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Review paper

ALTERNARIA spp. ON SMALL GRAINS

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ABSTRACT: As a consequence of climate changes and global warming, high emergence of mycobiota on small grains may have negative impact on quality and safety of food and feed. Genus *Alternaria* is ubiquitous and includes large number of saproprobes and pathogens. *Alternaria* spp. are one of the major contaminants of small grains causing "black point" disease. Beside yield losses, *Alternaria* spp. are responsible for spoilage of commodities during transport, storage and in processing, which may lead to the reduction of technological quality and serious economic losses. There is a growing concern of *Alternaria* spp. due to their ability to produce secondary metabolites with different toxicological properties, which are harmful for human and animal health. Accurate identification of *Alternaria* spp. and their metabolites is a crucial phase in the implementation of preventive measures and controls in the system from farm to fork. Considering the importance of *Alternaria* spp. occurrence on small grains and *Alternaria* toxins risk assessment, additional studies in this area are indispensable.

Key words: *Alternaria* spp*, small grains, identification of species, toxins*

INTRODUCTION

Alternaria spp. are cosmopolitan mould fungi and can be found in soils, plants, food, feed and indoor air. The genus *Alternaria* includes both saprobes and plant pathogens which have been reported worldwide infecting crops in the field and causing post harvest decay of many plant products (Thomma, 2003). *Alternaria* species are frequently found on small grains, causing yield losses in production and processing. Due to their growth even at low temperature, *Alternaria* spp. are well known post harvest pathogens, responsible for spoilage of food during refrigerated transport and storage (Ostry, 2008). Economical losses are mainly related to quality reduction due to decreased nutritive value, discoloration and insipidness (Kosiak et al., 2004). In addition to losses in food and feed production, many *Alternaria* species are mycotoxin producers with different toxicological properties. The most important *Alternaria* toxins are alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), tenuazoic acid (TEA) and altertoxins (ATX-I, II, III) (Logrieco et al., 2009). The occurrence of *Alternaria* toxins in small grains and cereal-based products is a global issue of high concern, due to their potential health risks for humans and/or livestock. It has been reported that some *Alternaria* toxins might have even the carcinogenic effect (Liu et al., 1992). Based on the requests from the European Commission (EC) in order to highlight the need for possible follow up actions, the European Food Safety Authority (EFSA) provided a scientific opinion on the risks for animal and human health related to the presence of *Alternaria* toxins in food and feed (EFSA, 2011). Moreover, *Alternaria* spores are considered to be one of the most

prolific fungal allergens, which has been associated with respiratory allergies and skin infections (Corden et al., 2003; Kilic et al., 2010; Pavon et al., 2010). Since the great importance of the genus *Alternari*a related to food safety and quality of small grains and cereal-based products, this paper presents the extensive overview of *Alternaria* species on small grains with focus on mycotoxin risks in food chain.

OCCURANCE OF *ALTERNARIA* SPP. ON SMALL GRAINS

Since it has been originally described in 1816 by Nees (Nees von Esenbeck 1816-1817) with *A.tenuis* as the only type member of the genus, *Alternaria* species are widely distributed infecting a broad range of economically important crops. Cereal grains are frequently infected by species of the genus *Alternaria*, in particular *A.alternata*, which can cause disease called "black point". Discoloration of ears and grains (particularly embryo end of the grain) is due to the presence of mycelia and conidial masses with dark pigment, melanin, which is characteristic for *Alternaria* genus (Thomma, 2003). High humidity or frequent rainfall from the milky ripe to soft dough stage can often favor infection by these fungi and may cause serious losses (Logrieco et al., 2003). The importance of "black point" disease does not reflect to yield reduction as much as on the quality of milling wheat, barley and oats for processing. The majority of mycobiota in wheat flour are derived from initial infected kernels in the field (Plavšić et al., 2007). Poor flour and bran color due to discoloration of the grain can have a significant economic impact. Decreased nutritive value, discoloration and insidipidness reduce technological quality of cereal products (Kosiak et al., 2004). Besides damages on ears and grains, disease may occur on leaves in the form of leaf blight lesions, usually caused by species *A.triticina*. *Alternaria* spp. may occur in storage causing spoilage of small grains and small-grains based products. After harvest, mainly physical factors dictate whether or not fungi will grow and/or produce mycotoxins. The primary factors influencing fungal growth in stored food products are the moisture content (more precisely, the water activity) and the temperature of the commodity. However, *Alternaria* growth may be favored in grains stored in moisture storage, but infection may also spread from affected plant products to adjacent healthy ones by secondary infections (Barkai-Golan&Nachman, 2008).

There is a wide range of *Alternaria* spp. distributed worldwide in both humid and semi-arid regions. Besides wheat, the prevalence of *Alternaria* spp. was recorded on barley (Medina et al., 2006; Hudec, 2007), oat (Kwasna & Kosiak, 2003) and rye (Grabarkiewicz Szczesna et al.,1989). *A.alternata* and *A.tenuissima* are the most frequently reported on wheat in the Mediterranean countries (Logrieco et al., 1990, Bensassi et al., 2009), followed by Estonia (Kütt et al., 2010), Slowakia (Maškova et al., 2012), and Argentina (Patriarca et al, 2007), while *A.infectoria* was predominate in Norway (Kosiak et al., 2004) *Alternaria triticina* causes significant yield losses in wheat on the Indian subcontinent, whence it originates (Prasada & Prabhu, 1962) and has been reported in Argentina as well (Perelló and Sisterna, 2006). Since this species has been considered as quarantine pathogen in many countries, it will be necessary to investigate the incidence and importance of this disease in wheat areas worldwide. Recently, Toth et al. (2011) reported a new species on the Hungarian wheat, *A.hungarica,* considering it a minor foliar pathogen with small economical importance. In Serbia the detected species on wheat are *A. alternata* and *A. logipes* (Ivanovic et al.), while in researches of mycobiota on spelt wheat predominant species were *A. alternata* and *A. tenuissima* (Vučković et al., 2012). The main *Alternaria* species of phytopathological interest of small grains are listed in Table 1.

Table 1.
Distribution of *Alternaria* species on small grains across the world

Country	Alternaria spp.	References
Mediterranean countries	*A.triticina* *A.alternata*	Logriecco et al., 1990
Norway	*A.infectoria*	Kosiak et al., 2004

Estonia	*A.tenuissima* *A.alternata* *A.alternata*	Kütt et al., 2010
Tunisia	*A.tenuissima* *A.alternata* *A.tenuissima* *A.japonica*	Bensassi et al., 2009
Argentina	*A.tenuissima* *A.alternata* *A.longipes* *A.arborescens* *A.gaisen* *A.mali* *A.triticimaculans*	Patriarca et al., 2007; Perelló & Sisterna., 2007
Serbia	*A.alternata* *A.tenuissima* *A.longipes*	Ivanović et al., 2011; Vučković et al., 2012

DETECTION METHODS OF *ALTERNARIA* SPECIES

The anamorphic genus *Alternaria* comprises nearly 300 described taxa (Simmons, 2007). Considering a specific mycotoxin profile of different *Alternaria* spp., an accurate identification is highly important for proper risk assessment. It is, however, problematic to isolate the fungi of interest due to the presence of competing fungi that may grow faster or produce antagonistic compounds. To overcome this problem fully selective media are desirable in detection procedures. According to literature data, most of the authors recommend Potato Carrot Agar (PCA) and V-8 juice agar which are selective for *Alternaria* species and encourage abundant sporulation (Hoog and Horre, 2002, Chou & Wu, 2002; Kosiak et al., 2004, Vergnes et al., 2006, Hudec, 2007, Pavón et al., 2010, Maškova et al., 2012). Dichloram Chloramphenicol Malt Extract Agar (DCMA) showed to be very effective for detection of *Alternaris* strains according to Patriarca et al. (2007). Sørensen et al. (2009) proved that modified PCA (PCA-Mn) based on easy available manganese has several advantages compared to previously-developed semi-selective media for isolation of small-spored *Alternaria.* This medium makes it easier to detect, otherwise overlooked or suppressed, *Alternaria* isolates in environmental samples. For further detection the majority of authors are following instructions by Simmons (2007).

Molecular techniques, such as polymerase chain reaction (PCR) based system, have been applied as alternative assays replacing troublesome and time spending microbiological and chemical methods for detection and identification the most serious fungal toxin producers (Niessen, 2007). In PCR method 'universal' primers are used to isolate specific DNA regions followed by comparison of the sequence of the target species with that of other species within databases. The coding portions of many fungal 18S, 5.8S and 28S rDNA genes are highly conserved and primers to these regions have been generated (White *et al.*, 1990). These allow the isolation of the internal transcribed spacer sequences (ITS-1 and ITS-2), which lie between the coding regions and are generally responsible for polymorphism at the level of species. The ITS region is amplified from the target fungus and sequenced to identify regions of DNA unique to the fungus of interest (Magan & Olsen, 2004). The ITS region was

also used to design primers to differentiate large spectrum of *Alternaria* species (Pryor & Gilbertson, 2000; Pryor & Michailides, 2002; Hoog & Horre, 2002; Choui & Wu, 2002; Zur et al, 2002, Vergnes et al.,2006.; Bensassi et al., 2009; Pavón et al., 2011, Toth et al., 2010)

ALTERNARIA TOXINS

The problem of mould damage and the hazard of consuming damaged grains have been recognized since historical times, but mycotoxins have attracted considerable attention especially over the last three decades (Bhaat and Miller, 1991). Mycotoxins are secondary metabolites, produced by a range of fungal species. Generally, mycotoxins are chemically and thermally stable compounds, surviving storage and the most food processng conditions and therefore, persist to the final products (Matić et al., 2008). Mycotoxins in cereal-based foods and feeds are a global issue of high concern, due to their potential health risks for humans and/or livestock (Köppen et al., 2010). The *Alternaria* genus produces more than 70 mycotoxins and phytotoxins but only few occur naturally in foodstuffs or are of major toxicological significance. *A. alternata* is considered as the most important toxin producing species (Battilani et al., 2009).

The most important *Alternaria* toxins are divided into three mains structural classes according to Ostry (2008), Logriecco et al. (2009) and Battilani et al. (2009):

- dibenzo-α-pyrone derivatives: alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), altenuisol (AS);
- tetramic acid derivates: tenuazoic acid (TEA)
- perylene derivatives: altertoxins I, II, III (ATX-I,-II,-III)

Alternaria toxins have been detected in wide range of cereal grains and small-grains based products such as bread and rolls, musli, fine bakery wares, pasta etc. (EFSA, 2011).
There are reports of AOH, AME and TEA in “black point wheat” on German market (Siegel et al., 2009, Asam, 2011), AOH, AME and ALT in Slovakian (Maškova et al., 2012) and Czech grains (Malachova et al., 2011), AOH and AME in small cereal grains in Poland Grabarkiewicz Szczesna et al.,1989) and AOH was detected in Estonian grains (Kütt et al., 2010). Li & Yoshizawa (2000) analyzed wheat kernels in China which were significantly invaded by *Alternaria* spp., mostly *A. alternata*, with an average infection frequency of 87.3%. AOH was detected in 20 of 22 tested samples between 116-731 μg/kg and AME at a mean level of 443 μg/kg (range= 51-1426 μg/kg) in 21 samples. The presence of TEA, as major *Alternaria* toxin in terms of quantity, was detected with an average level of 2419 μg/kg and with a maximum quantity of 6432 μg/kg. The toxigenic potential of *Alternaria* strains isolated from Argentinean wheat, showed that TEA was the toxin produced at the highest concentration, but in lower frequency (72%), compared to AOH (87%) and AME (91%) (Patriarca et al., 2007). A comprehensive overview of the most common *Alternaria* toxins in small grains is presented in Table 2.

Table 2.
Toxigenic profile of *Alternaria* spp. in small grains reported across the world[a]

Country	Mycotoxin	N	n>LOQ	LOD/LOQ (μg/kg)	Mean (μg/kg)	Maximum	Method	Reference
Germany	AOH	13	1	1.05	-	4.01	HPLC-MS/MS	Asam et al., 2011
	AME	13	1	0.03	-	0.06		
	TEA	27	2	50	49	851		Siegel et al., 2009
Belgium	AOH	1	0	12/23	n.d.	n.d.	LC-MS/MS	Monbaliu et al., 2010

	AME	1	0	18/39	n.d.	n.d.		
Czech Republic	AOH	8	0	12/23	n.d.	n.d.	LC-MS/MS	Monbaliu et al., 2010
	AME	8	0	18/39	n.d.	n.d.		
Denmark	AOH	14	0	12/23	n.d.	n.d.	LC-MS/MS	Monbaliu et al., 2010
	AME	14	0	18/39	n.d.	n.d.		
Estonia	AOH	4	3	100	-	340	HPLC-DAD	Kütt et al., 2010
Hungary	AOH	7	0	12/23	n.d.	n.d.	LC-MS/MS	Monbaliu et al., 2010
	AME	7	0	18/39	n.d.	n.d.		
Sweden	AOH	18	16	35/45	-	335	HPLC-UV	Häggblom et al., 2007
	AME	18	7	35/45	-	184		
	TEA	18	18	100/135	-	4310		
Argentina	AOH	64	4	50	1054	1388	HPLC-UV	Azcarate et al., 2008
	AME	64	15	50	2118	7451		
	TEA	64	12	80	2313	8814		
Egypt	AOH	15	4	50	-	2320	HPLC-UV	Abd El-Aal et al., 1997
	AME	15	2	300	-	1890		
	ALT	15	2	100	-	1480		
	ATX-I	15	2	200	-	1678		
	TEA	15	5	100	-	658		
Russia	AOH	28	4	20	98	192	ELISA	Burkin & Kononenko, 2011
China	AOH	22	20	50	335	731		
	AME	22	21	50	443	1426	HPLC-FLD	Li & Yoshizawa, 2000
	ALT	22	0	100	n.d.	n.d.		
	ATX-I	22	0	200	n.d.	n.d.	HPLC-UV	
	TEA	22	22	100	2419	6432		
Australia	AOH	5	0	10	n.d.	n.d.		
	AME	5	0	10	n.d.	n.d.	HPLC-UV	Webley at al., 1997
	TeA	5	1	10	-	15		

[a] *Source: European Food Safety Authority (2011); Abbreviations: AOH:alternariol; AME: alternariol monomethyl ether; ALT:altenuen; TEA:tenuazoic acid; ATX:altertoxin I; N: number of tested samples; n: number of samples>LOQ; LOQ: limit of quantification; LOD: limit of detection, n.d: not detected*

The database concerning toxicological effects of *Alternaria* toxins in experimental animals and/or in humans is currently too limited to be used as a basis for detection of reference

points for different toxicological effects. Experiments performed in rodents with purified *Alternaria* toxins indicate that the acute toxicity is in the following order: ALT > TeA > AME and AOH. However, these data are not suitable for the risk assessment of *Alternaria* toxins since the risk for public health related to these toxins is not expected to result from acute exposures (EFSA, 2011). Mycotoxins such as AOH and AME are found to be mutagenic and genotoxic and in certain areas in China might be responsible for oesophageal cancer (Liu et al., 1992). The mycotoxingenic potential depends on species and strains of the fungus, composition of matrix and environmental factors, such as temperature and moisture, and particularly water activity (*a*w) (Fernandez-Cruz et al., 2010). According to Magan et al. (1984) *A. alternata* needs rather high water activity (a_w=0.98) to produce mycotoxins on wheat grain. Knowledge of mycotoxin production under marginal or sub-optimal temperature and a_w conditions for growth can be important since improper storage conditions accompanied by elevating temperature and moisture content in the grain can favor further mycotoxin production and lead to reduction in grain quality (Oviedo et al., 2011)
Several methods have been reported in the literature for the determination of *Alternaria* toxins from food commodities. In particular, analytical methods are largely based on procedures, involving clean up by solvent partitioning or solid phase extraction, followed by chromatographic separation techniques, in combination with ultraviolet, fluorescence electrochemical and mass spectroscopic detection (Ostry, 2008). Since none of the mentioned techniques have been validated by interlaboratory studies and because of the lack of certified reference materials or proficiency studies available for the determination of *Alternaria* toxins, validated analytical methods for the quantification of *Alternaria* toxins are needed as a prerequisite for a survey on their occurrence in feed and food (Battilani et al., 2009).

PREVENTION AND FUTURE CONCERNS

The occurrence of mycotoxins in the food chain is an unavoidable and serious problem the world is facing with. Once the foodstuffs are contaminated with toxins it is impossible to eliminate them. Certainly, the best protection against mycotoxins is monitoring their presence in feed and food (Matić et al., 2008). Prevention of fungal contamination and thereby toxin production can be achieved either during preharvest stages by good agricultural practice and the use of a HACCP plan, as well as during postharvest stages by the application of proper drying, storage, and transport procedures (FAO, 2001). Application of fungicides at field might reduce fungal infection resulting in the decrease of mycotoxins production. However the modern trends are toward environmentally friendly alternatives at the field level rather than relying on chemicals (Bhaat et al., 2010). Development of resistant cultivars with the application of modern biotechnological methods would prove to be effective way for prevention and control hazardous fungi and their mycotoxins. A proven system of storage management which includes drying, avoiding grain damages and ensuring proper storage conditions is needed. To reduce or prevent production of most mycotoxins, drying should take place as soon as possible after harvest and as rapidly as feasible. Fungi cannot grow (or mycotoxins cannot be produced) in properly dried foods, so efficient drying of commodities and maintenance of the dry state is an effective control measure against fungal growth and mycotoxin production (FAO, 1989). Damaged grain is more prone to fungal invasion and mycotoxin contamination, thus it is important to avoid damage before and during drying, as well as in storage. Insect pests and storage pests may attack grain and due to their activities accumulated moisture provides ideal condition for fungal growth.
However, besides toxicity and occurrence, the chemical behavior of mycotoxins during food processing needs to be understood when assessing risks associated with the consumption of food made from contaminated raw materials. Siegel et al. (2010) investigated the stability of AOH, AME and ALT upon bread baking using a spiked wholemeal wheat flour. The obtained results indicated that the *Alternari*a mycotoxins are barely degraded during wet baking, while significant degradation occurs upon dry baking, with the stability decreasing in the following order AME >AOH > ALT.

Further studies are needed to clarify the possible tranfer of the toxins into wheat flour after milling and their fates during food processing and coocking (Li and Yoshizawa, 2000) Additionally, more work is needed towards impact of *Alternaria* spp. on technological quality of small grains and small-grains based products. There is relatively large number of studies on the impact of fungal infection on food safety and yield parameters (Plavšić et al., 2010), but information on the effect of mycobiota infection on technological parameters is rather scant. A significant decrease in technological wheat quality in kernels attacked by *Fusarium* spp. and *Alternaria* spp. was proved in the researches of Šarić et al. (1997). According to these authors, increased enzyme activity of field moulds in samples with severe infection has negative impact on physical dough properties which leads to a complete wheat uselessness for further processing. Bodroža et al. (2012) found out that flour obtained from wheat affected with *Fusarium* spp. and *Alternaria* spp. showed less water and less stability during mixing and higher protein weakening during heating in comparison to the flour from wheat treated with fungicide.
Research gaps include also official validated methods for *Alternaria* metabolites analysis in order to carry out surveys of food and obtain an estimate of human exposure to *Alternaria* toxins. Additional toxicological work with purified *Alternaria* toxins, such as subacute toxicity and cancer studies, are also required (Magan and Olsten, 2004). Since there are no specific regulations for any of the *Alternaria* toxins in food, surveys to check the occurrence of these metabolities in order to ensure that contamination level do not pose a significant hazard to human health are strongly suggested.

CONCLUSION

Contamination of food and agricultural commodities by various types of toxigenic molds is a serious and a widely neglected problem. *Alternaria* species are ubiquitous plant pathogens and saprobes and are often found on small grains and small-grains based products. *Alternaria* spp. are also well known as post-harvest pathogens causing considerable economic losses to growers and the food-processing industry. They are of particular interest because suitable conditions may lead to production of a number of mycotoxins, such as AOH, AME, TeA, which may be implicated with human and animal health disorders. Most *Alternaria* mycotoxins exhibit considerable cytotoxic, carcinogenic, foetotoxic, teratogenic, antitumoral, antiviral and antibacterial activity. Prevention of fungal invasion ongrains is by far the most effective method of avoiding mycotoxin problems. It should consider an integrated management program, focusing on the good agricultural practice and food quality from the field to the consumer. Since there is currently no regulations set on *Alternaria* toxins in food and feed in the Europe nor worldwide, more attention is needed in monitoring of production of food safety products.

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ALTERNARIA spp. **НА СТРНИМ ЖИТИМА**

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Сажетак: Као последица климатских промена и глобалног загревања, интензивна појава микобиота на стрним житима може имати негативне последице на квалитет и безбедност прехрамбених производа и храну за животиње. Врсте рода *Alternaria* су широко рас-

простањене и обухватају велики број сапробних и фитопатогених врста. *Alternaria* spp. су главни контаминанти стрних жита и произроковачи карактеристичног обољења „црне пегавости“. Поред штета на пољу, *Alternaria* spp. могу узроковати и кварљивост производа у процесу прераде, током транспорта и складиштења, што може довести до великих економских губитака. Осим фитопатогеног аспекта, *Alternaria* врсте имају све већи значај због способности да продукују секундарне метаболите различите токсичности који могу имати штетан утицај на здравље људи и животиња. Правилна идентификација *Alternaria* врста и њихових токсина, кључна је фаза при примени превентивних и контолних мера у систему од њиве до трпезе. С обзиром на значај појаве *Alternaria* spp. на стрним житима и утицаја *Alternaria* токсина на квалитет и безбедност хране, неопходна су додатна истраживања у овој области

Кључне речи: *Alternaria* spp.*, стрна жита, идентификација врста,токсини*

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