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Light regulation of mycotoxin biosynthesis: new perspectives for food safety

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REVIEW ARTICLE

Abstract

Mycotoxins are secondary metabolites produced by toxigenic fungi contaminating foods and feeds in pre-, post-harvest and processing, and represent a great concern worldwide, both for the economic implications and for the health of the consumers. Many environmental conditions are involved in the regulation of mycotoxin biosynthesis. Among these, light represents one of the most important signals for fungi, influencing several physiological responses such as pigmentation, sexual development and asexual conidiation, primary and secondary metabolism, including mycotoxin biosynthesis. In this review we summarise some recent findings on the effect of specific light wavelength and intensity on mycotoxin biosynthesis in the main toxigenic fungal genera. We describe the molecular mechanism underlying light perception and its involvement in the regulation of secondary metabolism, focusing on VeA, global regulator in *Aspergillus nidulans*, and the White-Collar proteins, key components of light response in *Neurospora crassa*. Light of specific wavelength and intensity exerts different effects both on growth and on toxin production depending on the fungal genus. In *Penicillium* spp. red (627 nm) and blue wavelengths (455-470 nm) reduce ochratoxin A (OTA) biosynthesis by modulating the level of expression of the ochratoxin polyketide synthase. Furthermore a mutual regulation between citrinin and OTA production is reported in *Penicillium* toxigenic species. In *Aspergillus* spp. the effect of light treatment is strongly dependent on the species and culture conditions. Royal blue wavelength (455 nm) of high intensity (1,700 Lux) is capable of completely inhibit fungal growth and OTA production in *Aspergillus stenyii* and *Penicillum verrucosum*. In *Fusarium* spp. the effect of light exposure is less effective; mycotoxin-producing species, such as *Fusarium verticillioides* and *Fusarium proliferatum*, grow better under light conditions, and fumonisin production increased. This review provides a comprehensive picture on light regulation of mycotoxin biosynthesis and discusses possible new applications of this resource in food safety.

Keywords: light, mycotoxins, *Aspergillus*, *Penicillium*, *Fusarium,* food safety

1. Introduction

Mycotoxins are secondary metabolites (SM) produced by toxigenic fungi that contaminate foods and feeds in pre-, post-harvest and processing and represent a great concern worldwide either for the economic implications and for public health. International trade in agricultural commodities such as wheat, rice, barley, corn, sorghum, soybeans, groundnuts and oilseeds amounts to hundreds of millions of tons each year. Many of these commodities are subjected to a high risk of mycotoxin contamination. The Food and Agriculture Organization of the United Nations (FAO) estimated that each year, between 25 and 50% of the world's food crops are contaminated by mycotoxins (FAO, 1988; Mannon and Johnson, 1985).

The mycotoxins of most concern from a food safety perspective include aflatoxins, ochratoxin, toxins produced by *Fusarium* spp., such as fumonisins, trichothecenes and zearalenone (ZEA) and *Alternaria* toxins (Desjardins and Proctor, 2007). These molecules have different chemical structures and biosynthetic pathways and can cause a variety of diseases in human and animals with a wide range of severity (Bennett and Klich, 2003).

Being SM, mycotoxin production is not directly related to growth or to sexual development of fungi (Betina, 1984; Fox and Howlett, 2008). The reason of their synthesis is thought to be connected to the interaction with the host (Desjardins *et al.*, 1989) and the environment (Sanchis and Magan, 2004), enabling a better adaptation under certain conditions. The conditions favouring mycotoxin production are related to the development of fungal biomass and the colonisation of the host by the pathogen (Arias *et al.*, 2012; Desmond *et al.*, 2008; Xu *et al.*, 2007). Stressing conditions, such as non-optimal temperature, osmotic pressure and pH, also play a role in the activation of the mycotoxin biosynthetic pathway (Schmidt-Heydt *et al.*, 2008).

One of the most intriguing aspect which has recently aroused great interest among scientific community is the influence of light on secondary metabolism. Light is one of the most important signals for fungi, since it influences several physiological responses such as pigmentation, sexual development, asexual conidiation and the circadian clock. (Lee *et al.*, 2000; Purshwitz *et al.*, 2006). The connection among these pathways was demonstrated at genetic level in model organisms, such as *Aspergillus nidulans* (Busch *et al.*, 2003; Kato *et al.,* 2003; Kim *et al.*, 2002). In recent years the knowledge on molecular aspects of light sensing in fungi greatly increased. This review summarises some recent findings on light regulation of mycotoxin biosynthesis in the major toxigenic fungal genera (*Penicillium*, *Aspergillus* and *Fusarium*), providing a comprehensive picture on this topic and discussing a possible new application of this resource in food safety.

2. Mycotoxins: a worldwide concern

According to the FAO (FAO, 1988) mycotoxin contamination causes global losses of foodstuffs in the range of billion tons per year (Boutrif and Canet, 1998) with annual losses of around 1 billion metric tons of foods and food products. Strategies for controlling mycotoxin are mainly preventive. Good agricultural practices in both pre and post-harvest underlies all the guidelines for reducing mycotoxin contamination. These practices were coded and reported in official documents (e.g. Commission Recommendation 2006/583/EC and Commission Regulation (EC) no. 1881/2006 (EC, 2006a,b)). Besides performing risk assessments at the beginning of the sowing time, in these practices the choice of more resistant varieties, the use of appropriate drying techniques, the control of weed and insect population, the use of fertilisers and fungicides, a timely harvesting and the maintenance of proper storage facilities and conditions are recommended.

Methods of detoxification were proposed in the past but they are virtually non-usable due to the high stability of mycotoxin compounds and the uncertainty about the residual toxicity of the reaction products. As example, ammoniation, which was demonstrated to be efficient in reducing the toxicity of aflatoxin in cottonseed and cottonseed meal (Bailey *et al.*, 1994), is only allowed in some states and has not been approved by the US Food and Drug Administration, due to the potential toxicity and carcinogenicity of the reaction product. Furthermore, except for biological detoxification approaches, treatments required for chemical or physical detoxification would necessarily lead to quality reduction and alteration of the final products.

Among the new technologies proposed for mycotoxin reduction, light treatments could represent an useful approach, considering several advantages, including the limited cost and the safety use of light-emitting devices, below discussed. The main mycotoxins treated in this study (Figure 1) are aflatoxins, ochratoxin A (OTA), fumonisins and citrinin (CIT).

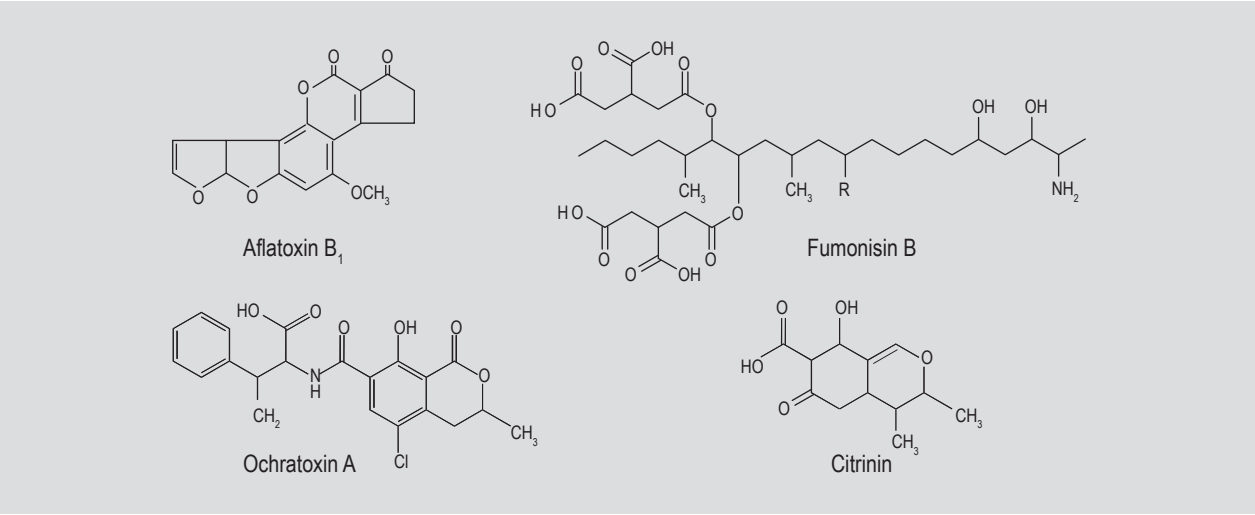

Figure 1. The main mycotoxins described in this review.

Aflatoxins are potent liver carcinogenic toxins produced by *Aspergillus* spp., in particular by *Aspergillus flavus*, *Aspergillus parasiticus* (Codner *et al.*, 1963; Schroeder, 1966). Aflatoxins are structurally-related difuranocoumarin derivates produced through 23 enzymatic reactions and 15 intermediate precursors (Meyers *et al.*, 1998). The 25 genes involved in aflatoxins biosynthesis, clustered in a 75-kb DNA region (Bhatnagar *et al.*, 2006) on chromosome 3 in *A. flavus* (Chang *et al.*, 1993; Payne *et al.*, 2006; Woloshuk *et al.*, 1994; Yu *et al.*, 2004). The sequences of the aflatoxin genes are highly conserved among *A. flavus* and *A. parasiticus* (Yu *et al.*, 1995).

OTA is a nephrotic mycotoxin produced by several species belonging to the genera *Aspergillus* and *Penicillium*. OTA is a stable compound which is not destroyed by common food production procedures. Indeed, even a heat treatment at temperatures above 250 °C for several minutes is insufficient to completely destroy the molecule (Boudra *et al.*, 1995), demonstrating its high stability.

Fumonisins are a group of mycotoxins associated with several mycotoxicoses, including equine leukoencephalomalacia, porcine pulmonary oedema and experimental kidney and liver cancer in rats (Ross *et al.*, 1993). The main source of fumonisin contamination worldwide is *Fusarium verticillioides* (Desjardins *et al.*, 1995), although fumonisin production has been associated with several other fungal species, belonging mainly to the genus *Fusarium* (*Fusarium napiforme, Fusarium fujikuroi, Fusarium proliferatum, Fusarium subglutinans, Fusarium sacchari*) (Nelson *et al.*, 1993; Shephard *et al.*, 1996). Analogues of this toxin are also synthetised by *Aspergillus niger* (Frisvad *et al.*, 2007) and *Alternaria alternata* (Chen *et al.*, 1992). At molecular level fumonisin production is achieved by a biosynthetic (*FUM*) cluster that consists of 17 co-regulated genes (Brown *et al.*, 2007; Proctor *et al.*, 2003; Seo *et al.*, 2001). The *FUM* cluster is activated under fumonisin producing inducible conditions (Proctor *et al.*, 2003). Many environmental factors affect fumonisin production. Among these a pivotal role is attributed to temperature, water availability and moisture conditions during the growing season and during storage (Fanelli *et al.*, 2013; Frisvad *et al.*, 2007; Marin *et al.* 1995; Mogensen *et al.*, 2009).

CIT was first isolated from *Penicillium citrinum* in 1931 (Hetherington and Raistrick, 1931) and is produced also by several other species of *Penicillium*, by some species of *Aspergillus* (Ciegler *et al.*, 1977; Deruiter *et al.*, 1992; Nelson *et al.*, 1980; Pollock, 1947; Reiss, 1977; Turner, 1971) and recently isolated from *Monascus ruber* and *Monascus purpureus* (Manabe, 2001). Most genes of the biosynthetic pathway of CIT biosynthesis in *Monascus* are known (Shimizu *et al.*, 2007). CIT is a polyketide and structurally it closely resembles the polyketide part of OTA, which carries the amino acid phenylalanine as an additional ligand via a peptide bridge. In addition, OTA contains chlorine at position 5 of the polyketide moiety. As OTA, CIT is also a nephrotoxin with varying toxicological effects depending on the target species (Flajs and Peraica, 2009).

3. Light sensing in fungi

The ability to sense light is crucial for many organisms determining timing and modality of development and orienting primary and secondary metabolic processes (for reviews: Bayram and Braus, 2012; Corrochano, 2011; Purschwitz *et al.*, 2008; Rodriguez-Romero *et al.*, 2010, Tisch and Schmoll, 2010). Due to the increased number of sequenced fungal genomes, numerous genes encoding proteins involved in light sensing and downstream gene regulation have been identified. This expedited understanding the mechanisms by which the fungus activates physiological and morphological reactions (Corrochano, 2007; Herrera-Estrella and Horowitz, 2007) in response to light.

The first knowledge about the influence of light on growth and physiology of fungi was obtained on *Neurospora crassa*. The circadian-regulated generation of asexual spores in race tubes was one of the first reasons encouraging researchers to analyse the influence of light on fungi (Maheshwari, 1991). This initial observation finally led to the unravelling of the molecular background of light regulation (Dunlap, 1993). *N. crassa* is the model organism for studying the light sensing system; in this species light perception and the response to blue light incubation (Ballario and Macino, 1997; Ballario *et al.*, 1996) have been elucidated at molecular level.

Light sensing in fungi involves several proteins, including photoreceptors, oscillators, signalling proteins and transcriptional activators, acting in concert to switch and regulate pathways belonging to primary and secondary metabolism, circadian rhythm, sexual development, conidiation, etc. In the last 50 years, hundreds of fungal species were found to react to light, having perception mechanism for blue, near UV, green and red light (Herrera-Estrella and Horowitz, 2007; Marsh *et al.*, 1959; Purschwitz *et al.*, 2006). Below are reported the main actors in fungal light response, characterised in two model organism: VeA in *A. nidulans* and White-Collar (WC) proteins in *N. crassa*.

The velvet protein VeA

The velvet gene (*veA*) was firstly characterised by Käfer (1965) in *A. nidulans*. The molecular analysis of this gene revealed that its gene product, VeA, is not a light sensor (Kim *et al.*, 2002), but a light-dependent global regulator of asexual/sexual development (Champe *et al.*, 1981; Yager *et al.*, 1992) and secondary metabolism (Calvo *et al.*, 2004, 2008; Cary *et al.*, 2007; Duran et al, 2007; Kato *et*

al., 2003). VeA regulates the expression of genes involved in the synthesis of sterigmatocystin (STE) in *A. nidulans* and aflatoxins in *A. flavus* (Kato *et al.*, 2003). Both SM are synthesised by the same biosynthetic pathway. Indeed STE, the end-product in *A. nidulans,* is the next-to-last precursor of aflatoxin biosynthetic pathway found in the closely related fungi *A. flavus* and *A. parasiticus* (Keller *et al.*, 1994). The VeA protein has an N-terminal domain which is highly conserved across fungal genera and a putative PEST domain in the C-terminal region (Kim *et al.*, 2002). Although VeA is not a photoreceptor, it shows light-dependent mobility in *A. nidulans.* Under light exposure it occurs abundantly in the cytoplasm, associated with filamentous bodies, while in the dark it is transferred into the nuclei by the aid of integrins. There it is functionally active and interacts in concert with other proteins forming the velvet complex (Stinnett *et al.*, 2007); it supports sexual reproduction and secondary metabolism in the dark and sporulation under light conditions (Fischer, 2008). The complex is constituted by the phytochrome-like red-light receptor FphA (Purschwitz *et al.*, 2008) and LreA and LreB, which are orthologues of *N. crassa* blue-light responsive elements White-Collar1 (WC-1) and White-Collar2 (WC-2) (Bayram *et al.*, 2008). FphA (Purschwitz *et al.*, 2008) is the boundary between VeA, LreA and LreB, with whom it interacts directly. In *A. nidulans* FphA represses the sexual development and the formation of STE (Blumenstein *et al.*, 2005). An overview of the interaction among VeA and other proteins during light perception is given in Figure 2.

The *veA* gene is highly conserved among the fungal genera, where orthologues of the velvet gene have been identified and functionally characterised (Bayram *et al.*, 2008; Dreyer *et al.*, 2007; Li *et al.*, 2006;) in the genome of several filamentous fungi, especially ascomycetes. In *F. verticillioides* (Li *et al.*, 2006) the VeA othologue (FvVE1) is involved in the regulation of the cell wall integrity, cell surface hydrophobicity, hyphal polarity and conidiation. Although VeA and FvVE1 present differences in the function and in the cellular processes they modulate, both regulate secondary metabolism (Wieman *et al.*, 2010). Indeed in *F. verticillioides* FvVE1 deletion mutants the production of fumonisins and fusarins is repressed (Myung *et al.*, 2009).

In *Penicillium chrysogenum* components of a velvet-like complex were firstly identified by Hoff *et al.* (2010). Core components of this complex are *P. chrysogenum* VelA (PcVelA) and LaeA (PcLaeA), which regulate secondary metabolite production, hyphal morphology, conidiation, and pellet formation. *P. chrysogenum* VelB (PcVelB), VelC (PcVelC), and VosA (PcVosA) are subunits of this velvet complex (Kopke *et al.*, 2013) with opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation. PcVelB acts as an activator and PcVelc as a repressor of conidiospore formation, while they exerted the

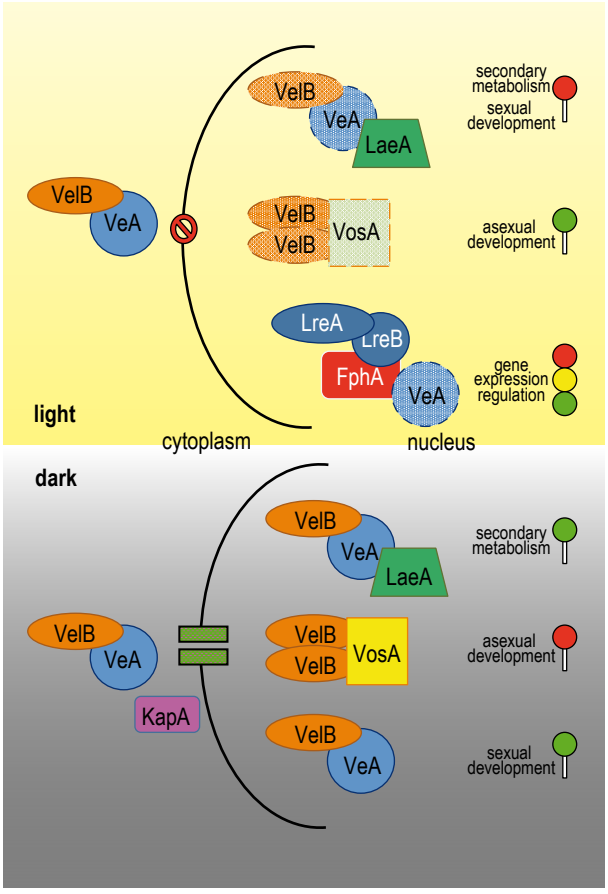

Figure 2. Model for VeA localisation and function in *Aspergillus nidulans* (adapted from Palmer *et al.*, 2013). Under dark conditions VeA is imported in the nucleus by the interaction with the importin α (KapA). In the nucleus VelA-VelB dimer activates sexual development, VelB-VosA dimer represses asexual development, LaeA-VelA-VelB heterotrimeric velvet complex activates secondary metabolism. Under light condition VeA is retained in the cytoplasm. The cellular levels of VosA and VelB decreased, allowing asexual reproduction. Secondary metabolism and sexual development are repressed. The interaction between VelA and the phytochrome-like red light sensor FphA, which in turn interacts with the photoreceptor homolog LreB, connecting VeA to the equivalent of the *Neurospora crassa* WCC. This complex regulates gene expression. Colour scheme: red = down-regulation; green = up-regulation; traffic light = modulation.

complete reverse functional role in penicillin biosynthesis (Kopke *et al.*, 2013).

One veA orthologue was also found in *N. crassa* (*ve*-1; Bayram *et al.*, 2008) and in *Acremonium chrysogenum* (AcveA; Dreyer *et al.*, 2007). In both species these genes control sexual conidiation, hyphal morphology and secondary metabolism. Recently Crespo-Sempere *et al.* (2013) demonstrated that VeA from *Aspergillus carbonarius* also plays a role in OTA regulation. After inactivation of

the velvet gene by *Agrobacterium tumefaciens* mediated transformation, the resulting transformant was still able to produce OTA but to reduced amounts.

White collar proteins

Characterised in 1996 and 1997 in *N. crassa* (Ballario *et al.*, 1996; Linden and Macino, 1997) the two white collar proteins, WC-1 and WC-2, are the key components of light response. WC-1 is a blue light receptor and PAS domain GATA-type transcription factor that dimerises with a second PAS domain GATA-type transcription factor, WC-2, forming the White Collar complex (WCC) (Ballario *et al.*, 1996). WCC acts as a transcription factor complex for downstream clock controlled genes (CCGs); it is responsible for all the blue-light responses in *N. crassa*, including the induction of sporulation, the protoperithecia development (Innocenti *et al.*, 1983), the induction of carotenoids synthesis (Nelson *et al.*, 1989) and, by interaction with the frequency gene (*frq*), for the regulation of circadian clock (Ballario and Macino, 1997). The WCC, upon light exposure, binds transiently to the promoters of the light-inducible genes, activating their transcription (Froehlich *et al.*, 2002; He *et al.*, 2005). This mechanism also underlies the circadian clock regulation in *N. crassa* which is regulated by a sophisticated negative feedback mechanism: WCC regulates the rhythmic expression of the *frq* gene (Crosthwaite *et al.*, 1997), which encodes for two frequency clock proteins (FRQ). FRQ proteins (large FRQ; lFRQ and small FRQ; sFRQ, resulting from alternative translation initiation) (Baker *et al.*, 2012; Brunner and Káldi, 2008; Dunlap, 2006) function as the negative elements in the loop (Aronson *et al.*, 1994; Garceau *et al.*, 1997; Liu *et al.*, 1997). Starting in the late subjective night, the WCC binds to the *frq* promoter inducing *frq* expression. The synthesised FRQ form functional homodimers (Cheng *et al.*, 2001) which enter the nucleus (Lee *et al.*, 2000; Luo *et al.*, 1998); there, they repress their own transcription by forming a complex with the WC proteins, phosphorylating it and preventing WCC from binding to the *frq* promoter and thus activating *frq* transcription (Cheng *et al.*, 2001; Denault *et al.*, 2001; Froehlich *et al.*, 2003; Merrow *et al.*, 2001).

Beside its role in the circadian clock, WC-1 is a blue light receptor (Froehlich *et al.*, 2002; Olmedo *et al.*, 2010), sensing the proportion of blue light wavelength in the light spectrum and transferring the signal towards the transcriptional level of light regulated genes. Orthologues of the *wc-1* and *wc-2* were also identified in *A. alternata* (Pruβ *et al.*, 2014), *F. fujikuroi* (*wcOA*; Estrada and Avalos, 2008) and *Fusarium oxysporum* (Ruiz-Roldán *et al.*, 2008).

Applying standard BLAST research on the *A. alternata* genome (unpublished data) Pruβ *et al.* (2014) found orthologues of WC-1 and WC-2, one orthologue of a cryptochrome, a phytochrome and an opsin regulated protein, named LreA and LreB respectively. LreA was reported to control the expression of several polyketide synthases upon light exposure (white light, 10 W).

In *F. fujikuroi* the predicted WcoA polypeptide is highly similar to WC-1; the major difference between both proteins is the lack in WcoA of some protein segments close to the carboxy end of WC-1, that include the second poly-glutamine domain of this protein. Although in *F. fujikuroi* the photoinduction of structural genes and gene involved in SM production (e.g. fusarins and carotenoids) suggests a possible involvement of a WC complex, contrary to *N. crassa*, there is no relevant effect of light on the expression of *wcOA* (Estrada and Avalos, 2008). Also in *F. oxysporum* mutants of the orthologue of WC-1 conserved the photoinduction of the carotenoid pathway, pointing to the participation of a different photoreceptor system (Ruiz-Roldán *et al.*, 2008).

A recent genome analysis of *P. chrysogenum* identified homologues of the *N. crassa* blue light receptors WC-1 and WC-2, known as PcLreA and PcLreB (Van den Berg *et al.*, 2008). In other *Penicillium* spp. parts of the sequences of the *wc*-1 genes in the two ochratoxin producing species *P. verrucosum* and *Penicillium nordicum* were determined. The genes in both species are very similar, but showed lower homology to the genes of *P. chrysogenum, Penicillium roqueforti* and *P. citrinum*. The lowest homology was found to the genes of *A. nidulans* and even *Fusarium verticilloides*. These genes however show great sequence divergence towards other *Penicillium* species (R. Geisen, personal communication).

4. Effect of light on fungal metabolism

In fungi, light signals could be detected to evaluate the optimal conditions for spore dispersal, and activate defence mechanisms against UV light radiation (Corrochano, 2007; Rodriguez-Romero *et al.*, 2010). The entire fungal metabolism is regulated by light, from fatty acid to nucleic acids metabolism, as well as the synthesis of cell wall components (for a review see: Tish and Schmoll, 2010).

In recent years, several studies report a regulation of transcribed genes activated by light (Chen *et al.*, 2009; Fuller *et al.*, 2013; Idnurm and Heitman, 2005; Ruger-Herreros *et al.*, 2014; Sommer *et al.*, 1989). However, the nature of light response is influenced by numerous factors, among which we can account the carbon source, the culture conditions, the species considered, the nature of the light source, and the intensity of the light stimuli.

Light induction of carotenoid biosynthesis in Fusaria was first described in *Fusarium aquaeductuum* (reviewed by Rau, 1980). These pigments, acting as antioxidants and photoprotectants, generally absorb light of blue wavelength.

The regulation occurs at transcriptional level: mRNA levels of *car*RA, *car*B, *car*T, and *car*X, genes of the carotenoid pathway, are transiently induced by illumination in *F. fujikuroi* (white fluorescent lamps of 5 W/m^2 at 22 °C and 25 W/m^2 30 °C (Estrada and Avalos, 2008; Prado *et al.*, 2004; Prado-Cabrero *et al.*, 2007). The same response was also described in *N. crassa* (lamps in the blue region at 2.5 µW/cm^2; Nelson *et al.*, 1989).

In *Aspergillus fumigatus,* light response involves a variety of metabolic processes which regulate growth, respiration, carbohydrate metabolism and oxidative stress resistance, the latter two known to be involved in virulence (Fuller *et al.*, 2013). In *Aspergillus ornatus* light exposure (cool white fluorescent bulbs providing 4·04 to 4·31kl x (375 to 400 ft-candle), promotes conidia formation (Hill, 1976) but inhibits growth, glucose uptake and phosphorylation. Carbohydrate metabolism is influenced by light (blue light (400-530 nm) fluorescent tubes of 80 erg/cm^2/s at the culture surface) in *Penicillium isariiforme* (Graafmans, 1974, 1997) and in *Phycomyces blakesleeanus* (white fluorescent tubes 32 W) (Rua *et al.*, 1987) by the regulation of the enzymes responsible for metabolite production.

In the human fungal pathogen *Cryptococcus neoformans* light (white fluorescent light 1,500-3,500 lux and filters to provide blue, green, or red light) inhibits mating and haploid fruiting body formation (Idnurm and Heitman, 2005), demonstrating the involvement of blue-red light perception mechanism also in the *Basidiomycete* phyla.

5. Light influence on mycotoxin production

Besides regulating primary fungal metabolism, light also modulates mycotoxin production, and may have promoting or inhibiting effects depending on the species. In *A. alternata* the first studies about the effect of light on secondary metabolism revealed that light exposure (fluorescent tubes of 23 W at 21.8 µE/m^2/s and multiple filters) inhibited the biosynthesis of alternariol (AOH) and alternariol monomethyl ether (AME) (Häggblom and Unestam, 1979; Söderhäll *et al.*, 1978). The mechanism of light perception in this species was recently studied at molecular level by Pruβ *et al.* (2014). As already reported by Häggblom and Unestam (1987) they confirmed that blue light in this species inhibited the sporulation. However, in contrast with the above mentioned study, Pruβ and coworkers demonstrated a stimulating effect of blue light (light emitting diodes (LED) 450 nm 0.72 W/m^2) on altertoxin (ATX), AOH and AME. Moreover, while AOH was already produced under constant dark conditions, ATX production was strictly dependent on light exposure. It should be considered that these contrasting results may be due to the use of a different kind of light source.

Light is the most critical factor for cercosporin production in *Cercospora* spp. (Daub *et al.*, 2005). This toxin is unique among the well-characterised fungal toxins, since it is classified as a photosensitiser (Daub, 1982), which is a compound activated by visible wavelengths of light and generating activated oxygen species toxic to living cells (Spikes, 1989).

Red light promotes SM production in *Monascus,* while blue light enhances only gamma minobutyric acid production (Miyake *et al.*, 2005). CIT biosynthesis by *Monascus* species decreased in blue light culture condition (Babitha *et al.*, 2008; Miyake *et al.*, 2005) and the toxin can be directly degraded by blue light (Wang *et al.*, 2009; Schmidt-Heyd *et al.*, 2012a), although in *Monascus X* the CIT biosynthetic gene cluster was found to be up-regulated (Wang *et al.*, 2012).

The influence of light on mycotoxin biosynthesis is not univocal. The effect depends both on the quality and intensity of illumination (with can be applied as a broad spectrum or with specific wavelength) and the species analysed. Furthermore, data collected by different research groups are often discordant, due to differences in experimental procedure and strain variability.

Although the molecular background of light perception has been elucidated in several species, the knowledge about the influence of light, with the exception of STE (see below), on mycotoxins more important from the food safety perspective, is very limited. Furthermore, despite the current data show that the biosynthesis of STE is inhibited by light, that must not always be the case. While the biosynthesis of some mycotoxins, such as OTA, is strongly inhibited by light, the production of other toxins, such as fumonisins or CIT, is promoted under certain light conditions. Also the growth behaviour of the corresponding mycotoxin-producing fungi differs depending on the species analysed (for a general picture of the effect of light on growth of different fungal species, see Figure 3).

In the past the analysis of light regulation of secondary metabolism has been mainly focused on the effect of white light on physiological and metabolic response and on the circadian rhythm. Only recently systematic studies on the light regulation of secondary metabolism highlighted that not only light and darkness can influence fungal metabolism, but also that light of different wavelengths and intensity has specific effect on the activation of mycotoxin biosynthetic pathways (Schmidt-Heydt *et al.*, 2011).

Below we reported the results of recent studies about the influence of light on the main toxigenic fungal genera, *Penicillium*, *Aspergillus* and *Fusarium*.

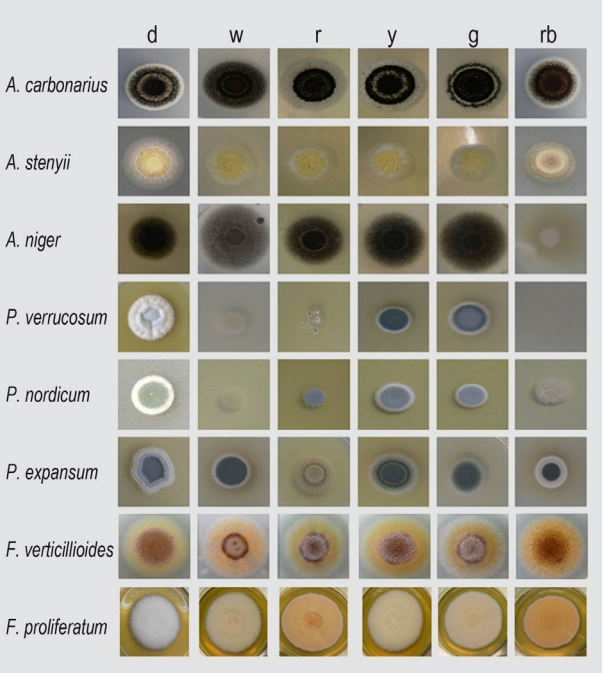

Figure 3. Growth of different fungal species under light wavelengths: d = dark; w = white: r = red; y = yellow: g = green: rb = royal blue. A. = *Aspergillus*; P. = *Penicillium*; F. = *Fusarium* (adapted from Schmidt-Heydt *et al.*, 2011).

Influence of light on mycotoxin biosynthesis in *Penicillium*

Although light responsive elements in *Penicillium* have been only recently characterised, the effect of light on mycotoxin production has been reported in different species of this genus. Beside the recent identification of a velvet-like complex in *P. chrysogenum*, Kopke *et al.* (2013) reported that light (unspecified) has no effect on penicillin biosynthesis in this species, unlike what happens in *A. nidulans*.

On the contrary, other mycotoxin biosynthetic pathways are strongly influenced by light exposure. Schmidt-Heydt *et al.* (2010) reported that in *P. nordicum* light has an inhibiting effect on OTA biosynthesis: when *P. nordicum* was incubated under constant dark conditions OTA levels were 20-30% higher compared to constant day-light conditions (daylight-emitting neon tubes 36-58 W, 6,500 kelvin, 1,600 and 2,800 lux respectively). Interestingly under light/dark fluctuating conditions the amount of OTA oscillated correspondingly, being higher in the dark and lower under light exposure. This indicates, in agreement with previous results of Gillman *et al.* (1998), that OTA is degraded under light exposure, either by the photo-irradiation itself or by enzymatic activities, and then produced again under dark conditions.

To deepen the light response and the regulation of OTA biosynthesis in this genus, LED of different wavelengths were used: for this analysis red (627 nm, 7700 lux), green (530 nm, 7,250 lux) yellow (590 nm, 6,400 lux) blue (470 nm, 2,357 lux) and royal blue (455 nm, 3,350 lux) wavelengths were tested on cultures of two OTA-producing *Penicillium* species: *P. nordicum* and *P. verrucosum*. Interestingly, red, and to a greater extent, blue light inhibited the growth of both species. This inhibition was also reported in other OTA non-producing *Penicillium* species, such as *Penicillium expansum*, but to a lesser extent compared to the two OTA-producing species (Schmidt-Heydt *et al.*, 2011); thus indicating that the ability to produce OTA emphasised the negative effect of light exposure on growth. Generally the biosynthesis of OTA under light conditions, especially under red and blue light, is drastically reduced. This reduction is regulated at transcriptional level: when the culture is shifted from dark to blue light conditions (390-450 nm), the levels of expression of the ochratoxin polyketide synthase gene rapidly decreased (Schmidt-Heydt *et al.*, 2010). Depending on the light intensity a complete inhibition could be achieved (450 nm, 1,700 lux measured at surface area). In contrast to red and blue light, yellow and green wavelengths partly supported OTA biosynthesis.

OTA was reported to be toxic for the producing *P. nordicum* under light conditions (Schmidt-Heydt *et al.*, 2010). Therefore, under light exposure, this species reduced OTA biosynthesis shifting the secondary metabolism towards the production of a less toxic compound, ochratoxin B (OTB). OTB is an OTA precursor, it has the same structure as OTA, but without chlorine at position 5. OTB is less toxic than OTA by a factor of up to 10 (Stander *et al.*, 2000). In addition, non-halogenated coumarins have antioxidative properties (Fylaktakidou *et al.*, 2004) and their increased production under light conditions may counteract the oxidative effect of light. Indeed, the addition of external OTA reduced the growth rate of *P. nordicum*, whereas externally added OTB did not induce this effect (Schmidt-Heydt *et al.*, 2010). Because of this flexible situation the amount of OTA or OTB produced cannot be predicted. Weather the biosynthesis is shifted towards OTA or OTB strongly depends on the particular light conditions. This of course has also implications to food safety aspects. A *Penicillium* culture grown in the dark will produce much more of the toxic OTA than of the less toxic OTB. OTA is a mycotoxin typically produced in cereal and cereal products under storage conditions (EC, 2002): e.g. wheat, one of the major corps contaminated with OTA, is often stored in silos, which means in the dark. According to the described above data this storing condition favours the biosynthesis of OTA (Schmidt-Heydt *et al.*, 2015) by *Penicillium*.

While *P. nordicum* is reported to produce only OTA, *P. verrucosum* is also able to produce CIT. Also the latter species shifts secondary metabolite production under light conditions, in this case from OTA towards CIT (Schmidt-Heydt *et al.*, 2010). CIT, again, is an antioxidant (Heider

et al., 2006) and since irradiation by intense light induces oxidative stresses (Fuller *et al.*, 2013; Miyzaki *et al.*, 2001), it should be surprising that CIT is described as a potential light protectant (Stormer *et al.*, 1998): indeed it was experimentally demonstrated that highly CIT-producing species, such as *P. citrinum* and *P. expansum*, are much more resistant towards light stress with regard to growth rate than CIT non-producing *Penicillium* spp. (Stoll, 2013).

The toxic effect of OTA under light conditions on the producing organism itself is obviously one of the reasons why OTA producing Penicillia are most sensitive against blue light, compared to the moderately sensitive OTA non-producing Penicillia. Schmidt-Heydt *et al.* (2012a) recently reported a wavelength-dependent degradation of ochratoxin and CIT, both *in vitro* and *in vivo,* in *Penicillium*. They reported that white light (LED of mixed wavelength with a sharp peak at 440 nm and a smooth peak from 475 to 780 nm, 366 μW/cm^2) and blue light of 470 nm (345.1 μW/cm^2) and 455 nm (490.4 μW/cm^2) are able to degrade OTA, OTB and CIT after 5 days of illumination. Interestingly, and fitting to the points above, OTA is degraded to chlorine containing derivatives, which might be more toxic, while OTB is degraded to non-chlorinated derivatives.

These toxins are differently sensitive to light exposure, indicating a different absorbance of energy. CIT is completely degraded by blue light, indicating that CIT absorption range includes this wavelength. In the same study, the authors also evaluated the effect of light treatments on OTA-producing culture of *P. verrucosum* in wheat samples. After a pre-incubation under high relative humidity condition, inducing OTA production, blue light exposure was able to degrade OTA to levels up to 50% compared to the pre-incubated sample, and up to 90% compared to the sample incubated under dark conditions. Thus light degrading effect is exerted both on artificial and on natural substrate, suggesting the usefulness of blue light to control surface-growing of *P. verrucosum*.

In conclusion, the two OTA producing *Penicillium* species are very sensitive especially against blue light. At increasing light intensities both OTA biosynthesis and growth can be completely stopped. This effect is, at least partly, due to the toxicity of degradation products formed by the action of short-wave light. In order to avoid the formation of these toxic products both species react by shifting the secondary metabolism either to the production of OTB or CIT, since both these SM are less toxic and have antioxidant properties.

Influence of light on mycotoxin biosynthesis in *Aspergillus*

Discordant data were collected on the effect of light on aflatoxin biosynthesis in *Aspergillus* genera: in *A. flavus*: Joffe and Lisker (1969) reported an inhibiting effect by continuous light exposure (diffused laboratory light), while Bennett *et al.* (1981) demonstrated a promoting effect of continuous illumination (six General Electric cool-white fluorescent Bulbs) on *A. flavus* and *A. parasiticus*. However this effect was temperature-dependent: indeed they showed that only at medium temperature light exposure has an increasing effect, while at 15 and 30 °C the production was decreased. Similarly, Aziz and Moussa (1997) reported that aflatoxin B_1 (AFB_1) by *A. flavus* was enhanced upon cultivation in light (white neon lamps of 320 W) compared to the dark incubation, and that UV-grown cultures (fluorescent 40 nm lamp at 206/10^{6}J/cm^{2}s) produced the highest amount of AFB_1.

Not only temperature, but also the culture medium plays a role modulating the influence of light on aflatoxin biosynthesis. Schmidt-Heydt *et al.* (2011) showed that cultures of *A. parasiticus* on yeast extract with supplements (YES) medium are not influenced by white light, while on malt extract medium there is a clear influence of light exposure on growth, and aflatoxin biosynthesis was strongly reduced under white light exposure (17,750 Lux).

In *A. nidulans* the biosynthesis of penicillin, the most important secondary metabolite with respect to pharmaceutical use, is regulated by VeA, which acts as a repressor. In this species Spröte and Brakhage (2007) demonstrated that light (unspecified) positively influenced the production of penicillin.

Most of the molecular mechanisms that are now known to be involved in the regulation of mycotoxins by light, have been unravelled in *A. nidulans* in conjunction with the regulation of STE biosynthesis (Bayram *et al.*, 2008, 2010; Fischer, 2008; Stinnett *et al.*, 2007). In this species the effect of light (unspecified) on the production of STE was reported to be medium dependent (Kato *et al.*, 2003): Atoui *et al.* (2010) and Guzman-de-Pena *et al.* (1998; room illuminated at 40 W/m^2/seg) showed that glucose concentration modulates the effects of light on STE production: it is enhanced upon light exposure in rich medium, revealing a relation between light response and nutritional component. The regulation by light on STE biosynthesis has been deeply investigated as a kind of model system to disclose the molecular background of the link among light, sexual development and secondary metabolite production (Bayram *et al.*, 2008, 2012; Fischer, 2008). It was demonstrated that light (white fluorescent light) decreases STE biosynthesis by reducing the amount and the transport of the VeA/VelB complex into the nucleus

(Bayram *et al.*, 2008). Moreover Purschwitz *et al.* (2008) demonstrated that also the red light sensor FphA and the blue light sensing complex LreA/LreB (the *A. nidulans* homologue of WC-1/WC-2) bind to VeA under blue light conditions (LED of 450 nm, 25 mE/m^2s) and thereby reduce STE biosynthesis (Fischer, 2008).

The effect of light on OTA production was firstly studied by Aziz and Moussa (1997) who demonstrated that in *Aspergillus ochraceus* the biosynthesis of this toxin is considerably higher under light exposure (8 neon lamps of total 320 W Power) than in the dark, while in *A. carbonarius* Belli *et al.* (2006) reported unclear data on the effect of light (white light bulbs, 1,500 lumen) and photoperiod, however, describing a non-inhibitory effect of continuous light.

When incubated under specific light wavelengths, *Aspergillus* species from Section *Nigri*, such as *A. niger* and *A. carbonarius*, generally seem to be more resistant to light treatment than *Penicillium*. While red (LED, 627 nm, 7,700 Lux) and blue light (LED, 450 nm, 2,357 Lux) have an overall inhibitory effect on growth, contrary to *Penicillium,* white light (LED, 17,750 Lux) and blue light, of lower wavelength and lower intensity than the one above mentioned (LED, 455 nm, 1,700 Lux), support growth and sporulation of *Aspergillus* spp. compared to the dark. (Fanelli *et al.*, 2012a; Schmidt-Heydt *et al.*, 2011). The black colour of these spores, which are abundantly produced under these conditions, could exert a protective role against the stress induced by high intensity illuminating condition.

As in *Penicillium,* OTA production in *A. carbonarius* was strongly reduced under blue light conditions, depending on light intensity (Schmidt-Heydt *et al.*, 2011). Moreover, also in this species a mutual regulation between two SM could be observed: under blue light exposure (LED, 450-470 nm) *A. carbonarius* shifted its metabolism from OTA towards a new metabolite, called here MX. First analysis of the chemical formula of this metabolite revealed similarity with ochratoxin β (unpublished data). The shift induced by blue light exposure could be explained as a protection mechanism against the toxicity of OTA towards the microorganism itself. It could also be considered as a light protection mechanism, since blue light especially exerts an oxidative effect (Miyzaki *et al.*, 2001; Schmidt-Heydt *et al.*, 2011).

A further mutual regulation of mycotoxin production has been reported in one *A. niger* strain able to produce OTA and fumonisin B_2 (FB_2) (Fanelli *et al.*, 2012a). In this case OTA and FB_2 levels were conversely modulated in relation to light exposure. OTA was highly produced under dark conditions, and strongly inhibited under white (17,750 Lux), blue (470 nm, 2,357 lux) and red (627 nm, 7,700 lux) light. Just the opposite was reported for FB_2 biosynthesis: under all tested light wavelength, FB_2 biosynthesis was higher than in the dark, and, interestingly, red and blue light were reported to be the most effective in promoting FB_2 production.

It may be possible that FB_2 has some protective properties under these light conditions as suggested for CIT and OTB in *Penicillium*. However in *A. niger* FB_2 production was less influenced by light exposure, while the effect of light on OTA biosynthesis was remarkable: this can be explained by the assumption that FB_2 is not the main toxin produced by this fungal species and thus its biosynthetic pathway may be less responsive to changes in environmental light conditions.

Influence of light on mycotoxin biosynthesis in *Fusarium*

Compared to other previously described fungal genera, the effect of light on *Fusarium* toxins is less effective. Light response in this fungal genus has been studied in four main mycotoxin-producing species, such as *F. verticillioides, Fusarium graminearum* and *F. proliferatum* (Fanelli *et al.*, 2012b,c; Schmidt-Heydt *et al.*, 2011), and recently also in *F. fujikuroi* (Matić *et al.*, 2013).

The effect of light was weak both on growth and morphology, but all the analysed species grew better under light condition compared to the dark. The stimulation was more effective under white and short wavelength light (390-450 nm). Light stimuli had also a positive effect on mycotoxins biosynthesis. Deoxynivalenol biosynthesis in *F. graminearum* increased under blue and royal blue light (470 and 455 nm respectively) (Schmidt-Heydt *et al.*, 2011).

In both *F. verticillioides* and *F. proliferatum* fumonisin B production increased under light conditions. This effect was more specific in *F. proliferatum*, where the most effective light wavelengths in promoting fumonisin production were red (LED, 627 nm, 7,700 lux), and blue (LED, 450 nm, 2,357 Lux) light. These are exactly the light wavelengths leading to a reduction of OTA biosynthesis in *Penicillium* and *Aspergillus*, confirming that light response could be extremely different among fungal genera.

The induction of FB biosynthesis in these two above mentioned *Fusarium* spp. is regulated at transcriptional level via the modulation of *FUM1* and *FUM21* gene expression, two key genes of the fumonisin biosynthetic pathway (Fanelli *et al.*, 2012b,c).

Being a mixture of all wavelength with a broad spectrum of radiation, white light had no remarkable effect on fumonisin biosynthesis in *Fusarium* when applied via continuous irradiation (24 h/day). By contrast, a pulsed white light with high luminous emittance (25,000 lx, pulse rate of 1/s), was able to inhibit both fungal growth and fumonisin production in *F. verticillioides*. Thus could indicate that,

in this case, rather than the light wavelength itself, the inhibition was ascribable to the stressing condition due to light pulse (Fanelli *et al.*, 2012b).

The promoting effect of light (bulbs of 72 lm) on mycotoxin production by *Fusarium* was also reported by Matić *et al.* (2013) in strains of *F. proliferatum, F. verticillioides* and *F. fujikuroi* isolated from rice, which indicates that this effect is not related to the host origin of the *Fusarium* strain.

6. Conclusion and perspectives

As a general consideration, even if light response has been demonstrated to be very different among fungal genera, we can affirm that the most effective light wavelengths in regulating mycotoxin production are blue and red. This is in accordance with the presence of blue and red light perception mechanism acting in several fungal species, such as the blue light receptors WC-1/WC-2 or their homologues in *Aspergillus* LreA/LreB, and the phytochrome red light receptor FphA (Herrera-Estrella and Horowitz, 2007; Purschwitz *et al.*, 2006). These receptors regulate mycotoxins biosynthesis by modulating gene transcription. This mechanism has been demonstrated both in *F. verticillioides* and *F. proliferatum* (Fanelli *et al.*, 2012b,c), where, after light exposure, the transcriptional levels of key genes (e.g. *FUM1* and *FUM21*) involved in fumonisin biosynthesis increased with a concurring phenotypic regulation of fumonisin levels, and in *P. nordicum*, where *otapks*PN levels were modulated in a circadian way by light exposure, with the concurring regulation of OTA levels (Schmidt-Heydt *et al.*, 2010).

Light can influence mycotoxin biosynthesis at several levels (Table 1 gives an overview about the influence of light on certain important mycotoxins). Firstly, depending on the absorbance spectrum of the molecule, light may degrade the toxin either to degradation products, as in the case of OTA and OTB, or it can lead to a more or less complete degradation of the molecule, as was shown for CIT (Schmidt-Heydt *et al.*, 2012a). Secondly, the most important influence is the direct action on the fungus. Due to the involvement of blue and red light receptors as signal transduction molecules, light acts at transcription level, influencing the expression of the mycotoxin biosynthetic genes. This action can be inhibiting, as in the case of STE (Bayram *et al.*, 2009), OTA (Schmidt-Heydt *et al.*, 2010), AOH (Häggblom and Unestam, 1987) and trichothecenes (Schmidt-Heydt *et al.*, 2010), or can be supporting as for CIT (Schmidt-Heydt *et al.*, 2010) and fumonisin B (Fanelli *et al.*, 2012a,c).

As a general observation, we can conclude that pathways that are induced by light exposure lead to the formation of molecules with possible protectant functions, which counteract the oxidative stress derived from these unfavourable conditions, as light itself could be. Although this role was demonstrated only for CIT (Heider *et al.*, 2012; Stormer *et al.*, 1998), it can be expected that other mycotoxins, whose production is promoted by light exposure, have protective functions against the unfavourable activity of light. That is indeed also the case of other SM such as carotenoids (Iigusa *et al.* 2005; Rodriguez-Ortiz *et al.* 2009; Zhirong *et al.*, 2012).

The effect of light on mycotoxin production is both wavelength specific and species specific, and its role in fungal life must be considered in an ecological perspective, as a more fine-tuned adaptation to the environment, increasing the competitiveness of the producing fungus under certain conditions. Mycotoxin biosynthesis has been recently associated with pathogenicity or with the host-pathogen interaction (Graf *et al.*, 2012; Sanzani *et al.*, 2012) helpful for the fungus to colonise living plant tissue (Desjardin *et al.*, 1993), and plant material after harvest; considered as playing a role in response to several type of stress, including predation (Saari *et al.*, 2010), oxidative stress (Jayashree and Subramanyam, 2000) high NaCl conditions (Schmidt-Heydt *et al.*, 2012b) or non-favourable light conditions (Fanelli *et al.*, 2012b), assisting the fungus in creating a suitable environment for survival, growth and reproduction.

Table 1. Overview about the influence of light/dark conditions on mycotoxin biosynthesis.[1]

Dark	Light
VeA in the nucleus in *Aspergillus nidulans*	VeA in the cytoplasm in *A. nidulans*
Activation of secondary metabolism by VeA/VelB/LaeA in *A. nidulans*	Activation of the white collar complex in *Neurospora crassa*
Enhanced production of OTA	Reduced production or complete inhibition of OTA
Enhanced production of STE/aflatoxins	Reduced production of STE/aflatoxins
	Enhanced production of OTB/CIT/MX
	Degradation of OTA/OTB/CIT
	Enhanced production of fumonisin B by *Aspergillus* and *Fusarium*

[1] CIT = citrinin; OTA = ochratoxin A; OTB = ochratoxin B; STE = sterigmatocystin; MX = new metabolite, likely ochratoxin β.

Light response involved a well-structured system, based on the ability to recognise changes in the environment and by consequence to activate complex molecular pathways. This system consists of receptors for light wavelengths and intensity, proteins for signal transduction and transcriptional regulation. Since light response was demonstrated to be so different among fungal genera and species, we are led to assume that these differences are linked with features developed during the evolution, specifically related to the ecological niche, including the host and substrate colonised, the habitat and the environmental conditions that characterise the optimum for fungal growth and spreading. The currently available results argue that mycotoxins, whose production is decreased during exposure to light, act somehow negatively on the fungus under these conditions. On the other hand, mycotoxins whose biosynthesis is increased may act as protective substances. This view is supported by the fact that the production of SM, synthesised preferably in the dark (OTA, STE, aflatoxin), is shifted to others, which has, or it is thought to have, a possible protective or anti-oxidative activity (CIT, OTB).

One of the biggest problems dealing with mycotoxins is related to their high chemical stability, which makes their reduction very challenging. At certain conditions, food processing operations, such as heat treatment, were shown to be efficient in reducing mycotoxin contamination significantly. For example roasting of green coffee beans at 200 °C for 12 min can reduce aflatoxin content up to 79% (Levi, 1980); heating of CIT-contaminated maize to 105 °C for 16 min eliminated the diuretic effect of this toxin (Kirby *et al.*, 1987). Other mycotoxins, such as OTA and *Fusarium* mycotoxins, are much more resistant against thermal inactivation. Heat treatment was demonstrated to be insufficient to achieve a significant reduction in food commodities. The effectiveness of using physical, chemical or biological decontamination procedures, e.g. heat treatment, irradiation or adsorption, solvent extraction, enzymatic activities, detoxification by microorganism, is often limited and must be evaluated together with the influence on food quality and nutritional value.

According to the above reported data, light could be a new physical parameter for controlling the production of some mycotoxins by at least surface-growing fungi, or it could further be used directly to degrade mycotoxins. Pulsed light (PL) and specific wavelength exposure could be easily applied in food processing and during distribution. The degradation of toxins by light of specific wavelength (Schmidt-Heydt *et al.*, 2012a), or by pulsed light exposure (Moreau *et al.*, 2011) on ZEA, deoxynivalenol, AFB_1 and OTA, opened new perspective for the use of light as toxin decontaminant in the whole production process.

The use of PL has been proposed to replace chemicals in decontamination operations in the food industry. So far, the PL technology selected to achieve this aim was characterised by intense flashes of white light rich in ultraviolet light. The inactivation achieved by this PL is commonly attributed to DNA damage caused by UV radiations. The ability of PL to kill microorganism is documented, especially towards pathogens such as *Staphylococcus aureus* (Kristhnamurthy *et al.*, 2004) or *Listeria monocytogenes* (Fernandez *et al.*, 2009). Fungal spores, such as *A. niger* spores, are more resistant to inactivation by PL or UV treatment (Levy *et al.*, 2012). Other kind of PL, such as white light PL (Fanelli *et al.*, 2012b) was able to inhibit both fungal growth and FB production in *F. verticillioides*. Thus PL technology could be considered for several application, especially related to food safety. Short term treatments could be introduced in food processing industries (e.g. over conveyor belt). This technology is not expensive, also considering the brevity of the treatment needed to inhibit proliferation of undesirable microorganism.

Beside short term treatment, long term treatment could be realised developing packaging with coloured filter to extend shelf life of stored products and avoid contamination by surface growing fungi, e.g. pasta, bread, cheese or fruit products.

The possibility to set specific wavelength of light and intensity makes LED technology very helpful to study fungal response to light and develop new application in food safety. The vast majority of devices containing LED are safe under all conditions of normal use. LED fluorescent lamps do not contain mercury, and are not hazardous except for low-intensity red LEDs, which leached Pb and could be of concerning toxicity when treated as waste (Lim *et al.*, 2011). LEDs can be very small (smaller than 2 mm^2 (Dialight, Newmarket, UK)) and are easily attached to printed circuit boards. All the advantages of this technology let them be considered one of the greatest invention of the last century (Crawford, 2009). The ductility of LED technology and the fact that they are relatively inexpensive, especially if valuating their high efficiency and their long useful life, encourage LED application for long time treatments associated with prolonged process, such as aging in cheese or meet, or storage even at low temperature (fridge, transportation, etc.).

All the technologies above proposed, conveniently optimised, could be effective in reduce mycotoxin contamination in food, both inhibiting fungal growth and down-regulating the biosynthetic pathways responsible for mycotoxin production. They can be easily integrated in food processing and preservation with limited cost and safe procedures. However, it must be considered that in some case light can foster growth and toxin biosynthesis depending on the species. Hence, the choice of the area

of interest of this technology should be made carefully, avoiding the use of light in those food commodities usually contaminated by fungi towards which light exerts a promoting effect.

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