, 2 = 2012, 3 = 2013); ζ_k = area; k = 1, …, 5 (North, Centre-East, Centre-West, South, Sicily); ε = error term. The goodness of fit of GLZ model could be based on the deviance statistic, approximated by a chi-square distribution. We used the log-likelihood value to measure the goodness of fit of the regression models. To assess association within DON and T2+HT2 mycotoxins and agronomic/grain quality variables (yield, test-weight, 1000-kernel weight, and protein content), Spearman's non-parametric correlation was used. Statistical significance levels for the correlation tests were set at $p < 0.01$ (**) and at $p < 0.05$ (*).

Results

The diffusion of monitored mycotoxins during the three-year period was reported in Fig. S2 as percentage (incidence %) of positive samples (concentration ≥ LOD). In the North it could be noted a high incidence of DON positive samples (from 79% to 100%) and a general low incidence of T2+HT2 (from 4% to 29%) with greater presence of DON and less diffusion of T2+HT2 in the year 2013. In Central Italy, where an exceptional amount of rain in the year 2013 occurred (Quaranta et al. 2013), a general heavy diffusion of both classes of mycotoxins was assayed. In the South lower incidence of DON (from 44% to 63%) and no negligible presence of T2+HT2 (from 25% to 44%) were pointed out. Data referred to the unique field of Sicily showed no presence of T2+HT2 all over the three-year period whereas DON incidence ranged from 0 to 63%, even if corresponding to negligible concentration level (Fig. S2).

The different trend of DON and T2+HT2 toxins was also confirmed by the contamination level (mean) of positive samples for each year especially in the North, where the highest value of DON (14452 µg/kg) was reached in the year 2013 (Fig. S3). In Central Italy (both in East and West sides) the average levels of DON for positive samples were lower than 500 µg/kg, with a maximum value of 1080 µg/kg in Centre-West during 2011. In the South and Sicily, DON levels were always below 150 µg/kg (Fig. S3a). A clear presence of T2+HT2 was found in Centre-East (average values from 67 µg/kg to 106 µg/kg) and in the South (average values from 33 µg/kg to 96 µg/kg) (Fig. S3b). These toxins were not detected in the Central West side only in the year 2013, whereas in the field of Sicily all over the three years. Although the conditions for the *in vivo* production of T2 and HT2 toxins in cereals are not entirely clear, data collected in several European countries outline that the occurrence of wet summer could represent a more favourable condition for the production of the main *Fusarium*-toxins (e.g.: DON). On the contrary T2 and HT2 toxins reach high levels in warm and dry seasons (Langseth and Rundberget 1999; Hietaniemi et al. 2004; Imathiu et al. 2013). These weather conditions generally occur both in late spring and in summer seasons in the Centre-South of Italy and in Sicily. Therefore, even though the implicated *Fusarium* species were not assayed in this survey by using microbiological and/or molecular methods from the milled samples, the low

presence of T2+HT2 toxins in the North overall the three-year period, especially in the wettest year 2013, and the absence of the same toxins in the Centre-West in the same rainy year suggest the absence of suitable conditions for development of T2+HT2 toxins producer fungi and/or the competition of these types of fungi with other *Fusarium* species (e.g.: *F. graminearum*). Limited to the North area, an opposite trend between DON and T2+HT2 emerged. The field considered in Sicily showed the lowest mycotoxins risk for durum wheat, according to previous surveys on DON contamination (Aureli et al. 2009; Quaranta et al. 2010). Moreover, the data concerning the incidence and the contamination levels of T2+HT2 toxins in the central-southern areas of Italy resulted in agreement with the presence of *F. langsethiae* previously reported (Santori et al. 2010) (Fig. S3).

Tables 1, 2 and 3 showed some descriptive statistics (mean, standard deviation, minimum, maximum, 25th percentile Q1, median Q2, 75th percentile Q3 and 95th percentile P95) of DON and T2+HT2 toxins with regard to cultivar, year and cultivation area, respectively. With regard to cultivar, the box plot of DON (Fig. S4a) and T2+HT2 (Fig. S4b) concentrations are reported. The distribution referred to cultivar is strongly asymmetric. The T2+HT2 values were distributed close to zero with the exception of Sculptur, Simeto and Duilio and the last two cultivars were also sensitive to the DON (Table 1, Fig. S4). Particularly, Simeto showed the greatest 75th percentile values both of DON and T2+HT2 (428 and 77 ppb, respectively). On the contrary the cultivar Ramirez achieved the best performance with regard both to the T2+HT2 (P95 < LOD) and DON (P95 < 773 µg/kg) occurrence. On the whole the DON values were distributed over a wide range: from < LOD to 1080 µg/kg in 2011, from < LOD to 2799 µg/kg in 2012 and from < LOD to 14452 µg/kg in 2013 (Table 2). T2+HT2 contamination was similar all

Table 1. Descriptive statistics: number of samples (N), mean (µg/kg), standard deviation (SD), minimum (µg/kg), maximum (µg/kg), 25th percentile Q1, median Q2, 75th percentile Q3 and 95th percentile P95 of DON and T2+HT2 toxins contamination by cultivar. Limit of detection (LOD) = 18.5 µg/kg for DON and 25.0 µg/kg for T2+HT2

Cultivar	Toxin	N	Mean	SD	Min	Max	Q1	Q2	Q3	P95
Claudio	DON	30	557	1957	<LOD	10704	21	78	179	2036
	T2+HT2	30	<LOD	–	<LOD	161	<LOD	<LOD	<LOD	84
Duilio	DON	30	518	1562	<LOD	8496	<LOD	103	385	2129
	T2+HT2	30	<LOD	–	<LOD	211	<LOD	<LOD	35	101
Dylan	DON	30	272	577	<LOD	2900	22	52	345	1272
	T2+HT2	30	<LOD	–	<LOD	153	<LOD	<LOD	<LOD	85
Iride	DON	30	313	876	<LOD	4713	<LOD	82	152	1373
	T2+HT2	30	<LOD	–	<LOD	103	<LOD	<LOD	<LOD	100
Kanakis	DON	30	328	746	<LOD	3275	<LOD	96	221	2610
	T2+HT2	30	<LOD	–	<LOD	64	<LOD	<LOD	<LOD	41
Ramirez	DON	30	179	344	<LOD	1755	<LOD	48	242	773
	T2+HT2	30	<LOD	–	<LOD	72	<LOD	<LOD	<LOD	<LOD
Sculptur	DON	30	271	690	<LOD	2799	<LOD	50	132	2744
	T2+HT2	30	28	43	<LOD	185	<LOD	<LOD	47	115
Simeto	DON	30	791	2623	<LOD	14452	24	165	428	2231
	T2+HT2	30	52	65	<LOD	212	<LOD	<LOD	77	178

Table 2. Descriptive statistics: number of samples (N), mean (µg/kg), standard deviation (SD), minimum (µg/kg), maximum (µg/kg), 25th percentile Q1, median Q2, 75th percentile Q3 and 95th percentile P95 of DON and T2+HT2 toxins contamination by year. Limit of detection (LOD) = 18.5 µg/kg for DON and 25.0 µg/kg for T2+HT2

Year	Toxin	N	Mean	SD	Min	Max	Q1	Q2	Q3	P95
2011	DON	80	147	219	<LOD	1080	<LOD	52	183	683
	T2+HT2	80	<LOD	–	<LOD	185	<LOD	<LOD	<LOD	87
2012	DON	80	278	597	<LOD	2799	<LOD	50	223	2083
	T2+HT2	80	26	48	<LOD	212	<LOD	<LOD	35	157
2013	DON	80	786	2264	<LOD	14452	33	133	364	3994
	T2+HT2	80	<LOD	–	<LOD	174	<LOD	<LOD	<LOD	94

over the three-year period even if in 2012 the greatest values were obtained (mean, max, Q3 and P95). Northern Italy was more sensitive to DON occurrence than the other areas, whereas the Centre-East to T2+HT2 toxins occurrence (average value equal to 45 µg/kg), followed by the South (average value equal to 24 µg/kg) (Table 3).

The results of the GLZ analysis concerning the effect of cultivar, year and cultivation area on DON and T2+HT2 contamination degrees (Table 4) showed that all of them were highly significant ($p < 0.0001$) whether as single factors or in the two-way interactions. Year ($\chi^2 = 24387$, $p < 0.0001$), cultivation area ($\chi^2 = 16234$, $p < 0.0001$) and their interaction ($\chi^2 = 16259$, $p < 0.0001$) were the most important factors which strongly affected DON occurrence. The genotype, although statistically significant ($\chi^2 = 25$, $p < 0.0001$), showed a low influence on DON, while cultivar had the highest influence on T2+HT2 contamination ($\chi^2 = 158$, $p < 0.0001$). For T2+HT2 toxins the most important interaction between cultivar and year was shown ($\chi^2 = 159$, $p < 0.0001$) while cultivation area seemed to have low influence on T2+HT2 toxins contamination degree ($\chi^2 = 38$, $p < 0.0001$).

Spearman's non-parametric correlation among DON and T2+HT2 toxins concentrations and the other kernel traits (yield, test weight, 1000-kernel weight, and protein con-

Table 3. Descriptive statistics: number of samples (N), mean (µg/kg), standard deviation (SD), minimum (µg/kg), maximum (µg/kg), 25th percentile Q1, median Q2, 75th percentile Q3 and 95th percentile P95 of DON and T2+HT2 toxins contamination by cultivation area. Limit of detection (LOD) = 18.5 µg/kg for DON and 25.0 µg/kg for T2+HT2

Area	Toxin	N	Mean	SD	Min	Max	Q1	Q2	Q3	P95
North	DON	72	1070	2386	<LOD	14452	84	220	814	4713
	T2+HT2	72	<LOD	–	<LOD	178	<LOD	<LOD	<LOD	52
Centre-East	DON	48	196	196	<LOD	752	31	143	317	583
	T2+HT2	48	45	59	<LOD	212	<LOD	29	70	167
Centre-West	DON	48	187	224	<LOD	1080	41	108	244	641
	T2+HT2	48	<LOD	–	<LOD	84	<LOD	<LOD	<LOD	62
South	DON	48	26	36	<LOD	137	<LOD	<LOD	33	100
	T2+HT2	48	<LOD	–	<LOD	185	<LOD	<LOD	44	88
Sicily	DON	24	<LOD	–	<LOD	68	<LOD	<LOD	21	39
	T2+HT2	24	<LOD	–	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table 4. χ^2 regression for two models referred to DON and T2+HT2 toxins (µg/kg)

Factor	DON		T2+HT2	
	χ^2	Pr > χ^2	χ^2	Pr > χ^2
CV	25	<0.0001	158	<0.0001
Year	24387	<0.0001	97	<0.0001
Area	16234	<0.0001	38	<0.0001
CV*Year	25	<0.0001	159	<0.0001
CV*Area	12	0.0005	66	<0.0001
Year*Area	16259	<0.0001	38	<0.0001

Note: CV = cultivar; Area = cultivation area.

Table 5. Spearman's non-parametric correlation coefficient among agronomic/grain quality variables, DON and T2+HT2 concentrations (µg/kg)

Mycotoxin	DON	T2+HT2	Yield	Test-weight	1000-kernel weight	Protein content
DON	1	0.098 (ns)	0.117 (ns)	–0.319(**)	0.020 (ns)	0.314(**)
T2+HT2		1	0.147(*)	0.048 (ns)	0.250(**)	0.057 (ns)

** Correlation is significant at the 0.01 probability level; *Correlation is significant at the 0.05 probability level. ns: not significant.

tent) are given in Table 5. No significant association between DON and T2+HT2 toxins was found. The DON concentration showed a positive correlation with the protein content ($\rho = 0.314$, $p < 0.01$) and a negative correlation with the test-weight ($\rho = –0.319$, $p < 0.01$); a positive relationship between T2+HT2 toxins and yield ($\rho = 0.147$, $p < 0.05$) was found and lightly higher with 1000-kernel weight ($\rho = 0.250$, $p < 0.01$). On the whole the correlation between the mycotoxins concentration and agronomic/grain quality parameters showed a different trend for DON and T2+HT2 toxins. In fact the negative correlation rate ($\rho = –0.319$, $p < 0.01$) between DON and test-weight would suggest a damaged grain kernels related to the protein content (Boyacioğlu and Hettiarachchy 1995; Dexter et al. 1997; Nightingale et al. 1999).

Discussion

The results showed that year, cultivation area and their interaction were the most important factors which strongly affected DON occurrence in agreement with previous surveys on wheat (Edwards et al. 2009; Aureli et al. 2009). As regards T2+HT2 toxins the data obtained seemed to be in agreement with recent surveys carried out in other European countries which outlined the importance of the year in the accumulation of T2 and HT2 in cereals at least partly related to the warm and dry weather conditions but also in relation to varieties (Edwards 2009; van der Fels-Klerx and Stratakou 2010).

The positive correlation with protein content would be explained by a concentration effect in damaged kernel. Although no significant correlation was found between DON

and yield and 1000-kernel weight, these preliminary results seemed at least partially in agreement with the effect caused by FHB attacks on grains (shrivelled kernels) when DON producers fungi, mainly *F. graminearum* and *F. culmorum,* are involved.

On the other hand, the significant and positive correlation of T2+HT2 toxins with the grain yield and the 1000-kernel weight seemed to suggest a general healthy kernel condition which substantially agrees with previous observations on the lack of clear disease and/or visual symptoms on cereal heads in field although the presence of *F. langsethiae* was confirmed (Imathiu et al. 2013; Opoku et al. 2013). In fact, this fungus is known for its inability to cause severe disease symptoms on cereals (e.g.: wheat and oat) without apparent loss of grain yield (Torp and Adler 2004; Imathiu et al. 2010; Divon et al. 2011). Nowadays the relation between grain characteristics and the presence of DON and T2+HT2 are not sufficient to draw comprehensive conclusions.

However, available data from the literature concerning various types of cereals (wheat, barley and oat) does not seem to show a positive correlation between the concentration levels of DON and T2+HT2 but rather a trend to mutual exclusion between the two types of mycotoxins. A sign of mutual exclusion among different types of mycotoxins is probably due to the competition and/or different environmental requirements of the different species of *Fusarium* mycotoxin producers (Barrier-Guillot 2009; Edwards 2009).

The coexistence of the two toxins in cereals, however, was already reported in other areas of northern Europe (Langseth and Rundberget 1999). Moreover, the impact of agronomic practices on the concentration of T2+HT2 in cereals has not been clearly identified but surveys carried out in some European countries revealed the influence on the *F. langsethiae* spreading and T2+HT2 production of some agronomic practices, such as presence of crop debris and minimum-tillage, in a way similar to the FHB disease (Imathiu et al. 2013). On the whole, this preliminary investigation on the co-occurrence of DON and T2+HT2 mycotoxins on durum wheat samples, collected in several growing areas of Italy over a three-year period, pointed out several aspects concerning their diffusion in the assayed growing areas.

The different trend of the incidence percentage and the contamination rate of the two types of mycotoxins were highlighted in relation to the cropping year and to the examined cultivation areas. The assayed cultivars showed different rates of sensibility to DON and T2+HT2 mycotoxins accumulation in grains, and this indication could suggest the opportunity to perform further investigations about this aspect within the durum wheat breeding programs. Among the environmental factors which were assayed, both year and its interaction with the cultivation area showed the main influence on DON contamination levels, whereas T2+HT2 toxins were mostly influenced by the cultivar and its interaction with the year. DON and T2+HT2 contamination rates were not significantly correlated and each type of these mycotoxins was differently related with several agronomic/grain quality parameters. The latter aspect might perhaps be connected to the behavior of the respective producing fungi on wheat plant with particular regard to the disease effects on grains. Further information about the relationship between the co-occurrence of DON and T2+HT2 toxins on durum wheat necessarily implies an in-depth investigation on the characteristics of competitiveness and/or adaptability to environmental conditions of the fungal species involved.

Acknowledgements

The authors acknowledge M. Perenzin (CRA-SCV, S. Angelo Lodigiano – LO), P. Viola (Apsovsementi, Voghera – PV), A. De Montis (Co.Na.Se., Conselice – RA), R. Santilocchi (University of Ancona, Agugliano – AN), A. Petrini (CERMIS, Tolentino – MC), P. Bottazzi (Tuscany Region, Grosseto), P. Codianni (CRA-CER, Foggia and Calitri – AV), A. S. Frenda (University of Palermo) for carring out the field trials, and C. Cecchini and C. Ripa for technical support. This work was carried out in the frame of the project "MICOPRINCEM" supported by the Italian Ministry of Agricultural, Food and Forestry Policies and coordinated by the Agricultural Research Council (CRA).

References

Aureli, G., Belocchi, A., Pascale, M., Amoriello, T., D'Egidio, M.G., Desiderio, E. 2009. Progetto regionale MICOCER: Monitoraggio dei livelli di deossinivalenolo nella granella di frumento duro (*Triticum durum* Desf.). /MICOCER Project: Monitoring of the deoxynivalenol levels in durum wheat grain (*Triticum durum* Desf.)/. Rapporti ISTISAN **10/32**:84–92.

Barrier-Guillot, B. 2009. T-2 and HT-2 in cereals in France. 6th Fusarium Toxin Forum, 9–10 February 2009, Brussels, Belgium. www.micotossine.it/public/pag_542.pdf.

Berthiller, F., Schuhmacher, R., Adam, G., Krska, R. 2009. Formation, determination and significance of masked and other conjugated mycotoxins. Anal. Bioanal. Chem. **395**:1243–1252.

Boyacioğlu, D., Hettiarachchy, N.S. 1995. Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearum*. J. Cereal Sci. **21**:57–62.

Commission Recommendation (2013/165/EU). Official J. of the European Union L. 91/12.

Commission Regulation (EC) No. 1126/2007. Official J. of the European Union L. 255/14.

Commission Regulation (EC) No. 1881/2006. Official J. of the European Union L. 364/5.

Dexter, J.E., Marchylo, B.A., Clear, R.M., Clarke, J.M. 1997. Effects of fusarium head blight on semolina milling and pasta-making quality of durum wheat. Cereal Chem. **74**:519–525.

Divon, H.H., Razzaghian, J., Udnes-Aamont, H., Klemsdal, S.S. 2011. *Fusarium langsethiae* (Torp and Nirenberg), investigation of alternative infection routes in oats. Eur. J. Plant Pathol. **132**:147–161.

Edwards, S.G., Imathiu, S.M., Ray, R.V., Back, M., Hare, M.C. 2012. Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. Int. J. of Food Microbiology **156**:168–175.

Edwards, S.G. 2009. *Fusarium* mycotoxin content of UK organic and conventional wheat. Food Addit. Contam. **26**:496–506.

Edwards, S.G., Barrier-Guillot, B., Clasen, P-E., Hietaniemi, V., Pettersson, H. 2009. Emerging issues of HT-2 and T-2 toxins in European cereal production. World Mycotoxin J. **2**:173–179.

EFSA 2011. Scientific opinion on the risk for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J. **9**:2481.

Gauthier, T., Waché, Y., Laffitte, J., Taranu, I., Saeedikouzehkonani, N., Mori, Y., Oswald, I.P. 2013. Deoxynivalenol impairs the immune functions of neutrophils. Mol. Nutr. Food Res., Doi:10.1002/mnfr.201200755.

Häller, G.B., Munich, M., Kleijer, G., Mascher, F. 2008. Characterisation of kernel resistance against *Fusarium* infection in spring wheat by baking quality and mycotoxin assessments. Eur. J. Plant Pathol. **120**:61–68.

He, J., Zhou, T., Young, J.C., Boland, G.J., Scott, P.M. 2010. Chemical and biological transformations for detoxifications of trichothecene mycotoxins in human and animal food chains: a review. Trends in Food Sci. & Technol. **21**:67–76.

Hietaniemi, V., Kontturi, M., Rämö, S., Eurola, M., Kangas, A., Niskanen, M., Saastamoinen, M. 2004. Contents of trichothecenes in oats during official variety, organic cultivation and nitrogen fertilization trials in Finland. Agricultural and Food Sci. **13**:54–67.

Imathiu, S.M., Edwards, S.G., Ray, R.V., Back, M.A. 2013. *Fusarium langsethiae* – a HT-2 and T-2 toxins producer that needs more attention. J. Phytopathol. **161**:1–10.

Imathiu, S.M., Hare, M.C., Ray, R.V., Back, M., Edwards, S.G. 2010. Evaluation of pathogenicity and aggressiveness of *F. langsethiae* on oat and wheat seedlings relative to known seedling blight pathogens. Eur. J. of Plant Pathol. **126**:203–216.

Infantino, A., Pucci, N., Conca, G., Santori, A. (2007). First report of *Fusarium langsethiae* on durum wheat kernels in Italy. Plant Disease **91:**1362.

Lancova, K., Hajslova J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova, H., Vaniva, M. 2008. Fate of trichothecene mycotoxins during the processing: Milling and baking. Food Additives and Contaminants **25**:650–659.

Langseth, W., Rundberget, T. 1999. The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. Mycopathologia **147**:157–165.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterházy, Á., Krska, R., Ruckenbauer, P. 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium Head Blight resistance in wheat. MPMI. **18**:1318–1324.

Maresca, M. 2013. From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins **5**:784–820.

Miller, J.D. 2008. Mycotoxins in small grains and maize: Old problems, new challenges. Food Addit. Contam. **25**:219–230.

Nazari, L., Pattori, E., Terzi, V., Morcia, C., Rossi, V. 2014. Influence of temperature on infection, growth, and mycotoxin production by *Fusarium langsethiae* and *F. sporotrichioides* in durum wheat. Food Microbiology **39**:19–26.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E., Preston, K.R. 1999. Fusarium Head Blight: Effect of fungal proteases on wheat storage proteins. Cereal Chem. **76**:150–158.

Opoku, N., Back, M., Edwards, S.G. 2013. Development of *Fusarium langsethiae* in commercial cereal production. Eur. J. Plant. Pathol. **136**:159–170.

Pascale, M., Haidukowski, M., Lattanzio, V.M.T., Silvestri, M., Ranieri, R., Visconti, A. 2011. Distribution of T-2 and HT-2 toxins in milling fractions of durum wheat. J. of Food Protection **74**:1700–1707.

Pestka, J.J., Smolinski, A.T. 2005. Deoxynivalenol: toxicology and potential effects on humans. J. of Toxicology and Environmental Health Part. B. **8**:39–69.

Quaranta, F., Amoriello, T., Aureli, G., Belocchi, A., D'Egidio, M.G., Fornara, M., Melloni, S., Desiderio, E. 2010. Grain yield, quality and deoxynivalenol (DON) contamination of durum wheat (*Triticum durum* Desf.): Results of National Networks in Organic and Conventional Cropping Systems. Ital. J. of Agronomy **4**:353–366.

Quaranta, F., Belocchi, A., Fornara, M., Mazzon, V., Camerini, M., Cecchini, C., Gosparini, E., D'Egidio, M.G. 2013. Varietà di grano duro per le semine 2013 (The durum wheat cultivars suitable for sowing in the year 2013). L'Informatore Agrario, Suppl. **32**:5–11.

Rocha, O., Ansari, K, Doohan, F.M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Addit. Contam. **22**:369–378.

Russel, R., Paterson, M., Lima, N. 2010. How will climate change affect mycotoxin in food? Food Research International **43**:1902–1914.

Santori, A., De Felice, S., Aureli, G., Scala, V., Belocchi, A., Infantino, A. 2010. *Fusarium langsethiae* in Italy: geographical distribution, pathogenicity and toxin production. Petria **20**:459.

SCF 2002. Opinion on *Fusarium* toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol (http://www.europa.eu.int/comm/food/fs/sc/scf/out123_en.pdf).

Shephard, G.S. 2008. Impact of mycotoxins on human health in developing countries. Food Addit. Contam. **25**:146–151.

Schmidt, H., Adler, A., Holst-Jensen, A., Klemsdal, S.S., Logrieco, A., Mach, R.L. 2004. An integrated taxonomic study of *Fusarium langsethiae*, *Fusarium poae* and *Fusarium sporotrichioides* based on the use of composite datasets. Int. J. Food Microbiol. **95**:341–349.

Terzi, V., Morcia, C., Faccioli, P., Faccini, N., Rossi, V., Cigolini, M., Corbellini, M., Scudellari, D., Delogu, G. 2007. *Fusarium* DNA traceability along the bread production chain. Int. J. of Food Sci. and Technol. **42**:1390–1396.

Tittlemier, S.A., Gaba, D., Chan, J.M. 2013. Monitoring of *Fusarium* trichothecenes in Canadian cereal grain shipments from 2010 to 2012. J. Agr. and Food Chem. **61**:7412–7418.

Torp, M., Adler, A. 2004. The European Sporotrichiella project: a polyphasic approach to the biology of a new *Fusarium* species. Int. J. of Food Microbiol. **95**:241–245.

van der Fels-Klerx, H.J., Stratakou, I. 2010. T-2 toxin and HT-2 toxin in grain and in grain-based commodities in Europe: occurrence, factors affecting occurrence, co-occurrence and toxicological effects. World Mycotoxin Journal **3**:349–367.

Váňová, M., Klem, K., Míša, P., Matušinsky, P., Hajšlová, J., Lancova, K. 2008. The content of *Fusarium* mycotoxins, grain yield and quality of winter wheat cultivars under organic and conventional cropping systems. Plant Soil Environ. **9**:395–402.

Wu, Q., Dohnal, V., Huang, L., Kuča, K., Yuan, Z. 2010. Metabolic pathways of trichothecenes. Drug Metabolism Reviews **42**:250–267.

Yazar, S., Omurtag, G.Z. 2008. Fumonisins, trichothecenes and zearalenone in cereals. Int. J. Mol. Sci. **9**:2062–2090.

Zain, M.E. 2011. Impact of mycotoxins on human and animals. J. of Saudi Chem. Soc. **15**:129–144.

Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Figure S1*. Map of Italy showing the cultivation areas (left) and latitude and longitude of the locations (right) assayed

Electronic Supplementary *Figure S2*. The positive samples (concentration ≥ LOD) percentage referred to the total samples (incidence %) for DON and T2+HT2 toxins (three-year period, 2011–2013)

Electronic Supplementary *Figure S3*. The average and the highest concentrations (ppb) of DON and T2+HT2 toxins in positive samples (three-year period, 2011–2013)

Electronic Supplementary *Figure S4*. The box plot of DON concentration (µg/kg) and T2+HT2 concentration (µg/kg) of cultivars. Anomalous data or suspected outlie.rs are displayed as unfilled circles, outliers as filled stars