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Evaluation of Agronomic Practices for Mitigation of Natural Toxins

Report

Commissioned by the ILSI Europe Process-related Compounds and Natural Toxins Task Force

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EVALUATION OF AGRONOMIC PRACTICES FOR MITIGATION OF NATURAL TOXINS

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REPORT

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INTRODUCTION

1.1 Background

The problem of contamination by natural toxins and the hazards to health of consuming spoilt foodstuffs have been recognised since historical times. Natural toxins have been studied widely but have received particular attention in the last three decades. Levels of natural toxins (inherent plant toxins[1], mycotoxins and some phycotoxins in freshwater) in agricultural commodities are likely to be affected by the agronomic practices that the crops or their environments are subject to. It is important to recognise that Good Agricultural Practice (GAP) represents the primary line of defence against the contamination of food products by inherent plant toxins and mycotoxins. GAP must be accompanied by the implementation of good manufacturing practice (GMP) during the handling, storage, processing and distribution of cereals for human food and animal feed. In order to explore this important field, this report considers examples of the influence of different factors on two major food crops and of some specific mycotoxins, as well as available data on inherent plant toxins. Attention is drawn to those areas where data are scarce. The term "inherent plant toxins" is used in this paper because most of the examples presented focus on plant compounds that cause a toxic effect at the levels found in the plant.

1.2 Objectives

Several preventive and corrective measures to minimize toxin contamination in agricultural commodities, particularly by mycotoxins, have been attempted. These can be divided into three broad categories: plant breeding, good agronomic practice and detoxification. The focus of the current review is the evaluation of agronomic practices aimed to modulate "natural toxins". In order to identify what is known about the agronomic effects and how they might potentially be manipulated to reduce toxin levels, it is necessary to analyse the relationship between toxins, crops and agricultural practices. In this discussion paper we will deal firstly with two major food crops (cereals and potatoes) and their food safety. We will then deal with agronomic factors and their effects on different crops, and also on fungal strains, and the occurrence of mycotoxins or the occurrence of inherent toxins on different food crops. Detoxification is considered to be outside the scope of this review.

As the starting point the two key food crops in Europe, cereals and potatoes, were selected. The intention was to develop an inventory of the current agronomic practices that are applied in order to reduce the concentration of inherent plant toxins and mycotoxins. With respect to food processing, only those closely related to harvesting, storage, or domestic food preparation will be considered. Industrial food processing is outside the remit of this paper. Other topics considered include masked mycotoxins, mycotoxin boundaries and the environmental conditions that influence fungal growth.

This review considers different fungal strains, the influence of climate (temperature), growth conditions, toxicity, data gaps and research needs, toxin interaction with other compounds, kinetics, sensitivity of the fungal population and possible polymorphism. Additional topics include the definition and comparison of agronomic factors, the application of plant protection products, hereafter referred to as pesticides, and the occurrence of mycotoxins.

1. Inherent plant toxins are considered to be compounds that occur naturally in the plant and under certain conditions (e.g., increased concentration) exert toxic effects. The definition is used with some flexibility in this paper to include for example nitrate, which as a source of nitrogen can cause toxic effects or a chemical that may provide the precursor of a contaminant of processed food.

Other food crops will be also considered in relation to mycotoxins and inherent toxins, and to the influence of agronomic factors on the formation of such toxins. These include the mycotoxins, *Fusarium* toxins (particularly trichothecenes such as T-2, HT-2 and deoxynivalenol (DON), ochratoxins and phenyl hydrazine derivatives (cultivated mushroom) and cyanogenic glycosides (cassava).

With respect to the occurrence of mycotoxins and inherent plant toxins in food crops, this review considers only a selection of several examples and cannot therefore be regarded as a comprehensive review.

2. MYCOTOXINS

Mycotoxins are natural secondary metabolites produced by fungi belonging mainly to *Aspergillus*, *Penicillium* and *Fusarium* genera (Anonymous, 2003). Their role in pathogenesis is generally unknown, but they can cause acute and chronic effects in humans and animals; carcinogenic compounds are included, with aflatoxin B_1 confirmed as carcinogenic for humans (Kelly *et al.*, 1997; Williams *et al.*, 2004). Mycotoxins were recently defined as the main chronic health risk related to food, but they can also cause acute effects (Kuiper-Goodman, 2004).

Cereals, including maize, are the main host crop for mycotoxin-producing fungi, but fruits, grapes, nuts, coffee and spices can contain one or more such toxins (Barug *et al.*, 2004) and their permitted levels are regulated in the European Union by EC Regulation 1881/2006, updated for *Fusarium* toxins in EC Regulation 1126/2007 (European Commission, 2006a,b; 2007). Other regulations are in force in many other countries all over the world (van Egmond *et al.*, 2007). The only regulated product of animal origin is milk; animals fed with aflatoxin B-contaminated commodities can excrete another toxin, aflatoxin M1, into milk (Galvano *et al.*, 1996). The list of products/toxins included in the EU regulations is summarised in Table 1.

Table 1. Products and mycotoxins included in EC Regulations. Maximum levels are defined for all the products, and frequently for their derived goods, except for T-2 and HT-2 toxins.

Product	**Toxin**						
	Aflatoxins	**Ochratoxin A**	**Patulin**	**Deoxy-nivalenol**	**Zearalenon**	**Fumonisins**	**T-2, HT-2**
Cereals	•	•		•	•		•
Maize	•	•		•	•	•	
Nuts	•						
Fruits		•*			•+x		
Apples				•+x			
Grapes			•*+				
Spices	•						
Coffee		•					
Wine	•	•					
Milk	•						

* dried; + juice; x solid

Prevention of mycotoxin contamination of food raw materials is considered more important than subsequent cure. Thus HACCP[2] approaches are being developed to examine the critical control points at which mycotoxigenic moulds and mycotoxins may enter a range of food chains. In this respect, diagnostic methods for field analyses are important tools. Some of the key pre-harvest aspects have been the influence of agrochemical inputs on the control of mycotoxigenic pathogens, the effects of environmental factors, the absence of obvious symptoms with toxin contamination and the lack of cereal cultivars with very effective resistance to both pathogen infection and toxin production. Furthermore, it has been recently discovered that some metabolites from known mycotoxins in food crops may be masked as they are conjugated (see 2.5 masked mycotoxins). Therefore, current analytical methods may not provide accurate measurements of total contamination in foodstuffs and may underestimate the true value.

2. Hazard Analysis and Critical Control Points

Recently, high concentrations of HT-2 and T-2 (Type A trichothecenes) have been detected in barley and oats in some European countries. These are produced by *Fusarium* species different to those producing DON in wheat. The impacts of climate and agronomy on HT-2 and T-2 are largely unknown. The limited available information suggests that the impacts of climate and agronomy on HT-2 and T-2 are different from those on DON in wheat. Current European recommendations for Good Agricultural Practice to reduce *Fusarium* mycotoxins in cereals may not be applicable to HT-2 and T-2. Thus, the European Commission is examining setting legislative limits on these two mycotoxins.

There are key environmental factors, both pre- and post-harvest, such as prevailing climatic conditions, water availability and temperature, especially during ripening of cereals, which are key determinants of the level of risk of exceeding the legislative limits already set for some of the mycotoxins (Magan *et al.*, 2003; Magan and Aldred, 2007; Magan *et al.*, 2008). Thus, climatic factors have been used to develop models for predicting the risk of mycotoxin contamination pre-harvest for both DON (cereals) and fumonisins (maize) (Battilani *et al.*, 2006). The relationship between these important environmental factors and marginal and optimum conditions for germination, growth and mycotoxin production have also been examined and used to develop integrated profiles for determining the conditions that increase the risk of mycotoxins exceeding the legislative limits. The relationship between water activity and moisture content of different cereals is given in Table 2. This is important as information presented in this section of the report will be based on water availability (represented by water activity, a_w) and can be interpolated to approximate moisture contents (mc).

Table 2. Relationship between moisture content (wet weight basis, %) and water activity (a_w) for some key cereals at 25°C. Safe storage requires a_w <0.70 a_w.

Moisture content (% w/w)					
a_w	**Maize**	**Wheat**	**Sorghum**	**Rice**	**Groundnuts**
0.98	30–32	30–34	31–32	26–28	16–17
0.95	26–27	26–28	26–27	23–24	14.5–15
0.90	23–24	21–22	22.5–23	20–21	12.5–13.5
0.80	16–17	16–17	18–19	17–18	9–10
0.70	15–16	14–14.5	16–17	14–14.5	7–8

2.1 Use of pesticides and occurrence of mycotoxins in different crops

Mycotoxin-producing fungi differ in their ecological growing conditions, mainly in terms of temperature and available water. *Fusarium* species are favoured by high levels of available water (Doohan *et al.*, 2003), while *Aspergilli* are xerophilic and take advantage of higher temperatures (Scheidegger and Payne, 2005). As a consequence, different geographic areas and crops are commonly associated with different fungi and mycotoxins (Battilani *et al.*, 2006; Battilani *et al.*, 2008; Marasas *et al.*, 2008). In addition, some fungi, like *Fusaria*, are more favoured by field conditions, and others, like *Aspergilli* by storage conditions. Nevertheless, the contamination of products always starts in the field and fungi can be isolated during the growing season (Munkvold, 2003; Payne, 1998). Mycotoxins are commonly detectable pre-harvest, in early stages or close to ripeness, depending on the pathosystem; only patulin is normally detected post-harvest. The pre-harvest stage is crucial in determining later mycotoxin contamination, but in dried products, like nuts or raisins, it is common to observe an increase in contamination during drying, especially in the sun.

With the exception of *Fusaria*, that penetrate cereal ears starting at the flowering stage (Parry *et al.*, 1995; Munkvold, 2003), mycotoxin-producing fungi are not such strong pathogens and they take advantage of wounds and damage to plant organs as the way to penetrate and initiate infection. This can be caused by abiotic agents, like strong rain and wind, hail, water stress, or by biotic agents, pests and other diseases of plants (Le Bars *et al.*, 1994). Abiotic agents cannot be managed by human intervention but pesticides can help in the control of pests and diseases.

Some studies of pathosystems have shown a significant effect of pest control in reducing the risk of mycotoxin contamination. The main example is European corn borer (ECB – *Ostrinia nubilalis*) in maize, whose control reduces fumonisin and aflatoxin contamination, with variable success depending on meteorological conditions and the severity of the fungal and pest attacks (Munkvold and Desjardin, 1997; Dowd, 1998).

Ochratoxin A contamination in grapes has been shown to be related to damage by grape berry moth (*Lobesia botrana*) (Cozzi *et al.*, 2006) and powdery mildew (*Uncinula necator*) (Minguez *et al.*, 2004) and in general the good management of pest and disease allows the production of safe grapes.

Navel orangeworm (larvae) (*Amyelois transitella*) is cited as the main insect involved in tree nut damage in the USA and the lesser cornstalk borer (*Elasmopalpus lignosellus*) as the main insect damaging peanuts; their presence has been associated with aflatoxin contamination of the nuts, but their control is not a common practice and no data are available on the possible reduction of mycotoxins (Lynch and Wilson, 1991; Mehrnejad and Panahi, 2006).

The direct control of mycotoxin-producing fungi has recently started to be included among Good Agricultural Practices. The crops where it was first introduced are small cereals with the aim of controlling *Fusaria*, which infect ears at flowering. The window for infection and for fungicide sprays is narrow but the application of effective chemicals at the right time has produced interesting results (Wiersma and Motteberg, 2005; Yoshida *et al.*, 2008). The control of *Aspergillus carbonarius* in grapes can be managed with fungicides, which are also active against grey mould (*Botrytis cinerea*) following the same schedule (Tjamos *et al.*, 2004; Veronesi *et al.*, 2006; Valero *et al.*, 2007). In this case, because grey mould is commonly controlled by chemicals, the success of the new approach of spraying to control mycotoxin-producing fungi depends on the choice of active ingredient.

Maize is another crop where the direct control of *F. verticillioides*, a fumonisin producer, is under study. However, until now it is only a research issue because no fungicides are registered in Europe for foliar sprays in this crop. Nevertheless, preliminary results are encouraging because this operation helps in producing maize with contamination below the legal limit (Battilani *et al.*, personal communication).

Limited experience in the direct control of mycotoxin-producing fungi has shown some evidence of increases in mycotoxins due to the choice of active ingredient. The first evidence has been shown in wheat where a complex of fungi is involved in *Fusarium* head blight (FHB) including *Microdochium nivale*. Azoxystrobin, an active ingredient used for FHB control, is more effective against this species than the other *Fusaria*, including those relevant for mycotoxin production. This compound modifies the balance between microorganisms and the result is a higher DON contamination in kernels (Simpson *et al.*, 2001).

In trials *in vitro*, which were managed to test the efficacy of different active ingredients towards black *Aspergilli* ochratoxin A (OTA) producers in grapes, with the main target *Aspergillus carbonarius*, several compounds enhanced OTA production. A specific study with carbendazim showed that the stimulation of OTA synthesis increased with the dosage (Medina *et al.*, 2007). The indirect control of mycotoxins, through the control of pests, has never been shown to increase mycotoxin production.

The interest of researchers has not only focused on chemicals, but also on biological control agents (BCA). The best example is the use of *A. flavus*. Several studies have shown very good results in using non-toxigenic competitive strains of this fungus as BCAs. Initial research was on cotton, but later investigations extended the practice to pistachio nuts, peanuts and maize (Dorner and Lamb, 2006). Other BCAs, mainly yeasts, were considered *in vitro* and in field trials, in some cases with positive results (Dimakopoulou *et al.*, 2008). The impact of the farming system, organic or conventional, has been investigated for its possible effect on mycotoxins. However, available data are limited and relate principally to cereals, like wheat and oats, contaminated with trichothecenes. In general, contamination levels are limited, below the legal limits when applicable, and lower or similar in organic farming compared to conventional farming (Berleth *et al.*, 1998; Biffi *et al.*, 2004; Jestoi *et al.*, 2004; Anselme *et al.*, 2006; Granado *et al.*, 2008; Versari *et al.*, 2008). These data are interesting but should not be considered conclusive because they were from only a few studies, with modest contamination. More data under conditions more conducive to mycotoxin synthesis and on different crops are needed to complete the scenario. The data available underline that potential mycotoxin problems must be considered when protocols for different farming systems are prepared and should be considered in preparing legislation of pesticides.

From the literature review, it is clear that reducing the application of pesticides, particularly fungicides, can in some cases lead to an increased risk of agricultural commodities and products becoming infected by mycotoxin-producing fungi and increased levels of mycotoxin contamination. As the biotic and abiotic conditions are of great importance and can vary considerably, such influence on the reduction of pesticides can only be assessed on a case-by-case basis.

2.2 Mycotoxins boundaries and environmental conditions in different crops

Alternaria toxins

The genus *Alternaria* is widely distributed in both soil and on aerial plant surfaces, with many known to be plant pathogens. Species are known to grow at low temperatures and have been predominantly associated with spoilage of fruit and vegetables during cooled transport and storage. *A. alternata* is a common spore found in the air spora (Lacey, 1986), especially in areas under arable crop production. Ripening cereal grains are colonised rapidly with it becoming the most common sub-epidermal fungus in wheat grain (Hyde and Galleymore, 1951). *A. alternata* and species such as *A. triticina* can also cause black or brown colouration of the wheat kernels called black point disease, which can affect yield and grain quality. Other *Alternaria* species are important pathogens of sunflower seeds, tomato, carrots and brassicas.

The most important secondary metabolites with mammalian toxicity produced by *Alternaria* species are the dibenzo-α-pyrones: altenuene (AE), alternariol (AOH), alternariol monomethyl ether (AME) and a derivative of tetramic acid, tenuazonic acid (Meronuck *et al.*, 1972; Pero *et al.*, 1973a,b; Harvan and Pero, 1976). It has been demonstrated that some or all of these mycotoxins can be produced by *Alternaria* species growing on wheat (Magan and Lacey, 1984a), tomato (Harwig *et al.*, 1979), sorghum (Sauer *et al.*, 1978; Magan and Baxter, 1994), pecans (Schroeder and Cole, 1977) and on cotton (Young *et al.*, 1980). However, few of the studies have attempted to build two-dimensional profiles for germination, growth and mycotoxin production by *Alternaria* species (Young *et al.*, 1980; Magan and Lacey, 1984b, 1985; Magan *et al.*, 1984). The available information is for *A. alternata* and *A. tenuissima* only.

Overall, water activity × temperature limits for *A. alternata* germination are lower than for growth and for production of AE, AME and AOH. Furthermore, comparisons between growth and mycotoxin production have shown that the temperature range is narrower for AE and AME production than for AOH. The absolute water activity a_w (a_w of 1.00 means free available water) limit for germination is about 0.86, for growth and mycotoxin production about 0.88–0.89 for all three mycotoxins. Optimum production was at about 25°C and >0.97 a_w for all three mycotoxins (Sanchis and Magan, 2004).

Some ecological studies have been carried out on the production of toxins of *Altenaria* species (TA) by *A. alternata* and *A. tenuissima* species on sorghum and cottonseed (Young *et al.*, 1980; Magan and Baxter, 1994). Minimum water availability conditions for production were found to be about 0.90–0.93 a_w *in vitro* on sorghum-based media.

On cottonseed the optimum environmental conditions for TA production by *A. tenuissima* were 20°C/37.5% mc (= freely available water (1.00 a_w)). The absolute limiting condition of water availability was 14.9% (= 0.85 a_w). However, a_w was not accurately controlled in these studies. At intermediate moisture levels (=0.95 a_w) a 50% reduction was recorded. The results suggested that > 0.90 a_w and 20°C were required for TA production. Different temperatures favour biosynthesis of these different mycotoxins by *Alternaria* species. Visconti *et al.* (1992) found similar TA production levels for *Alternaria* from rapeseed, but no temperature or water availability aspects were considered in detail.

Fusarium toxins

Fumonisins

The *Fusarium* section *Liseola* species are responsible for the production of fumonisins in maize, and more recently in wheat. *F. verticillioides* isolates produce primarily fumonisin B_1 (FB_1) and lower amounts of FB_2, FB_3 and FB_4. Only a few isolates have been isolated that do not produce any measurable fumonisins when grown on corn in the laboratory. The range of a_w × temperature conditions conducive to growth was much wider than that for fumonisin B_1 production. Growth occurred at 4–37°C with an optimum at about 30°C (Marin *et al.*, 1999a), while FB_1 accumulation took place at 10–37°C, with 15–30°C being the optimum temperatures, depending on the isolates (Marin *et al.*, 1999b). Both growth and fumonisin B_1 accumulation increased with increasing a_w levels, with 0.90 being the minimum a_w for growth and 0.93 the minimum for FB_1 production (Marin *et al.*, 1999a b). Fumonisin production has been tested in other cereals, such as wheat and barley; however, negligible amounts of fumonisins were accumulated, suggesting that substrate composition affects fumonisin biosynthesis and is the reason for the low natural incidence in those cereals (Marín *et al.*, 1999a). Recent work in the USA has suggested that black point symptoms can be produced by these *Fusarium* species on wheat.

Agronomic practices are important as fumonisins are almost always formed in maize pre-harvest. It is critical that maize hybrids are selected properly and that soft kernel hybrids are avoided. Delay in sowing dates (in Europe, in May) can cause a problem and avoidance of high cropping density, good balanced fertilisation and avoidance of late harvesting are critical. Effective control of pests such as the insect corn borer is also required. It is also important to minimise the time between harvesting and drying.

Deoxynivalenol, nivalenol and zearalenone

In recent years much attention has been focussed on understanding the ecophysiology of *F. culmorum* and *F. graminearum* in relation to infection of ripening cereals (*Fusarium* head blight; Magan *et al.*, 2002). Contamination of cereal grain with deoxynivalenol (DON) and nivalenol (NIV) has been a particular problem for the flour milling and baking industry and to some extent for the brewing industry.

Early studies by Magan and Lacey (1984a) established comparative contour maps of water and temperature relations for growth of *F. culmorum, F. poae, F. avenaceum* and *F. tricinctum.* These showed that growth optima were at 20–25°C, with marginal conditions at 5–10°C and 35°C. The water availability optimum was close to 0.98–0.995 a_w, with minima around 0.90–0.91 a_w over the optimal temperature range. Germination thresholds were about 0.88 a_w for these *Fusarium* species (Magan and Lacey, 1984a). Versonder *et al.* (1982) demonstrated that *F. graminearum* and so-called *F. roseum* (= *F. culmorum*) strains produced DON optimally at 29–30°C and 25–26°C, respectively on cracked moist maize (30% water content = 0.99 a_w). Minimum temperatures for production of DON were about 11°C, dependent on the time of incubation for both strains. These contour maps were very useful, but excluded the interaction

with water availability. Cycling of temperature can have a significant impact on both DON and NIV production. Studies by Ryu and Bullerman (1999) using rice cultures showed that temperature cycling between 15°C and 30°C over a 6 week period resulted in the highest biomass. However, steady-state incubation at 25°C for two weeks resulted in the highest DON and zearalenol (ZEA) production. There was also a correlation between DON and ZEA production, but none between fungal biomass and production of either. This suggests that environmental stress has an important influence on toxin production, often unrelated to total fungal biomass.

More recently, Lacey *et al.* (1999) showed that wet periods at anthesis critically influenced the amount of DON and other related mycotoxins produced in ripening wheat. Hope *et al.* (2005) developed two-dimensional temporal production profiles for DON and NIV by *F. culmorum* which showed that 0.995 a_w was optimum for growth on wheat-based media, with 0.90 being the limit at both 15°C and 25°C. Production of both DON and NIV was over a much narrower range than growth (Hope *et al.*, 2005).

Less information is available on the ecological profiles of the production of zearalenone. Recently, Vellutti and Sanchis (unpublished data) examined production by *F. graminearum* on maize grain and found that the limit of production was at between 0.90–0.91 a_w over a 12-week incubation period at 25–30°C. Optimum conditions for production were at > 0.98 a_w at optimum temperature for growth.

Overall, while fully resistant cultivars are not available, it is essential that the partially resistant cereal cultivars should be used. The recommended rates of fungicide application should be observed to avoid stimulation of trichothecene production, harvesting or drying to 14–14.5% for wheat/barley/oats is essential, and "approved supplier systems" should be operated, requiring the setting of specifications for acceptance and rejection. Agronomic practices will also be significantly influenced by weather conditions during anthesis and during harvesting. Thus fungicide targeting needs to be related to prevailing rainfall and temperatures, which are most conducive to *Fusarium* head blight.

Ochratoxins

OTA has been shown to be produced by several species in the *A. ochraceus* group (Ciegler, 1972) and by *A. alliaceus, A. albertensis* (in section Circumdati), *A. niger, A. carbonarius* (in section Nigri) (Abarca *et al.*, 1994; Varga *et al.*, 1996) and *Penicillium verrucosum* (Northolt *et al.*, 1979). They contaminate different crops with a different distribution depending on climatic conditions. *Aspergillus* predominates in warm and temperate regions while *Penicillium* isolates are frequent in colder regions. Recently, the key responsible *Aspergillus* species (*A. ochraceus*) has been taxonomically reclassified as *A. westerdijkiae*.

Aspergillus westerdijkiae

A. westerdijkiae (=*A. ochraceus*; see Leong *et al.*, 2007) has been reported in a wide range of foods around the world, mainly those having an intermediate water availability, including cereals, beans, spices, dried fruits, nuts and oilseeds (Mislivec and Bruce, 1977; Sanchis *et al.*, 1986; Shrisvastava and Jain, 1992; Cvetnic, 1994). For this species the range of a_w × temperature conditions conducive to growth was much wider than that for ochratoxin A (OTA) production. Growth occurred over the temperature range of 8–37°C (ICMSF, 1996) with an optimum of about 30°C on barley grains (Ramos *et al.*, 1998). The highest amounts of OTA were obtained at 0.98 a_w, regardless of the temperature, with maximum OTA production between 25–30ºC, depending on the isolates. Both growth and OTA accumulation increased with increasing a_w values until 0.96–0.98, with 0.80 (at 25–30ºC) being the minimum a_w for growth on maize-based media (Marin *et al.*, 1998) and 0.83–0.87 the minimum for OTA production (Northolt *et al.*, 1979). The food matrix and nutritional status is very important in determining OTA production (Madhyastha *et al.*, 1990). Recently, the ecological similarity and differences between *Aspergillus ochraceus* group species (including *A. westerdijkiae*) in terms of growth and OTA and Ochratoxin B (OTB) have been compared (Abdel-Hadi and Magan, 2009).

Penicillium verrucosum

P. verrucosum effectively colonises food matrices, particularly in cooler climatic regions of the world. It is a particular problem in cool damp climatic regions of Northern Europe in wheat and barley, especially those not efficiently dried to safe moisture contents of about 14.5% (= 0.70 a_w). Where high temperature drying systems are used, grain can be effectively dried and the risk from contamination is low. However, short-term buffer storage on farm and ambient air-drying can lead to moulding and contamination with *P. verrucosum* and OTA.

P. verrucosum can grow over a wide range of temperatures 0–35°C. While 25°C is optimum for OTA production on grain, at 5–10°C it is produced during long-term storage under conducive moisture conditions. Interestingly, optimum colonisation rates of stored grain are at 0.95 a_w. However, optimum OTA production is between 0.90 and 0.95 a_w and amounts increase with time. The minimum a_w for OTA production is about 0.83–0.85 a_w and is dependent on the duration of storage (Cairns-Fuller *et al.*, 2006).

Overall, pre-harvest agronomic practices do not have a big effect on the contamination of cereal grain with OTA, especially in northern Europe. OTA predominantly contaminates grain during the harvesting and drying process. No OTA is produced at 0.80 a_w, although some is produced at 0.85 a_w (approx. 19% moisture) at 15–20°C. Efficient, rapid drying of damp grain to 14–14.5% moisture content is required. Regular and accurate determinations of moisture content are necessary.

2.3 HT-2 and T-2 toxins in cereals

Fusarium mycotoxins are common natural contaminants of cereals and cereal products as a result of *Fusarium* infections of cereal crops in the field. Consequently, methods of reducing their presence in cereal products must be focussed on controlling *Fusarium* infections in the growing crop. Extensive research has been conducted on the contamination of wheat by deoxynivalenol (DON, a Type B trichothecene), as this is the most common mycotoxin produced by *Fusarium* species worldwide. European legislative limits for the *Fusarium* mycotoxins DON, zearalenone and fumonisins were set in 2006.

The European Commission is currently considering setting legislative limits for HT-2 and T-2, which are two closely related Type A trichothecenes, in cereals and cereal products. HT-2 and T-2 are routinely detected in maize and small grain cereals; however, they usually occur at low frequency and at low concentrations. The SCOOP 3.2.10 Task calculated that the combined t-TDI (temporary-tolerable daily intake) for HT-2 and T-2 (0.06 µg/kg body weight) was, in most cases, exceeded in intake estimates (European Commission, 2003). However, a large proportion of the intake estimate came from the 80% of samples below the limits of detection. Therefore, the limit of detection strongly influenced the intake estimate.

According to Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, "…the development of a reliable and sensitive method, collection of more occurrence data and more investigations/research in the factors involved in the presence of T-2 and HT-2 toxin in cereals and cereal products, in particular in oats and oat products, is necessary and of high priority." (European Commission, 2006b).

Recent surveys have identified high concentrations of HT-2 and T-2 in barley in France and in oats in UK and Nordic countries (Edwards *et al.*, 2009; Pettersson *et al.*, 2008). The species responsible for these mycotoxins is believed to be *Fusarium langsethiae* (Imathiu, 2008). This is a newly identified *Fusarium* species, for which limited information is available.

Limited studies have been conducted on the impact of agronomic practices on HT-2 and T-2 contamination of cereals. In France, Arvalis and Syngenta have conducted surveys of barley and analysed HT-2 and T-2 content against agronomic practices (Barrier-Guillot, 2008; Gautier, 2007). In the UK, Harper Adams University College has conducted similar work on wheat, barley and oats (Edwards, 2007a; Edwards, 2007b). In the UK study, only an adequate high frequency of positive samples on oats allowed modelling of HT-2+T-2 concentration. The results from both studies are summarised and compared to the known impact of agronomy on deoxynivalenol (DON) in wheat in Table 3.

Table 3. Impact of agronomy on HT-2 and T-2 in French barley and UK oats and on DON in wheat.

Agronomic practice	**HT-2+T-2 in French Barley**	**HT-2+T-2 in UK Oats**	**DON in Wheat**
Previous crop	High after cereals† Low after maize Low after non-cereals	High after cereals Low after maize Low after non-cereals	Moderate after cereals High after maize Low after non-cereals
Cultivation	Ploughing beneficial after non-cereal	Ploughing beneficial after non-cereal	Ploughing beneficial after all crops, particularly maize and cereals
Variety	Higher in Spring barleys (difference between varieties unknown)	Higher in Winter oats (large differences between varieties)	Large differences between varieties but differences limited within some countries
Irrigation at flowering	Increases mycotoxins	No data (Higher in dryer summers)	Increases mycotoxins
Fungicides	Decrease mycotoxins	No data (Swedish field experiments indicated slight or no decrease*)	Triazoles decrease (Some strobilurins can increase DON)

† cereals refers to small grain cereals – wheat, barley and oats
*(Pettersson *et al.*, 2008)

The table shows that many of the agronomic factors are similar for HT-2 and T-2 in barley and oats, but different to what is known for DON in wheat. Why HT-2 and T-2 are high in spring barleys in France and winter oats in the UK is not known. Differences may be genetic or based on prevailing weather conditions when the different crops are in flower. Flowering is believed to be the most susceptible growth stage and winter and spring varieties flower at different times.

Studies have tended to show that there is no significant difference between DON concentration in organic and conventional samples. In the UK study, much lower levels of HT-2 and T-2 were detected in organic compared to conventional oat samples, the reasons for this are unclear, but are likely to be a combination of several factors including the facts that organic growers generally:

1. Grow oats after a non-cereal
2. Plough before oats
3. Grow less susceptible varieties including more Spring oats
4. Use less intense cereal rotations

There is extensive knowledge on the impact of agronomy on DON contamination of wheat and this is well-documented and has been used to formulate Good Agricultural Practices (GAP) to reduce *Fusarium* toxins. The principles of GAP are outlined in the Commission Recommendation of 17 August 2006 on the prevention and reduction of *Fusarium* toxins in cereals and cereal products (European Commission, 2006a), and have been used to formulate National Codes of GAP to reduce *Fusarium* mycotoxins in cereals. However, as differences in Table 3 indicate, GAP to reduce DON in wheat is not directly transferable to reduce HT-2 and T-2 contamination of cereals.

In addition to the urgent need for a better insight into the distribution of HT-2 and T-2 in cereals worldwide and to identify the impacts of agronomy on their occurrence, further toxicological research on T-2 and HT-2 is also needed. The toxicity data available are limited and mainly on T-2 and rarely on HT-2. In the repeated dose toxicity studies, microscopical changes such as dystrophia, necrosis or hyperplasia were seen in liver, heart and kidneys. The changes in the kidneys and heart were most pronounced. T-2 also caused cardiovascular effects. Doses up to 3 mg/kg diet of T-2 had hardly any effect in the reproduction study. Developmental effects of T-2 toxin were seen at doses of 1.5 mg/kg body weight. Agrelo and Schoental (1980) reported induction of DNA damage *in vitro* at concentrations of 6 ng/ml or higher. However, the overall conclusion of the Joint Expert Committee on Food Additives (JECFA) of FAO and WHO (FAO, 2001) was that T-2 was not genotoxic. T-2 had an effect on the haematological, immunological and nervous systems. T-2 toxin is a potent inhibitor of protein synthesis. As no toxicity studies with a clear No Observed (Adverse) Effect Level (NO(A)EL) were available, the JECFA (FAO, 2001) took the Lowest Observable Effect Level (LOEL), which was considered to be close to the NOEL. The LOEL was 0.029 mg/kg body weight/day and using a safety factor of 500, JECFA established a provisional maximum tolerable daily intake of 60 ng /kg body weight/day for both T-2 and HT-2 toxins (FAO, 2001).

2.4 Ergot alkaloids

Ergot alkaloids are produced by *Claviceps purpurea* and the disease caused by its alkaloids has been a problem for mankind for thousands of years. This mould is most famous for the epidemics it caused in the Middle Ages. However, outbreaks of ergotism still occur today. The growth of the mould causing Saint Anthony's fire and the infection of rye by the mould, is dependent on environmental conditions. The spores are called ergot bodies or ergot and the infection with these spores is called ergotism.

The ergot alkaloids included in the ergot bodies formed by the fungus *C. purpurea* are somewhat different from other mycotoxins in that they mimic the corn kernel of rye, oats and wheat, and can even hibernate in winter crops. Infection with *C. purpurea* is called ergotism and occurs during the growing season of the crops, so the contamination happens pre-harvest. However, the possible safety issue of the ergot alkaloids arises during post-harvest milling, when the ergot bodies are not removed but are also milled and processed. Modern techniques enable the milling company in principle to remove the ergot bodies. Nevertheless, prevention of ergotism should not be forgotten, and the adoption of Good Agricultural Practice (GAP) should keep ergotism to a minimum.

Agronomic conditions that influence the growth of C. purpurea

Fungi of the *Claviceps* genus occur in nearly all climate zones. However, the range of distribution of *C. purpurea* is limited to the moderate climates of Europe, the USA and Northern Africa. It has a diversity of hosts and can grow on more than 400 different plant species, most of which belong to the grasses. Of the plants that are agriculturally used by humans, rye and triticale (a hybrid of female wheat and male rye plants) run the highest risk of becoming infected. Wheat, barley and oats can also be infected; however, this does not happen often.

There are different factors that contribute to the probability of an infection by *C. purpurea*. First, it is important for the mould that there are temperatures of 2–4°C for 6–8 weeks in the winter. Like a lot of other plants and fungi, *C. purpurea* needs this cold period in order to prevent premature sprouting.

With regard to the plants that can be the host of *C. purpurea*, it can generally be said that the duration and form of flowering is of importance. Plants, which flower openly, have an aggravated risk of being infected. The same is true for late-blooming rye, which has longer than average flowering periods. Climatic circumstances can also lead to long flowering periods. If the weather in the begining of summer is wet and relatively cool, the flowers of rye are opened several times and for longer times than in hot and dry weather. Individual rye plants that grow near to the side of a field run a higher infection risk than plants growing in the middle of the field. This is most likely because other grasses, which may be hosts of *C. purpurea* themselves, grow at the sides of fields. Another factor contributing to a higher incidence of *C. purpurea* infection is the use of hybrid ryes. Hybrid ryes are obtained by crossing two rye varieties that have been degenerated by inbreeding them to the point where they are nearly not able to grow anymore. The result in the F1 generation is a variety, which may have (for example) an extraordinarily high yield or resistance to parasites etc. due to heterosis. However, hybrid ryes usually have smaller antheridia, where the male gametes are produced, than conventional cultivars. This effect results in a lower amount of pollen or even complete male sterility. This, in turn, allows the spores of *C. purpurea* a greater opportunity to be spread to a flower that has not been fertilized yet. As well as the variety of rye used, the soil and environment, in which it is cultivated, can contribute to the spread of *C. purpurea*. Generally, the mould can grow on all kinds of soil; however, it has been found that *C. purpurea* prefers to grow on moor or sandy soils.

When considering the conditions for optimal growth and spread of *C. purpurea*, the different agricultural practices also have to be taken into account. The mould occurs most often in rye that is cultivated in monocultures without other plants in a crop rotation. A crop rotation with other grasses or potatoes also increases the growth and the rate of subsequent infections of *C. purpurea*. Furthermore, the infection risk is increased if the soil is not ploughed after the plants are harvested. Not ploughing before seeding again causes the ergot to stay on the surface, where it can reproduce. For the same reason it is not advisable to harvest very late. Sclerotia may fall to the ground and reproduce.

Taking all aspects of *C. purpurea* into account, it becomes clear that nowadays the focus should be on the prevention of occurrence of *C. purpurea*, and on research into the possibile applications of its alkaloids and their derivatives in medicine. Furthermore, it would be advisable to learn more about the toxicological properties of all 40 ergot alkaloids as only a few of them have been investigated properly so far. In modern times, epidemics of ergotism caused by *C. purpurea* are rare. In cases where ergotism occurs it can be treated successfully. Consequently, it is not necessary to invest in research on further prevention or treatment of ergotism.

The biggest outbreaks recently have been in developing countries. It can be assumed that the populations of these countries are not well-informed about how to prevent and treat the diseases caused by *Claviceps* species. It appears that public information campaigns are necessary to protect the local population from these intoxications.

In Western industrial countries, the occurrence of ergotism is usually prevented by the sophisticated filtering systems in the rye mills. The prevention of occurrence of *C. purpurea* is also the responsibility of farmers, who are advised to use agronomic practices that will reduce the prevalence of infected rye plants. As this approach is only partially successful in combating *C. purpurea*, high economic losses continue to occur, because the filtering systems in mills always also filter out a proportion of good uninfected rye grains. Today there are still no rye cultivars that are really resistant to infection by this mould. Consequently, in order to improve the economic circumstances of rye farmers and mills – as well as the availability of affordable rye grain and its products for the consumer – the development of new rye cultivars, which are more or even completely resistant to infection by *C. purpurea*, is a priority.

2.5 Masked mycotoxins

Masked or conjugated mycotoxins first came to the attention of toxicologists in the late 1980s because in some cases of mycotoxicoses, clinical observations in animals did not correlate with the low mycotoxin content determined in the corresponding feed. The unexpected high toxicity was therefore attributed to the occurrence of undetected, conjugated forms of mycotoxins that hydrolysed to the precursor toxins in the digestive tracts of the animals (Gareis *et al.*, 1990).

It was shown that plants can reduce the toxicity of mycotoxins either by chemical modification and/or by inclusion into the plant matrix. Living plants are capable, as part of their metabolism, of transforming xenobiotic compounds into conjugated forms (Cole and Edwards, 2000). This detoxication process includes the conjugation of mycotoxins to polar substances such as sugars, amino acids, or sulphate and subsequent storage of the conjugate in vacuoles. In this way a huge variety of conjugated forms can be expected. The total quantity of mycotoxins available in foodstuffs, therefore, might currently be underestimated.

However, little is known about the natural occurrence of these altered mycotoxins, their bioavailability and further metabolism. So far, the natural occurrence of zearalenone glucoside in wheat, deoxynivalenol glucosides in wheat, maize and beer, *N*-(carboxymethyl)fumonisin B_1 in corn have been reported (Schneweis *et al.*, 2002; Berthiller *et al.*, 2005; Lancova *et al.*, 2008). Ochratoxin A has been shown to be transformed in wheat and maize cell suspension cultures to ochratoxin α, ochratoxin A methyl ester, hydroxyochratoxin A as well as glucoside and methyl esters of hydroxyochratoxin A (Berthiller *et al.*, 2007).

Besides metabolism in the plant, processing of food, e.g., heat treatment and extrusion cooking, has been shown to induce partial degradation of fumonisins by the loss of tricarballylic acid groups (hydrolyzed fumonisins), Maillard-type reactions with reducing sugars (*N*-carboxymethylfumonisins) and possible reaction with protein amino groups or starch hydroxyl groups to yield bound derivatives (Howard *et al.*, 1998; Dall'Asta *et al.*, 2008). However, in a strict sense this reaction during cooking or processing does not produce masked mycotoxins.

No information was found about the occurrence of other masked mycotoxins. A summary of available data is given in Table 4. Recently, a review was written on the formation, determination and significance of masked and other conjugated mycotoxins (Berthiller *et al.*, 2009).

Berthiller *et al.* (2006) detected an array of 17 different metabolites, most prominently glucosides, malonylglucosides, di-hexose- and hexose-pentose disaccharides of zearalenone and α- and β-zearalenol in an test plant, *Arabidopsis thaliana*.

It is generally assumed that zearalenone-4-β-glucopyranoside (naturally present in wheat) is hydrolysed/digested in the gastrointestinal tract. The aglucone is released and might be implicated in the development of a mycotoxicosis. However, toxicity data are scarce for many other altered mycotoxins.

So far EU limits do not take conjugated mycotoxins into account. More data on the occurrence of conjugated mycotoxins in food and feed should be generated. Environmental factors should be studied. According to Howard *et al.* (1998) *N*-(carboxymethyl)fumonisin B_1 can be naturally present in corn if sufficient metals and phosphate are present in the corn to catalyze the reaction. Metabolism of xenobiotics is different in different plants. Biotransformation studies should be performed in different crops in different agronomic situations.

The consequence for risk assessment of the masked mycotoxin depends on the toxicokinetic behaviour of this modified/included/conjugated mycotoxin. If these plant metabolites do contribute to the toxicity there is a need to reconsider previously performed hazard, exposure and risk assessments. Further research is needed to illuminate the possible effects. Based on generated bioavailability and toxicity data on these mycotoxin metabolites, risk assessments (and probably also tolerable daily intakes (TDIs)) should be reconsidered. Furthermore, the influence of climate change should be investigated (Miraglia *et al.*, 2009).

Table 4. Summary of available data on masked mycotoxins

Mycotoxin	**Conjugate type**	**Occurrence of the conjugate**			**Analytical method**	**References**
		Artificial (Process)	**Natural**	**Matrix**		
Deoxynivalenol	Glucosides	Yes (chemical, enzymatic)	Yes	Wheat, maize, beer	LC/MS of DON glucoside	Berthiller *et al.*, 2006 Berthiller *et al.*, 2007 Lancova *et al.*, 2008 Vendl *et al.*, 2009
Zearalenone	Glucosides	Yes (enzymatic)	Yes	Wheat	LC/MS of ZEA glucoside	Berthiller *et al.*, 2006 Berthiller *et al.*, 2007 Poppenberger *et al.*, 2006 Schneweis *et al.*, 2002 Vendl *et al.*, 2009
Ochratoxin A	Glucosides	Yes (enzymatic)	No information available	Wheat, maize	No information available	Berthiller *et al.*, 2007
	Methyl esters	Yes (enzymatic)	No information available	Wheat, maize		
Fumonisins	Glucosides	Yes (thermal treatment)	No information available	Starch, corn	LC/MS	Berthiller *et al.*, 2007 Seefelder *et al.*, 2003 Poling *et al.*, 2002 Howard *et al.*, 1998 Dall'Asta *et al.*, 2008
	Amino acids	Yes (thermal treatment)	No information available	Proteins, corn		
	N- (Carboxy-methyl)-fumonisin	Yes (Maillard type of reaction with reducing sugars)	Yes	Corn		

3. INHERENT PLANT TOXINS

3.1 Glycoalkaloids in potatoes

Introduction

Potatoes are usually planted in spring into relatively widely spaced ridges. After planting, the tubers are immediately covered with a layer of soil. Shallow planting can increase the proportion of green tubers at harvest and planting too deep may result in harvesting problems. Therefore, tubers are usually planted at a depth of 15 cm.

To control soil-born pathogens, fumigant chemicals (metham sodium) are sometimes applied some weeks before planting, non-fumigant chemicals are incorporated immediately prior to planting to control nematodes and soilborn fungi such as *Rizoctonia solani* to prevent disease and stress, which can have impact on the concentration of potato-glycoalkaloids. Similarly, next to these pre-planting treatments, fungicides and insecticides can be applied during crop growth. Late blight (*Phytophthora infestans*) is the most important disease worldwide and requires sometimes 15 or more foliar applications. As a result, application of fungicides may begin shortly after emergence and be repeated at 7-day intervals.

To end the growth of the crop and enable the harvesting of the tubers, defoliation is often carried out. This is usually done chemically using the herbicides glufosinate ammonium or diquat; defoliation can also be done mechanically or by burning. Such treatments can cause stress and induce enzymes that synthesise potato glycoalkaloids. After defoliation the tuber periderm becomes progressively more resistant to damage during removal, but depending on variety and conditions an interval of 2–6 weeks may be required before significant damage by harvesters can be avoided.

Mechanised harvesters separate tubers from haulm, soil, clods and stones on a series of rollers and webs and collect the tubers for transport.

A significant proportion of the potato tubers is consumed or processed soon after harvest, but most are stored prior to use. Potatoes are washed to remove the soil. For some products, hydro-cooling is used to reduce the temperature of the potatoes within hours of harvest before transport to packers and retailers. Potatoes are stored in bulk stores and box stores. In the first few hours of storage, it is very important to ventilate the potatoes to remove surface moisture. Ideally, the tubers are stored at 10°C for the first 10–14 days to reduce the risk of disease resulting from unset skins and wounds. However, sometimes this is not possible because of the risk of quality loss through the development of blemishing disease. After this period, potatoes are stored at temperatures of 0.5°C. The quality of the stored potatoes is dependent on the control of sprouting, dehydration and diseases. Over long periods of storage, changes in tuber composition, temperature, relative humidity, ventilation and composition of the storage atmosphere can all be adjusted in such a way that optimal storage conditions are achieved (Firman *et al.*, 1992; Bradshaw *et al.*, 2000; Bowen *et al.*, 1996; Wigginton, 1974; Firman and Allen, 2007).

Chemical composition; particularly glycoalkaloids

The potato genotype is the most important factor determining glycoalkaloid concentrations in the tubers of both wild species and cultivated potatoes. However, factors such as temperature, light conditions and tuber damage may alter these levels significantly (Valkonen *et al.*, 1996). Green tubers, being exposed to light, contain higher levels of glycoalkaloids, although the magnitude of increase is variety dependent. Immature tubers form larger levels of glycoalkaloids after exposure to light than matured tubers.

Most of the environmental variation seems to result from climate influences (Maga, 1980; Jadhav *et al.*, 1981; Van Gelder, 1989). In particular, it has been suggested that an unusually cool growing season accompanied by an abnormally high number of overcast days, probably resulting in an immature potato crop, is the cause of excessive levels of solanidine glycosides. Tuber size, as well as maturity, is inversely related to the content of solanidine glycosides within the cultivar, as these alkaloids are formed early during tuber development and are "diluted" during the process of enlarging and maturation of the tubers. Moreover, the potato alkaloids are particularly concentrated in the peel, which is a relatively larger part of the tubers when they are smaller.

Storage conditions can influence the concentration of solanum alkaloids considerably. When harvested potatoes are exposed to light, the solanum alkaloid content increases. This increase depends on the cultivar and is positively related to the light intensity and duration of exposure (Maga, 1980; Jadhav *et al.*, 1981; Van Gelder, 1989). During exposure to light, the content of solanum alkaloids in the tubers increased more strongly in immature and small tubers (up to 480 mg/kg) than it did in mature and larger tubers. Light exposure also leads to the formation of chlorophyls, which results in green potatoes. A large number of physical and chemical treatments are used in post-harvest packaging: packaging in coloured polyethylene bags, ionizing radiation, submerging in water to which chemicals are added, heating the tubers and treating them with waxes, chemicals or oils, some of which inhibit both the solanum alkaloid formation and the greening.

Tuber injury, resulting from dropping, puncturing, cutting, hammering, brushing or physical injury caused by diseases or by animals, stimulates the synthesis of solanum alkaloids (Olsson, 1986; Mondy *et al.*, 1987; van Gelder, 1989). Such injury of the potatoes induces the enzyme system that synthesises the solanum alkaloids.

Solanum alkaloids are fairly heat stable compounds. It is therefore not surprising that steaming, boiling, baking, cooking, frying and microwaving of potatoes did not affect their content of solanum alkaloids (van Gelder, 1989). When, during the manufacturing of potato crisps and French fries, half products such as slices or stripes (injured potatoes and exposed to light) are left some time (3 hr to 3 days) before frying or cooking, the total glycoalkaloid content of the potato can increase by 100% (Maga, 1981; van Gelder, 1989).

One possible way to obtain tubers with low glycoakaloid levels is by selective breeding. At the same time many potato-breeding programmes are aimed at achieving pathogen resistance. Highly resistant cultivars usually have high glycoalkaloid levels. Glycoalkaloids are used as a defence mechanism against, for instance, the Colorado beetle *Leptinotarsa decemlineata*. Defoliation of potato plants by Colorado beetles induced a 58.1% and a 48.3% increase of glycoalkaloids in the skin and inner tissue of tubers compared to control plants (Pariera-Dinkins *et al.*, 2008), while manual defoliation did not increase the concentrations. An enhanced glycoalkaloid content in new varieties to increase resistance against, for instance, Colorado beetles is only feasible when leaf and tuber glycoalkaloid are under independent genetic control.

The content of total glycoalkaloid (α-chaconine and α-solanine), in conventionally and organically grown potatoes (*Solanum tuberosum* L.), was studied by Hajslova *et al.* (2005). Tubers of eight varieties grown in controlled field trials at one or two geographical sites in the Czech Republic in four consecutive harvest years from 1996–1999 were analysed. The mean total glycoalkaloid content seemed to be slightly higher in organically grown tubers pooled together (80.8 ± 44.5 mg/kg) compared to conventionally grown ones (58.5 ± 44.1 mg/kg). However, the total glycoalkaloid levels varied considerably from variety to variety. The content of glycoalkaloids in organically grown potatoes ranged between 16–157 mg/kg fresh weight and 47–97 mg/kg at the two locations whereas the content in conventionally

grown potatoes ranged between 15–245 mg/kg and 34–108 mg/kg. Only one variety showed statistically significant higher levels of total glycoalkaloids when grown organically at both locations throughout the experiment. Two other varieties also showed a trend towards having higher glycoalkaloid content in organically grown potatoes. A survey investigating the distribution and content of total glycoalkaloids in potatoes on the Danish market over six years revealed that concentrations not only vary between varieties, but also within the same variety between years (Knuthsen *et al.*, 2008). However, only three of the 386 varieties tested contained more than the recommended 200 mg/kg fresh weight of potatoes. The majority of glycoalkaloids found in potatoes are linked to the acute toxicity of maltreated potatoes (also observed in humans) and to reproductive and developmental effects, which in some cases were linked to blight growing on the potatoes. The data available on exposure to and hazards of potato glycoalkaloids are insufficient for a full risk assessment – only a provisional risk assessment of α-solanine can be justified. The key data are an exposure varying from 0.17 to 1.4 mg/kg bodyweight/day, a lowest effect level from a reproduction study, a NOAEL from sub-chronic study equivalent to 22.5 mg α-solanine and the lowest reported dose causing acute toxicity in humans of 1.2 mg total glycoalkaloids (TGA)/kg bodyweight. The margin of safety between exposure and the NOAEL ranges from 16–130 (Speijers, 1998). Kuiper-Goodman and Nawrot (1993) also considered the data not adequate to perform a safety assessment.

3.2 Cyanogenic glycosides in cassava

Introduction

Cassava or manihot (*Manihot exculenta* Crantz) is a tropical shrub, the starchy storage root of which is important as food. It was domesticated by Indians living in north-eastern South America several thousand years ago. Cassava is still an important crop in Asia and in Africa where its roots provide 30% of the staple food (De Bruin and Fresco, 1989). The crop's agronomic advantages, such as high productivity, ease and flexibility of cultivation, tolerance to drought and ability to grow well on relatively poor soils, led to its rapid and extensive adoption in Africa. Drawbacks of cassava roots as a food are their bulkiness, their perishability, their low protein content and their potential toxicity. The roots contain about 65% water and they deteriorate within 4 days after harvest. This makes them difficult to transport and market. As the protein is lower than that of other staple foods, cassava roots have to be supplemented with protein-rich foods to give a balanced diet. Leaves contain higher amounts of proteins. The toxicity is related to the capacity of all parts of the plant to release hydrogen cyanide from stored cyanogenic glycosides in a process known as cyanogenesis (Esser, 1995).

Character of cassava and agronomic factors

The cyanogenic potential of cassava results from the presence of two cyanogenic glycosides, linamarin and lotaustralin in a ratio of about twenty to one respectively (De Bruijn, 1971; Esser, 1995). Other compartments in the same tissue contain the enzyme linamarase, which can hydrolyse these glycosides to yield the respective cyanohydrins and glucose. Disruption of the cell structure initiates this hydrolysis. The term "bitter" cassava, as opposed to "sweet" cassava, refers to the taste of the root parenchyma. Bitterness is associated with higher levels of cyanogenic glycosides (Cock, 1985).

Certain ecological stress factors, such as pest attacks, prolonged drought and low phosphorus and potassium levels in the soil may cause roots to acquire bitterness, and this coincides with an increase in the levels of cyanogenic glycosides (Ayanru and Sharma, 1984/85; De Bruijn, 1971). Reported levels of cyanogenic glycosides in parenchyma of fresh roots are 10 to 2000 mg HCN equivalent per kg dry weight (Coursey, 1973).

Linamarin and linamarase levels vary widely between cassava cultivars, between plants of the same cultivar, between different tissues of the same plant, between roots of the same plant and even within the root parenchyma (De Bruijn, 1971; Boudreaux *et al.*, 1980). This heterogeneity in the distribution of cyanogenic glycosides makes research on cassava cyanogen levels demanding in terms of the number and size of samples in both experimental and observational studies (Esser, 1995).

Processing and cyanogens removal

Cassava starchy roots are processed to make them storable, to reduce their bulk, to increase their palatability and to reduce their potential toxicity. The large range of processing methods and products has been reviewed (Lancaster *et al.*, 1982; Padmaja, 1995). Sweet roots can be eaten raw or boiled, but a more elaborate processing is applied when roots are considered bitter or toxic.

A cassava paste can be obtained by mashing boiled cassava roots, steaming the dough of soaked roots, mixing roasted granules with hot or cold water, or by mixing flour obtained from the dried root with boiling water, The easiest way to obtain flour is sun-drying of the peeled roots. Drying can be preceded by soaking in water, by crushing or keeping roots covered, or by various combinations of these procedures (Esser, 1995; Padmaja, 1995; Ravi and Padmaja, 1997).

Applying only sun-drying to peeled roots was found to diminish the level of total cyanogens insufficiently from cassava roots with high initial cyanogenic potential (Pieris *et al.*, 1974). Nevertheless, sun-drying remains the main processing method in most of Africa. Studies on the effects of drying on cyanogen levels in cassava roots were reviewed by Coursey (1973). In some areas of Africa cassava roots are processed in such a way that they get covered with moulds. The lack of purity (Douglas, 1966) has raised concern among the health and agricultural authorities, but no adequate studies have been performed to assess the effectiveness of this method in reducing cyanogen levels.

Toxic effects of cassava cyanogenic glycosides

Several health problems have been attributed to cyanide exposure from insufficiently processed cassava. Occasional acute poisonings following meals of cassava have been known for centuries. This is the result of cyanogenic glycosides that have been hydrolysed by the inherent linamarase (intracellular β-glucosidase) in cassava yielding hydrogen cyanide (HCN), which causes the acute toxicity. Cyanogenic glycosides are not themselves very acutely toxic (Speijers, 1993). Thiocyanate is the main metabolite in mammals normally produced during detoxification.

Chronic toxic effects of the metabolite HCN in plant material have been associated with long-term exposure to low levels. HCN had been identified as aggravating iodine deficiency and increasing the prevalence of goitre in some of the African populations (Ermans *et al.*, 1980). Osuntokun (1981) found an association between a neurological degenerative disease, tropical ataxic neuropathy (TAN) as well as konzo and long-term moderate cyanide exposure from cassava consumption (Teles, 2002). An epidemic of spastic paraparesis took place in Nigeria and the uptake of dietary cyanogens from insufficiently processed cassava was suggested as the main etiological factor (Esser, 1995; Speijers, 1993).

The JECFA[3] (Speijers, 1993) concluded that because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be estimated. However, the Committee concluded that a level of up to 10 mg/kg hydrogen cyanide in the CODEX[4] standard for Cassava Flour (CAC, 1991) is not associated with acute toxicity.

3. Joint FAO/WHO Expert Committee on Food Additives

4. FAO/WHO Codex Alimentarius Commission

3.3 Phenylhydrazine derivatives in cultivated mushrooms

Introduction

Many scientific synonyms exist for the cultivated mushroom (*Agaricus bisporus* J.E. Lange) Pilát a.o., *Agaricus hortensis* (Cooke) Pilát and *Agaricus hortensis* (Cooke) S. Imai.

Agaritin, (β-*N*-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine), is the major phenylhydrazine derivative found in the cultivated mushroom (*Agaricus bisporus* (J.E. Lange) Pilát. The average values of agaritin range from 200–500 mg/kg fresh weight (Andersson and Gry 2004). Other phenylhydrazine derivatives are found in small quantities in the mushrooms. The average content of 4-(carboxy)phenylhydrazine was 11 mg/kg fresh mushroom, the average content of β-*N*-[γ-L-(+)-glutamyl]-4-(carboxy)phenylhydrazine was 42 mg/kg, and the average content of 4 (hydroxymethyl) benzenediazonium ion was 0.6–4 mg/kg (Chauhan *et al.*, 1984; Chauhan *et al.*, 1985; Ross *et al.*, 1982; Toth *et al.*, 1997).

Composition, particularly the phenylhydrazine derivatives

The cultivated mushroom and some of the phenylhydrazine derivatives found in the mushroom have shown a carcinogenic effect in a single mouse strain, Swiss albino mice. Agaritin was not found to be carcinogenic when administered in drinking water whereas 4-(carboxy)phenylhydrazine, β-*N*-[γ-L-(+)-glutamyl]-4-(carboxy)phenylhydrazine and the 4-(hydroxymethyl)benzenediazonium ion were all carcinogenic when administered orally in high doses (Toth *et al.*, 1981; McManus *et al.*, 1987; Toth 1986; Toth *et al.*, 1982). Carcinogenicity has also been observed when Swiss mice were fed fresh mushrooms or baked mushrooms in high doses (Toth and Erickson, 1986; Toth *et al.*, 1997). These studies are not guideline studies and their design has been criticised for many reasons (Andersson and Gry, 2004). Based on the available data, the Danish food authorities recommend that intake of phenylhydrazines should be limited and, especially, that the consumers should not eat raw mushrooms in high quantities. Cooking of mushrooms leads to lower levels of agaritins.

Some data on the agaritin content in the mushrooms grown under different cultivation practices and after post-harvest storage have been published whereas no data on the other phenylhydrazine derivates exist.

Compost

The cultivated mushroom grows on mushroom compost. The major ingredient of "natural composts" is wheat straw-embedded horse manure. The term "synthetic composts" are used for composts where horse-manure is not the main ingredient. Instead they consist of hay and crushed corn cobs. "Blended composts" are mixtures of natural and synthetic composts. All types of composts need to undergo a sort of fermentation whereby chemical and biological alteration of the starting materials takes place to provide the mycelium with the proper nutrients for growth (Andersson and Gry, 2004). Speroni and co-workers (Speroni *et al.*, 1983) found a higher level of agaritin in mushrooms grown on synthetic compost compared to mushrooms grown on natural or blended composts. Two samples of mushrooms grown on synthetic compost contained about 4100 and 7500 mg/kg agaritin respectively. Samples grown on blended compost contained 2700 or 3100 mg/kg, and mushrooms grown on natural composts contained 2400 and 2700 mg/kg. All concentrations in this study were given on a dry weight basis.

Strain

Liu *et al.* (1982), who studied the concentration of agaritin in 14 lots of fresh mushrooms from ten different growers, found more than a 4-fold difference in the content of agaritin. They saw no indication of strain influence of the content of agaritin when studying strain types of different colours: white, off-white, golden, white, cream and brown.

Maturity

Younger and smaller fruiting bodies contained a statistically significant higher content of agaritin than larger mushrooms (4–5 days old, with a cap diameter of 4–6 cm) ready to be harvested (Schulzova *et al.*, 2002). This is in agreement with studies by Fischer *et al.* (1984) and Chiarlo *et al.* (1979), whereas Sharman *et al.* (1990) did not see much difference between agaritin levels in mushrooms in the early button state compared to the later states.

Flushes

During the harvesting period, mushrooms are produced in flushes of 3–5 days' duration followed by a period with few available mushrooms. Growers harvest from 3–8 flushes on the same mushroom bed (Andersson and Gry, 2004). Speroni *et al.* (1983) found a strong tendency to an increased concentration of agaritin in mushrooms harvested from later flushes. Sharman *et al.* (1990) did not find such a difference between flushes when studying the agaritin content in two strains of mushrooms in four flushes. Additionally, Schulzova *et al.* (2002) found high levels of agaritin from mushrooms from an early flush.

Seasonal variation

Liu *et al.*, (1982) found no indication of a seasonal trend in the content of agaritin.

Pesticides

Pesticides like chlorfenvinphos, chlorpyrifos, cyromazine, diflubenzuron, methoprene and prochoroz are used for controlling insects and competing fungi (Andersson and Gry, 2004). Data on whether pesticide use has an influence on the agaritin levels have not been reported.

Post-harvest storage

The level of agaritin is influenced by post-harvest storage. Schulzova *et al.* (2002) followed the content of agaritin in mushrooms obtained directly from the grower and stored in a refrigerator at 5°C. The initial agaritin concentration was 393 mg/kg fresh weight. During the first three days the agaritin content increased slightly (around 4%); thereafter, the content of agaritin substantially decreased. A 25% decrease in the amount of agaritin was found after 6 days of storage, and the amount had decreased to 50% of the original content after storage for 14 days. However, the mushroom quality deteriorated with storage time.

In conclusion, the content of agaritin (β-*N*-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine) is influenced by agronomic practices like the choice of compost type. Other parameters, like maturity of the fruiting body and whether the mushrooms are harvested at early or later flushes, have influenced the agaritin content in some studies but not in all. The post-harvest period, the period from when the mushrooms are harvested until they are eaten by the consumer, has a strong effect on the agaritin content.

3.4 Alkenylbenzene levels in spice and herb crops (including basil, tarragon, nutmeg, mace and allspice)

Introduction

Exposure to alkenyl benzenes from food occurs predominantly from the use of herbs and spices including basil (*Ocimum basilicum* L.), tarragon (*Artemisia dracunculus* L), nutmeg or mace (*Myristica fragans* Houtt.) and allspice (*Pimenta dioica* L.). Known alkenyl benzenes such as safrol, estragole and methyleugenol have been classified as genotoxic carcinogens. The concentrations of estragole or methyleugenol in a spice show significant variations which, in large part, are a function of plant maturity at harvest, harvesting techniques, storage conditions, processing (e.g., drying) and method of measurement (e.g., extraction with CO_2 vs CH_2Cl_2).

Composition: alkenylbenzenes

Geographical origin influences the presence of methyleugenol in sweet basil (*Ocimum basilicum* L.) and exotic (i.e. Reunion-type as found in the Comoro Islands) basil. The methyleugenol content of the volatile oil from commercial samples of sweet and exotic basil is 0–0.5% and 1.3–2.0%, respectively (Lawrence and Shu, 1993). Other factors affecting methyleugenol content include distribution within the plant, plant maturity, harvest time and drying conditions. The methyleugenol content for the same cultivar of sweet basil was in the range of 0.6–2.4% for the leaf, flower and stem with highest concentrations found in the leaf (Tsai and Sheen, 1987; Sheen *et al.*, 1991). Methyleugenol content of sweet basil leaves of the Genovese Gigante variety is inversely proportional to plant height (maturity). Methyleugenol content in the essential oil of sweet basil decreased from essentially 100% to 10% as the plant matured from 3–6 cm to 12–60 cm (Miele *et al.*, 2001). Therefore, early harvested basil provides significantly increased exposure to methyleugenol. For basil harvested at the optimum time and stored over various periods, the concentration of methyleugenol in fresh leaves was 0.05%, in leaves stored 2 months it was 20.4% and in leaves crushed and frozen at -20°C it was 1.6% (Bobin *et al.*, 1991).

Large variations in the methyleugenol concentrations in the fresh herb were also found by Ribnicky *et al.* (2004). This was ascribed to sample heterogeneity, variable rates of decomposition, or variations of content within the plant due to a variety of factors such as age or environmental conditions. Both estragole and methyleugenol are volatile oils and do decrease within the herb by evaporation as observed from the lower content of methyleugenol and absence of estragole in the air-dried herb.

The effects of harvesting time, temperature and drying period on the yield and chemical composition of *Ocimum basilicum* essential oil, with eugenol as one of the main components, was studied by Carvalho *et al.* (2006). Transplanted seedlings were harvested after 40 and 93 days. Harvesting time, drying temperature and period of drying appeared to be important factors in the yield of the oil.

Chang *et al.* (2008) found an effect of daylight on the content of eugenol. There were three chemical compounds in basil leaves, namely linalool, eugenol and methyleugenol, influenced by shading treatments. Linalool and eugenol, which contribute to the characteristic taste of basil, were significantly increased by high daily light integrals, whereas methyleugenol was increased by lower daily light integrals.

In a review paper of Figueiredo *et al.* (2008) many factors are mentioned as having influence on the yield and composition of essential oils. These factors include: a) physiological variations; (b) environmental conditions; (c) geographic variations; (d) genetic factors and evolution; (e) political/social conditions; and also (f) amount of plant material/space and manual labour needs.

4. NITRATES

Nitrate itself is regarded to be of low toxicity. Its metabolites particularly nitrite, nitric oxide and reaction products with secondary amines and amides, e.g., *N*-nitroso compounds have however given rise to health concerns due to their implications for adverse health effects like methaemoglobinaemia, where small children are especially sensitive, and carcinogenesis (EFSA 2008a). Human consumption of nitrate stems from vegetables, and to a lesser extent from water and other foods (EFSA 2008a). Up to 80–85% of the dietary nitrate intake comes from vegetables. The bioavailability of nitrate from raw lettuce and cooked vegetables like spinach and beetroot is around 100% (van Velzen *et al.*, 2008). The acceptable daily intake (ADI) for nitrate of 0–3.7 mg/kg body weight was established in 1995 and was recently reconfirmed by the European Food Safety Authority (EFSA, 2008a). This EFSA opinion considers both the potential adverse and beneficial health effects of consumption of vegetables and states that "overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risk, therefore, the recognised beneficial effects of consumption of vegetables prevail."

However, unfavourable local production conditions of vegetables may give rise to levels of nitrate resulting in intake levels beyond the ADI. Also high nitrate content in some species may give rise to intakes of nitrate exceeding the ADI. Intake of approximately 50 g rucola with the median nitrate concentration may in itself exceed the ADI level even without taking other sources of nitrate into consideration (EFSA, 2008a).

4.1 EU regulation

The maximum levels of nitrate in certain foodstuffs are regulated by Commission Regulation no. 1881/2006 (European Commission, 2006). This regulation sets maximum levels for nitrate in frozen and fresh spinach (*Spinacea oleracea* L.) and in lettuce (*Lactuca sativa* L.). For iceberg-type lettuce the maximum level is lower than for other types of lettuce. Seasonal variation in nitrate levels is reflected in this legislation so higher levels are accepted for lettuce and spinach grown from 1 October to 31 March than for these vegetables grown from 1 April to 30 September.

4.2 Nitrate levels in food plants

The content of nitrate varies considerably in different vegetables. Generally, the nitrate content is higher in vegetables where the used plant parts are the leaves, e.g., spinach or lettuce, compared to those plants where roots, tubers, bulbs or fruits are the edible parts. However, considerable differences in nitrate levels are also found within species where the same plant parts are eaten. The median nitrate content in red beet (*Beta vulgaris* var. *vulgaris*) was 1110 mg/kg compared to a median level in carrots (*Daucus carota* L. ssp. *sativa*) of 125 mg/kg (EFSA, 2008a).

The nitrate level may vary two-fold between various lettuce varieties, e.g., higher levels were found in romaine types compared to crisphead types. The nitrate level may differ even within a specimen, e.g., inner (younger) leaves of lettuce and head chicory (*Cicorium intybus* L. ssp *intybus* Foliosum Group) contained lower levels of nitrate than outer (older) ones (Santamaria *et al.*, 1999).

The nitrate concentration varies greatly between cultivars of lettuce. Therefore, it may be possible to select cultivars that accumulate less nitrate (Escobar-Gutierrez *et al.*, 2002). Also nitrate level in potatoes is partly dependent on variety (Hajslova *et al.*, 2005).

Rucola accumulates nitrate to a very high extent (up to 9300 mg/kg of fresh product) (Santamaria *et al.*, 2002). The median level was as high as 4800 mg/kg (EFSA, 2008a). Two species, *Eruca sativa* L. and *Diplotaxis tenuifolia* (L.) DC, are sold under the same vernacular names, rucola or rocket salad (Santamaria *et al.*, 2002; Pilegaard *et al.*, 2007). Comparison of the nitrate level in the two species is currently lacking. Under experimental conditions (hydroponic growth in growth chamber) higher levels of nitrate were found in *D. tenuifolia* (Santamaria *et al.*, 2002).

4.3 Environmental and agronomic factors

Light intensity, temperature and soil moisture are environmental factors that influence nitrate levels in leaf vegetables. Therefore, seasonal variations in the content of nitrate in lettuce exist with the highest levels found in the winter period and the lowest in summer (Petersen and Stolze, 1999). Geographical differences between nitrate contents of leaf vegetables exist with findings of higher nitrate levels in Northern Europe compared to Southern Europe.

The nitrate levels in lettuce grown in glasshouses are higher compared to lettuce grown in fields, due to the reduced light intensity. Use of nitrogen fertilizers increases the concentration of nitrate in leaf vegetables, but little effect is observed in vegetables where the edible parts are storage organs like peas, beans and roots. Schemes for Good Agricultural Practice have been developed in European countries to help farmers reduce the nitrate levels in food plants and to respond to nitrate regulations (EFSA, 2008a). Merino *et al.* (2006) suggest that the Good Agricultural Practice used by Swedish farmers may explain why the nitrate contents of more than 95% of samples of lettuce and spinach monitored in Sweden from 1995–2005 were below the EU maximum levels.

Comparison of nitrate between organically produced different food plants and conventional ones is variable. Williams (2002) mentions that out of 40 comparisons of organically versus conventionally grown crops, 25 organic crops had decreased nitrate levels, 10 had the same levels and in five higher nitrate levels were found. A Dutch study found that the nitrate level was much lower in organically grown head lettuce from the field compared to conventionally grown. However, no difference was seen when this lettuce was produced in greenhouses, or when iceberg lettuce was grown in the field. Organic carrots had statistically significant higher levels of nitrate compared to conventional carrots (Hoogenboom *et al.*, 2008). Hajslova *et al.* (2005) studied the content of nitrate in organically and conventionally grown potatoes in two fields in the Czech Republic for four years. The conventionally grown potatoes contained statistically higher levels of nitrates than the organically grown ones in three years out of four. However, considerable year-to-year variation in nitrate content was seen for both farming systems.

Since nitrate content in vegetables is highly influenced by agronomic practices, schemes for Good Agricultural Practices developed in European countries have helped farmers to reduce the nitrate levels in food plants.

Consumers wanting to reduce their intake of nitrates may eat less leaf vegetables like lettuce, spinach and more vegetables where the plant parts used are roots, tubers, bulbs or fruits. Replacing some sort of leaf vegetables like rucola with lettuce may reduce the intake of nitrate substantially.

5. PROCESS-RELATED TOXINS

5.1 Acrylamide formation during heat processing of products of several crops

Recently an EFSA report appeared on acrylamide carcinogenicity (EFSA, 2008b) and the role of epidemiological studies in relating dietary exposure levels or biomarker levels to site-specific cancers. In this report it is stated that national differences in levels of acrylamide in foods and different consumption patterns make it difficult to give meaningful values for the relative contribution from different dietary sources of exposure at the pan-European level. A very rough estimate suggests similar contributions from potato products, cereal based products and coffee. The possibility and efficacy of mitigation measures was discussed. It was concluded that a 40% maximum reduction might be possible, based on measures taken by food producing industries. It was agreed that measures have to be taken in home cooking, by catering companies and, perhaps most importantly, by consumers by changing consumption patterns, in order to achieve a substantial decrease in exposure.

The commodities giving the highest exposure to acrylamide in the western diet are potatoes, cereals and coffee. About a third of the acrylamide exposure via foods comes from these three commodities. However, it is still important to identify specific risk groups with high exposure, e.g., children or "exceptional"consumers of specific food commodities or specific ethnic foods containing relevant levels of acrylamide

As asparagine is a main determinant in the formation of acrylamide, prospective epidemiological studies could focus on a better exposure assessment by analytical measurement of asparagine in combination with food frequency questionnaires.

Acrylamide production in foods is a consequence of the Maillard reaction between asparagine and reducing sugars. Therefore, factors affecting the concentration of these precursors in food, together with processing conditions during food preparation and storage will affect the final concentration of acrylamide in food (CIAA, 2009).

Potatoes

Reducing sugars are the key reactants in potatoes for the formation of acrylamide. The sugar content of the tuber correlates well with the acrylamide concentration in the product. Controlling sugar is currently the primary measure employed by industry to reduce acrylamide levels in crisps and French fries.

Future opportunities include: 1) breeding new potato varieties with lower reducing sugar content and/or less cold sweetening effect, 2) further optimising the agricultural practices to reduce sugars and asparagine. The nitrogen fertilizer regime appears to influence the reducing sugar concentration of the potato tuber, i.e. reducing sugars increased by 60–100% upon lowering the field *N*-fertilisation (Swiniarski, 1970; Kumar, 2004).

So far no control of asparagine levels in potatoes has been established. Potential leads being explored are 1) the breeding of lower asparagine varieties, 2) the impact of storage on free asparagine levels and 3) the impact of farming practices (e.g., fertilizer regimes) on asparagine levels.

Cereals

Asparagine is the key determinant of acrylamide formation in cereal products. The sugar composition of cereal grain has not been considered relevant to breakfast cereal manufacture. In soft wheat varieties asparagine formation showed no relation to total reducing sugars or to individual sugar concentration.

Free asparagine within and between cereal types varies widely. Year to year variations from one harvest to another are considerable and more fundamental knowledge is needed concerning the impact of agronomical practices and cereal varieties on asparagine levels. Sulphur-deprived soils have been shown to have a considerable impact on the free asparagine concentrations in certain cereal crops. The choice of wheat with lower asparagine has led to products with lower acrylamide levels. The major soft wheat varieties in use in the UK are those with the lowest asparagine concentration. In France more variation is seen and soft wheat varieties have been selected. There is a large environmental variation between growing sites.

Refined grain cereal products dominate cereal consumption. Fine bakery products have high acrylamide concentrations and so can contribute significantly to acrylamide intake. Recent recommendations to increase the consumption of wholegrain foods are likely to add to this intake since whole grains contain higher concentrations of asparagine and will lead to higher concentrations of acrylamide in wholegrain foods.

Coffee

For coffee, acrylamide formation does not appear to be affected by reducing sugars and is only weakly correlated with asparagine concentration in green beans. Acrylamide does not accumulate during coffee roasting. The limiting factor for acrylamide formation seems to be the content of free asparagine. Only 20% of the initially formed acrylamide survives the roasting process and further decay is observed during storage, predominantly through the binding of acrylamide to constituents of the ground and roasted coffee matrix.

Post-harvest changes in precursor concentrations will affect the potential for acrylamide formation.

It may be concluded that acrylamide levels may vary a lot due to seasonal effects, fertilisation and/or plant variety. Depending on the key determinant, management decisions to reduce acrylamide can be made.

6. EXECUTIVE SUMMARY

6.1 Mycotoxins

From the literature review it is clear that the knowledge of the influence of agronomic factors on the occurrence of mycotoxins in food crops is variable. Only for a few mycotoxins, does near-adequate data on agronomic factors exist. For most others there are limited data on the influence of pre-harvest, harvest and storage conditions. The information is summarised in Box 1 (page 30).

Agronomic field conditions are important in a number of cases where fungi have occurred and mycotoxins have formed, e.g., in maize during pre-harvest and harvest conditions, but for other cases the post-harvest conditions, storage conditions in particular, are more important for the formation of mycotoxins.

It is critical that proper selection of cultivars is made and soft kernel hybrids are not used. Delaying the sowing dates (in Europe, in May) can cause a problem, and the avoidance of high cropping density, good balanced fertilisation and avoiding late harvesting are critical. Effective control of pests, such as the European corn borer in maize, is required. It is also important to minimise the time between harvesting and drying. There is also interaction with prevailing weather conditions during silking.

From the literature review, it is clear that reducing the application of pesticides, particularly fungicides, can in some cases lead to an increased risk of agricultural products becoming infected by mycotoxin-producing fungi and contaminated with increased levels of mycotoxins. As the biotic and abiotic conditions are of great importance and can vary considerably, the impact of reducting pesticides can only be assessed on a case-by-case basis. Thus it is critical that sustainable fungicide use is implemented at the recommended rates of application and at the key effective times, according to the label instructions. Limited evidence on the direct control of mycotoxin-producing fungi by pesticides has shown an increase in mycotoxins due to the choice of active ingredient. Biological control agent (BCA) studies have shown very good results in using non-toxigenic strains of this fungus as BCA. Other BCAs, mainly yeasts, were considered, in some cases with positive results.

The impact of the farming system, i.e. organic, anticipated sustainable or conventional, has been examined for its possible effect on mycotoxins. However, available data are limited and relate principally to cereals like wheat and oats, for contamination by trichothecenes, fumonisins and the ergot alkaloids. These data are interesting but should not be considered conclusive because they result from only a few studies and with modest contaminations. Further data on favourable conditions for mycotoxin synthesis and on different crops are needed.

Plants hosting fungi and containing mycotoxins can either reduce the toxicity of mycotoxins or increase the toxicity by the biotransformation of mycotoxins to altered mycotoxins (masked), which are not as easily detected. Most analytical and toxicity studies address well-known mycotoxins. However, little is known about the natural occurrence of these altered mycotoxins, their bio-availability and further metabolism.

Box 1: Summarising the influence of agronomic factors and conditions on mycotoxins in food crops

Mycotoxins	**Amount of data**	**Preharvest practices**	**Harvest conditions**	**Storage conditions**	**Pest control**	**Biocontrol**	**Masked mycotoxins**	**Crop rotation**	**Organic versus conventional**
Altenaria toxins	+	Weather/ climate, water activity, temperature	Water activity humidity, temperature, mechanical harvesting	Water activity, temperature, air-drying	Fungicides, insecticides, other pesticides		Production of masked mycotoxins, little data	Not growing crops at the same location each harvest season	No data
Fumonisins	+++	Treatment with insecticides, climate	No data	Not very relevant	Fungicides, insecticides	Biological	Production data, little data	Varying location for each harvest season	Some data
DON, NIV, ZEA	+++	Temperature, climate, water activity	Temperature, climate, water activity	Temperature, climate, water activity	Fungicides, insecticides, other pesticides	Biological	Production data, little data	Varying location for each harvest season	Some data
HT-2 and T-2 toxins	++	Temperature, climate, water activity	Temperature, climate, water activity	Temperature, climate, water activity	Fungicides, insecticides, other pesticides	No data		Varying location for each harvest season	
Ochratoxins; OTA	+++		Temperature, climate, water activity	Temperature, climate, water activity	Fungicides	Biological	Production data, little data	Varying location for each harvest season	
Ergot alkaloids	+++	Temperature, climate, water activity	Less relevant	Less relevant	No data, possibly use of fungicides in field	No data	Organic higher content than conventional grown crops	Varying location for each harvest season	Higher ergot contents in crops grown and processed organically
Other *Fusarium* toxins, e.g. *F. moniliforme*	++	Temperature, climate, water activity	No data	Less relevant	Fungicides, insecticides	No data	Production data, little data	Varying location for each harvest season	Some data

+ little data ++ some data +++ many data

6.2 Inherent plant toxins

Most data on the influence of agronomic factors on the composition and contamination of plant crops arise from studies of the growth of pathogenic fungi and the production of mycotoxins. However, it is known that stress, such as disease or wounding, causes the formation or increased formation of inherent plant toxins under certain conditions.

These examples of the influence of agronomic factors on the concentration of inherent plant toxins present are discussed and summarised in Box 2 (page 32).

Examples include glycoalkaloids in several crops of the family of solanaceae (e.g. tomatoes, potatoes, sweet peppers), cyanogenic glycosides in several crops such as linseed, stone fruits and cassava, phenylhydrazine derivatives in cultivated mushroom, oestrogenic compounds in plants such as red clover, alkenyl benzene levels in herbs and spices (tarragon, nutmeg, mace and allspice).

From studies on growing potato crops, several agronomic factors are evidently important to prevent the reduction of quality and food safety. Some of these agronomic factors might be specific to potatoes but others may have similar impacts on other food crops, which may be already established or might be expected on the basis of plant physiology.

For cereal crops there is no issue of inherent plant toxins since there are no reports of secondary plant metabolites causing adverse health effects.

The example of cassava illustrates the influence of agronomic factors that have an impact on toxicity and the influence of the direct preparation of food.

In the cultivated mushroom (*Agaricus bisporus* and *Agaricus hortensis*) agaritin (β-*N*-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine) is the major phenylhydrazine derivative (see section 3.3). Phenylhydrazines have been demonstrated to be carcinogenic. The choice of compost type and the maturity of the fruiting body are major agronomic factors influencing the subsequent concentration of phenylhydrazines.

The methyleugenol content within the same cultivar of sweet basil may vary markedly with the highest concentrations found in the leaf. Furthermore, it was shown for some varieties that the methyleugenol content of sweet basil leaves is inversely proportional to plant height (maturity).

Both estragole and methyleugenol are volatile oils and may decrease within the herb by evaporation as found in air-dried herbs. Harvesting time, drying temperature and the period of drying appear to be important factors in the yield of basil oil. Also, an effect of daylight on the content of eugenol was noted. Overall many factors have been identified to have an influence on the yield and composition of essential oils. These factors include: (a) physiological variations; (b) environmental conditions; (c) geographic variations; (d) genetic factors and evolution; and (e) the amount of plant material/space and manual labour needs.

Acrylamide production in foods is a consequence of the Maillard reaction between asparagine and reducing sugars. Therefore, factors affecting the concentration of these precursors in food, together with processing conditions during food preparation and storage, will affect the final concentration of acrylamide in food. The commodities giving the highest exposure to acrylamide in the western diet are potatoes, cereals and coffee. A third of the total acrylamide exposure via foods comes from potato products, cereal products and coffee.

Box 2: Summarising the influence of agronomic factors and conditions on inherent plant toxins in food crops

Inherent plant toxins/ food crop	Amount of data	Preharvest practices	Harvest conditions	Storage conditions	Pest control	Cultivar selection	Food processing	Crop rotation	Organic versus conventional
Potato glycoalkaloids	+++	Growth conditions, weather, climate, soil conditions	Temperature, mechanical harvesting, wounding, exposure to daylight, maturity	Humidity, sprouting wounding, exposure to daylight	Fungicides, insecticides, other pesticides, biocontrol of insect pests	Cultivar, strain, variety, GMO	Cutting of slices, crisps, heating, (acrylamide) composition, extracting,	Not growing crops at the same location each harvest season	No data
Cassava	++	Little data on growth conditions, weather/climate soil conditions	No data	Not very relevant	Fungicides, insecticides	Cultivar, strain, variety, GMO	Masceration, cutting, crushing induce enzyme system releasing HCN	Varying location for each harvest season	Some data
Agaritin	+++	Some data on growth conditions, weather, climate, soil conditions, humidity, compost, manure	Temperature method of harvesting, wounding, exposure to daylight	Little data no long-term storage	Fungicides, insecticides other pesticides, no data on biological pest control	Cultivar, strain, variety,	Method of cooking and baking of mushrooms important, cutting raw	Varying location for each harvest season	Some data
Alkenyl in herbs and spices	++	Little data on growth conditions, weather, climate, soil conditions	Maturity, method of harvesting	Temperature, climate, humidity	Fungicides, insecticides, other pesticides, gas treatment	No data	No data	Varying location for each harvest season	No data
Nitrate	+++	Growth conditions, weather, climate, soil conditions, daylight, light intensity, use of fertilizer/manure	Temperature, climate	Temperature, climate	Fungicides, insecticides, other pesticides, biocontrol of insect pests	Cultivar, strain, variety, GMO	Processing in which nitrate is converted to nitrite, concomitant availability of *N*-nitrosable compound (e.g., secondary amines or amides)	Varying location for each harvest season	No adequate data. Possibly somewhat lower nitrate concentration inorganic grown crops
Cereals	++	Growth conditions, weather, climate, soil conditions, daylight, light intensity, use of fertilizer, manure	Maturity, method of harvesting, Temperature, climate,	Humidity	Fungicides, insecticides, other pesticides, biocontrol of insect pests	Cultivar, strain, variety, GMO	No data	Varying location for each harvest season	Higher ergot contents in crops grown and processed organic

+ little data ++ some data +++ many data

Light intensity, temperature and soil moisture are environmental factors that can influence nitrate levels in leaf vegetables. Therefore, seasonal variations in the content of nitrate in lettuce are found with the highest levels in the winter period and the lowest in summer. Geographical differences exist between the nitrate content of leaf vegetables with higher nitrate levels in Northern Europe compared to those in Southern Europe.

Conventionally grown crops focus on the use of chemically produced pesticides such as fungicides, insecticides and to a lesser extent herbicides compared with organically grown crops, which are only treated with so-called biological pesticides. The chemically derived pesticides all undergo regulatory safety assessment, whereas for the biological pesticides there is a limited list of chemicals that have never been subject to a safety assessment by official international expert panels. The issue of organically grown crops and conventionally grown crops is a complicated one as analysis and review of the data published so far does not give a clear answer, with respect to the presence of contaminants such as mycotoxins and inherent plant toxins, due to different treatment and related agronomic measures. For mycotoxins there are data available, but for inherent plant toxins hardly any comparative data exist on this aspect.

The content of glycoalkaloids in organically grown potatoes varied considerably; only one variety showed statistically significant higher levels of total glycoalkaloids when grown organically at both locations throughout the experiment. Two other varieties of potatoes also showed a trend towards having higher glycoalkaloid content in organically grown potatoes.

Comparison of nitrate levels in organically produced different food plants with conventionally grown ones gave variable results. From the limited number of studies available it seems that in some vegetables like carrots, organically grown had higher nitrate concentrations than conventionally grown carrots, whereas in potatoes, those grown organically had lower nitrate concentrations than the conventionally grown ones. Other studies comparing different crops including lettuce showed variable results where sometimes the nitrate concentration in organically grown plants was lower, sometime similar to and sometimes higher than conventionally grown food plants.

Processing may have a major impact on the presence of toxins in food. Moreover, it may also give rise to new toxins.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Studying the potato reveals that several agronomic factors can have an impact on the disease, injury or stress of the plant and as such can play a role in the contamination with mycotoxins (toxicogenic fungi) or inherent plant toxins (glycoalkaloids) and also may affect the the nutritional composition of the tuber. For other crops which can suffer from infestation with toxicogenic fungi it is also clear that agronomic factors can play an important role, although their impact might differ from crop to crop and from fungal species to fungal species, as was illustrated by the different sections on mycotoxins.

Overall, to avoid fungi producing mycotoxins, it is critical that the correct cultivar is selected, based on previous cropping experience, that Good Agricultural Practices are employed and where agrochemicals are used these are applied at recommended rates to minimise the potential to stimulate mycotoxin contamination. Rain episodes and temperatures that may be conducive for the germination and infection by mycotoxigenic fungi pre-harvest, especially during flowering and grain setting, should be taken into account.

From several studies it is clear that the reduction of pesticides, particularly fungicides, in some cases can lead to an increased risk of agricultural food products becoming infected by mycotoxin-producing fungi with subsequent occurrence of mycotoxins. As the biotic and abiotic conditions are of great importance and these can vary considerably, the consequences of the reduction of pesticides can only be assessed on a case-by-case basis.

For mycotoxins certain post-harvest agronomic factors, such as conditions of storage, can have an impact on both contamination later in the processing and on the formation of masked mycotoxins.

There are less extensive data on the levels of inherent plant toxins; however, as detailed in earlier sections, agronomic factors can have an important role. Stress and disease can increase certain inherent plant toxins as already seen for fungal growth and mycotoxin production. It might, therefore, be concluded that agronomic factors are also important for the control of the levels of inherent plant toxins. As data on the occurrence of inherent plant toxins in relation to agronomic factors are currently limited, research on these aspects should have the highest priority. This should include the different influences of agronomic factors under conventional and organic regimes. In the case of cultured mushrooms some specific agronomic factors are important such as the type of compost. Another factor influencing the agaritin content is the maturity of the fruiting body and whether the mushrooms are harvested at early or later flushes. The post-harvest period, the interval between mushroom harvest and consumption, has a strong effect on the agaritin content.

For herbs and spices and derived essential oils, our literature review revealed that there are many factors that can have influence on the yield and composition of plant and essential oils. These factors include: (a) physiological variations; (b) environmental conditions; (c) geographic variations; (d) genetic factors and evolution, and (e) amount of plant material/space and manual labour needs.

Vegetables are a major source of dietary nitrate. Nitrate in vegetables provide an example of a plant food toxin that may be mitigated by environmental factors including light intensity and temperature and by agronomic practices such as cultivation in glasshouses, choice of cultivars and Good Agricultural Practices. Additionally, nitrate content varies considerably between different food plants. So changes in consumption pattern, e.g., replacement of some vegetables with others or the increased popularity of a plant with a high nitrate content, may influence the human dietary nitrate intake considerably.

There are also limited data on the occurrence of many mycotoxins. More research is needed, with a lower priority for DON and OTA where a lot of data are already available.

7.2 Recommendations

The following recommendations are given in a random order and do not present a priorisation.

- More data are needed on the occurrence of *Alternaria* toxins and the role of agronomic factors as well as more data on the toxicity of *Altenaria* toxins.
- For zearalenone reasonable data on toxicity are available, but there is a lack of information on the ecological profiles for its production. More data on the role of agronomic factors in the production of zearalenone in cereals and maize are needed.
- There is an urgent need to determine the distribution of HT-2 and T-2 within cereals worldwide and to identify the impacts of climate and agronomy on their occurrence.
- In addition, further toxicological research on T-2 and HT-2 is needed.
- OTA predominantly contaminates grain during the harvesting and drying process and so regular and accurate moisture determinations are required.
- A very recent publication on the genotoxic properties of OTA by Mantle *et al.* (2010) suggests that OTA can form covalent adducts with DNA in experiments carried out *in vivo*. A clarification of whether this effect could be carcinogenic would be extremely welcome. OTA has a long half-life in humans and can accumulate in organs such as liver and kidneys.
- No rye cultivar which is really resistant to infection by mould has yet been developed. Consequently, in order to ensure good economic circumstances for rye farmers and the mills as well as to ensure the availability of affordable rye grain and its products for the consumer, focus should be on the development of new rye cultivars, which are more – or even completely – resistant to an infection with *Claviceps purpurea*.
- Overall, in order to mitigate mycotoxin contamination, it is critical that the correct cultivar choice is made (on the basis of previous cropping experience), that Good Agricultural Practices are employed and, where they are used, agrochemicals are applied at recommended rates to minimise the potential stimulation of mycotoxin contamination. Rain episodes and temperatures that may be conducive to germination and infection by mycotoxigenic fungi pre-harvest, especially during flowering and grain setting, have to be taken into account.
- Clear specifications and traceability from field to store is essential. Since almost no information is available on the influence of agronomic practices on masked mycotoxins, this needs further research.
- Furthermore, the influence of climate change should be investigated.
- Further toxicological studies with masked mycotoxins (mycotoxin metabolites) are needed to establish whether, and if so how, the risk of adverse effects is related to the parent mycotoxin.
- There are few research data on agronomic factors and their influences on the concentrations of inherent plant toxicants in a few food plants. It is therefore recommended that these crops and other food crops be studied to investigate systematically the effects of agronomic factors on the concentration of their inherent plant toxicants.
- Cultivars have to be produced with low toxicant levels within ecological and environmental acceptable ranges.
- The influence of climate on several agronomic factors and thus on the level of occurrence of mycotoxins or inherent plant toxins should be investigated.

- Further studies on the effects of pre-harvest conditions on the formation of agaritin, such as choice of compost and post-harvest storage conditions, should be performed.
- For spices and herbs and derived essential oil some information is available, but more research is needed to study the influence of agronomic factors.
- Light intensity, temperature and soil moisture all influence the content of nitrate, especially in leaf vegetables, and should be investigated further.
- For process-related toxins the single example of processing is acrylamide and levels may vary considerably due to seasonal effects, fertiliser use and/or plant variety. Depending on the key determinant, decisions on how to reduce acrylamide can be made.

8. REFERENCES

Abarca, M.L., Bragulat, M.R., Castella, G. and Cabañes, F.J. (1994). Ochratoxin A production by strains of *Aspergillus niger* var. niger. *Applied Environmental Microbiology*, **60**: 2650–2652.

Abdel-Hadi, A. and Magan, N. (2009). Influence of environmental factors on growth, sporulation and ochratoxin A and B production of the new grouping of the *A. ochraceus* group. *World Mycotoxin Journal*, **2**: 429-434.

Agrelo, C.E. and Schoental, R. (1980). Synthesis of DNA in human fibroblasts treated with T-2 toxin and HT-2 toxin (the trichothecene metabolites of *Fusarium* species) and the effects of hydroxyurea. *Toxicology Letters*, **5**: 155–160.

Andersson, H.C. and Gry, J. (2004). Phenylhydrazines in the cultivated mushroom (*Agaricus bisporus*) – occurrence, biological properties, risk assessment and recommendations. *TemaNord*: 558. ISBN 92-893-1080-4.

Anonymous. (2003). Mycotoxins: risk in plant, animal, and human systems. Task Force Report No. 139, Council for Agricultural Science and Technology (CAST), Ames, Iowa.

Anselme, M., Tangni, E.K., Pussemier, L., Motte, J.C., Hove, F. van, Schneider, Y.J., van Peteghem, C. and Larondelle, Y. (2006). Comparison of ochratoxin A and deoxynivalenol in organically and conventionally produced beers sold on the Belgian market. *Food Additives and Contaminants,* **23**: 910–918.

Ayanru, D.K.G. and Sharma, V.C. (1984/5). Changes in total cyanide content of tissues from cassava plants infested by mites (*Mononychellus tanajoa*) and meallybugs (*Phenacoccus manihoti*). In: "Agriculture, Ecosystems and Environment", Elsevier Science Publishers, Amsterdam, pp. 35–46.

Barrier-Guillot, B. (2008). T-2 and HT-2 in cereals grown in France, Fifth *Fusarium* Toxin Forum. European Commission, Brussels.

Barug, D., van Hegmond, H.P., Lopez-Garcìa, R., van Osenbruggen, W.A. and Visconti, A. (Eds.) (2004). Meeting the mycotoxin menace, Wageningen Academic Publishers, Wageningen, The Netherlands.

Battilani, P., Barbano, C., Marin, S., Sanchis, V., Kozakiewicz, Z. and Magan, N. (2006). Mapping of *Aspergillus* section *Nigri* in Southern Europe and Israel based on geostatistical analysis. *International Journal of Food Microbiology*, **111**: S72-–S82.

Battilani, P., Barbano, C. and Logrieco, A. (2008). Risk assessment and safety evaluation of mycotoxins in fruits. In: "Mycotoxins in fruits and vegetables", Barkai-Golan, R. and Paster, N. (Eds), Academic Press, New York, pp. 1–26.

Berleth, M., Backes, F. and Kramer, J. (1998). Mould spectrum and mycotoxins (deoxynivalenol and ochratoxin A) in grain samples from ecological and integrated cultivated sites. *Agribiological Research*, **51**: 369–376.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G. and Krska, R. (2005). Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography – tandem mass spectrometry, *Journal of Agricultural and Food Chemistry*, **53**: 3421–3425.

Berthiller, F., Werner, U., Sulyok, M., Krska, R., Hauser, M.T. and Schuhmacher, R. (2006). Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) determination of phase II metabolites of the mycotoxin zearalenone in the model plant *Arabidopsis thaliana*. *Food Additives and Contaminants,* **23**: 1194–1200.

Berthiller, F., Sulyok, M., Krska, R. and Schuhmacher, R. (2007). Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals. *International Journal of Food Microbiology,* **119**: 33–37.

Berthiller, F., Schuhmacher, R., Adam, G. and Krska, R. (2009). Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry*, **395**: 1243–1252.

Biffi, R., Munari, M., Dioguardi, L., Ballabio, C., Cattaneo, A., Galli, C.L. and Restani, P. (2004). Ochratoxin A in conventional and organic cereal derivatives: a survey of the Italian market, 2001–02. *Food Additives and Contamination*, **21**: 586–591.

Bobin, M.F., Gau, F., Pelltier, J. and Cotte, J. (1991). Etude de L'Arome Basilic. *Rivista Italiana EPPOS*, 3–13.

Bourdoux, P., Mafuta, M., Hanson, A. and Ermans, A.M. (1980). Cassava toxicity: the role of linamarin. In:"Role of cassava in the etiology of endemic goitre and cretinism", Ermans, A.M., Bulamoko, N.M., Delange, F. and Ahluwalia, R. (Eds), Ottawa: International Development Research Centre, (IDRC monograph 136e.), pp. 15–27.

Bowen, S.A., Muir, A.Y. and Dewar, C.T. (1996). Investigations into skin strength in potatoes: factors affecting skin adhesion strength. *Potato Research*, **39**: 313–321.

Bradshaw, N.J., Elcock, S.J., Turner, J.A. and Hardwick, N.V. (2000). Are potato blight fungicides being used rationally? British Crop Protection Conference, *Pests and Diseases*, **3**: 847–852.

Brown, C.R. (2005). Antioxidants in potato. *American Journal of Potato Research*, **82**: 163–172.

CAC. (1991). Codex Alimentarius, Vol. XII,suppl.4. Codex Standard for Edible Cassava Flour (African Regional Standard)., Food and Agriculture Organization of the United Nations (CODEX Stan 176), Rome.

Cairns-Fuller,V., Aldred, D. and Magan, N. (2005). Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of Penicillium verrucosum on wheat grain. *Journal of Applied Microbiology*, **99**: 1215–1221.

Carvalho Filho, J.L.S.C., Blank, A. F., Alves, P. B., Ehlert, P.A.D., Melo, A.S., Cavalcanti, S.C.H., de Fátima Arrigoni-Blank, M. and Silva-Mann, R. (2006). Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil. *Brazilian Journal of Pharmacognosy*, **16**: 24–30.

Chang, X., Alderson, P.G. and Wright, C. J. (2008). Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. *Environmental and Experimental Botany*, **63**: 216–223.

Chauhan,Y., Nagel, D., Issenberg, P. and Toth, B. (1984). Identification of p -hydrazinobenzoic acid in the commercial mushroom *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*., **32**: 1067-–1069.

Chauhan, Y., Nagel, D., Gross, M., Cerny, R. and Toth, B. (1985). Isolation of N^2-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine in the cultivated mushroom *Agaricus bisporus*. *J Agric Food Chem*., **33**: 817–820.

Chiarlo, B., Cajelli, E. and, Acerbo, C.. (1979). The presence of agaritin in a mushroom (*Agaricus bisporus*) commonly cultivated in Italy. *Fitoterapia*, **50**: 111–113.

CIAA (Confederation of the food and drink industries of the EU) (2009) The CIAA Acrylamide"Toolbox", Revision 12. http://ec.europa.eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf.

Ciegler, A. (1972). Bioproduction of ochratoxin A and penicillic acid by members of the *Aspergillus ochraceus* group. *Canadian Journal of Microbiology*, **18**: 631–636.

Cock, J.H. (1985). Cassava, New potential for a neglected crop. Westview Press Inc., Boulder, Colorado.

Cole, D.J. and Edwards, R. (2000). Secondary metabolism of agrochemicals in plants. In:"Metabolism of agrochemicals in plants", Roberts, T. (Ed). Wiley, New York, pp. 107–154.

Coursey, D.G. (1973). Cassava as food: toxicity and technology. In:"Chronic Cassava Toxicity", Nestel, B. and MacIntyre, R. (Eds), Proceedings of an International Workshop, London, England 29–30 January 1973. International Development and Research Centre, Ottawa, Canada, pp. 89–96.

Cozzi, G., Pascale, M., Perrone, G., Visconti, A. and Logrieco, A. (2006). Effect of *Lobesia botrana* damages on black *Aspergilli* rot and ochratoxin A content in grapes. *International Journal of Food Microbiology*, **111**: S88–S92.

Cvetnic, Z. (1994). 'Cyclopiazonic acid and aflatoxin production by cultures of *Aspergillus flavus* isolated from dried beans and maize'. *Nahrung*, **38**: 21–25.

Dall'Asta, C., Galaverna, G., Aureli, G., Dossena, A. and Marchelli, R. (2008). A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize-based products. *World Mycotoxin Journal,* **1 (3)**: 237-246.

De Bruijn, G.H. (1971). Etude du charatere cyanogenetique du manioc. *Mededelingen van de Landbouwhogeschool Wageningen*, **71**: 13–16.

De Bruijn, G.H. and Fresco, L.O. (1989). The importance of cassava in world food production. *Netherlands Journal of Agricultural Science*, **37**: 21–34.

Dimakopoulou, M., Tjamos, S.E., Antoniou, P.P., Pietri, A., Battilani, P., Avramidis, N., Markakis, E.A. and Tjamos, E.C. (2008). Evaluation and effectiveness of phyllosphere grapevine yeasts in reducing sour rot incidence, *Aspergillus carbonarius* presence and ochratoxin A contamination in wine producing vineyards in Greece. *Biological Control*, **46**: 158–165.

Doohan, F.M., Brennan, J. and Cooke, B.M. (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. *European Journal of Plant Pathology*, **109**: 755–768.

Dorner, J.W. and Lamb, M.C. (2006). Development and commercial use of afla-guard, an aflatoxin biocontrol agent. *Mycotoxin Research,* **21**: 33–38.

Douglas, M. (1966). Purity and danger. Penguin Books Ltd, London.

Dowd, P. F. (1998). Involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions. In:"Mycotoxins in agriculture and food safety", Sinhaand, K.K. and Bhatagnar, D. (Eds.) Marcel Dekker, New York, pp. 307–350.

Edwards, S.G. (2007a). Investigation of *Fusarium* mycotoxins in UK barley and oat production, HGCA, London. Available at: http://www.foodbase.org.uk/admintools/reportdocuments/48_91_CO4033_Final_report-_Investigation_of_fusarium_toxins_in_UK_.pdf

Edwards, S.G. (2007b). Investigation of *Fusarium* mycotoxins in UK wheat production, HGCA, London. Available at: http://www.foodbase.org.uk/admintools/reportdocuments/47_90_C04022_Final_report_-_Investigation_of_fusarium_toxins_in_UK.pdf

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

EFSA. (European Food Safety Authority) (2008a). Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. *The EFSA Journal*, **689**: 1–79.

EFSA. (2008b). Acrylamide carcinogenicity – New evidence in relation to dietary exposure. 11th EFSA Scientific Colloquium, 22 and 23 May 2008, Tabiano (PR), Italy.

Ermans, A.M., Mbuloamoko, N.M., Delange, F. and Ahluwalia, R. (Eds.) (1980). Role of cassava in the etiology of endemic goitre and cretinism. International Development and Research Centre, IDRC-136e, Ottawa, Canada, pp. 3–87.

Escobar-Gutierrez, A.J., Burns, I.G., Lee, A. and Edmonson, R.N. (2002). Screening lettuce cultivars for low nitrate content during summer and winter production. *Journal of Horticultural Science and Biotechnology*, **77**: 232–237.

Esser, A.J.A. (1995). Removal of cyanogens from cassava roots. PhD thesis, The Wageningen Agriculture University, Wageningen, The Netherlands. pp. 1–27.

European Commission. (2003). SCOOP TASK 3.2.10. Collection of occurrence data of *fusarium* toxins in food and assessment of dietary intake by the population of EU member states. European Commission, Brussels.

European Commission. (2006a). Commission recommendation of 17 August 2006 on the prevention and reduction of *Fusarium* toxins in cereals and cereal products. *Official Journal of the European Union*, **L234**: 35–40.

European Commission. (2006b). Commission Regulation (EC) No 1881/2006 setting maximum levels of certain contaminants in foodstuffs. *Official Journal of the European Union*, **L364**: 5–24.

European Commission. (2007). Regulation N° 1126/2007 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of European Union*, **255**: 14 –17.

FAO. (2001). Safety evaluation of certain mycotoxins in food. Prepared by the fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). FAO Food and Nutrition Paper No. 74. Rome, Italy.

Figueiredo, A.C., Barroso, J.G., Pedro, L.G. and Scheffer, J.J.C. (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavor and Fragrance Journal*, **23**: 213–226.

Firman, D.M. and Allen, E.J. (2007). Agronomic practices. In:"Potato biology and biotechnology, advances and perspectives,"Vreugdenhil, D (Ed.), Elsevier, Amsterdam, pp. 719–737.

Firman, D.M., O'Brien, P.J. and Allen, E.J. (1992). Predicting the emergence of potato sprouts. *Journal of Agricultural Science,* **118**, 55–61.

Fischer, B., Lüthy, J. and Schlatter, C. (1984). Gehaltbestimmung von Agaritin im Zuchtchampignon (*Agaricus bisporus*) mittels Hochleistungsflüssigchromatographie (HPLC). *Zeitschrift für Lebensmittel-untersuchung und -Forschung,* **A 179**: 218–223.

Galvano, F., Galofaro, V. and Galvano, G. (1996), Occurrence and stability of aflatoxin M1 in milk and milk products: a world wide review. *Journal of Food Protection*, **59**: 1079–1090.

Gareis, M., Bauer, J., Thiem, J., Plank, G., Grabley, S. and Gedek, B. (1990). Cleavage of zearalenone-glycoside, a masked mycotoxin, during digestion in swine, *Journal of Veterinary Medicine,* **B 37**: 236–240.

Gautier, P. (2007). Les 1ers enseignements des enquêtes parcellaires pluri annuelles en orges, 4éme Colloque Qualites de Céréales, Syngenta Agro, Versailles.

Granado, J., Thurig, B., Kieffer, E., Petrini, L., Fliessbach, A., Tamm, L., Weibel, F.P. and Wyss, G.S. (2008). Culturable fungi of stored 'Golden Delicious' apple fruits: a one-season comparison study of organic and integrated production systems in Switzerland. *Microbial Ecology*, **56**: 720–732.

Hajslova, J., Schulzova, V., Slanina, P., Janné, K., Hellenäs, K.E. and Andersson, Ch. (2005). Quality of organically and conventionally grown potatoes: four-year study of micronutrients, metals, secondary metabolites, enzymic browning and organoleptic properties. *Food Additives and Contaminants,* **22**: 514–534.

Harvan, D.I. and Pero, R.W. (1976). The structure and toxicity of *Alternaria* metabolites. *Advances in Chemistry,* **149**: 344–355.

Harwig, J., Scott, P., Stolz, D.R. and Blanchfield, B.J. (1979). Toxins of moulds from decaying tomato fruit. *Applied Environmental Microbiology,* **38**: 267–274.

Hoogenboom, L.A.P., Bokhorst, J.G., Northolt, M.D., van de Vijver, L.P.L., Broex, N.G.J., Mevius, D.J., Meijs, J.A.C. and van de Roest, J. (2008). Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products. *Food Additives and Contaminants*, **25**: 1195–1207.

Hope, R., Aldred, D. and Magan, N. (2005). Comparison of the effect of environmental factors on deoxynivalenol production by *F.culmorum* and *F.graminearum* on wheat grain. *Letters in Applied Microbiology*, **40**: 295–300.

Howard, P.C., Churchwell, M.I., Couch, L.H., Marques, M.M. and Doerge, D.R. (1998). Formation of *N*-carboxymethyl-fumonisin-B_1, following the reaction of fumonisin B_1 with reducing sugars. *Journal of Agricultural Science,* **46**: 3546–3557.

Hyde, M.B. and Galleymore, B.J. (1951). The sub-epidermal fungi of cereal grains II. The nature, identity and origin of the mycelium in wheat. *Annals of Applied Biology*, **38**: 348–356.

ICMSF (International Commission on Microbiological Specification for Foods). (1996). Toxigenic fungi: *Aspergillus*. In: "ICMSF, Microorganisms in foods. 5. Characteristics of food pathogens", Blackie Academic and Professional, London, pp. 347–381.

Imathiu, S.M. (2008). *Fusarium langsethiae* infection and mycotoxin production in oats. Ph D thesis, Harper Adams University College, Newport, Shropshire.

Jadhav, S.J., Sharma, R.P. and Salunkhe, D.K. (1981). Naturally occurring toxic alkaloids in foods. CRC Critical Reviews in Toxicology, **9**: 21–104.

Jestoi, M., Somma, M.C., Kouva, M., Veijalainen, P., Rizzo, A., Ritieni, A. and Peltonen, K. (2004). Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets. *Molecular Nutrition Food Research*, **48**: 299–307.

Kelly, J.D., Eaton, D.L., Guengerich, F.P. and Coulombe, R.J. (1997). Aflatoxin B_1 activation in human lung. *Toxicology and Applied Pharmacology,* **144**: 88–95.

Knuthsen, P., Jensen, U., Schmidt, B. and Larsen, I.K. (2008). Glycoalkaloids in potatoes: content of glycoalkaloids in potatoes for consumption. *Journal of Food Composition and Analysis*, **22**: 577–581.

Kuiper-Goodman, T. (2004), Risk assessment and risk management of mycotoxins in food. In: "Mycotoxins in food: detection and control", Magan, N. and Olsen, M. (Eds.), Woodhead Publishing, Cambridge, England, pp. 244–261.

Kuiper-Goodman, T. and Nawrot, P.S. (1993). Toxicological evaluation of certain food additives and naturally occurring toxicants; alfa-solanine and alfa-chaconine. *WHO Food Additives Series*, **30**: 339–372.

Kumar, D., Singh, B.P. and Kumar, P. (2004). An overview of the factors affecting sugar content of potatoes. *Annals of Applied Biology,* **145**: 247-256.

Lancaster, P.A., Ingram, J.S., Lime, M.Y. and Coursey, D.G. (1982). Traditional cassava based foods: survey of processing tchniques. *Economic Botany*, **36**: 12–45.

Lacey, J. (1986), Water availability and fungal reproduction: patterns of spore production, liberation and dispersal. In:"Water, Fungi and Plants", Ayres, P.G. and Boddy, L. (Eds.), Cambridge University Press, Cambridge, UK.

Lacey, J., Bateman, G.L. and Mirocha, C.J. (1999). Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. *Annals of Applied Biology*, **134**: 277–283.

Lancova, K., Hajslova, J., Poustka, J., Krplova, A., Zachariasova, M., Dostalek, P. and Sachambula, L. (2008). Transfer of *Fusarium* mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. *Food Additives and Contaminants*, **25**: 732–744.

Lawrence, M. and Shu, C.K. (1993). Essential oils as components of mixtures: their method of analysis and differentiation. In: "Flavor Measurement", Ho, C.T. and Manley, C.M. (Eds.), Marcel Dekker, New York, pp. 267–328.

Le Bars, J., Le Bars, P., Dupuy, J. and Boudra, H. (1994). Biotic and abiotic factors in fumonisins B_1 production and stability. *Journal of AOAC International*, **77**: 517–521.

Leong, L.T., Hien, T.V., An, N.T. and Trang, A.D. (2007). Ochratoxin A-producing *Aspergilli* in Vietnamese green coffee beans. *Letters in Applied Microbiology*, **45**: 301–306.

Liu, J.W., Beelman, R.B., Lineback, D.R. and Speroni, J.J. (1982). Agaritin content in fresh and processed mushrooms [*Agaricus bisporus* (Lange) Imbach]. *Journal of Food Science,* **47**: 1542–1544 and 1548.

Lynch, R.E. and Wilson, D.M. (1991). Enhanced infection of peanuts, *Arachis hypogea* L., seeds with *Aspergillus flavus* group due to external scarification of peanuts pods by the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). *Peanut Science,* **18**: 110–116.

Madhyastha, S.M., Marquardt, R.R., Frohlich, A.A., Platford, G. and Abramson, D. (1990). Effects of different cereal and oilseed substrates on the growth and production of toxins by *Aspergillus alutaceus* and *Penicillium verrucosum*'. *Journal of Agricultural and Food Chemistry*, **38**: 1506–1510.

Maga, J.A. (1980). Potato glycolakaloids. *CRC Critical Reviews in Food Science and Nutrition*, **12**: 371–405.

Maga, J.A. (1981). Total and individual glycolalkaloid composition of stored potato slices. *Journal of Food Processing and Preservation,* **5**: 23–29.

Magan, N. and Aldred, D. (2007). Post-harvest control strategies: minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, **119**: 131–139.

Magan, N. and Baxter, E.S. (1994). Environmental factors and tenuazonic acid production by *Alternaria* spp. isolated from sorghum. In:"Stored Product Protection", Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R., (Eds.), CABI BioSciences, Wallingford, U.K., pp. 1043–1046.

Magan, N. and Lacey, J. (1984a). Water relations of some *Fusarium* species from infected wheat ears and grain. *Transactions of the British Mycological Society*, **83**: 281–285.

Magan, N. and Lacey, J. (1984b). The effect of temperature and pH on the water relations of field and storage fungi. *Transactions of the British Mycological Society*, **82**: 71–81.

Magan, N. and, Lacey, J. (1985). The effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. In: "Trichothecenes and other mycotoxins", J.Lacey, J. (Ed.), Wiley and Sons, Oxford, U.K. pp. 243–256.

Magan, N., Cayley, G. and Lacey, J. (1984). The effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. *Applied Environmental Microbiology*, **47**: 1113–1117.

Magan, N., Hope, R., Colleate, A. and Baxter, E.S. (2002). Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. *European Journal of Plant Pathology,* **108**: 685–690.

Magan, N., Hope, R., Cairns, V. & Aldred, D. (2003). Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. *European Journal of Plant Pathology,* **109**: 723-730.

Magan, N., Olsen, M. and Aldred, D. (2008). Prevention strategies for trichothecenes and ochratoxin in cereals. In: "Mycotoxins: detection methods, management, public health and agricultural trade", Leslie, J., Bandyopadhyay, R. and Visconti, A. (Eds.), CABI BioSciences, Wallingford, U.K., pp. 369–383.

Mantle, P.G., Faucet-Marquis, V., Manderville, R.A., Squilaci, B. and Pfohl-Leszkowicz, A. (2010). Structures of covalent adducts between DNA and ochratoxin A: a new factor in debate about genotoxicity and human risk assessment. *Chemical Research in Toxicology,* **23**: 89–98.

Marin, S., Sanchis, V., Saenz, R., Ramos, A.J., Vinas, I. and Magan, N. (1998). 'Ecological determinants for germination and growth of some *Aspergillus* and *Penicillium* spp. from maize grain'. *Journal of Applied Microbiology*, **84**: 25–36.

Marin, S., Magan, N., Serra, J., Ramos, A.J., Canela, R. and Sanchis, V. (1999a). Fumonisin B_1 production and growth of *Fusarium moniliforme* and *Fusarium proliferatum* on maize, wheat, and barley grain. *Journal of Food Protection*, **64**: 921–924.

Marin, S., Magan, N., Belli, N., Ramos, A.J., Canela, R. and Sanchis, V. (1999b). Two-dimensional profiles of Fumonisin B_1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain'. *International Journal of Food Microbiology*, **51**: 159–167.

Marasas, W.F.O., Gelderblom, W.C.A., Shepard, G.S. and Vismer, H.F. (2008). Mycotoxins: a global problem. In: "Mycotoxins: detection methods, management, public health and agricultural trade". Leslie, J.F., Bandyopadhyay, R. and Visconti, A. (Eds.), CABI BioSciences, Wallingford, U.K., pp. 29–39.

McManus, B.M., Toth, B. and Patil, K.D. (1987). Aortic rupture and aortic smooth muscle tumors in mice. Induction by *p*-hydrazinobenzoic acid hydrochloride of the cultivated mushroom *Agaricus bisporus*. *Laboratory Investigations*, **57**: 78–85.

Medina, A., Mateo, R., Valle-Algarra, F.M., Mateo, E.M. and Jimenez, M. (2007). Effect of carbendazim and physicochemical factors on the growth and ochratoxin A production of *Aspergillus carbonarius* isolated from grapes. *International Journal of Food Microbiology*, **119**: 230–235.

Mehrnejad, M.R. and Panahi, B. (2006). The influence of hull cracking on aflatoxin contamination and insect infestation in pistachio nuts. *Applied Entomology and Phytopathology*, **73**: 39–42.

Merino, L., Darnerud, P.O., Edberg, U., Åman, P. and Castillo, M.D.P. (2006). Levels of nitrate in Swedish lettuce and spinach over the past 10 years. *Food Additives and Contaminants*, **23**: 1283–1289.

Meronuck, R.A., Steele, J.A., Mirocha, C.J. and Christensen, C.H. (1972). Tenuazonic acid, a toxin produced by *Alternaria alternate. Applied Microbiology*, **23**: 613–617.

Miele, M., Dondero, R., Ciarallo, G. and Mazzei, M., 2001. Methyleugenol in *Ocimum basilicum* L. Cv, Genovese Gigante. *Journal of Agricultural and Food Chemistry*, **49**: 517–521.

Minguez, S., Cantus, J.M., Pons, A., Margot, P., Cabanes, F.X., Masque, C., Accensi, F., Elorduy, X., Giralt, L.L., Vilavella, M., Rico, S., Domingo, C., Blasco, M. and Capdevila, J. (2004). Influence of the fungus control strategy in the vineyard on the presence of Ochratoxin A in the wine. *Bulletin de l'OIV*, **77**: 821–831.

Miraglia, M., Marvin, H.J.P., Kleter, G.A., Battilani, P., Brera, C., Coni, E., Cubadda, F., Croci, L., De Santis, B., Dekkers, S., Filippi, L., Hutjes, R.W.A., Noordam, M.Y., Pisante, M., Piva, G., Prandini, A., Toti, L., van den Born, G.J. and Vespermann, A. (2009). Climate change and food safety: an emerging issue with special focus on Europe. *Food Chemistry and Toxicology,* **47**:1009–1021.

Mislevic, P.B. and Bruce, V.R. (1977). Direct vs dilution plating in qualitatively determining the mould flora of dried beans and soy beans. *Journal of the Association of Official Analytical Chemists*, **60**: 742–743.

Mondy, N.I., Leja, M. and Gosselin, B. (1987). Changes in total phenolic, total glycoalkaloid, and ascorbic acid content of potatoes as a result of bruising. *Journal of Food Science*, **52**: 631–633.

Munkvold, G.P. and Desjardin, A.E. (1997). *Fumonisin* in maize. Can we reduce their occurrence? *Plant Disease*, **81**: 556–565.

Munkvold, G.P. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology*, **109**: 705–713.

Northolt, M.D., van Egmond, H.P. and Paulsch, W.E. (1979). Ochratoxin A production by some fungal species in relation to water activity and temperature. *Journal of Food Protection*, **42**: 485–490.

Olsson, K. (1986). The influence of genotype on the effects of impact damage on the accumulation of glucoalkaloids in potato tubers. *Potato Research*, **29**: 1–12.

Osuntokun, B.O. (1981). Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. *World Review of Nutrition and Dietetics*, **36**: 141–173.

Padmaja, G. (1995). Cyanide detoxification in cassava for food and feed use. *Critical Reviews in Food Science and Nutrition*, **35**: 229–239.

Pariera Dinkins, C.L., Peterson, R.K.D., Gibson, J.E., Hu, Q. and Weaver, D.K. (2008). Glycoalkaloid responses of potato to Colorado potato beetle defoliation. *Food and Chemical Toxicology,* **46**: 2832–2836.

Parry, D.W., Jenkinson, P. and McLeod, L. (1995). *Fusarium* ear blight (scab) in small grain cereals – a review. *Plant Pathology,* **44**: 207–238.

Payne, G.A. (1998). Process of contamination by aflatoxin-producing fungi and their impact on crops. In:"Mycotoxins in agriculture and food safety", Sinha, K.K. and Bhatnagar, D. (Eds.), Marcel Dekker Inc, NewYork, USA, pp. 279–306.

Pero, R.W., Posner, H., Harvan, D. and Spalding, J.W. (1973a). Toxicity of metabolites produced by the *Alternaria. Environmental Health Perspectives*, **4**: 87–94.

Pero, R.W., Harvan, D and Blois, M. (1973b). Isolation of the toxin altenuisol, from the fungus *Alternaria tenuis* Auct. *Tetrahedron Letters*, **12**: 945–948.

Petersen, A. and Stoltze, S. (1999). Nitrate and nitrite in vegetables on the Danish market: content and intake. *Food Additives and Contaminants*, **16**: 291–299.

Pettersson, H., Börjesson, T., Persson, L., Lerenius, C., Berg, G. and Gustafsson, G. (2008). T-2 and HT-2 toxins in oats grown in nothern Europe. *Cereal Research Communications*, **36**: 591–592.

Pieris, N., Jansz E.R. and Kandage, R. (1974). Cyanogenic glucosides content of manioc. 1, enzymic method of determination applied to processed manioc. *Journal of Nutritional Science Council of Sri Lanka*, **2**: 67–76.

Pilegaard, K., Eriksen, F.D., Soerensen, M. and Gry, J. (2007). EuroFIR-NETTOX Plant List. European Food Information Resource Consortium (EuroFIR). ISBN 0 907 667 570.

Poling, S.M., Plattner, R.D. and Weisleder, D. (2002). *N*-(1-deoxy-D-fructose-1-yl) fumonisin B_1, the initial reaction product of fumonisin B_1 and D-glucose. *Journal of Agricultural and Food Chemistry,* **50**: 1318–1324

Poppenberger, B., Berthiller, F., Bachmann, H., Lucyshyn, D., Peterbauer, C., Mitterbauer, R., Schuhmacher, R., Krska, R., Glössl, J. and Adam, G. (2006). Heterologous expression of Arabidopsis UDP-glucosyltransferases in *Saccharomyces cerevisiae* for production of zearalenone-4-O-glucoside. *Applied Environmental Microbiology*, **72**: 4404–4410.

Ramos, A.J., Labernia, N., Marin, S., Sanchis, V. and Magan, N. (1998). Effect of water activity and temperature on growth and ochratoxin production by three strains of *Aspergillus ochraceus* on a barley extract medium and on barley grains. *International Journal of Food Microbiology*, **44**: 133–140.

Ravi, S. and Padmaja, G. (1997). Mechanism of cyanogen reduction in cassava roots during cooking. *Journal of the Science of Food and Agriculture,* **75**: 427–432.

Ribnicky, D.M., Poulev, A., O'Neal, J., Wnorowski, G., Malek, D.E., Jager, R. and Raskin, I. (2004). Toxicological evaluation of the ethanolic extract of *Artemisia dracunculus* L. for use as a dietary supplement and in functional foods. *Food and Chemical Toxicology,* **42**: 585–598.

Ross, A.E., Nagel, D.L. and Toth, B. (1982). Evidence for the occurrence and formation of diazonium ions in the *Agaricus bisporus* mushroom and its extracts. *Journal of Agricultural and Food Chemistry*, **30**: 521–525.

Ryu, D. and Bullerman, L.B. (1999). Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. *Journal of Food Protection*, **62**: 1451–1455.

Sanchis, V. and Magan, N. (2004). Environmental conditions affecting mycotoxins. In: "Mycotoxins in food: detection and control", Magan, N. and Olsen, M. (Eds.) Woodhead Publishing Ltd, Cambridge, U.K. pp. 174–189.

Sanchis, V., Sala, N., Palomes, A., Santamarina, P. and Burdaspal, P. (1986). Ocurrence of aflatoxin and aflatoxigenic molds in foods and feed in Spain. *Journal of Food Protection*, **49**: 445–448.

Santamaria, P., Elia, A., Serio, F. and Todaro, E. (1999). A survey of nitrate and oxalate content in fresh vegetables. *Journal of the Science of Food and Agriculture*, **79**: 1882–1888.

Santamaria, P., Elia, A. and Serio, F. (2002). Effect of solution nitrogen concentration on yield, leaf element content, and water and nitrogen use efficiency of three hydroponically-grown rocket salad genotypes. *Journal of Plant Nutrition*, **25**: 245–258.

Sauer, D.B., Seitz, L.M., Burroughs, R., Mohr, H.E. and West, J.L. (1978). Toxicity of *Alternaria* metabolites found in weathered sorghum grain at harvest. *Journal of Agricultural and Food Chemistry,* **26**: 1380–1383.

Scheidegger, K.A. and Payne, G.A. (2005). Unlocking the secrets behind secondary metabolism: a review of *Aspergillus flavus* from pathogenicity to functional genomics. In: "Aflatoxin and food safety", Abbas, H.K. (Ed.), CRC Taylor and Francis, Boca Raton, FL, pp. 137–165.

Schneweis, I., Meyer, K., Engelhardt, G. and Bauer, J. (2002). Occurrence of zearalenone-4-β-d-glucopyranoside in wheat. *Journal of Agricultural and Food Chemistry*, **50**: 1736–1738.

Schroeder, H.W. and Cole, R.J. (1977). Natural occurrence of alternariols in discoloured pecans. *Journal of Agricultural and Food Chemistry*, **25**: 204–206.

Schulzova, V., Hajslova, J., Peroutka, R., Gry, J. and Andersson, H.C. (2002). Influence of storage and household processing on the agaritin content of the cultivated *Agaricus* mushroom. *Food Additives and Contaminants*, **19**: 853–862.

Seefelder, W., Knecht, A. and Humpf, H.U. (2003). Bound fumonisin B_1: analysis of fumonisin-B_1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry,* **51**: 5567–5573.

Sharman, M., Patey, A.L. and Gilbert, J. (1990). A survey of the occurrence of agaritin in U.K. cultivated mushrooms and processed mushroom products. *Food Additives and Contaminants*, **7**: 649–656.

Sheen, L.Y., Tsai Ou, Y.H. and Tsai, S.J. (1991). Flavor characteristic compounds found in the essential oil of *Ocimum basilicum* L. with sensory evaluation and statistical analysis. *Journal of Agricultural and Food Chemistry*, **39**: 939–943.

Shrisvastava, A. and Jain, P.C. (1992). Seed mycoflora of some spices. *Journal of Food Science and Technology, India*, **29**: 228–230.

Simpson, D.R., Weston, G.E., Turner, J.A., Jennings, P. and Nicholson, P. (2001). Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European Journal of Plant Pathology*, **107**: 421–431.

Speijers, G.J.A. (1993). Cyanogenic glycosides, toxicological evaluation of certain food additives and naturally occurring toxicants. In:, "WHO Food Additives Series, 30", World Health Organization, Geneva, Switzerland, p. 299.

Speijers, G.J.A. (1998). Risk assessment of potato-glycoalkaloids. In: "Inherent Food Plant Toxicants, Report No. 7, Nettox Graz Seminar on Inherent Food Plant Toxicants", Gry, J., Aune, T., Busk, L., Speijers, G.J.A., Strigl, A. and Soborg, I. (Eds.), Danish Veterinary and Food Administration, Denmark, pp. 43–47.

Speroni, J.J., Beelman, R.B. and Schisler, L.S. (1983). Factors influencing the agaritin content in cultivated mushrooms, *Agaricus bisporus. Journal of Food Protection*, **46**: 506–509.

Swiniarski, E. and Ladenberger, D. (1970). The sugar content of potato tubers grown with different rates of nitrogen application. *Potato Research*, 13: 114-118.

Teles, F.F. (2002). Chronic poisoning by hydrogen cyanide in cassava and its prevention in Africa and Latin America. *Food and Nutrition Bulletin*, **23**: 407–412.

Tjamos, S.E., Antoniou, P.P., Kazantzidou, A., Antonopoulos, D.F., Papageorgiou, I. and Tjamos, E.C. (2004). *Aspergillus niger* and *Aspergillus carbonarius* in Corinth raisin and wine-producing vineyards in Greece: population composition, Ochratoxin A production and chemical control. *Journal of Phytopathology*, **152**: 250–255.

Toth, B. (1986). Carcinogenesis by *N2*-[γ-L-(+)-glutamyl]-4-carboxy-phenylhydrazine of *Agaricus bisporus* in mice. *Anticancer Research*, **6**: 917–920.

Toth, B. and Erickson, J. (1986). Cancer induction in mice by feeding of the uncooked cultivated mushroom of commerce *Agaricus bisporus. Cancer Research*, **46**: 4007–4011.

Toth, B., Raha, C.R., Wallcave, L. and Nagel, D. (1981). Attempted tumor induction with agaritin in mice. *Anticancer Research*, **1**: 255–258.

Toth, B., Nagel, D. and Ross, A. (1982). Gastric tumorigenesis by a single dose of 4 (hydroxymethyl) benzenediazonium ion of *Agaricus bisporus. British Journal of Cancer*, **46**: 417–422.

Toth, B., Erickson, J., Gannett, P. and Patil, K. (1997). Carcinogenesis by the cultivated baked *Agaricus bisporus* mushroom in mice. *Oncology Reports*, **4**: 931–936.

Tsai, S.J. and Sheen, L.Y. (1987). Essential oil of *Ocimum basilicum* L. cultivated in Taiwan. In: "Trends in Food Science" Proceedings of the 7th World Congress of Food Science and Technology, Singapore. Sze, L.W. and Woo, F.C. (Eds.) Institute of Food Science and Technology, Singapore, pp. 66–70.

Valkonen, J.P.T., Keskitalo, M., Vasara, T. and Pietila, L. (1996). Potato glycoalkaloids: a burden or a blessing? *Critical Reviews in Plant Science*, **15**: 1–20.

Valero, A., Marin, S., Ramos, A.J. and Sanchis, V. (2007). Effect of preharvest fungicides and interacting fungi on *Aspergillus carbonarius* growth and ochratoxin A synthesis in dehydrating grapes. *Letters in Applied Microbiology*, 45: 194–199.

van Egmond, H.P., Schothorst, R.C. and Jonker, M.A. (2007). Regulations relating to mycotoxins in food. Perspectives in a global and European context. *Analytical and Bioanalytical Chemistry*, **389**: 147–157.

van Gelder, W.M.J. (1989). Steroidal glycoalkaloids in *solanum* species: consequences for potato breeding and food safety. PhD thesis, Agriculture University Wageningen, Netherlands, pp. 9–184.

van Velzen, A.G., Sips, A.J.A.M., Schothorst, R.C., Lambers, A.C. and Meulenbelt, J. (2008). The oral bioavailability of nitrate from nitrate-rich vegetables in humans. *Toxicology Letters*, **181**: 177–181.

Varga, J., Kevei, E., Rinyu, E., Teren, J. and Kozakiewicz, Z. (1996). Ochratoxin production by *Aspergillus* species. *Applied Environmental Microbiology,* **62**: 4451–4464.

Vendl, O., Berthiller, F., Crews, C. and Krska, R. (2009). Simultaneous determination of deoxynivalenol, zearalenone, and their major masked metabolites in cereal-based food by LC-MS-MS. *Analytical and Bioanalytical Chemistry,* **395**: 1347–1354.

Veronesi, L., Camisa, M.G., Politi, A. and Serrati, L. (2006). Use of Switch against secondary moulds in the [grape] bunch. Impiego di SwitchReg. controli marciumi secondari del grappolo. *Vignevini*, **33**: 74–77.

Versari, A., Parpinello, G.P., Mattioli, A.U. and Galassi, S. (2008). Patulin contamination in organic and conventional apple juices. *Industrie Bevande*, **37**: 219–223.

Versonder, R.F., Ellis, J.J., Kwolek, W.F. and DeMarini, D.J. (1982). Production of vomitoxin on corn by *Fusarium graminearum* NRRL 5883 and *Fusarium roseum* NRRL 6101. *Applied Environmental Microbiology*, **43**, 967–970.

Visconti, A., Sibilia, A. and Sabia, C. (1992). *Alternaria alternata* from oilseed rape: mycotoxin production, and toxicity to *Artemia salina* larvae and rape seedlings. *Mycotoxin Research*, **8**: 9–16.

Wiersma, J.J. and Motteberg, C.D. (2005). Evaluation of five fungicide application timings for control of leaf-spot diseases and *Fusarium* head blight in hard red spring wheat. *Canadian Journal of Plant Pathology*, **27**: 25–37.

Wigginton, M.J. (1974). Effects of temperature, oxygen tension and relative humidity on the wound-healing process in the potato tuber. *Potato Research*, **17**: 200–214.

Williams, C.M. (2002). Nutritional quality of organic food: shades of grey or shades of green? *Proceedings of the Nutrition Society,* **61**: 19–24.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing counties: a review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition*, **80**: 1106–1122.

Yoshida, M., Nakajima, T., Arai, M., Suzuki, F. and Tomimura, K. (2008). Effect of the timing of fungicide application on *Fusarium* head blight and mycotoxin accumulation in closed-flowering barley. *Plant Disease*, **92**: 1164–1170.

Young, A.B., Davis, N.D. and Diener, U.L. (1980). The effect of temperature and moisture on tenuazonic acid production by *Alternaria tenuissima. Phytopathology*, **70**: 607–609.

LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
AE	Altenuene
AME	Altenariol monomethyl ether
AOH	Altenariol
a_w	Water activity
BCA	Biological control agents
CODEX	FAO/WHO Codex Alimentarius Commission
DON	Deoxynivalenol
EC	European Commission
ECB	European Corn Borer
EFSA	European Food Safety Authority
EU	European Union
FAO	Food Agriculture Organization of the United Nations
FB_1	Fumonisin B_1 (or 2,3,4)
FHB	*Fusarium* head blight
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
HT-2	HT-2 mycotoxin; trichotecene
JECFA	Joint Expert Committee on Food Additives of FAO and WHO
LOEL	Lowest Observable Effect Level
mc	Moisture content
NIV	Nivalenol
NO(A)EL	No Observed (Adverse) Effect Level
OTA	Ochratoxin A
OTB	Ochratoxin B
T-2	T-2 mycotoxin; trichotecenes
TA	Toxins of *Altenaria* species
TAN	Tropical ataxic neuropathy
TDI	Tolerable daily intake
TGA	Total glycoalkaloids
WHO	World Health Oragnisation of the United Nations
ZEA	Zearalenol

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