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Alternaria mycotoxins in wheat – A 10 years survey in the Northeast of Germany

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ABSTRACT

Although, the genus *Alternaria* is considered to be an important plant pathogen and capable to produce mycotoxins, the presence of *Alternaria* mycotoxins in cereals has been largely ignored both in Europe and overseas. This is very likely due to a lack of survey data. This study aimed at filling this gap by investigating a total of 1064 freshly harvested winter wheat samples from commercial farms in different regions of the State of Brandenburg (Germany) in the years from 2001 to 2010. We analysed alternariol (AOH), its monomethyl ether (AME), altenuene (ALT, since 2006) and tenuazonic acid (TeA) by a HPLC method with diode array and fluorescence detection. The most frequently found *A.* mycotoxin was TeA. An amount of 322 out of 1064 samples (30.3%) were naturally contaminated by TeA, 86 out of 1064 by AOH (8.1%), 33 out of 1064 by AME (3.1%) and 7 out of 267 samples (2.6%) were contaminated by ALT. The maximum toxin contents in all years were 4224 μg TeA kg^{-1}, 832 μg AOH kg^{-1}, 905 μg AME kg^{-1} and 197 μg ALT kg^{-1}. A co-occurrence of several *A.* mycotoxins in wheat samples was infrequent: only three samples were contaminated by all the four toxins, 14 by three toxins, 61 by two toxins and 273 samples by only one toxin. The contamination of wheat ears in the State of Brandenburg in the wet years 2010, 2009 and 2002 were most pronounced, whereas 2001 and 2008 were *A.* "toxin free" years. The accumulation of TeA in freshly harvested wheat kernels seems to depend on preceding crop and tillage. Minimum tillage practices and maize as well as winter wheat as preceding crops led to increased TeA concentrations in wheat. A co-occurrence of mycotoxins produced by several mycotoxigenic fungi, e.g. *Fusarium* and *Alternaria* spp., is discussed.

1. Introduction

The black mould genus *Alternaria* includes various saprophytic, endophytic and pathogenic species. They occur worldwide in different habitats such as surface of buildings, monuments, cellulose, paper, textiles, household dusts and in soil as well as on dead or dying plant tissues (Rotem, 1994). Moreover, the genus *Alternaria* is considered to be an important plant pathogen and can cause crucial economic losses by infection of agronomic and vegetable plants (potato, carrot, onion), fruits (tomato, apple, citrus), oilcrops, cereals and ornamentals. Stem cancer, leaf blight or leaf spot and black point are well known symptoms as a result of an infection of different host plants by *Alternaria* species (Bottalico & Logrieco, 1998; Logrieco, Moretti, & Solfrizzo, 2009). In addition to yield losses and changes in qualitative traits of the infected crop products, some *Alternaria* species are capable of producing a number of mycotoxins and host-specific phytotoxins. Pathotypes of *Alternaria* produce compounds that are selectively toxic for their host plants. Such host-specific toxins of *Alternaria alternata* are known for apple, pear, citrus, strawberry, tobacco and tomato (Otani & Kohmoto, 1992; Strange, 2007). In contrast, mycotoxins are non-host specific toxins and an infection by and the development of *Alternaria* fungi in several crop plants could lead to an accumulation of mycotoxins in raw or manufactured plant products. More than 30 mycotoxins have been isolated from *Alternaria* fungi. The most important *A.* mycotoxins can be grouped into three different structural classes: alternariol (AOH) and its monomethyl ether (AME) as well as altenuene (ALT), which are dibenzopyrone derivatives; tenuazonic acid (TeA), a tetramic acid derivative and altertoxins I, II and III (ATX I, II or III), which are perylene derivatives. Chemical characteristics and potential producers were reviewed by Montemurro and Visconti (1992), Ostry (2008), Scott (2001) and Visconti and Sibilia (1994). These toxins show cytotoxic, fetotoxic and/or teratogenic activity, they are mutagenic, clastogenic and oestrogenic in

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microbial and mammalian cell systems and tumorigenic in rats as well as they inhibit the cell proliferation (Brugger et al., 2006; Lehmann, Wagner, & Metzler, 2006; Logrieco et al., 2009; Tiemann et al., 2009). The consumption of contaminated cereals was related to risk of human esophageal cancer in China (Liu et al., 1992). Although a potential risk to consumer health as well as its natural occurrence in food was demonstrated, so far there are no specific international regulations for any of the *Alternaria* mycotoxins in food and feed.

The *A.* mycotoxins were frequently detected in tomatoes and tomato products, in apples and apple products, mandarins, citrus fruits, melons, grapes, blackberries, gooseberries and strawberries (and in its nectars, juices and wines), in carrots, lentils, peas and olives (reviewed by Ostry, 2008; Scott, 2001). In contrast, there is a remarkable lack of information about the natural contamination of cereals by *A.* mycotoxins although small-spored *A.* species *A. alternata* (Fr.) Keissler, *Alternaria tenuissima* (Kunze) Wiltshire, *Alternaria triticina* (Prasada & Prabhu, 1962) and *Alternaria infectoria* E.G. Simmons are belonging to the most predominant species found on wheat, oats and barley (Andersen, Thrane, Svendsen, & Rasmussen, 1996; Joshi & Miedaner, 2003; Kosiak, Torp, Skjerve, & Andersen, 2004; Mercado Vergnes, Renard, Duveiller, & Maraite, 2006). The biology, epidemiology and pathogenicity of the genus *Alternaria* were already described by Magan, Cayley, and Lacey (1984) and Rotem (1994). The authors point out *A. triticina* and *A. alternata* as pathogens of wheat. They are seedborne and airborne pathogens, appear on leaves, leaf sheaths, glumes and seeds of wheat. Its germ tubes form appressoria-like structures and penetrate the epidermis directly or through stomata. Germination of spores, distribution and infection already occurs during several short wetting periods even though interrupted by dry days. *A. alternata* is frequently abundant in the ripening ears from the anthesis in all grain filling stages up to the harvest.

These *A.* species, particularly *A. alternata*, have been shown to produce frequently AOH, AME, ALT and TeA in laboratory cultures (Grabarkiewicz-Szczęsna & Chełkowski, 1992; Li, Toyazaki, & Yoshizawa, 2001; Logrieco, Bottalico, Solfrizzo, & Mulè, 1990; Patriarca, Azcarate, Terminiello, & Fernández Pinto, 2007). So far, surveys on naturally contaminated grains have only rarely been reported. In Argentina (Azcarate, Patriarca, Terminiello, & Fernández Pinto, 2008), in Australia (Webley, Jackson, Mullins, Hocking, & Pitt, 1997) and in China (Li & Yoshizawa, 2000), AOH, AME and TeA were detected in weather-damaged wheat. Quantitative data on the AOH, AME and TeA content in unprocessed cereals in Europe are still limited: In Poland (Grabarkiewicz-Szczęsna & Chełkowski, 1992), Germany (Müller, Waydbrink, Peters, Umann, & Seyfarth, 2002), in the Czech Republic (Ostry, Skarkova, Nedelnik, Ruprich, & Moravcova, 2005) and in Sweden (Häggblom, Stepinska, & Solyakov, 2007), some small grain cereal samples only in few years were examined. Low levels of AOH, AME and ALT and slightly higher concentration of TeA were detected. An EFSA report (EFSA, 2011) summarised data on *Alternaria* toxins analysed in feed and agricultural commodities and based on the few available data from the literature only. The results were characterised by a high proportion of toxin concentrations below the limit of quantification (LOQ) ranging from 9% up to 66% for different *Alternaria* toxins.

Alternaria mycotoxin contents in consumable processed cereal products in Europe were also reported in the EFSA scientific opinion (2011): AOH, AME and TeA were found only in few samples of grains and grain-based products. For example, in Switzerland, Asam, Konitzer, and Rychlik (2011) and Asam, Lichtenegger, Liu, and Rychlik (2012) using a stable isotope dilution assay detected very low concentration of AOH only in one spelt flour and AME in two oat flakes samples out of 13 cereal products for human consumption as well as TeA in 11 out of 12 cereal samples. In Germany, Siegel, Rasenko, Koch, and Nehls (2009) examined 27 cereal samples obtained from a local supermarket and quantified TeA concentrations only in three samples.

Overall, so far there have been very limited reports in the literature of the natural contents of these mycotoxins, of the frequency of contamination and of the co-occurrence of some different *Alternaria* mycotoxins in cereals and cereal products. Our study aimed at filling this gap. We examined the natural occurrence of AOH, AME and TeA (since 2001) and ALT (since 2006) in freshly harvested wheat samples from fields in the State of Brandenburg in the Northeast of Germany between 2001 and 2010. Agricultural management data like preceding crop, tillage and wheat cultivars were collected according to the information of the farmers and related to the concentrations of the *A.* mycotoxins with a view to assessing important driving factors of the accumulation of these toxins.

2. Materials and methods

2.1. Sampling

Samples were taken at winter wheat fields (*Triticum aestivum L. emend. Fiori et Paol.*) of commercial farms in different regions of the State of Brandenburg (Germany) each year from 2001 to 2010. Freshly harvested and threshed wheat kernels at growth stage Z92 (Zadoks, Chang, & Konzak, 1974) were sampled as mixed bulk samples from at least three sites within the field. Samples were randomly selected without any symptoms of phytopathogenic infections. The amount of harvested kernels averaged from 1.5 to 3.0 kg at each field. Information regarding the crop management practices (crop rotation, tillage, wheat cultivar) was requested from the farmers. The tillage treatments are classified into (a) mouldboard ploughing 30 cm deep or (b) non-inverting soil techniques including shallow mixing 15 cm deep up to no-tillage or direct drilling (summarised as minimum tillage). The crops were managed according to standard agricultural procedures and good professional practice. The number of wheat samples per year and their corresponding crop management information is displayed in Table 1.

Brandenburg is one of the driest regions in North-Central Europe (Wessolek & Asseng, 2006) with long-term annual precipitation between 450 and 600 mm. We used data of actual annual precipitation and temperature of each year in comparison to the long-term annual data to characterize the differences between the years of investigation (Fig. 1). Data were interpolated from data measured at two weather stations in the two most important wheat growing areas in the State of Brandenburg (Uckermark, Märkisch-Oderland) operated by the Research Station of the Leibniz-Centre for Agricultural Landscape Research at Dedelow and Müncheberg.

2.2. Mycotoxin analysis

The harvested and threshed samples were dried at 60 °C for 48 h. The grain was ground to a fine powder in an ultra centrifugal mill with vibratory feeder (ZM 200, Retsch Haan Germany) to pass a 0.75-mm sieve for the following toxin extractions. The samples were extracted and purified according to Li and Yoshizawa (2000) and Visconti, Logrieco, and Bottalico (1986) with modifications (Tiemann et al., 2009). Briefly, the samples were extracted with acetonitrile/KCl solution for 30 min. After a protein precipitation, the filtrate was divided into two parts. One part was extracted three times with dichloromethane. The organic phases were combined, evaporated and redissolved in 2 ml of methanol for the HPLC analyses of AOH, AME and ALT. The second part of the crude extract