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Coffee, mycotoxins and climate change

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ABSTRACT

Coffee is very valuable. However, it is subjected to various pest and diseases of which mycotoxin contamination is of great importance in terms of the health of consumers and economic loss. The major compound of concern is ochratoxin A (OTA) which has statutory limits imposed on the commodity in, for example, the European Union. The concentrations of OTA appear often well within the limits and the situation is containable, although some surveys revealed levels higher than statutory limits and frequency of contamination was often high. The producing fungi are Aspergilli. Nevertheless, there remains some misidentification in the literature in relation to *Aspergillus ochraceus* and *Aspergillus westerdijkiae*: other important producers are *Aspergillus carbonarius* and *Aspergillus niger*. Coffee husks are high in OTA and are fortunately removed during processing. Beans obtained after falling on the soil and coffee which had been floated as part of processing, were reported as high in OTA and should be avoided. The presence of aflatoxins (AF) needs consideration. More work is required on the presence of fumonisins in coffee. The effect of climate change on mycotoxins in coffee requires urgent consideration as, for example, AF may become more problematic than OTA in 50 years. Finally, careful monitoring of coffee is essential to nurture this desirable commodity into the future.

1. Introduction

Coffee is valuable and enjoyable. The commodity is the most traded apart from oil and is consumed as a beverage in vast numbers of cafes and homes around the world. Furthermore, worldwide coffee consumption has nearly doubled over 40 years and is forecast to be greater than 9 million tonnes by 2019. More than 70 developing countries produce coffee of vital importance to their balance of payments and Brazil grew an average of 2.5 million tonnes/year from 2007 to 2011. The major consumers are all in developed regions, i.e. USA, Europe and Japan, apart from Brazil which is the second largest consumer. Coffee is important to social development and an excellent resource for rural employment, which provides work for 125 million people globally (Taniwaki, Teixeira, Teixeira, Copetti, & Lamanaka, 2014). Vietnam has dramatically increased coffee production recently to become the world's second biggest producer after Brazil. Coffee is produced in all continents in tropical regions of the world (Fig. 1), including more recently in Australia and Yunnan Province, China (Oestreich-Janzen, 2010). Lists of producing countries are available (FAO/WHO, 2008; Oestreich-Janzen, 2010; Table 1).

However, it is subjected to various pests and diseases of which mycotoxin contamination by fungi has been of great concern for decades (Levi, Trenk, & Mohr, 1974; Paterson, Baker, & van der Stegen, 2001). Furthermore, the importance of climate change (CC) (IPCC, 2013) to coffee production (Davis, Gole, Baena, & Moat, 2012) and the effect of CC on mycotoxin contamination (Paterson & Lima, 2010a, 2011, 2012), require urgent consideration. The commodity is susceptible to mycotoxigenic fungal growth because of the hot humid conditions encountered which are optimal for these fungi. There are two coffee species that are employed, *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee). The quality of the beans at the end of processing influences the price achieved when sold on the market and defects in coffee beans are undesired because they decrease the coffee quality. Coffee fruits in contact with the soil for long periods allow contamination by microorganisms and the formation of black and sour defective beans. Some of these microorganisms are fungi which can produce toxins (Taniwaki et al., 2014).

Furthermore, there are two optimal growing climates for Arabica beans: the (a) subtropical regions at high altitudes of 16–24° where rainy and dry seasons are well defined, and altitude must be between 550 and 1100 m. These environments result in one growing season and maturation season, usually in the coldest part of autumn. Examples are the Brazilian S. Paulo and Minas Gerais regions, Jamaica, Mexico and Zimbabwe; and (b) equatorial regions at latitudes lower than 10° and altitudes of 1100–1900 m where frequent rainfall causes almost

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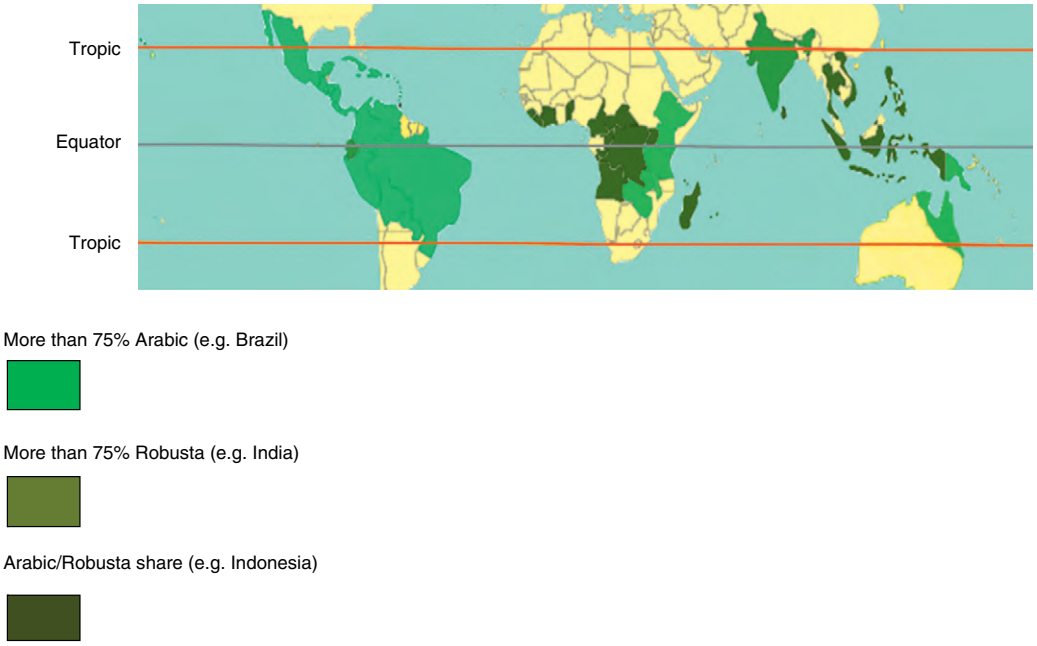

Fig. 1. Worldwide production of coffee. The Yunnan highland of China is not shown on this map which has become a recent producer.

continuous flowering, resulting in two coffee harvesting seasons. The highest rainfall period determines the principal harvesting period and mechanical dryers are used because rainfall is too frequent for patio drying in this environment. Kenya, Colombia, and Ethiopia have these coffee growing environments. Generally, Robusta coffee is grown at lower altitudes (sea level — 900 m) at 10° North and South of the equator.

Some inter-specific crosses of the two species are used in coffee production (FAO, 2006). This publication mentions that Arabica coffee requires altitudes of between 600 and 1600 m, whereas Robusta can be grown at sea level but is often grown in wet tropical highlands. In general, the guidelines for prevention of mould on coffee should be read and adhered to (FAO, 2006). Furthermore, the Codex Alimentarius Commission (CAC, 2009) states that Arabica coffee is grown at 600–2000 m with an average temperature between 18 and 22.5 °C and Robusta coffee is grown at below 600 m where average temperature is between 22 and 26 °C, both in the wet tropics, and these are humid conditions suitable for fungal growth. Fig. 2 indicates the various stages of wet and dry processed processing of coffee to assist in understanding critical points for fungal contamination (CAC, 2009). These involve preharvest, postharvest, storage and processing stages when reduced to the basic elements. The various climates and production systems confer different risks for the development of OTA producing fungi. In shaded coffee plantations, the soil remains moist even if there is a dry season. In some regions the harvest period (typically stretching over three months) coincides with a rainy season or humid conditions. Under these scenarios, there is a high risk of fallen coffee fruit becoming grossly contaminated with fungi. In unshaded production systems where the harvest is conducted in a dry season, the risk is reduced (FAO/WHO, 2008). Hence, in terms of CC (see later), a logical response would be to decrease the amount of shade if the weather becomes more humid and vice versa. A definition of mycotoxins will now follow which are the primary concerns of this review.

Mycotoxins are fungal compounds that cause low performance, sickness or death in people and/or animals when ingested, inhaled or absorbed. The most direct method to indicate if there is mycotoxin contamination is to analyse crops such as coffee, directly for particular mycotoxins using chromatography: mycological surveys of crops are useful to determine the potential for mycotoxin contamination. The principal mycotoxigenic *Aspergillus*, *Penicillium* and *Fusarium* spp. are present throughout coffee production (Batista, Chalfoun, Prado, Schwan, & Wheals, 2003; Nakajima, Tsubouchi, Miyabe, & Ueno, 1997), although there are normally few fungi in the final product on the supermarket shelf. However, mycotoxins may be detectable in the final product.

The toxicity of mycotoxins is classified by the International Agency for Research on Cancer (IARC, 1993), where aflatoxins (AF) are considered carcinogenic to humans and others are placed in categories reserved for the less toxic compounds (Paterson & Lima, 2010b; Venâncio & Paterson, 2007). Importantly, there are no statutory limits on fungi on crops — only for some mycotoxins. The most significant of these low molecular weight secondary metabolites are produced by a small number of fungal species. Mycotoxins occur naturally and are the most prevalent source of food-related health risk in field crops. Approximately 25% of the global food and feed crop output is affected.

2. Ochratoxin A producing fungi in coffee

Coffee cherries and beans are subjected to colonization by microorganisms during development, harvesting, preparation, transport and storage (Fig. 2). The cherries will also be exposed to damage by insects and birds and detrimental macro/and microenvironmental factors (Paterson & Lima, 2010a, 2011), which will influence how ochratoxigenic fungi develop. Coffee fruits and beans may be contaminated by (a) OTA, compromising safety and quality and/or (b) ochratoxigenic fungi, presenting a risk of OTA contamination (Batista et al., 2009). Interestingly, a reason for the low incidence and levels of mycotoxins in green coffee beans may be related to inhibition of fungal growth by the caffeine in coffee (Levi et al., 1974; Micco, Grossi, Miraglia, & Brera, 1989). However, some strains of *Aspergillus flavus* and *Aspergillus ochraceus* produced high AFB and OTA with 0.1–1.0% caffeine added to the growth media (Nakajima et al., 1997).

OTA is produced by some *Aspergillus* and *Penicillium* species with Aspergilli being the more common producers on crops in tropical climates (Venâncio & Paterson, 2007) because the fungi have adapted to the hot, humid climates (Paterson & Lima, 2010a, 2011, 2012). The sources of OTA in coffee are probably the known fungal OTA producers of *Aspergillus* sections Circumdati and Nigri (Batista et al., 2003; Joosten, Goetz, Pittet, Schellenberg, & Bucheli, 2001; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003). Two Penicillia known to produce OTA, *Penicillium verrucosum* and *Penicillium nordicum*, are less likely, as they are

Table 1
Incidence of ochratoxin A in commercial raw coffee worldwide.

Origin	No. of samples/no. of positive	Range of OTA (μg/kg)	Coffee type	Reference
Angola	4/0	<20[a]	N.S.[b]	Levi et al. (1974)
Brazil	7/3	Trace–360	"	"
Colombia	139/17	Trace–50	"	"
African countries	15/2	Trace–<20[a]	"	"
Unknown	102/7	Trace	"	"
Brazil	14/10	0.2–3.7	Arabica	Micco et al. (1989)
African countries	7/6	Traces–15	Robusta	"
African countries	2/0	<0.01[a]	Arabica	"
Colombia	2/1	3.3	Arabica	"
Costa Rica	2/1	Traces	Arabica	"
Mexico	2/1	1.4	Arabica	"
Brazil	5/3	2.0–7.4	N.S.[b]	Studer-Rohr et al. (1995)
Colombia	5/3	1.2–9.8	"	"
Central America	3/0	<0.5[a]	"	"
New Guinea	1/0	<0.5[a]	"	"
African countries	7/4	<0.5[a]–56	"	"
America, Africa, Papua New Guinea	153/31	0.2–9.0	Arabica	MAFF (1996)
America, Africa, Asia	75/55	0.2–27.3	Robusta	"
Yemen	10/7	0.7–17.4	Arabica	Nakajima et al. (1997)
African countries	10/5	<0.1[a]–7.2	Arabica	"
Indonesia	9/2	0.2–1.0	Robusta	"
Central America	6/0	<0.1[a]	Arabica	"
South America	12/0	<0.1[a]	Arabica	"
East Africa	42/33	0.2–62.0	N.S.[b]	Heilmann et al. (1999)
West Africa	9/9	0.3–5.0	"	"
Asia	29/20	0.2–4.9	"	"
Central America	15/6	0.2–0.8	"	"
South America	17/5	0.2–1.0	"	"
South America	19/9	0.1–4.9	N.S.[b]	Trucksess et al. (1999)
Africa	84/76	0.5–48.0	N.S.[b]	Romani et al. (2000)
Latin America	60/19	0.1–7.7	"	"
Asia	18/11	0.2–4.9	"	"
Brazil	37/17	0.2–6.2	Arabica	Gollücke, Taniwaki and Tavares (2004)
Brazil	132/27	0.7–47.8	Arabica	Leoni et al. (2000)
Brazil	40/5	0.6–4.4	Arabica	Batista et al. (2003)
Brazil	60/20	0.2–7.3	Arabica	Martins et al. (2003)
Africa	12/12	2.4–23.3	Robusta	Pardo et al. (2004)
America	31/31	1.3–27.7	Arabica	"
Asia	14/14	1.6–31.5	Arabica and Robusta	"
Vietnam	30/10	0.4–1.8	Robusta	Leong et al. (2007)
Thailand	32/28	<0.6–55	Arabica	Noonim, Mahakarnchanakul, Nielsen et al. (2008)
Thailand	32/32	1.3–8.9	Robusta	"
India	39/25	0.2–4.3	Arabica	Gopinandhan et al. (2008)
India	41/34	0.5–13.5	Robusta	"

[a] Corresponds to the detection limit of the method.
[b] Not specified.

associated with production on crops of temperate climates and have somewhat lower growth and OTA production optima.

In contrast, *Aspergillus carbonarius* and *A. ochraceus* are common in warmer climates. Strains identified as *A. ochraceus* have been isolated from coffee samples (Batista et al., 2003; Martins, Martins, & Gimeno, 2003; Suárez-Quiroz et al., 2004; Taniwaki et al., 2003; Urbano, Taniwaki, Leitão and Vicentini, 2001) and the species was proposed as the major cause of OTA in coffee beans (Frank, 1999; Taniwaki et al., 2003) before *Aspergillus westerdijkiae* was recognized as a member of section Circumdati: these closely related species can be misidentified. For example, 84% of isolates from Brazilian coffee identified as *A. ochraceus* were *A. westerdijkiae* as assessed from sequencing the β-tubulin gene (Morello et al., 2007). OTA is also produced by *Aspergillus sulphureus*, *A. carbonarius*, *Aspergillus niger* and *Aspergillus sclerotiorum*. *A. carbonarius* and *A. niger* have been isolated from coffee, strains of which are capable of producing OTA (Joosten et al., 2001; Nakajima et al., 1997; Pardo, Marin, Ramos, & Sanchis, 2004; Suárez-Quiroz et al., 2004; Taniwaki et al., 2003; Téren et al., 1997; Urbano, Taniwaki et al., 2001), indicating that these are also potential sources in coffee. These findings led to the consensus that: *A. ochraceus*, *A. carbonarius* and *A. niger* are responsible (Batista et al., 2003; Taniwaki, 2006) and to these *A. westerdijkiae* must be added.

OTA-producing fungi require favourable conditions for a period of time, to grow and produce the toxin. The level of available water and temperature are the most important factors and some relevant data are reproduced here of use for understanding and controlling contamination. *A. ochraceus* optimally (a) grows at 30 °C and (b) produces OTA at 25–30 °C, which compares to *P. verrucosum* at (a) 26 °C and (b) 25 °C respectively. In addition, *A. carbonarius* produced OTA at a higher rate at 30 °C than 20 °C and grew optimally at 25–30 °C, with some Greek strains growing optimally at 35 °C, although optimal OTA production was 15–20 °C (Paterson & Lima, 2011), and so these strains are unusual. In general, these data indicate that the Aspergilli will dominate when they occur on coffee and produce OTA. Furthermore, whereas 12.5% moisture is considered a maximum water content for coffee to avoid OTA contamination (CAC, 2009), *A. ochraceus*, *A. flavus* and to some extent Penicillia, can grow at lower moisture on crops such as sunflower, safflower, peanuts and copra (Paterson & Lima, 2011) and so great care is required with respect to moisture content (MC) for coffee. OTA-producing fungi are unlikely to grow at high water activity ($a_w > 0.95$), as fast-growing hydrophilic fungi and yeasts will outgrow them. OTA-producing fungi can be present at $a_w < 0.80$ but not produce the toxin, and at below 0.78–0.76 they cannot grow. The limiting a_w for growth of *A. ochraceus* (*A. westerdijkiae*) and *A. niger* is 0.79 and 0.77

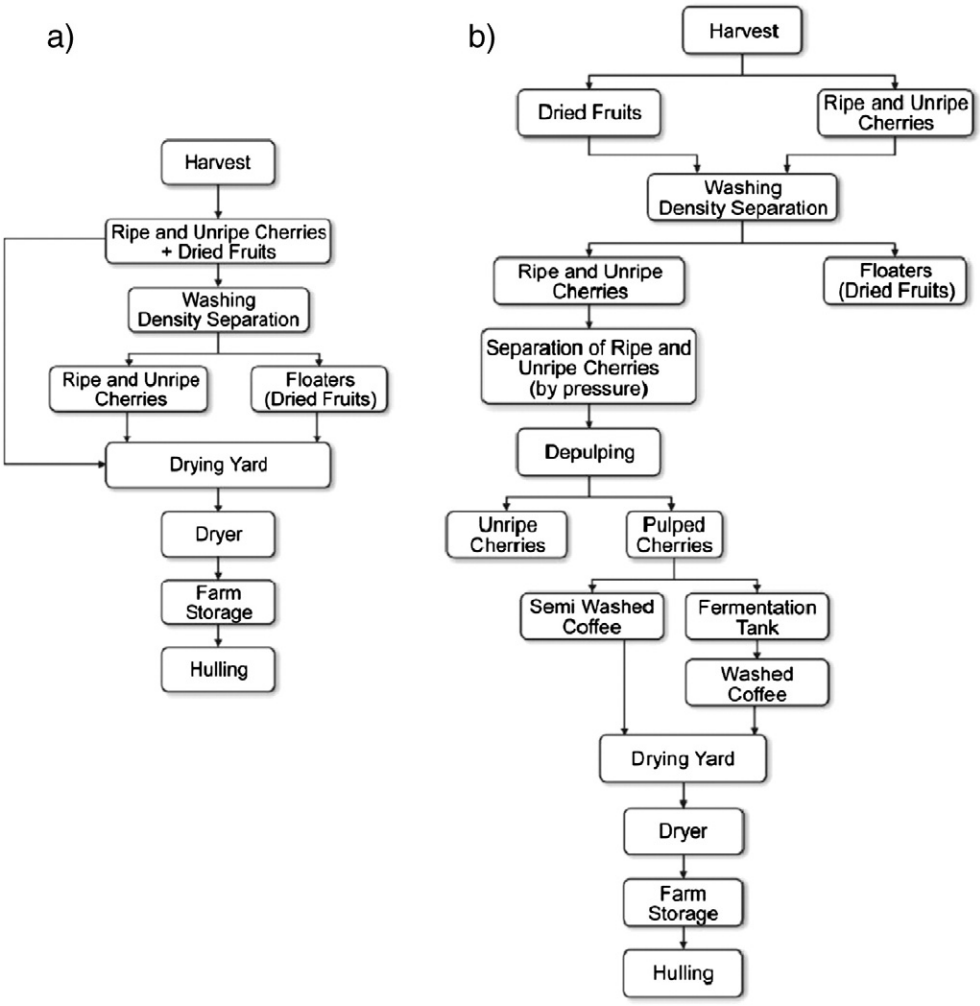

Fig. 2. Diagram to indicate the various stages of processing where contamination by fungi may occur. a. Represents dry processing and b. wet processing.

respectively (FAO/WHO, 2008; Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004). Finally, Silva, Batista, and Schwan (2008) state that the minimum a_w to produce ochratoxin is 0.85 (c. 20% humidity) in coffee grains for *A. ochraceus*, and the minimum and maximum temperatures of growth are between 8 and 12 °C and 24 and 31 °C, respectively, while for ochratoxin production, the temperature is between 25 and 31 °C.

Coffee production has three main steps (see Fig. 2): 1) pre-harvest; 2) post-harvest storage and transportation, and 3) raw coffee

Table 2
Incidence of ochratoxin A in commercial roasted coffee worldwide.

Retail country	No. of samples/no. of positives	Range of OTA (μg/kg)	Reference
Japan	68/5	3.2–17.0	Tsubouchi et al. (1988)
United Kingdom	20/17	0.2–2.1	Patel et al. (1997)
Europe	484/?	<0.5[a]–8.2	Van der Stegen et al. (1997)
Denmark	11/11	0.1–3.2	Jørgensen (1998)
Spain	29/29	0.22–5.64	Burdaspal and Legarda (1998)
United States	13/9	0.1–1.2	Trucksess et al. (1999)
Brazil	34/23	0.3–6.5	Leoni et al. (2000)
Brazil	47/41	0.99–5.87	Prado et al. (2000)
Germany	67/22	0.3–3.3	Wolff (2000)
Germany	490/273	0.21–12.1	Otteneder and Majerus (2001)
Canada	71/42	0.1–2.3	Lombaert et al. (2002)
Hungary	38/22	0.17–1.3	Fazekas, Tar, and Zomborszky-Kovács (2002)
India	2/0	<0.1[a]	Gopinandhan, Velmourougane, Panneerselvam, Keshamma, and Raghuramulu (2007)
Japan	9/3	0.11–0.33	Kumagai et al. (2008)
Japan	49/18	?–2.75	Aoyama et al. (2010)
France	30/25	≤0.025–11.9	Tozlovanu and Pfohl-Leszkowicz (2010)

?: no available data.
[a] Corresponds to the detection limit of the method.

processing to roasted/ground and soluble coffee, and contamination is possible throughout as discussed in the following (and see Tables 1–3).

2.1. Pre-harvest coffee

It is likely that OTA-producing fungi can infect coffee fruits remaining on the plant and produce OTA at or before harvest. This may involve two different contamination routes (a) through the flowers, without visible sign and/or (b) by coffee berry borers (CBB) (*Hypothenemus hampei*), that can take spores to the fruits, and make holes in the cherries and beans. Coffee fruits detach naturally from the plant, and/or through agricultural activities which may remain on the ground, and are more likely to be contaminated with fungi and OTA (FAO/WHO, 2008). Recommended practices to reduce the development and spore load from OTA-producing fungi in the plants, and CBB occurrence, are provided in FAO/WHO (2008).

To understand the inter-relationship between coffee and the contaminating fungi, it is mentioned here that Arabica coffee grows at 600–2000 m and an average temperature between 18 and 22.5 °C: Robusta coffee is grown at below 600 m where average temperature is between 22 and 26 °C, both in the wet tropics. As *A. ochraceus* optimally (a) grows at 30 °C and (b) produces OTA at 25–30 °C, whereas *P. verrucosum* optima are (a) 26 °C and (b) 25 °C respectively, there would be no reason to assume that only the Aspergilli were responsible for OTA in coffee. However, the coffee growth temperatures are averages and it is likely that much higher temperatures would occur which may inhibit or damage the *Penicillium* species, whereas the Aspergilli may grow well. In addition, *A. carbonarius* produced OTA at a higher rate at 30 °C than 20 °C and grew optimal at 25–30 °C, with some Greek strains growing optimally at 35 °C, although optimal OTA production was 15–20 °C (Paterson & Lima, 2011). In general, these data indicate that the Aspergilli will dominate when they occur on coffee plants and produce OTA. Furthermore, whereas 12.5% moisture is considered the maximum for coffee to avoid OTA (CAC, 2009) the fungi can grow at that level, and so great care is required with respect to MC for coffee.

The invasion of coffee by ochratoxigenic fungi is crucial to understanding OTA contamination and developing control strategies. Controlling these fungi preharvest is more difficult because the coffee is less containable in the field than in storage containers for example. The major risk factors and processing steps that can lead to contamination of raw coffee with OTA have been revised (Bucheli & Taniwaki, 2002). Taniwaki et al. (2003) found an average *A. ochraceus* infection of <0.6% to 4% on fruit on the plant. Sixteen and 35% were present in fruit (a) from the ground and (b) during drying and storage respectively. Furthermore, the discovery of endophytic fungi, including OTA-producing Penicillia, from coffee (Vega, Peterson, & Chaves, 2006, Vega et al., 2010) raises the possibility that apparently healthy coffee may contain OTA and that fungal growth could arise internally: OTA was produced by one endophytic isolate each of *Penicillium brevicompactum*, *Penicillium crustosum*, *Penicillium olsonii* and *Penicillium oxalicum*, although these species are not normally associated with OTA production. Finally, OTA being translocated from the soil into berries (Mantle, 2000) requires confirmation.

2.2. Post-harvested coffee

The opportunities for OTA accumulation can increase postharvest if poor procedures are employed such as slow drying. Equally, OTA will increase if inadequate storage conditions are employed, although will tend to be reduce during processing from (a) sorting, (b) analysis for OTA and segregation, and (c) roasting (Pitt, Taniwaki, & Cole, 2013) (see Fig. 2). As mentioned in the Ochratoxin A producing fungi in coffee section, OTA-producing fungi require favourable conditions for a period of time to grow and produce the toxin and this section should be consulted for details. Fungal sources of contamination are soil, equipment and drying-yard surfaces at the postharvest stage. There are various (a) ecological parameters involved in OTA biosynthesis and (b) types of coffee produced by different processing methods (Fig. 2) and localities (Fig. 1). Hence, it is important to evaluate the incidence of ochratoxigenic fungi and correlate this with OTA in beans at different stages of maturation and processing (Batista et al., 2009), although such correlations are seldom carried out and even fewer employ statistical methods. In addition, the interrelationship between other fungi and microorganisms is important in terms of which fungi will dominate and influence each other (Paterson & Lima, 2010a, 2011) and ideally the complete mycological spectrum on coffee requires consideration.

Post-harvest problems relate to unfavourable (a) climates for drying, (b) drying practice, (c) quality control and/or (d) storage conditions. Cherries contain sufficient water to support mould growth and OTA formation on the exterior during the initial 3–5 days of drying. Sun-drying of cherries can lead to OTA contamination if performed incorrectly and drying of fruits appears to carry a particular risk for the occurrence of black Aspergilli, perhaps linked to the resistance of the melanin (the black fungal pigment) to UV light. Drying is crucial to the development of *A. ochraceus* (cf. *A. westerdijkiae*) with the main factor being the time taken beyond a critical water activity (a_w) of c. 0.80. Berries should spend less than 4 days at a_w 0.97 to 0.80 and Palacios-Cabrera et al. (2004) showed that *A. ochraceus* produced 0.15 μg kg^{-1} in coffee beans at 0.80 a_w, but 2500 and >7000 μg kg^{-1} at 0.86 and 0.90, respectively. Again, these data are relevant to storage of beans, i.e. maintaining an a_w of 0.80 is crucial.

Bucheli and Taniwaki (2002) provided indirect evidence that the depulping process reduced OTA contamination. Suárez-Quiroz et al. (2004) evaluated OTA producing fungi during wet, mechanical and dry processing from harvest to storage (see Fig. 2). They concluded that there was little difference between the processes regarding the

Table 3
Incidence of ochratoxin A in commercial soluble coffee worldwide.

Retail country	No. of samples/no. of positives	Range of OTA (μg/kg)	Reference
Australia	22/7	0.2–4.0	Pittet et al. (1996)
United States	6/3	1.5–2.1	"
Germany	9/5	0.3–2.2	"
United Kingdom	80/64	0.1–8.0	Patel et al. (1997)
Europe	149/?	<0.5[a]–27.2	Van der Stegen et al. (1997)
Spain	9/9	0.19–1.08	Burdaspal and Legarda (1998)
Brazil	10/8	0.31–1.78	Prado et al. (2000)
Brazil	16/16	0.5–5.1	Leoni et al. (2000)
Germany	41/12	0.28–4.8	Otteneder and Majerus (2001)
Canada	30/20	0.1–3.1	Lombaert et al. (2002)
Brazil	82/81	0.17–6.29	Almeida et al. (2007)
India	17/13	0.1–3.0	Gopinandhan et al. (2007)
Japan	66/63	?–4.23	Aoyama et al. (2010)

[a] Corresponds to the detection limit of the method.

isolation of *A. ochraceus*, although the dry method promoted the presence of *A. niger* which may relate to the high melanin content of the fungus (Pitt & Hocking, 2009). However, the presence of ochratoxigenic fungi does not imply that OTA will be present for which biochemical analysis must be performed.

Of 269 isolates from post-harvest coffee, 75% at least were capable of OTA production, a higher percentage than was reported previously (Taniwaki et al., 2003), although this is dependent upon the detection system used for OTA. *A. carbonarius* was isolated much less frequently, but strains did produce OTA. Few cherries on the plant were contaminated with OTA species, indicating that infection was post-harvest predominantly, and the fungal sources were soil, equipment and drying yard surfaces. Variability in contamination rates was reflected in a wide range of OTA levels in samples from the drying yard and storage. *A. niger* was more common than *A. ochraceus* or *A. carbonarius*, but only 3% of isolates were capable of producing detectable OTA in vitro (Taniwaki et al., 2003). The situation in vivo requires determination.

More filamentous fungi were isolated from coffee (a) which had remained in the ground for long periods and (b) obtained by the floating technique (Batista et al., 2009): most of these were isolates of *Aspergillus* section Nigri from which OTA was not detected using the basic TLC method employed. However, OTA was detected from *A. ochraceus*, *A. sulphureus* and *A. sclerotiorum* which were isolated from other fractions. The interpretation that coffee from the ground and floated coffee may contain more OTA does not entirely follow from these observations. More work is required in obtaining correlations between the isolation of fungi and the determination of mycotoxins in the same samples of coffee (cf. enumeration of *A. flavus* and detection of AF in the same samples of chilies in Paterson (2007), where there was no correlation). Unsurprisingly, coffee beans obtained from being left on the ground are low quality and display more defects due to higher fungal proliferation, and associated OTA risk (Taniwaki et al., 2014). Beans from the "floated in water" coffee had higher fungal contamination, since they originated from malformed and damaged fruit: they had been exposed to climatic extremes in the plantation and to fungal contamination throughout the coffee production and preprocessing stages (Batista et al., 2009).

Furthermore, fungi (184 isolates) were obtained from coffee (Batista et al., 2003) and 155 isolates of *Aspergillus* and its teleomorphs, were distributed over 20 species, with five species (*A. ochraceus*, *A. sulphureus*, *Aspergillus tamarii*, *A. niger* and *A. flavus*) comprising 71% of the total. A large number of Aspergilli that produced OTA were detected, although more may have been detected if a more sensitive OTA analytic procedure had been employed. Low levels of OTA were detected in some of the processed beans, and there were limited attempts to correlate this with levels of ochratoxigenic fungi. Finally, the 25 *Penicillium* isolates comprised eight species and some species may have produced patulin (*Penicillium expansum*) and citrinin (*P. expansum* and/or *Penicillium citrinum*).

Species of *Aspergillus* sections Circumdati and Nigri were found in samples of coffee fruits and beans at all harvest and processing stages (Batista et al., 2009). *Aspergillus* section Circumdati representatives were detected in 80 and 41% of the fruits and beans respectively, although a significant reduction in this population was observed when processing began. OTA was detected from 85% of the 178 strains of *Aspergillus* section Circumdati (represented by 165 *A. ochraceus*, 3 *Aspergillus melleus*, 4 *A. sulphureus*, 2 *Aspergillus dimorphicus* and *A. sclerotiorum*, and 1 *Aspergillus auricomus*) by the basic TLC method. Ninety five, 25 and 66% of *A. ochraceus*, *A. sulphureus* and *A. sclerotiorum* strains were positive for OTA respectively. *A. ochraceus* was the most common in the case of section Circumdati species, accounting for c. 93% of the total; however, at least some of these may have been *A. westerdijkiae* as mentioned previously. OTA was not detected from *A. niger* (58) and *A. foetidus* (25) of *Aspergillus* section Nigri although these could be producers in vivo and OTA may have been detected using a more sophisticated analytical method.

Batista et al. (2009) state that fruit quality, climate during drying, drying procedure, presence of OTA-producing fungi, and farm conditions are more important than type of fermentation and drying platforms, with the conclusion that: (1) the bóia and varrição (which the current authors take to mean "floated" and "swept from the earth" respectively) presented the highest risk of contamination by ochratoxigenic fungi and OTA; (2) the fractions bóia and the mixtures of different coffees presented higher levels of contamination with OTA when dried in patios with earth, rather than with asphalt floors; and (3) the type of floor of the yard (i.e. asphalt, soil or cement) was irrelevant to contamination by OTA for coffee swept from the ground. The use of patios with earth floors should be avoided to produce the highest quality coffee.

Aspergilli and Penicillia were isolated from 11 samples of *C. arabica* and 1 sample of *C. canephora* coffee beans and OTA was detected from, particularly *A. ochraceus* and *Aspergillus ostianus* (Rezende, Couto, Borges, da Silva, & Batista, 2013). Black Aspergilli were predominant in green and dried coffee cherries from Thailand and the frequency was much higher than in beans from Vietnam or Brazil (Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2008; Taniwaki et al., 2003): *A. carbonarius* and *A. niger* were predominant in both types of beans. Similarly, Joosten et al. (2001) found that most green coffee samples from Southern Thailand were contaminated by black Aspergilli, of which 50% were *A. carbonarius*. Finally, *Aspergillus aculeatinus* and *Aspergillus sclerotiicarbonarius* (Noonim, Mahakarnchanakul, Varga, Frisvad, & Samson, 2008) were observed from Thai beans, which were previously undescribed black species.

Wet processing (Fig. 2) is applied to Arabica coffee beans and the mycobiota was highly influenced by (a) fermentation (water quality and equipment used) and (b) drying (time period, cleanliness of equipment and environment). Noonim, Mahakarnchanakul, Nielsen et al. (2008) observed a random distribution of filamentous fungi in Thai coffee bean samples from different growing sites in the same province (Chiang Mai). The year of harvest played a role in the mycobiota, with 2006 having a higher incidence of *Aspergillus* section Circumdati strains than 2007 and there were differences in ambient conditions from year to year.

2.2.1. Drying

Coffee yards with an asphalt floor for drying floated coffee presented lower risk than soil floors (Batista et al., 2009). Unsurprisingly, this was due to the moist samples being in contact with soil, favouring fungal infection of the coffee. However, high to moderate levels of OTA contamination were independent of the yard employed which apparently contradicts Good Agricultural Practices recommending cement-floors. For example, coffee cherries after harvest are dried directly, or after water sorting, in Thailand. Drying on a cement floor is done predominantly in this country, resulting in surface contact with soil and an increased chance of fungal contamination of damaged pulp. Re-wetting from rain is another important factor leading to more fungal infection in the North of Thailand than in the South, where the harvest is in the rainy season. The coffee beans have a thick pulp and if the drying process could be faster and more effective then fungal invasion would be prevented, resulting in less OTA contamination (Noonim, Mahakarnchanakul, Nielsen et al., 2008).

Thirty two dried coffee beans of (a) *C. arabica* and (b) *C. canephora* var. *robusta* from two different Thai plantations, were assessed for OTA producing fungi. Ninety eight percent of the coffee had fungal contamination which was reduced to 60% after surface disinfection (Noonim, Mahakarnchanakul, Nielsen et al., 2008). The Arabica beans had an average of 78% *Aspergillus* section Circumdati strains with *A. westerdijkiae* and *A. melleus* predominant; *Aspergillus* section Nigri species were found in 75% of the samples, whereas *A. ochraceus* was undetected. The identification of *A. westerdijkiae* is interesting as most other reports found the similar species, *A. ochraceus* (cf. Batista et al.,

2009; Taniwaki et al., 2003) and relates to the fact that the authors were aware of the current taxonomic revision.

Robusta coffee beans were 98–100% contaminated with predominantly *A. carbonarius* and *A. niger*: *A. westerdijkiae* was from only one sample. The diversity of the fungal population probably correlated with the geographical origin of the coffee, coffee cultivar, and processing methods. The lack of melanin in the Circumdati strain may have been a factor in reduced numbers from the Arabica coffee obtained from the North of the country, rather than the South, from where the Robusta was obtained. Representative isolates of section Circumdati (52) and Nigri (82) were analysed for OTA production using HPLC, which is a more sophisticated method than the TLC discussed elsewhere, and presumably more sensitive. Nevertheless, it has detection limits and may not have detected OTA production by some stains: *A. westerdijkiae* (42/42), *Aspergillus steynii* (13/13), and *A. carbonarius* (35/35) produced large amounts of OTA, while one isolate of *A. sclerotiorum* and 13% of *A. niger* produced intermediate quantities (Noonim, Mahakarnchanakul, Nielsen et al., 2008).

Noonim, Mahakarnchanakul, Nielsen et al. (2008) state that only *A. carbonarius* and *A. niger* produced OTA which appears to contradict their results as, for example, *A. westerdijkiae* was listed as a producer. All *A. carbonarius* strains produced high amounts of OTA with less ochratoxin B (ochratoxin B had not previously been analysed and is a less important mycotoxin). Other reports indicate that *A. carbonarius* from coffee is an efficient OTA producer (Joosten et al., 2001; Pardo et al., 2004). Taniwaki et al. (2003) observed that only 77% of the *A. carbonarius* isolates from Brazilian coffee beans produced OTA but this level may not be significantly different to the other reports, especially when it is unknown if nonproducers were present rather than strains from which the OTA was simply not detected (see Paterson, Venâncio, and Lima (2004) for a discussion on detection of mycotoxins). Thirteen percent of the *A. niger* strains from Thai coffee could produce OTA and OTB but in small amounts compared to *A. carbonarius*, in contrast to other studies where 2–3% of *A. niger* isolates could produce OTA (Noonim, Mahakarnchanakul, Nielsen et al., 2008; Taniwaki et al., 2003). Noonim, Mahakarnchanakul, Nielsen et al. (2008) suggested that the fungi with the greatest potential to produce OTA in Thai coffee beans were *A. westerdijkiae* and *A. carbonarius* in Northern Arabica and Southern Robusta coffee respectively. Also, these two species were predominant on each type of bean and all isolates produced detectable OTA. *A. niger* was also common in both types of coffee, although the authors consider it unlikely as an important source of OTA contamination because only 13% of the *A. niger* isolates could produce (detectable) OTA. Silva et al. (2008) found that species of OTA-producing fungi become prevalent in the final parts of the 22 day fermentation and drying involved in the 'dry' processing of coffee beans.

However, data were unavailable previously concerning the mycobiota in defective coffee beans, which could be a serious source of mycotoxins, until Taniwaki et al. (2014) investigated these types of samples. All the beans were dried and samples included green (immature), black, black green, sour and normal, which were investigated for (i) fungi and their capacity to produce OTA as detected by the agar plug method and (ii) OTA in the beans. The coffee came from two Brazilian coffee producing regions: a) Cerrado Mineiro, in the Western part of Minas Gerais State (average of 19.1 °C and 14.8 mm/month rainfall at altitude 1100 m), and produced good quality coffee, and b) Sorocabana in South Western São Paulo State (average of 17.7 °C and 64.9 mm/month rainfall at 800 m), and generally produced a low quality beverage.

The fungi detected were (a) *A. carbonarius*, (b) other members of *Aspergillus* section Nigri, (c) *A. westerdijkiae* and (d) other members of section Circumdati. *A. carbonarius* was found in low numbers and only in the Cerrado — MG region, although all isolates produced OTA. This species is common in Robusta coffee, in, for example, Thailand but is uncommon in Brazil. *A. westerdijkiae* and isolates from section Circumdati, were found at higher numbers in both regions and these may be the main source of OTA in defective beans, since most of the section Nigri strains were negative for OTA: as mentioned, this might relate to the method employed (Taniwaki et al., 2014).

The green (immature) bean was the most common defective sample at 38%, found from Cerrado — MG and Sorocabana — SP. However, the presence of ochratoxigenic fungi and OTA was low. The black green beans were also low in OTA from both regions. The black beans from the Cerrado — MG region had the highest OTA (11.3 μg/kg) and the reason may be due to these being (a) overripe cherries which were left on the tree, and/or (b) beans fallen on the ground: The fungi then had the opportunity to grow and produce the toxin in the coffee. The sour beans from the Sorocabana — SP region had the highest OTA concentration, due to deterioration from excessive fermentation causing the sour taste in the beverage. Overall, the reduction of defective beans in coffee, lowers ochratoxigenic fungi, reduces OTA and improves considerably beverage quality (Taniwaki et al., 2014).

Finally, it is important to appreciate that all steps from pre/harvest are inter-connected and that if drying is not performed properly, then this is more likely to lead to contamination during storage. Conversely, if the coffee is dried efficiently, then further contamination during storage can be minimized if effective storage conditions are maintained.

2.2.2. Storage

The conditions for storage to maintain low OTA levels in the tropics relate especially to water activity and temperature as discussed above in the Ochratoxin A producing fungi in coffee section, and these should be referred to for more information. Green coffee can be stored for c. 3 years under optimal conditions, although colour and flavour change even under the best conditions (Bucheli, Meyer, Vuataz, & Viani, 1998): Storage should be at 10–12% MC and good-quality green coffee requires to be used. Deterioration is minimized when the temperature is maintained below 26 °C and the relative humidity (RH) of the storage atmosphere ranges between 50 and 70%. Green Robusta coffee storage conditions are particularly variable under the conditions in southeast Asia (Bucheli et al., 1998). The MC of stored monsooned coffee is required to be 14.5% to prevent fungal growth, although this is higher than 10–12% recommended above; 14% MC and 75% RH were the limits for fungal spoilage of green coffee. Finally, *A. ochraceus* and *Penicillium* spp. were found to affect bean colour and beverage quality in stored coffee.

Silva et al. (2008) discovered that the number of isolated fungi in coffee grains increased during storage, and *A. niger* and *A. flavus* dominated. The number of species isolated from jute sacks was higher than from polystyrene bags which are less permeable and re-absorption of water occurs to a lesser extent that in the jute sacks: humidity and temperature were not favourable for the growth of toxigenic species. Furthermore, cold storage chambers at 3 °C are at a much lower temperature than the temperature cited for growth and toxin production and should be safe. Although several species of toxigenic fungi were found in the study by Silva et al. (2008) during coffee processing, neither OTA nor AF were detected in any sample. The use of good hygiene practices and management during processing minimize the production of mycotoxins in coffee (Silva et al., 2008).

The growth of fungi and OTA formation in stored Robusta raw coffee were investigated (Bucheli et al., 1998) and at 30 °C/60% RH, using aerated and non-aerated silos, neither the growth of ochratoxigenic fungi nor OTA production was observed. This study demonstrated that safe storage of coffee under humid tropical conditions can be achieved, even in the rainy season, if the initial a_w of beans stored in bags was 0.72 and never exceeded 0.75.

2.3. Methods for isolating fungi

The conditions employed for isolating and identifying fungi from coffee varied considerably in the above studies which may have affected which were isolated as, for example, taxa that produce few conidia will

be isolated less than those that produce many. OTA-producing endophytes in coffee (Vega et al., 2006) would not generally be isolated by the conventional methods. Furthermore, Paterson and Lima (2013) discussed how the presence of mutagens in the isolation media could mutate wild type fungi: Some fungal secondary metabolites are mutagenic and are produced in growth media (i.e. "self" produced mutagenic secondary metabolites). The range of mutagenic compounds from fungi is presented in Paterson and Lima (2013, 2014) and they include OTA and AF. Often dissimilar methods are employed for isolating fungi from food as discussed in Paterson and Lima (2014). Each growth condition will lead to quantitative and qualitative differences of the mutagenic secondary metabolites produced by fungi and these may interact with the fungi. Novel taxa from coffee could be artefacts because of mutagens and hence great care is required when describing new species. The current authors recommend that work is undertaken to limit contact between secondary metabolites in growth media and fungal isolates from coffee with the objective of ensuring wild type strains are obtained, as discussed in Paterson and Lima (2013, 2014).

For example, the time of growth requires minimizing when isolating from mixed samples on antibiotic-containing media, because the antibiotics used to control contaminants are often mutagenic. Similarly, the time on media used to identify the fungi and undertake PCR needs reduction (e.g. halved) as the fungi may produce self mutagenic compounds (e.g. ochratoxin A, AF). This could be problematic in some cases as some identification methods stipulate that fungi are grown for specific periods (e.g. 7 days). Media require analyses (usually by chromatography) to determine if mutagenic compounds were produced. Furthermore, the DNA of individual fungi should be analysed on different media and for various time periods to determine if mutation occurs. The physiology of secondary metabolite production could be determined so that fungi can be harvested for DNA analyses during trophophase (growth phase) rather than idiophase (metabolite production phase) to avoid contact with mutagens. Also, standard isolation methods are necessary to enable more accurate comparisons between studies. Paterson and Lima (2013, 2014) can be consulted for more information. Finally, solutions to these problems of experimental design and interpretations of results are not straight forward and will involve a considerable amount of research.

3. Ochratoxin A in coffee

OTA is the mycotoxin of most concern that is detected currently in coffee (Taniwaki, 2006). If levels of OTA are above, for example, the EU limits set for coffee, the commodity is liable to be rejected causing economic loss. Surveys on the presence of OTA in coffee have been undertaken in European countries on imported raw, roasted and soluble coffee produced and sold in Europe (Taniwaki, 2006); coffee production is governed increasingly by EU legislation. The EU limits published in 2006 (No. 1881/2006) are (a) 5 μg OTA kg^{-1} for roasted coffee beans and ground roasted coffee and (b) 10 μg kg^{-1} for soluble coffee (instant coffee) and it is interesting that some samples in surveys (Tables 2, 3) had higher levels. The European Coffee Federation (http://www.ecf-coffee.org/) (a) follows legislation, (b) defines the position of the coffee sector and (c) maintains contacts with the EU institutions. In addition, countries such as Brazil, Cuba, Indonesia, Islamic Republic of Iran, and Singapore have national regulations for the marketing of coffee (ANVISA, 2011; Vanesa & Pacin, 2013).

Biochemical analysis is undertaken to determine if OTA is present in coffee, frequently involving chromatography. The fate of OTA during processing of coffee beans is not fully understood, but the results of surveys for OTA in retail roasted, and soluble coffees (Tables 2, 3) indicate that it is not a major source of OTA in the diet, with estimated intakes being well within safety limits (Taniwaki, 2006). Nevertheless, limits are placed on coffee which require adherence, otherwise rejection of the commodity can result from importing countries. It is apparent that high levels in coffee were reported originally in some cases (Batista et al., 2003; Micco et al., 1989; Nakajima et al., 1997; Romani, Sacchetti, Chaves López, Pinnavaia, & Dalla Rosa, 2000; Trucksess, Giler, Young, White, & Page, 1999) and that vigilance is required. Extensive sampling of raw Arabica and Robusta coffee from all origins has shown that OTA contamination may be more frequent in some areas, but that no producing country is entirely free from contamination (Table 1).

Only some fractions of coffee contain the mycotoxin and levels were from 0.5 to 23.0; 9.9 to 46; 0.2 to 5.5; 0.1 to 17.4; 0.1 to 4.6 and 0 to 48 μg kg^{-1} (Batista et al., 2003; Micco et al., 1989; Nakajima et al., 1997; Romani et al., 2000; Trucksess et al., 1999) which are high in some cases and beyond EU limits. Green and roasted coffee beans have been analysed (Batista et al., 2009; Lombaert et al., 2002; Patel, Hazel, Winterton, & Gleadle, 1997; Studer-Rohr, Dietrich, Schlatter, & Schlatter, 1995; Urbano, Taniwaki et al., 2001; Van der Stegen et al., 1997; Vecchio, Mineo, & Planeta, 2012) and 18% of green coffee beans had between 9.9 and 46 μg kg^{-1} (Nakajima et al., 1997). OTA was detected at low levels in 2 of 7 samples in a paper dedicated to assessing the Matrix Solid Phase Dispersion method (Rubert, Soler, & Mañes, 2010). Correlation studies on the OTA content of coffee and the presence of ochratoxigenic fungi from the same coffee sample are rare (although see Urbano, Freitas Leitão, Vicentini and Taniwaki, 2001; Urbano, Taniwaki et al., 2001), but essential, for a full understanding of which fungi produce the mycotoxin in coffee (Batista et al., 2003).

The presence of OTA in Brazilian coffee beans has been evaluated extensively with contamination levels varying significantly (Batista et al., 2009). Only low levels of contamination were found and only in a few samples, for example, an average of 2.4 μg kg^{-1} was found in 50 coffee bean samples (Leoni, Valente Soares, & Oliveira, 2000). However, a high incidence of OTA contamination was observed in Thai coffee beans but the amounts were low. No significant differences in OTA were observed between parchment and green coffee beans for Arabica coffee and the average OTA concentration in dried coffee cherries was higher than that found in green coffee beans. In contrast, OTA in dried coffee cherries was lower than in green coffee beans in the case of Robusta coffee samples. High contamination of *A. carbonarius* in Robusta dried cherries was probably the source of OTA contamination (Noonim, Mahakarnchanakul, Nielsen et al., 2008). A study carried out in Brazil demonstrated that "sour" and "black" defective beans had the highest contamination by ochratoxigenic fungi and OTA concentrations compared to other defective beans (Taniwaki et al., 2014). The levels of OTA were 11.3 μg/kg and 25.7 μg/kg for sour and black beans, respectively.

Wet processing (Fig. 2) gave the lowest level of OTA, although, perhaps surprisingly, the presence of *Aspergillus* section Circumdati strains was high, from which OTA was detected in vitro (Batista et al., 2009). Dry processing yielded higher levels of OTA in some cases. However, the number of samples analysed was often considerably higher than the case for wet processing which may have biased results. Highest OTA concentrations were detected in the coffee which had been lying on the ground for long periods and in "floated" coffee to a lesser extent: Mixed samples were also high in OTA. Interestingly, only *Aspergillus* Section Nigri strains were isolated at high levels from the dry-processed coffee, but none was positive for OTA (this may have reflected the detection limits of the methods employed). Wet processing appears more effective at producing low OTA levels, however, the presence of the fungi from which OTA was detected is a concern and presumably the conditions were not conducive to high OTA levels in vivo (Batista et al., 2009).

OTA in coffee beans was analysed using ELISA kits (and confirmed by LC–MS/MS in some cases): Ninety eight percent of 64 coffee bean samples were contaminated with OTA from 0.6 to 5.5 μg kg^{-1} (Arabica) and 1–27 μg kg^{-1} (Robusta) (Noonim, Mahakarnchanakul, Nielsen et al., 2008), making the Robusta concentrations notably higher. Furthermore, 58% of 40 bean samples were infected with potentially ochratoxigenic fungi, but only 22% of these beans were contaminated with OTA with

levels from 0.47 to 4.82 ng g^{-1}, and an average level of 2.45 ng g^{-1} (Batista et al., 2003), hence there was no correlation between the two factors.

The mean levels of OTA in Colombian coffee were 10 and 6.8 μg kg^{-1} for green and soluble coffee respectively (Vanesa & Pacin, 2013). Also, these authors determined the occurrence of OTA in coffee beans, ground/roasted coffee and soluble coffee, which were manufactured in Argentina. Fifty one samples were analysed by HPLC and a high percentage (69%) of the coffee was contaminated, although the medians obtained were 2.7, 0.24 and 0.43 μg kg^{-1} respectively, which are low values. OTA has been detected in coffee from many other countries (Vanesa & Pacin, 2013) (Tables 1–3).

3.1. De-husking and drying

Effective control measures require steps for contamination avoidance which includes using good quality coffee and processing conditions: The husks of coffee beans are a significant source of OTA, thus their removal is effective in reducing OTA. (It may be worth mentioning that technologies involved in utilizing husks as a byproduct (e.g. Mussatto, Machado, Martins, & Teixeira, 2011) need to consider that they may be high in OTA.) A 90% reduction of OTA can occur during the processing of beans to roasted and soluble coffee (Table 4) (Batista et al., 2009). The optimal conditions of water activity and temperature to control growth and OTA production by fungi are discussed in the Ochratoxin A producing fungi in coffee section and should be referred to.

The toxin is produced during the sun drying of coffee, and overripe cherries appear more susceptible than green ones, as demonstrated in coffee produced in Thailand over three seasons (Bucheli, Kanchanomal, Meyer, & Pittet, 2000). OTA was formed in the coffee cherry pericarp (i.e. the pulp and parchment) which is the part of the cherry removed as husk in the dehulling process (Fig. 2). This often produces dust containing OTA which may be transferred to the raw coffee and requires to be avoided (e.g. by workers). Furthermore, coffee beans had only 1% of the level of OTA found in husks. More than 90% of OTA contamination was concentrated in the husks and so cleaning, grading and hygienic storing of raw coffee are paramount. Apparently, fraudulent addition of husks to bulk out coffee is undertaken and which may lead to high levels of OTA contamination (Pittet, Tornare, Huggett, & Viani, 1996) and this needs to be discouraged. Suárez-Quiroz et al. (2004) reported that OTA was not eliminated after dehulling coffee and the concentrations in the coffee beans, parchment and husk were similar, although this appears atypical: the contamination was from between trace to 0.3 μg kg^{-1}. However, too few samples were analysed to make general conclusions.

OTA contamination of raw coffee depended on cherry maturation, with overripe cherries being the most contaminated, in which case cherries with defects and husk inclusion were the most important sources of contamination (Taniwaki et al., 2003). The main producer of OTA was *A. carbonarius* in this study. In general, results demonstrated that (a) raw material quality, (b) weather conditions during drying, (c) drying management, (d) presence of OTA producing fungi, and (e) local farm conditions, played a more important role in OTA contamination in raw coffee than the drying methodology (e.g. bamboo tables, bare ground or concrete) if it was performed efficiently. OTA will not be produced if drying is effective (Taniwaki et al., 2003).

Table 4
Effect of roasting on ochratoxin A reduction.

Toxin origin	Roasting condition	% OTA reduction	References
Inoculation[a]	200 °C/10–20 min	0–12	Tsubouchi et al. (1988)
Natural[b]	5–6 min/dark roasting	90–100	Micco et al. (1989)
Natural[b]	252 °C/100–190 s.	14–62	Studer-Rohr et al. (1995)
Inoculation[a]	252 °C/100–190 s.	2–28	"
Natural[b]	223 °C/14 min	84	Blanc et al. (1998)
Inoculation[a]	200 °C/10 min (medium)	22.5	Urbano, Freitas Leitão et al. (2001)
"	200 °C/15 min (medium)	48.1	"
"	210 °C/10 min (medium dark)	39.2	"
"	210 °C/15 min (medium dark)	65.6	"
"	220 °C/10 min (dark)	88.4	"
"	220 °C/15 min (dark)	93.9	"
Inoculation[a]	180 °C/5 min (light)	8	Ferraz et al. (2010)
"	180 °C/8 min (light)	42	"
"	180°/12 min (light)	54	"
"	200°/5 min (light)	30	"
"	200°/8 min (light)	56	"
"	200°/12 min (light)	67	"
"	220°/5 min (light)	47	"
"	220°/8 min (light)	76	"
"	220°/12 min (medium)	87	"
"	240°/5 min (light)	77	"
"	240°/8 min (dark)	94	"
"	240°/12 min (dark)	99	"

[a] Coffee beans inoculated with toxigenic spores of *Aspergillus ochraceus*.
[b] Naturally contaminated beans.

3.2. Storage

Storage is a very important stage at which OTA contamination can occur. If ochratoxigenic fungi are present on coffee or are introduced through poor storage conditions then they will grow and could produce OTA. In general, the data provided above in the Ochratoxin A producing fungi in coffee section and Storage section are relevant and can be consulted to determine the temperatures and water activities suitable for growth and OTA production by fungi. The conditions for coffee that are required to avoid contamination are also listed. In addition, OTA formation during storage of Robusta raw coffee was investigated (Bucheli et al., 1998) and at 30 °C/60% relative humidity, using aerated and non-aerated silos, OTA production was unobserved, demonstrating that safe storage of coffee under humid tropical conditions can be achieved, even in the rainy season, if the initial a_w of beans stored in bags is c. 0.72 and never exceeds 0.75.

3.3. Transportation

Condensation can occur during raw coffee transportation to the consuming countries and lead to mould growth (the Ochratoxin A producing fungi in coffee section of this paper should be consulted for relevant information on water activity and temperatures that affect fungal growth and see the Storage section). Blanc, Vuataz, and Hilckmann (2001) reported the transport of raw coffee in bulk or bags in containers where condensation was recorded at the top of the container, for example in a European harbour in winter. The appearance of areas with high moisture favours mould growth and OTA formation as discussed above. Storage and transportation trials have shown that condensation and wetting of coffee occur mainly during transport overland to the harbour for shipping and/or upon arrival at the destination.

Furthermore, Palacios-Cabrera et al. (2007) investigated OTA production during transportation and verified that the temperature inside coffee containers changed drastically during the voyage from a coffee exporting country such as Brazil (in summer) to an importing country such as Italy (in winter). This induced condensation at the top of the containers, causing an increase in MC from 12.26 to 14.13% (dry weight) and led to OTA formation from <0.1 to 7.91 μg kg^{-1} in the coffee beans at the top of the container located on deck at the end of the voyage.

3.4. Roasting and decaffeination

Roasting involves subjecting raw coffee to 180–250 °C for 5 to 15 min under which conditions chemical changes occur that (a) affect

aromas and colour and (b) cause water and carbon dioxide loss (Clarke, 1987). The process generates volatile compounds (aromas and acids) and non-volatiles (melanoidins and their acid precursors) formed by the Maillard reaction and caramelization (Bonnlander, Eggers, Engelhardt, & Maier, 2005). Initial data suggested that almost complete destruction of OTA would result from the roasting process (Micco et al., 1989). However, occurrences in market samples of roasted coffee beverages were reported after an improved detection method was introduced (Studer-Rohr et al., 1995) and the European Commission Scientific Committee for Food (SCF) (1995) considered that OTA in coffee was a contaminant. OTA levels were determined to decrease during the roasting process (Castellanos-Onorio et al., 2011; La Pera et al., 2008), but OTA in green coffee beans was not completely degraded by roasting procedures, and most of the mycotoxin was transmitted into the beverage (Tsubouchi, Terada, Yamamoto, Hisada, & Sakabe, 1988). In addition, OTA was present in commercial roasted coffee beans in five out of 68 samples at 3.2–17.0 μg kg^{-1} (Tsubouchi et al., 1988), which were similar to levels found in green coffee beans (Nakajima et al., 1997).

Conflicting data are found on the influence of roasting: Blanc, Pittet, Muñoz-Box, and Viani (1998) investigated the behaviour of OTA and the production of soluble coffee, where the most significant reduction occurred during the roasting process (Table 4); final coffee contained only 16% of the original OTA. Leoni et al. (2000) studied 34, 14 and 2 samples of ground roasted, instant and decaffeinated coffee respectively and found between 0.3 and 6.5 μg kg^{-1} of OTA in 23 of the ground roasted samples, and an average of 2.2 μg kg^{-1} in the instant coffee. Coffee beverage was prepared from the roasted samples and OTA was found in 74 to 86%. Urbano, Freitas Leitão et al. (2001) tested 18 samples of coffee inoculated with *A. ochraceus* which produced OTA. The samples were submitted to 200 °C, 210 °C and 220 °C for 10 to 15 min (Table 4) and the destruction varied from 22 to 94% depending on the time/temperature. However, 220 °C for 10 to 15 min may produce an unacceptable beverage as it damages the coffee. The scientific literature provides evidence that the roasting process is efficient in reducing OTA (Gollücke, Tavares, & Taniwaki, 2004), but there is a lack of more conclusive research on the effects of the stages of roasting, grinding and beverage preparation on the stability of the toxin.

Ferraz et al. (2010) studied the kinetics of OTA destruction during coffee roasting using a vertical spouted bed roaster (SBR) at 180, 200, 220 and 240 °C for 5, 8 and 12 min and reductions from 8% to 98% OTA were observed. SBR was very efficient for OTA reduction in coffee, and was a temperature-dependent reaction that followed first order reaction kinetics and may become an important predicting tool in the coffee industry. However, relying on roasting to decontaminate beans will greatly affect the quality of the beverage and severe roasting of coffee beans will also directly affect the level of chlorogenic acid and compounds beneficial to human health. Coffee with low OTA content should always be used because roasting, or other processes, to reduce OTA may detract from the quality of the product.

The fate of OTA during decaffeination and roasting processes has also been investigated (Blanc et al., 1998; Micco et al., 1989; Vecchio et al., 2012). Concentrations were 0.66 and 1.46 μg kg^{-1} for roasted and green coffee in a German study. Sixty percent contamination (highest 5.66 μg kg^{-1}) and 66.6% (highest 8.35 μg kg^{-1}) for green and roasted coffee respectively was reported in Egyptian work which are atypical results. In Costa Rican, coffee average, median and highest levels of 0.33, 0.036 and 0.96 μg kg^{-1} respectively were detected in roasted coffee (Vanesa & Pacin, 2013).

3.5. Coffee products

The levels of OTA occurrences tend to be similar between countries in the case of final coffee products. Vecchio et al. (2012) demonstrated that coffee contamination was below the EU limit but the incidence was high. OTA was detected in 96% of samples, and was over 2 μg kg^{-1} in 12%. No significant difference in values between normal and decaffeinated instant coffee was observed. For coffee products the contamination ranged between 0.33 and 0.52 μg kg^{-1} with a lower frequency of 30 to 60%. The authors concluded that pure soluble coffee is not a major source of OTA in the diet of Italians (Vecchio et al., 2012). However, the high frequency of OTA occurrence in the instant coffee samples demonstrates the importance of effective control of this product which is undertaken by government authorities and the industry (Vecchio et al., 2012). Heilmann, Rehfeldt, and Rotzoll (1999) showed that OTA was reduced significantly during the industrial process, especially in decaffeinated coffee by solvent extraction.

4. Ochratoxin A consumption from coffee

The role of coffee consumption in OTA intake by humans has been investigated. JECFA has set a Provisional Tolerable Weekly Intake (PTWI) for OTA of 100 ng kg^{-1} bw $week^{-1}$. Patel et al. (1997) calculated an Estimated Weekly Intake of OTA from soluble coffee from a survey of coffee drinkers (Table 3). The average coffee drinker ingested 0.4 ng kg^{-1} body weight $week^{-1}$ based on a consumption of 4.5 g of soluble coffee per day, while the heavy consumer (97.5% percentile) consumed nearly 20 g soluble coffee per week to give an intake of 1.9 ng kg^{-1} body weight/week. Those figures translate into 3.5 ng and 17 ng intake of OTA per day and 0.4% or 2% of the PTWI respectively. The average OTA concentration in roasted coffee was 0.9 μg kg^{-1} (Table 2) using the data obtained by Leoni et al. (2000) in Brazil, where ground and roasted coffee is most used, as is the case for many coffee producer countries. The average Brazilian adult drinks five cups of coffee per day then this would correspond to 30 g of roast and ground coffee (Leoni et al., 2000). The probable daily intake of OTA by a 70 kg adult would be 0.4 ng kg^{-1} bw day^{-1} and this falls far below the JECFA PTDI. These results indicate that coffee is not a major dietary source of OTA and this situation should be similar to other coffee drinking countries.

5. Aflatoxin in coffee

The information on the occurrence of AF in green and ground roasted coffee beans is limited despite the obvious importance of it being the most dangerous mycotoxin, with statutory limits set by the EU at low μg kg^{-1} levels for many commodities (Regulation No. 1881/2006). AF are produced predominately by *A. flavus* and *Aspergillus parasiticus*. The temperature optima for *A. flavus* growth and AF production are 33 and 35 °C respectively (Paterson & Lima, 2011) which are higher than those for ochratoxigenic fungi, with implications for CC (see later). In addition, higher humidity levels tend to favour *A. flavus* growth and AF production (Paterson & Lima, 2011). The minimum a_w for AF production by *A. flavus* is 0.82 (c. 18.4% humidity) and the minimum and maximum temperatures of growth for *A. flavus* range between 6 and 10 °C and 25 and 37 °C respectively, however, for AFB_1 and B_2 production the optimum temperature is between 16 and 31 °C (Silva et al., 2008).

Some *A. flavus* strains isolated by Batista et al. (2003) produced detectable AF by a basic TLC method, although the coffee was not analysed. However, AF in coffee has been reported (Soliman, 2002), although at low levels. Aflatoxin was detected during the later stages of fermentation particularly and during storage, especially for longer periods (Silva et al., 2008). AFB1 was detected in 50% of samples at levels as high as 37 μg kg^{-1} in coffee from Guatemala in an early report and contamination of AFB1 in 1% of green coffee beans at 3–12 μg kg^{-1} was reported (Nakajima et al., 1997). AFB1 was in 32% of 47 samples of commercial green coffee beans at 2–32 ng kg^{-1} (Soliman, 2002). The frequency of *A. flavus* ranged between (a) 4 and 80% and (b) 1 and 71% in (a) green coffee beans, and (b) ground roasted coffee beans respectively. AF were detected in (a) 76.5 and (b) 54.6% of the infected samples, with averages of (a) 4.28 and (b) 2.85 μg kg^{-1} in (a) green

and (b) ground roasted coffee beans respectively. Interestingly, roasting lowered the concentration of AF in green coffee beans by approximately 50%. The highest levels of AF were detected in decaffeinated green coffee and roasted coffee beans at 24.29 and 16.00 μg kg^{-1} respectively (Soliman, 2002). Four (18.2%) *A. flavus* isolates produced AFB1, B2, G1, and G2, although these may not have been *A. flavus* as this species is considered not to produce all of these. They may have in fact been *A. parasiticus* which does and is morphologically similar to *A. flavus*. *A. flavus* was detected (a) predominantly in *C. arabica* (Rezende et al., 2013) and (b) frequently in coffee in other investigations (Batista et al., 2003, 2009). Finally, AF were detected from some *A. flavus* isolates from coffee (Rezende et al., 2013).

Nakajima et al. (1997) reported the effect of heat treatment on the destruction of AF in green coffee beans and several other studies have indicated that AF in coffee beans are degraded (Soliman, 2002). Roasting reduced the individual AF levels in coffee, although AFB1 was the most persistent followed by AFG2 and AFB2. Oven and microwave methods of roasting caused AF destruction of c. 47.8 and 42.2%, respectively whereas the traditional method caused a 55.9% reduction in total AF (Soliman, 2002). Finally, AF may become particularly important with CC over the next 50 years and hence continual monitoring is required: it is important to develop methods for preventing AF contamination.

6. Fumonisin in coffee

In the first report of fumonisin (FUM) from coffee, 64 and 32 Thai Arabica and Robusta dried coffee beans respectively were assessed. *Fusarium* species known to produce FUM were not detected, but black Aspergilli had high incidences on both coffees. Liquid chromatography with high-resolution mass spectrometric detection showed that 67% of *A. niger* isolates from coffee beans were capable of producing FUM B2 (FB2) and B4. Low concentrations of FB2 (1–9.7 μg kg^{-1}) were detected in seven of 12 coffee samples after ion-exchange purification and LC–MS/MS detection: two also contained FB4. This was the first record of *A. niger* strains producing FUM (Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2009). However, the levels were low in coffee and more work is required to determine if this is a more general problem.

7. Other mycotoxins in coffee

Other mycotoxins in coffee have been studied insufficiently for inclusion in this review.

8. Mixtures of mycotoxins in coffee

Synergistic effects of combinations of mycotoxins are important and they occur commonly in crops. Interactive effects can be classified as additive, less than additive, synergistic, potentiative, or antagonistic. Levels of (a) AFB1 and (b) OTA were (a) 32% (2.0–32.9 ng kg^{-1}), and (b) 30% (0.1–17.4 μg kg^{-1}) respectively in a Japanese study (Nakajima et al., 1997). Arabica coffee from Yemen and Tanzania and Robusta from Indonesia were found to contain both mycotoxins frequently but they were not detected in Arabica and some unnamed coffees from Central and South America.

9. Dietary supplements

In an interesting study on the use of green coffee extracts (GCE) in 50 dietary supplements, OTA and ochratoxin B, FUM and mycophenolic acid were detected in 36%, 32%, 10%, and 16% of tested products, respectively. The results are difficult to interpret in terms of the contribution of the GCE to the mycotoxin load, because of the inclusion of the other components which were, *inter alia*, cellulose, gelatin, rice bran, rice hull, maltodextrin, black pepper, coconut oil, beeswax, red cabbage, turmeric and raspberry (Vaclavik, Vaclavikova, Begley, Krynitsky, & Rader, 2013). In addition, the samples were obtained by ordering over the internet and the compositions were only "declared" rather than being independently determined.

10. Climate change and mycotoxins in coffee

The effects of CC on diseases of coffee has had limited attention and there is no information on the effects on mycotoxins in coffee, although some general points can be made. The data available on tropical crops in general is based on modelling studies: little empirical knowledge exists for the development of adaptation strategies (Ghini, Bettiol, & Hamada, 2011; Paterson, Sariah, & Lima, 2013). In general, there is insufficient information on the effects of CC on tropical crops. Coffee is grown in the wet tropics (see the Introduction section and Fig. 1) and at these environmental conditions there is less scope for adaption to, for example, higher temperatures as discussed for other tropical crops (e.g. Paterson & Lima, 2012; Paterson et al., 2013). In addition, there is high confidence that mean seasonal and annual temperatures will be larger in the tropics than mid-latitudes relative to natural internal variability (IPCC, 2013). Robusta coffee is grown at lower altitudes (sea level — 900 m) at 10° north and south of the equator (see Fig. 1) and is much more tolerant of hot conditions than Arabica coffee (International Coffee Organisation, 2012): the vigour and disease resistance of Robusta are superior to Arabica (FAO, 2006). Consequently, Robusta will be more resistant to CC and crosses between Robusta and Arabica (FAO, 2006) could be useful in combating CC.

Long-term datasets are rare in relation to tropical and plantation crop diseases, which are a prerequisite for finding fingerprints of inter-annual climatic variation on plant diseases (Jeger & Pautasso, 2008): multifactor studies of CC effects have not been attempted. Experimental studies of the long-term effects of increased CO_2 and O_3 concentrations on tropical and plantation crops in more realistic field settings have not been carried out comprehensively and the rule that high concentration of atmospheric CO_2 results in higher yield and plant development may not apply. New Open Top Chambers designs and environmental control systems are necessary to address the requirements of tropical plant pathosystems (Ghini et al., 2011), including those for coffee. Much of the literature concerns modelling approaches to determine how tropical disease distribution may change (Ghini, Hamada, & Bettiol, 2008) and Deutsch et al. (2008) observed that warming in the tropics is likely to have the worst effects compared to the situation in temperate regions for example.

Coffee farmers already suffer from increased climate warming (Baker & Haggar, 2007; Haggar & Schepp, 2011) where the sustainability of the coffee industry faces serious challenges in the coming decades (Baker & Haggar, 2007; Camargo, 2010; Haggar & Schepp, 2011; Jaramillo et al., 2011; Titus & Pereira, 2008; Zullo, Pinto, & Assad, 2006) from the response of the crop to high temperature. Arabica productivity is influenced highly by CC (Camargo, 2010) and fruit development is increased at above 23 °C, leading to reduced beverage quality (Camargo, 1985), although with satisfactory yields (e.g. in northeast Brazil) (DaMatta, 2004): this loss of quality could include higher mycotoxin contamination (Paterson & Lima, 2010a, 2011). Furthermore, continuous exposure to 30 °C leads to stress, such as low, abnormal growth including leaf-yellowing and stem-tumours (Franco, 1958). If an increase of 4–6 °C from CC is obtained in 50 years as predicted (Paterson & Lima, 2010a, 2011, 2012) then current growing regions will likely experience this temperature and mycotoxin contamination is more likely. Coffee growth is depressed below 17–18 °C (DaMatta & Ramalho, 2006) and frosts may limit economic success (Camargo, 1985; Davis et al., 2012). Finally, elevated atmospheric CO_2 concentration (as expected from CC) on coffee leaf rust was evaluated (Ghini et al., 2011) and the average latent period was c. 36 days at 400 p.p.m., reduced to 21, 21 and 19 days at 500, 700 and 900 p.p.m. CO_2, respectively.

Furthermore, indigenous Arabica coffee is fundamental to coffee production in Ethiopia (Teketay, 1999) with a value to the industry of 0.5 to 1.5 billion US$/year. The country is the principal African coffee producer and the fifth largest Arabica exporter. Indigenous populations of coffee are a key resource for (a) the medium- to long-term sustainability of Arabica production as a "future-proofing" resource, and (b) providing genetic material to mitigate CC (Davis et al., 2012; Hein & Gatzweiler, 2006). Also, the CBB pest poses a significant threat to Arabica in indigenous populations and plantations. The insect is able to complete one or two generations/year/coffee season (Jaramillo et al., 2009, 2011) from none/year in SW Ethiopia and the suitability of the climate for the CBB is predicted to increase in the region (Davis et al., 2012; Jaramillo et al., 2011). The increase in the occurrence of this insect is likely to bring associated mycotoxigenic fungi which will be able to grow as a result of the damage caused to the coffee. A study of East African Kihansi coffee which is restricted to the Kihansi Gorge in Tanzania (Davis & Mvungi, 2004), provides an example of how coffee is influenced by pests under accelerated CC. This local change coincided with a parasitic infestation which undermined the potential of this coffee, with negative consequences for survival of the coffee species (Rija, Mwamende, & Hassan, 2011). However, re-locating coffee plantations will bring economic benefits within a realistic time frame (Davis et al., 2012) and this may include new plantations having fewer pest and diseases from the "Parasites Lost" phenomenon (see Paterson & Lima, 2011, 2012; Paterson et al., 2013), where crops planted in novel locations often have fewer pests.

Some Arabica coffee might resist CC until 2080 suggesting they should be candidates for long-term in situ conservation (Davis et al., 2012), although the increase in hostile conditions means they may be more susceptible to mycotoxigenic fungi. The levels of mycotoxins may increase (Magan, Medina, & Aldred, 2011), as will those fungi more suited to growth at higher temperatures such as AF producers (Paterson & Lima, 2010a). Overall, the models show a profoundly negative influence of CC on indigenous Arabica and the most favourable outcome was a 65% reduction in the number of pre-existing suitable localities in terms of climate, and at worst, an almost 100% reduction, by 2080. Bioclimatic unsuitability would place coffee populations in peril, leading to severe stress and a high risk of extinction. Arabica coffee is a climate sensitive species, supporting data and inferences that existing plantations will be negatively impacted by CC (Davis et al., 2012).

Consequently, the general response of crops to CC means that the change in, for example, temperature will make growing the crop impossible in some areas and the mycotoxin issue will be irrelevant. This is likely to occur in large amounts of land normally suitable for coffee growth (see Paterson & Lima, 2011, 2012; Paterson et al., 2013). There will be an intermediate region where growing the crop is possible but the crop will be subjected to greater stress from suboptimal climatic conditions, poor soil, insects, and pest and disease microorganisms, which will make the process less profitable or unprofitable. In this scenario, mycotoxins will become a particularly serious problem as the toxigenic fungi will be able to invade the coffee crop more readily. More dangerous mycotoxins will probably become prevalent at the higher temperatures and, for example, AF will tend to supersede OTA as the major mycotoxin, because temperatures will become more suitable for the thermotolerant Aspergilli that produce AF (Paterson & Lima, 2010a). This is one of the major issues for future coffee production. In addition, FUM may become more prevalent as it is a higher temperature mycotoxin, as described for maize (Paterson & Lima, 2012). The third scenario is that coffee will be grown in new land which may allow low levels of mycotoxins because of the Parasites Lost phenomenon, where new growth crops often have fewer pests and diseases

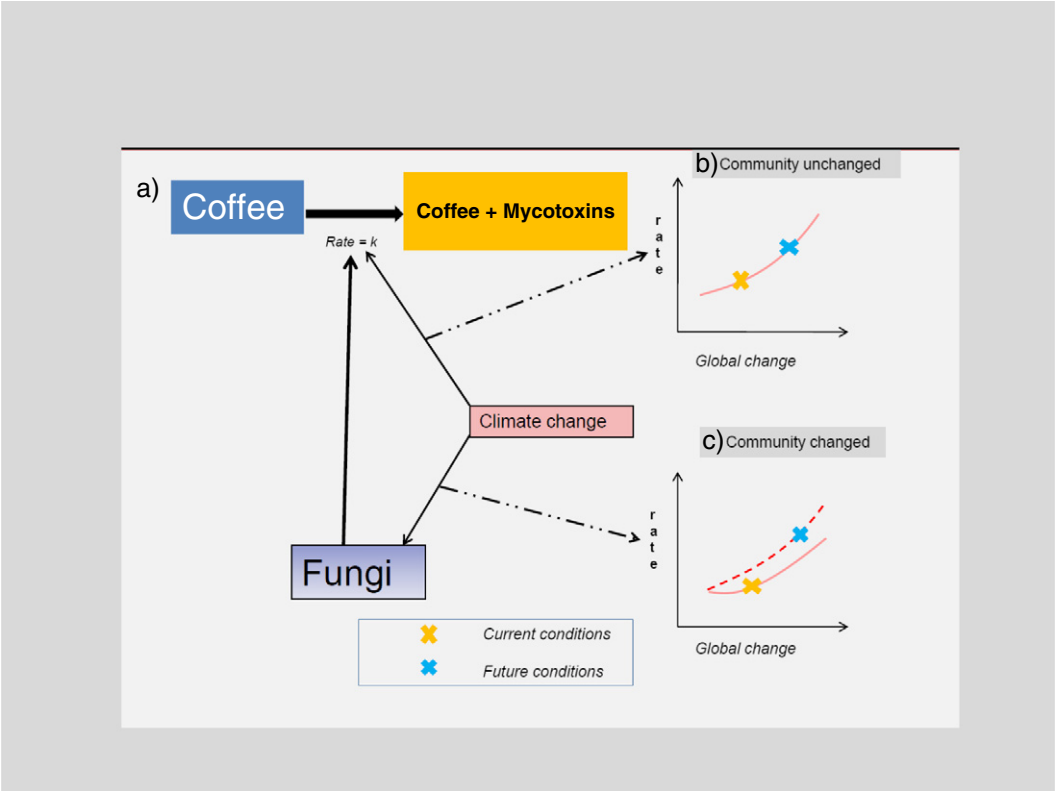

Fig. 3. This diagram indicates that fungal contamination of coffee proceeds at a certain rate "k" with established fungi which can be considered as members of the ochratoxigenic Aspergilli or other toxin producing fungi. When climate change (CC) occurs this rate may increase as indicated by "b" from increased production rates of existing fungi and by greater ingress of these fungi from stress to the coffee. However, the trajectory can move to a new unpredictable path "c" if novel fungi occur on the coffee because of CC and these fungi may include aflatoxigenic fungi which could grow more effectively at the higher temperatures for example. The novel fungi may occur simply by greater competition from better adapted fungi, and/or from mutated fungi from higher UV irradiation and/or from increased mutagenic fungal secondary metabolites (e.g. mycotoxins) as discussed in Paterson and Lima (2011).

(Paterson & Lima, 2010a, 2011, 2012; Paterson et al., 2013). However, an opposing factor may be a reduction of natural competing organisms which act as a form of biological control of the toxigenic fungi. Of course this basic analysis will be affected if more resistant coffee strains are developed which can adapt better to CC, perhaps by crosses of the two main species. Finally, the path of diseases may take a new, more devastating and unpredictable route, if the new climates allow the development of novel pest organisms (Paterson & Lima, 2011; Paterson et al., 2013) (Fig. 3).

The production of coffee may become optimal in currently sub-tropical regions such as the Southern United States of America as part of the general "movement of crops to the Poles" (Pritchard, 2011). The sub-tropical countries consist of the major developed countries such as the USA which will be able to cope better with increased mycotoxins in coffee, where the technological expertise is greater than in many currently tropical countries in which coffee is grown (Paterson & Lima, 2011).

11. Conclusions

OTA contamination can clearly be minimized by following good agricultural practice and post-harvest handling consisting of appropriate techniques for drying, grading, transportation and storage of raw coffee. The greatest OTA contamination of coffee appears in the (a) husks, (b) coffee from the soil, and (c) floated coffee obtained during processing. There is a requirement for more statistical experiments to determine the correlation between fungi isolated from, and the concentration of OTA in, coffee beans. The possibility of endophytic ochratoxigenic fungi within healthy beans requires greater consideration. Moreover, better quality raw material, appropriate dehulling procedures and reduction of defects using colour sorting to reject defective beans can substantially reduce the concentration of OTA in raw coffee. These procedures are well established. The effects of CC need urgent consideration. Finally, the European coffee associations and bodies published the Code of Practice "Enhancement of coffee quality through prevention of mould formation" (www.ecf-coffee.org). The objective is to assist operators throughout the coffee chain to apply Good Agricultural Practice, Good Practices in Transport and Storage and Good Manufacturing Practices hence preventing OTA contamination and formation. Preventive measures taken by all participants in the chain from tree to cup are the most appropriate ways to prevent OTA contamination in coffee.

References

Almeida, A. P., Alaburda, J., Shundo, L., Ruvieri, V., Navas, S. A., Lamardo, L. C. A., et al. (2007). Ochratoxin A in Brazilian instant coffee. *Brazilian Journal of Microbiology*, *38*, 300–303.

ANVISA- Agência Nacional de Vigilância Sanitária (2011). *Resolução RDC n° 7, de 18 de fevereiro de 2011- Dispõe sobre limites máximos tolerados para micotoxinas em alimentos.*

Aoyama, K., Nakajima, M., Takata, S., Ishikuro, S., Tanaka, T., Norizuki, H., et al. (2010). Four year surveillance for ochratoxin A and fumonisins in retail foods in Japan. *Journal of Food Protection*, *73*, 344–352.

Baker, P.S., & Haggar, J. (2007). Global warming: The impact on global coffee. *SCAA conference handout. Long Beach, USA.*

Batista, L. R., Chalfoun, S. M., Prado, G., Schwan, R. F., & Wheals, A. E. (2003). Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). *International Journal of Food Microbiology*, *85*, 293–300.

Batista, L. R., Chalfoun, S. M., Silva, C. F., Cirillo, M., Varga, E. A., & Schwan, R. F. (2009). Ochratoxin A in coffee beans (*Coffea arabica* L.) processed by dry and wet methods. *Food Control*, *20*, 784–790.

Blanc, M., Pittet, A., Muñoz-Box, R., & Viani, R. (1998). Behavior of ochratoxin A during green coffee roasting and soluble coffee manufacture. *Journal of Agricultural and Food Chemistry*, *46*, 673–675.

Blanc, M., Vuataz, G., & Hilckmann, L. (2001). Green coffee transport trials. *19th Proc. ASIC Coffee Confer., Trieste, Italy, 14–18 May*.

Bonnlander, B., Eggers, R., Engelhardt, U. H., & Maier, H. G. (2005). Roasting. In A. Illy, & R. Viani (Eds.), *Espresso coffee* (pp. 179–214). California: Elsevier Academic Press.

Bucheli, P., Kanchanomal, C., Meyer, I., & Pittet, A. (2000). Development of ochratoxin A during Robusta (*Coffee canephora*) coffee cherry drying. *Journal of Agricultural and Food Chemistry*, *48*, 1358–1362.

Bucheli, P., Meyer, I., Vuataz, A., & Viani, R. (1998). Industrial storage of green Robusta coffee under tropical conditions and its impact on raw material quality and ochratoxin A content. *Journal of Agricultural and Food Chemistry*, *46*, 4507–4511.

Bucheli, P., & Taniwaki, M. H. (2002). Research on the origin, and the impact of postharvest handling and manufacturing on the presence of ochratoxin A in coffee. *Food Additives and Contaminants*, *19*, 655–665.

Burdaspal, P. A., & Legarda, T. M. (1998). Ochratoxin A in roasted and soluble coffee marketed in Spain. *Alimentaria*, *296*, 31–35.

Camargo, A. P. (1985). Florescimento e frutificacão de café arábica nas diferentes regiões cafeeiras do Brasil. *Pesquisa Agropecuária Brasileira*, *20*, 831–839.

Camargo, M. B. P. (2010). The impact of climatic variability and climate change on Arabic coffee crop in Brazil. *Bragantia*, *69*, 239–247.

Castellanos-Onorio, O., Gonzalez-Rios, O., Guyot, B., Fontana Tachon, A., Guiraud, J. P., Schorr-Galindo, S., et al. (2011). Effect of two different roasting techniques on the ochratoxin A (OTA) reduction in coffee beans (*Coffee arabica*). *Food Control*, *22*, 1184–1188.

Clarke, R. J. (1987). Roasting and grinding. In R. J. Clarke, & R. Macrae (Eds.), *Coffee technology* (pp. 73–107). Essex: Elsevier Applied Science.

CAC (Codex Alimentarius Commission) (2009). Code of practice for the prevention and reduction of ochratoxin A contamination in coffee (CAC/RCP 69-2009). www.codexalimentarius.org/input/download/standards/…/CXP_069e.pdf.

DaMatta, F. M. (2004). Exploring drought tolerance in coffee: A physiological approach with some insights for plant breeding. *Brazilian Journal of Plant Physiology*, *16*, 1–6.

DaMatta, F. M., & Ramalho, J.D. C. (2006). Impacts of drought and temperature stress on coffee physiology and production: A review. *Brazilian Journal of Plant Physiology*, *18*, 55–81.

Davis, A. P., & Mvungi, E. (2004). Two new and endangered species of *Coffea* (Rubiaceae) from the Eastern Arc Mountains (Tanzania) and notes on associated conservation issues. *Botanical Journal of the Linnean Society*, *146*, 237–245.

Davis, A. P., Gole, T. W., Baena, S., & Moat, J. (2012). The impact of climate change on indigenous arabica coffee (*Coffea arabica*): Predicting future trends and identifying priorities. *PLoS ONE*, *7*(11), e47981, http://dx.doi.org/10.1371/journal.pone.0047981.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 6668–6672.

European Commission Scientific Committee for FoodWorking Group on Contaminants. (1995 January 12). *Opinion on aflatoxins, ochratoxin A and patulin, CS/CNTM/MYC/6 Rev. 3.*

FAO/WHO (2008). *Discussion paper on ochratoxin A in coffee. Food standards programme codex committee on contaminants in foods. Second session.* The Netherlands: The Hague (31 March–4 April ftp://ftp.fao.org/codex/meetings/CCCF/CCCF2/cf02_14e.pdf).

FAO (2006). Guidelines for the prevention of mould formation in coffee. http://dev.ico.org/documents/ed1988e.pdf.

Fazekas, B., Tar, A. K., & Zomborszky-Kovács, M. (2002). Ochratoxin A contamination of cereal grains and coffee in Hungary in the year 2001. *Acta Veterinaria Hungarica*, *50*, 177–188.

Ferraz, M. M., Farah, A., Iamanaka, B., Perrone, D., Copetti, M. V., Marques, V. X., et al. (2010). Kinetics of ochratoxin destruction during coffee roasting. *Food Control*, *21*, 872–877.

Franco, C. M. (1958). *Influence of temperature on growth of coffee plant.* New York: IBEC Research Institute.

Frank, J. M. (1999). HACCP and its mycotoxin control potential: Ochratoxin A (OTA) in coffee production. *Proc. 7th Intern. Comit. Food Microbiol. Hyg* (pp. 1222–1225). The Netherlands: Veldhoven.

Ghini, R., Bettiol, W., & Hamada, E. (2011). Diseases in tropical and plantation crops as affected by climate changes: Current knowledge and perspectives. *Plant Pathology*, *60*, 122–132.

Ghini, R., Hamada, E., & Bettiol, W. (2008). Climate change and plant diseases. *Scientia Agricola*, *65*, 98–107.

Gollücke, A. P. B., Taniwaki, M. H., & Tavares, D. Q. (2004). Survey on ochratoxin A in Brazilian green coffee destined for exports. *Ciência e Tecnologia de Alimentos*, *24*, 641–645.

Gollücke, A. P. B., Tavares, D. Q., & Taniwaki, M. H. (2004). Efeito do processamento sobre a ocratoxina A, em café. *Higiene Alimentar*, *18*, 38–48.

Gopinandhan, T. N., Kannan, G. S., Panneerselvam, P., Velmourougane, K., Raghuramulu, Y., & Jayarama (2008). Survey on ochratoxin A in Indian green coffee destined for export. *Food Additives and Contaminants*, *1*, 51–57.

Gopinandhan, T. N., Velmourougane, K., Panneerselvam, P., Keshamma, E., & Raghuramulu, Y. (2007). Occurrence of ochratoxin-A (OT-A) in green and commercial coffee samples. *Journal of Food Science and Technology*, *44*, 247–249.

Haggar, J., & Schepp, K. (2011). *Coffee and climate change. Desk study: Impacts of climate change in four pilot countries of the coffee and climate initiative.* Coffee and climate. Hamburg: University of Greenwich.

Heilmann, W., Rehfeldt, A. G., & Rotzoll, F. (1999). Behaviour and reduction of ochratoxin A in green beans in response to various processing methods. *European Food Research and Technology*, *209*, 297–300.

Hein, L., & Gatzweiler, F. (2006). The economic value of coffee (*Coffea arabica*) genetic resources. *Ecological Economics*, *60*, 176–185.

IARC (1993). *Monographs on the evaluation of carcinogenic risks to humans, some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins.* 56. (pp. 489–521). Lyon: International Agency for Research on Cancer, 489–521.

International Coffee Organization (ICO) (2012). 1. Trade Statistics. Available: http://www.ico.org/trade_statistics.asp?section=Statistics (Accessed 2013 Nov 13).

IPCC (2013). Summary for policymakers. In P.M. Midgley, T. F. Stocker, D. Qin, G. -K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, & V. Bex (Eds.), *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., et al. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: Predictions of climate change impact on a tropical insect pest. *PLoS ONE*, *4*, e6487, http://dx.doi.org/10.1371/journal.pone.0006487.

Jaramillo, J., Muchugu, E., Vega, F. E., Davis, A. P., Borgemeister, C., & Chabi-Olaye, A. (2011). Some like it hot: The influence and implications of climate change on coffee berry borer (*Hypothenemus hampei*) and coffee production in East Africa. *PLoS ONE*, *6*, e24528, http://dx.doi.org/10.1371/journal.pone.0024528.

Jeger, M. J., & Pautasso, M. (2008). Plant disease and global change and the importance of long-term data sets. *New Phytologist*, *177*, 8–11.

Joosten, H. M. L. J., Goetz, J., Pittet, A., Schellenberg, M., & Bucheli, P. (2001). Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries. *International Journal of Food Microbiology*, *65*, 39–44.

Jørgensen, K. (1998). Survey of pork, poultry, coffee, beer and pulses for ochratoxin A. *Food Additives and Contaminants*, *15*, 550–554.

Kumagai, S., Nakajima, M., Tabata, S., Ishikuro, E., Tanaka, T., Norizuki, H., et al. (2008). Aflatoxin and ochratoxin A contamination of retail foods and intake of these mycotoxins in Japan. *Food Additives and Contaminants*, *25*, 1101–1106.

La Pera, L., Avellone, G., Lo Turco, V., Di Bella, G., Agozzino, P., & Dugo, G. (2008). Influence of roasting and different brewing processes on the ochratoxin A content in coffee determined by high-performance liquid chromatography fluorescence detection (HPLC-FLD). *Food additives and contaminants, part A: Chemistry, analysis, control, exposure and risk assessment*, *25*, 1257–1263.

Leong, S. L., Hien, L. T., An, T. V., Trang, N. T., Hocking, A.D., & Scott, E. S. (2007). Ochratoxin A producing *Aspergilli* in Vietnamese green coffee. *Letters in Applied Microbiology*, *45*, 301–306.

Leoni, L. A.B., Valente Soares, L. M., & Oliveira, P. L. C. (2000). Ochratoxin A in Brazilian roasted and instant coffees. *Food Additives and Contaminants*, *17*, 867–870.

Levi, C. P., Trenk, H. L., & Mohr, H. K. (1974). Study of the occurrence of ochratoxin A in green coffee beans. *Journal of Association of Official Analytical Chemists*, *57*, 866–870.

Lombaert, G. A., Pellaers, P., Chettiar, M., Lavalce, D., Scott, P.M., & Lau, B. P. Y. (2002). Survey of Canadian retail coffees for ochratoxin A. *Food Additives and Contaminants*, *19*, 869–877.

Magan, N., Medina, A., & Aldred, D. (2011). Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. *Plant Pathology*, *60*, 150–163.

MAFF (1996). *Surveillance of ochratoxin A in green (unroasted) coffee beans*. Food surveillance information sheet 80. Ministry of Agriculture, Fisheries and Food.

Mantle, P. G. (2000). Uptake of radiolabelled ochratoxin A from soil by coffee plants. *Phytochemistry*, *53*, 377–378.

Martins, M. L., Martins, H. M., & Gimeno, A. (2003). Incidence of microflora and of ochratoxin A in green coffee beans (*Coffee arabica*). *Food Additives and Contaminants*, *20*, 1127–1131.

Micco, C., Grossi, M., Miraglia, M., & Brera, C. (1989). A study of the contamination by ochratoxin A of green and roasted coffee beans. *Food Additives and Contaminants*, *6*, 333–339.

Morello, L. G., Sartori, D., de Oliveira Martinez, A. L., Vieira, M. L. C., Taniwaki, M. H., & Fungaro, M. H. P. (2007). Detection and quantification of *Aspergillus westerdijkiae* in coffee beans based on selective amplification of β-tubulin gene by using real-time PCR. *International Journal of Food Microbiology*, *119*, 270–276.

Mussatto, S. I., Machado, E. M. S., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology*, *4*, 661–672.

Nakajima, M., Tsubouchi, H., Miyabe, M., & Ueno, Y. (1997). Survey of aflatoxin B_1 and ochratoxin A in commercial green coffee beans by high-performance liquid chromatography linked with immunoaffinity chromatography. *Food and Agricultural Immunology*, *9*, 77–83.

Noonim, P., Mahakarnchanakul, W., Nielsen, K. F., Frisvad, J. C., & Samson, R. A. (2008). Isolation, identification and toxigenic potential of ochratoxin A-producing *Aspergillus* species from coffee beans grown in two regions of Thailand. *International Journal of Food Microbiology*, *128*, 197–202.

Noonim, P., Mahakarnchanakul, W., Varga, J., Frisvad, J. C., & Samson, R. A. (2008). Two novel species of *Aspergillus* section Nigri from Thai coffee beans. *International Journal of Systematic and Evolutionary Microbiology*, *58*, 1727–1734.

Noonim, P., Mahakarnchanakul, W., Nielsen, K. F., Frisvad, J. C., & Samson, R. A. (2009). Fumonisin B_2 production by *Aspergillus niger* in Thai coffee beans. *Food Additives and Contaminants*, *26*, 94–100.

Oestreich-Janzen, S. (2010). Chemistry of coffee. In L. Mander, & H. -W. Liu (Eds.), *Comprehensive natural products II* (pp. 1085–1117). Amsterdam: Elsevier.

Otteneder, H., & Majerus, P. (2001). Ochratoxin A (OTA) in coffee: Nation-wide evaluation of data collected by German food control 1995–1999. *Food Additives and Contaminants*, *18*, 431–435.

Palacios-Cabrera, H. A., Menezes, H. C., Iamanaka, B. T., Canepa, F., Teixeira, A. A., Carvalhaes, N., et al. (2007). Effect of temperature and relative humidity during transportation on green coffee bean moisture content and ochratoxin A production. *Journal of Food Protection*, *70*, 164–171.

Palacios-Cabrera, H., Taniwaki, M. H., Menezes, H. C., & Iamanaka, B. T. (2004). The production of ochratoxin A by *Aspergillus ochraceus* in raw coffee at different equilibrium relative humidity and under alternating temperatures. *Food Control*, *15*, 531–535.

Pardo, E., Marin, S., Ramos, A. J., & Sanchis, V. (2004). Occurrence of ochratoxigenic fungi and ochratoxin A in green coffee from different origins. *Food Science and Technology International*, *10*, 45–49.

Patel, S., Hazel, C. M., Winterton, A. G. M., & Gleadle, A. E. (1997). Survey of ochratoxin A in UK retail coffees. *Food Additives and Contaminants*, *14*, 217–222.

Paterson, R. R. M. (2007). Aflatoxins contamination in chilli samples from Pakistan. *Food Control*, *18*, 817–820.

Paterson, R. R. M., & Lima, N. (2010a). How will climate change affect mycotoxins in food? *Food Research International*, *43*, 1902–1914.

Paterson, R. R., & Lima, N. (2010b). Toxicology of mycotoxins. *Experientia Supplementum*, *100*, 31–63.

Paterson, R. R. M., & Lima, N. (2011). Further mycotoxin effects from climate change. *Food Research International*, *44*, 2555–2566.

Paterson, R. R. M., & Lima, N. (2012 October 10–13thh). Climate change, fumonisins and animal feed. In E. M. Binder (Ed.), *NutriEconomics: Balancing global nutrition and productivity* (pp. 241–247). Singapore: Biomin World Nutrition Forum.

Paterson, R. R. M., & Lima, N. (2013). Biochemical mutagens affect the preservation of fungi and biodiversity estimations. *Applied Microbiology and Biotechnology*, *97*, 77–85.

Paterson, R. R. M., & Lima, N. (2014). Self mutagens affect detrimentally PCR analysis of food fungi by creating potential mutants. *Food Control*, *35*, 329–337.

Paterson, R. R. M., Baker, P.S., & van der Stegen, G. H. D. (2001). Ochratoxin A in coffee. In P.S. Baker (Ed.), *Coffee futures. A source book of some critical issues confronting the coffee industry* (pp. 16–25). The Commodities Press958-332356-X.

Paterson, R. R. M., Sariah, M., & Lima, N. (2013). How will climate change affect oil palm fungal diseases? *Crop Protection*, *46*, 113–120.

Paterson, R. R. M., Venâncio, A., & Lima, N. (2004). Solutions to *Penicillium* taxonomy crucial to mycotoxin research and health. *Research in Microbiology*, *155*, 507–513.

Pitt, J. I., Taniwaki, M. H., & Cole, M. B. (2013). Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of food safety objectives. *Food Control*, *32*, 205–215.

Pitt, J. I., & Hocking, A.D. (2009). *Fungi and food spoilage* (3rd ed.). New York: Springer.

Pittet, A., Tornare, D., Huggett, A., & Viani, R. (1996). Liquid chromatographic determination of ochratoxin A in pure and adulterated soluble coffee using an immunoaffinity column cleanup procedure. *Journal of Agricultural and Food Chemistry*, *44*, 3564–3569.

Prado, G., Oliveira, M. S., Abrantes, F. M., Santos, L. G., Veloso, T., & Barroso, R. E. S. (2000). Incidência de ocratoxina A em café torrado e moído e café solúvel consumido na cidade de Belo Horizonte, MG. *Ciência e Tecnologia de Alimentos*, *20*, 192–196.

Pritchard, S. G. (2011). Soil organisms and global climate change. *Plant Pathology*, *60*, 82–99.

Rezende, E. F., Couto, F. A., Borges, J. G., da Silva, D.M., & Batista, L. R. (2013). Potencial enzimático e toxigênico de fungos isolados de grãos de café. *Coffee Science*, *8*, 69–77.

Rija, A., Mwamende, K. A., & Hassan, S. N. (2011). The aftermath of environmental disturbance on the critically endangered *Coffea kihansiensis* in the Southern Udzungwa Mountains, Tanzania. *Tropical Conservation Science*, *4*, 359–372.

Romani, S., Sacchetti, G., Chaves López, C., Pinnavaia, G. G., & Dalla Rosa, M. (2000). Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. *Journal of Agricultural and Food Chemistry*, *48*, 3616–3619.

Rubert, J., Soler, C., & Mañes, J. (2010). Optimization of matrix solid-phase dispersion method for simultaneous extraction of aflatoxins and OTA in cereals and its application to commercial samples. *Talanta*, *82*, 567–574.

Silva, C. F., Batista, L. R., & Schwan, R. F. (2008). Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (*Coffea arabica* L.) beans. *Brazilian Journal of Microbiology*, *39*, 521–526.

Soliman, K. M. (2002). Incidence, level, and behavior of aflatoxins during coffee bean roasting and decaffeination. *Journal of Agricultural and Food Chemistry*, *50*, 7477–7481.

Studer-Rohr, I., Dietrich, D. R., Schlatter, J., & Schlatter, C. (1995). The occurrence of ochratoxin A in coffee. *Food and Chemical Toxicology*, *33*, 341–355.

Suárez-Quiroz, M., González-Rios, O., Barel, M., Guyot, B., Schorr-Galindo, S., & Guiraud, J. P. (2004). Study of ochratoxin A producing strains in coffee processing. *International Journal of Food Science and Technology*, *39*, 501–507.

Taniwaki, M. H. (2006). An update on ochratoxigenic fungi and ochratoxin A in coffee. In A.D. Hocking, J. I. Pitt, R. A. Samson, & U. Thrane (Eds.), *Advances in food mycology* (pp. 189–202). New York: Springer.

Taniwaki, M. H., Pitt, J. I., Teixeira, A. A., & Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Science and Technology*, *82*, 173–179.

Taniwaki, M. H., Teixeira, A. A., Teixeira, A.R. R., Copetti, M. V., & Lamanaka, B. T. (2014). Ochratoxigenic fungi and ochratoxin A in defective coffee beans. *Food Research International*, *61*, 161–166.

Teketay, D. (1999). History, botany and ecological requirements of coffee. *Walia, Journal of the Ethiopian Wildlife Natural History Society*, *20*, 28–50.

Téren, J., Palágyi, A., & Varga, J. (1997). Isolation of ochratoxin producing Aspergilli from green coffee beans of different origin. *Cereal Research Communications*, *25*, 303–304.

Titus, A., & Pereira, G. N. (2008). Global warming in coffee plantations. *Indian Coffee*, *72*, 19–24.

Tozlovanu, M., & Pfohl-Leszkowicz, A. (2010). Ochratoxin A in roasted coffee from French supermarkets and transfer in coffee beverages: Comparison of analysis methods. *Toxins*, *2*, 1928–1942.

Trucksess, M. W., Giler, J., Young, K., White, K. D., & Page, S. W. (1999). Determination and survey of ochratoxin A in wheat, barley and coffee — 1997. *Journal of the Association of Official American Chemistry, International*, *82*, 85–89.

Tsubouchi, H., Terada, H., Yamamoto, K., Hisada, K., & Sakabe, Y. (1988). Ochratoxin A found in commercial roast coffee. *Journal of Agricultural and Food Chemistry*, *36*, 540–542.

Urbano, G. R., Freitas Leitão, M. F., Vicentini, M. C., & Taniwaki, M. H. (2001). Preliminary studies on the destruction of ochratoxin A in coffee during roasting. *19th Proc. ASIC Coffee Confer., Trieste, Italy, 14–18 May*.

Urbano, G. R., Taniwaki, M. H., Leitão, M. F., & Vicentini, M. C. (2001). Occurrence of ochratoxin A producing fungi in raw Brazilian coffee. *Journal of Food Protection*, *64*, 1226–1230.

Vaclavik, L., Vaclavikova, M., Begley, T. H., Krynitsky, A. J., & Rader, J. I. (2013). Determination of multiple mycotoxins in dietary supplements containing green coffee bean extracts using ultra high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). *Journal of Agricultural and Food Chemistry*, *61*, 4822–4830.

Van der Stegen, G., Jorissen, U., Pittet, A., Saccon, M., Steiner, W., Vincenzi, M., et al. (1997). Screening of European coffee final products for occurrence of ochratoxin A (OTA). *Food Additives and Contaminants*, *14*, 211–216.

Vanesa, D., & Pacin, A. (2013). Occurrence of ochratoxin A in coffee beans, ground roasted coffee and soluble coffee and method validation. *Food Control*, *30*, 675–678.

Vecchio, A., Mineo, V., & Planeta, D. (2012). Ochratoxin A in instant coffee in Italy. *Food Control*, *28*, 220–223.

Vega, F. E., Peterson, S. W., & Chaves, T. J. G. F. (2006). *Penicillium* species endophytic in coffee plants and ochratoxin A production. *Mycologia*, *98*, 31–42.

Vega, F. E., Simpkins, A., Aime, M. C., Posada, F., Peterson, S. W., Rehner, S. A., et al. (2010). Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and Puerto Rico. *Fungal Ecology*, *3*, 122–138.

Venâncio, A., & Paterson, R. (2007). In A. McElhatton, & R. J. Marshall (Eds.), *Food safety — A practical and case study approach* (pp. 24–47). Brussels: Springer.

Wolff, J. (2000). *Forschungsbericht: Belastung des Verbrauchers und der Lebensmittel mit Ochratoxin.* A, study funded by. German Federal Ministry of Health (BMG vom 03. 02.2000. Gesch.Z. 415-6080-1/54).

Zullo, J., Jr., Pinto, H. S., & Assad, E. D. (2006). Impact assessment study of climate change on agricultural zoning. *Meteorological Applications*, *69–80* (Supplement).