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Climatic conditions influence emerging mycotoxin presence in wheat grown in Romania – A 2-year survey

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ABSTRACT

The correlation between the occurrence of four enniatins (ENA, ENA1, ENB, and ENB1) and beauvericin (BEA) and the weather parameters during anthesis and preharvest period was studied in 97 wheat samples collected in 2014 and 2015 across three counties from central and south Romania (Braşov, Dâmboviţa, and Teleorman). The highest mean values of ENA (16.1 μg kg^{-1}) and ENB (147.1 μg kg^{-1}) were measured in the samples from Braşov county in the harvest year 2015, whereas for ENA1 and ENB1 the highest means (55.2 μg kg^{-1}, and 108.0 μg kg^{-1}, respectively) were noted in samples from Teleorman county in 2014. Statistically significant differences ($P < 0.05$) were identified between ENA1 and ENB1 and the harvest year, coupled with a strong correlation with the weather parameters (ENA1: $r_s = 0.8745$ and $r_s = 0.9326$; ENB1: $r_s = 0.7814$ and $r_s = 0.8909$, for temperature and precipitation, respectively). Principal component analysis revealed that the influence of weather parameters on emerging mycotoxin concentrations in wheat samples varied by region. This study showed that the presence and levels of emerging mycotoxins are related to weather parameters from the respective Romanian region.

1. Introduction

Mycotoxins, the secondary metabolites of various fungi, enter into the food chain through naturally contaminated food and feed, mainly cereals. These toxins can cause biochemical, physiological, and/or pathological changes in both human and animal species (Stanciu et al., 2015). *Fusarium* spp. produce mycotoxins that are legislated in the European Union (EU) as trichothecenes, zearalenone, and fumonisins (EC, 2013, 2006a) in addition to a group of "emerging" mycotoxins such as fusaproliferin, beauvericin (BEA), enniatins (ENs: ENA, ENA1, ENB, and ENB1) and moniliformin. In the past years, ENs and BEA gained a high interest and recently toxicity and risk assessment have been performed (EFSA, 2014; Escrivá et al., 2015). The toxic effects of BEA and ENs cause a negative effect on food commodities including cereals such as wheat (Covarelli et al., 2015).

There are many factors that influence the occurrence of mycotoxins in cereals, e.g., plant substrate (composition, pH, and water activity), management factors (tillage, harvesting, storage and processing conditions), topographic factors (relief position and topographic wetness index), but weather parameters (rainfall, humidity and temperature) represent the key determinants for fungal colonization and mycotoxin production (Milani, 2013; Müller et al., 2010). In this context, climate changes might influence crop yield and the degree to which the crops are contaminated with mycotoxins or could increase the development of fungi not identified previously within a given area (EC, 2007; Magan et al., 2011).

Wheat (*Triticum aestivum* L.) is the main strategic crop worldwide (USDA, 2016). The EU is the world's largest wheat producer. Romania is the fifth biggest producer of wheat in the EU, after

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France, Germany, Poland, and Spain, with an annual production of 5.2–7.8 millions of tons in the last years (RINS, 2016), more than half of it being exported to different countries around the world. In addition, for Romanian population, a high consumption of "wheat and wheat products" has been recorded (133.09 kg/capita/year), which is more than the European annual average (102.86 kg/capita/year) and the double the global average (65.26 kg/capita/year) (FAOSTAT, 2016).

Romania is the largest country in south-eastern Europe, with an agricultural land cover of 62% (Dumitrescu and Birsan, 2015). The main production area for wheat is the Danube plane in the south of the country. Other important wheat growing areas are Transylvania, the northern part of Moldova (in north-eastern Romania), and the Banat region in the south-west (Bălan, 2015).

Fusarium spp. are the most prevalent mycotoxin-producing fungi in cereals in the temperate regions of America, Asia, and Europe (Medina et al., 2015). The most frequently identified species of *Fusarium* in Romania are *F. graminearum, F. culmorum, F. oxysporum, F. verticillioides*, and *F. poae*, the last one being recognized to produce emerging mycotoxins (Stroia et al., 2010). The Romanian climate is transitional temperate-continental, with oceanic influences from the west, Mediterranean modulations from the south-west, and excessive continental effects from the north-east. Climatic variations are modulated by geographical elements, such as the Carpathian Mountains chain and the location of the Black Sea (Marin et al., 2014).

Analysis of data from the last 50 years shows that there is a general warming signal over Romania, with the air temperature and the number of sunshine hours presenting significantly increasing trends. The precipitation amount stayed rather stable. However, extreme meteorological events have been frequently registered, such as excessive dryness, tropical days, substantial rainfall and high humidity (Bojariu et al., 2015; Dumitrescu et al., 2015).

Researchers have studied wheat samples from different areas or used logistic models to predict mycotoxin contamination during wheat anthesis and the preharvest period and concluded that fungi and their specific mycotoxins are climate-dependent; thus, when changes in normal weather occur, mycotoxins are affected (Wegulo et al., 2015). These aspects were studied in particular for trichothecenes and zearalenone, whereas for emerging mycotoxins there is a lack of information about their presence in wheat kernels and their regional distribution (Bernhoft et al., 2012; Xu et al., 2013).

The aims of this work were to determine by liquid chromatography tandem mass spectrometry (LC-MS/MS) the levels of ENs and BEA in wheat cultivated in three different regions of Romania and two different harvest years and correlate the measured mycotoxin levels with weather conditions during the grain-growing season. To the best of our knowledge, this is the first survey on the presence of emerging mycotoxins in wheat produced in Romania correlated with environmental parameters.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile and methanol were supplied by PanReac AppliChem (Castellar del Vallés, Spain), and LC-MS/MS-grade methanol (≥99.9% purity) was supplied by VWR International Eurolab (Llinars del Vallés, Barcelona, Spain).

For mobile phases, ammonium formate (99%) and formic acid (98%) were obtained from Sigma Aldrich (St. Louis, USA). Deionized water (<10 MΩ cm^{-1} resistivity) was manufactured in the laboratory using a Milli-Q SP® Reagent Water System (Millipore, Bedford, MA, USA).

Whatman No. 4 filter papers (Maidstone, England) were used to filter the extract samples. Polypropylene syringes (2 mL) and nylon filters (13 mm diameter, 0.22 μm pore size) were purchased from Análisis Vínicos S.L. (Tomelloso, Spain).

The certified standards of ENs (A, A1, B, and B1) and BEA were purchased from Sigma Aldrich (Madrid, Spain). The individual stock solutions of ENs and BEA were prepared in acetonitrile at 500 μg mL^{-1}. Matrix-matched calibration curves at concentrations between 2 and 1000 μg kg^{-1} were used to quantify emerging mycotoxins in samples. The solutions were stored in glass-stoppered bottles and darkness in safety conditions at −20 °C.

2.2. Sampling

A total of 97 whole unprocessed wheat samples were obtained to investigate the presence of mycotoxins. Wheat samples were collected during the 2014 (n = 29) and 2015 (n = 68) harvesting season from three different Romanian counties: Braşov (BV, n = 8) from central Romania and Dâmboviţa (DB, n = 35) and Teleorman (TR, n = 54) from the south of the country (Fig. A.1.). The criterion used to include a sample in the study was that wheat must be grown in one of the three regions and it must be dedicated to human consumption. The number of samples per county was influenced by the frequency of wheat cultivation in that area. Information about growing area (county and city), sowing and harvest periods were considered. Normally, in Romania, wheat is sown in the first ten days of October and it is harvested in the last ten days of July. If it is necessary, wheat is aerated and dried.

Sampling was performed according to the EU guidelines (EC, 2006b) for the official control of legislated mycotoxins for lots of cereals and cereal products of less than 50 tons. Consequently, three incremental samples of 1 kg of wheat were collected in the 7 days after harvesting, obtaining an aggregate sample of 3 kg. After homogenization, samples were packed in plastic bags and stored at −20 °C in a dark and dry place until analysis. Before the analysis, for all the samples, subsamples of 300 g were milled with a blender and divided into three bulks of 100 g each.

2.3. Extraction

Extraction was performed according to the method of Juan et al. (2013). Subsamples of each sample were weighed (2.00 g) and placed into 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes, followed by the addition of 10 mL acetonitrile/water (84:16, v/v). The tubes were stirred for 1 h at 300 shakes min^{-1} using a horizontal shaking device (IKA KS260 basic Stirrer, Staufen, Germany), then centrifuged for 5 min at 5 °C and 4500 rpm using an Eppendorf Centrifuge 5810R (Eppendorf, Hamburg, Germany), and filtered with Whatman filter paper. Following this, 5 mL of supernatants were placed in 15 mL PTFE centrifuge tubes and evaporated to dryness at 35 °C under a gentle stream of nitrogen using a multisample Turbovap LV Evaporator (Zymark, Hoptkinton, USA). The residue was reconstituted to a final volume of 1 mL with methanol/water (70:30, v/v) and filtered through a nylon syringe filter. All experiments were performed in triplicate.

2.4. LC-MS/MS

ENs and BEA were analyzed using a LC-MS/MS system consisting of a LC Agilent 1200 using a binary pump and an automatic injector and coupled to a 3200 QTRAP ® AB SCIEX (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo-V™ source interface (electrospray ionization). The chromatographic separation of the compounds was performed at 24 ± 1 °C on a C_{18} reverse-phase