Food Research International 103 (2018) 478–491

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Predominant mycotoxins, mycotoxigenic fungi and climate change related to wine

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ARTICLE INFO

Keywords:
Aspergillus
Fumonisin
Ochratoxin a
Aflatoxin
Climate change

ABSTRACT

Wine is a significant contributor to the economies of many countries. However, the commodity can become contaminated with mycotoxins produced by certain fungi. Most information on mycotoxins in wine is from Spain, Italy and France. Grapes can be infected by mycotoxigenic fungi, of which *Aspergillus carbonarius* producing ochratoxin A (OTA) is of highest concern. Climate is the most important factor in determining contamination once the fungi are established, with high temperatures being a major factor for OTA contamination: OTA in wine is at higher concentrations in warmer southern Europe than northern. Contamination by fumonisins is a particular concern, related to *Aspergillus niger* producing these compounds and the fungus being isolated frequently from grapes. Aflatoxins can be present in wine, but patulin is seldom detected. *Alternaria* mycotoxins (e.g. alternariol) have been frequently observed. There are indications that T-2 toxin may be common. Also, the combined effects of mycotoxins in wine require consideration. No other mycotoxins are currently of concern. Accurate fungal identifications and mycotoxin detection from the fungi are important and a consideration of practical methods are required. There is a diversity of wines that can be contaminated (e.g. red, white, sweet, dry and fortified). The occurrence of OTA is higher in red and sweet than white wines. Steps to control mycotoxins in wine involve good agriculture practices. The effect of climate change on vines and mycotoxins in wine needs urgent consideration by well-constructed modelling studies and expert interpretation of existing data. Reliable models of the effect of climate change on vines is a priority: the health of vines affects mycotoxin contamination. A modelling study of OTA in grapes at higher temperatures over 100 years is required. Progress has been made in reducing OTA in wine. The other mycotoxins require consideration and the effects of climate change will become crucial.

1. Introduction

Wine is defined by the Organisation Internationale de la Vigne et du Vin (OIV) (http://www.oiv.int; 10.07.17) as being obtained from grape berries (OIV, 2016a). The commodity is important, contributing greatly to the economies of countries such as France, Italy, Spain, USA, Argentina, and South Africa, which are the primary wine producers and in that order (OIV, 2016b). France was the highest consuming country followed by Italy (Mateo, Medina, Mateo, Mateo, & Jiménez, 2007), although OIV (2016b) places the USA as the highest for the 2014 vintage.

The quality of wine includes considering the microbiological and chemical purity by avoiding contamination with compounds such as mycotoxins. Grapes are susceptible to fungal diseases and in some cases the fungi produce mycotoxins (Paterson & Lima, 2010a; Paterson & Lima, 2010b; Pena, Cerejo, Silva, & Lino, 2010). Freire et al. (2017) discovered a correlation between (a) physiochemical qualities of wine grapes such as pectin content and total sugars, and (b) the presence of all fungi isolated, except for *Aspergillus flavus*. Mycotoxins are fungal secondary metabolites that contaminate crops from fungal growth and are associated with causing human and animal diseases. A list of mycotoxigenic fungi and the chemical structures of some mycotoxins are provided in Table 1 and Fig. 1 respectively.

Many countries have established regulations for control of mycotoxins in food (Venâncio & Paterson, 2007) and there is much toxicological information regarding mycotoxins (Liu & Wu, 2010; Paterson & Lima, 2010b) which are relevant to wine. This current review concentrates on the most important mycotoxins referred to as conventional mycotoxins, rather than the large list of potential mycotoxins (Paterson & Lima, 2017). The most important mycotoxin in wine

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http://dx.doi.org/10.1016/j.foodres.2017.09.080
Received 14 July 2017; Received in revised form 22 September 2017; Accepted 25 September 2017
Available online 03 October 2017

Table 1
Principal and other examples of mycotoxigenic fungi and the mycotoxins detected from each (N.B. these are not necessarily the only species capable of producing particular mycotoxins).

Filamentous fungi	Mycotoxins	Reference
Aspergillus		
A. carbonarius, A. westerdijkiae, A. niger	Ochratoxin A	Cabañes et al. (2002); Medina, Mateo, Lo, and Valle-algarra (2005); Díaz, Torres, Vega, and Latorre (2009); Paterson, Lima, and Taniwaki (2014); Garmendia and Vero (2016); Freire et al. (2017); Kizis, Natskoulis, Nychas, and Panagou (2014); Barberis, Merlera, Reynoso, Chulze, and Torres (2014)
A. niger	Fumonisins B2 and B4	Logrieco et al. (2009)
A. parasiticus	Aflatoxin B1, B2, G1, G2	Rodrigues, Venâncio, and Lima (2009)
A. flavus	Aflatoxin production strain dependent	Rodrigues et al. (2009)
Byssochlamys		
B. fulva, B. nivea	Patulin	Wright (2015)
Fusarium		
F. cerealis	Nivalenol	De Lucca and Walsh (2015)
F. culmorum, F. graminearum	Deoxynivalenol, Nivalenol	De Lucca and Walsh (2015)
F. equiseti	Zearalenone	De Lucca and Walsh (2015)
F. poae	Nivalenol	De Lucca and Walsh (2015)
F. sporotrichioides	T-2 toxin	De Lucca and Walsh (2015)
F. verticillioides (= *F. moniliforme*), *F. globosum*, *F. nygami*, *F. proliferatum*	Fumonisin B1	Mogensen, Møller, Von Freiesleben, et al. (2011)
Penicillium		
P. expansum	Patulin	Frisvad and Samson (2004); Wright (2015)
P. verrucosum, P. nordicum	Ochratoxin A	Frisvad and Samson (2004)

is ochratoxin A (OTA) (Amézqueta, González-Peñas, Murillo-Arbizu, & López de Cerain, 2009; Varga & Kozakiewicz, 2006). Wine consumption is an important source of OTA (Coronel, Marín, Cano-Sancho, Ramos, & Sanchis, 2012) and is the second most frequent source in the European diet (Miraglia & Brera, 2002).

The objectives of this paper are to (a) review the literature on the important mycotoxins, (b) establish the producing fungi, (c) suggest amelioration strategies, and (d) discuss how climate change will affect mycotoxins in wine.

2. Ochratoxin A and producing fungi from grapes

OTA is a cyclic, chlorinated pentaketide dihydroisocoumarin derivate linked to L-β-phenylalanine by an amide bond and which has been detected from many agricultural products. Several nephropathies affecting animals and humans have been attributed to OTA. It is the etiological agent of Danish porcine nephropathy and renal disorders in other animals and is often cited as the causative agent of Balkan endemic nephropathy in humans, although other compounds may be involved. The mycotoxin exhibits immunosuppressive, teratogenic, hepatotoxic and carcinogenic properties. OTA may cause genotoxic affects, although the evidence remains unclear and OTA-mediated DNA-adduct formation has been demonstrated. The compound may be involved with chronic interstitial nephropathy, urothelial tumours and testicular cancer: IARC classify OTA as a possible human carcinogen (Varga, Kocsubé, Szigeti, Baranyi, & Tóth, 2015).

The predominant OTA producing fungi in grapes are from the genus *Aspergillus* (Dachery, Manfroi, Berleze, & Welke, 2016; Leong, Hocking, & Scott, 2006a; Leong, Hocking, & Scott, 2006b; Rousseaux, Diguta, Radoï-Matei, Alexandre, & Guilloux-Bénatier, 2014) (Table 1) and particularly *A. carbonarius* and *A. niger* (Frisvad et al., 2007). Samson et al. (2014) demonstrated that *A. tubingensis* and *A. niger/welwitschiae* clades each contain strains of high similarity, but the clades are well separated from each other as determined by the calmodulin sequences. These two clades formed the *A. niger* aggregate clade. The *A. carbonarius* clade consisted of more heterogeneous strains perhaps forming two clusters within the clade. These three clades form the biseriate *Aspergillus* species, together with a forth clade *A. heteromorphus*, consisting of only two strains. *A. heteromorphus* has not been reported from grapes in the current authors´ review of the literature.

OTA is produced in higher quantities by *A. carbonarius* than *A. niger* in general and in vitro, although some strains of *A. niger* can be higher producers than *A. carbonarius* (Perrone et al., 2006). *A. tubingensis* were lower producers still on average, but equally some strains were higher producers than a few of *A. carbonarius*. Using different conditions may affect the relative production of OTA of strains of these species. A study of grape berries from 107 vineyards in different European countries led to the identification of four main populations of aspergilli which included *A. aculeatus, A. japonicus, A. uvarum*, *A. ellipticus, A. heteromorphus, A. carbonarius, A. ibericus, A. brasiliensis, A. niger*, *A. foetidus* and *A. tubingensis* (Perrone et al., 2007), a small proportion of which are OTA producers. Studies of black aspergilli occurring in wine grapes and vineyards from predominantly the Mediterranean, South America and Australia have clarified that the (a) biseriate species aggregate, *A. niger* and *A. carbonarius*, and (b) uniseriate species, *A. aculeatus* and *A. japonicus* are prevalent (Garmendia & Vero, 2016). Freire et al. (2017) isolated OTA-producing *A. carbonarius* from Brazilian grapes.

The frequency of ochratoxigenic strains of *A. carbonarius* and *A. niger* "aggregate" on grapes were similar in the Mediterranean countries and Australia (Leong et al., 2006a; Leong et al., 2006b; Leong, Hocking, & Scott, 2007) and a decrease in black *Aspergillus* spp. was apparent in cool regions such as Tasmania (Hocking, Leong, Kazi, Emmett, & Scott, 2007). However, *A. niger* was reported as the main ochratoxigenic species on grapes in South America (Da Rocha Rosa et al., 2002; Magnoli, Violante, Combina, Palacio, & Dalcero, 2003). The *A. niger* aggregate group of strains was also the most frequent from Argentinean grapes, with 27% of the isolates producing detectable OTA (Perrone et al., 2007). A study in Uruguay indicated that (a) *A. uvarium* (uniseriate) and *A. welwitschiae* were prevalent and (b) *A. carbonarius* was undetected (Garmendia & Vero, 2016). Barberis et al. (2014) isolated predominantly *A. tubingensis* amongst others in Argentinian vineyards. *A. carbonarius* was isolated to a lesser degree. All *A. carbonarius* produced OTA which was not detected in the other taxa.

There appears an association between the isolation of *A. carbonarius* from grape berries of southern Europe and the higher concentrations of OTA in wines (Mateo et al., 2007). *A. carbonarius* is prevalent in southern parts of France, Italy, Portugal and Greece, although *A. tubingensis* and *A. niger* proved the dominant species elsewhere (Perrone et al., 2007). *A. carbonarius* dominated the mycotoxigenic fungi in Portugal (Serra, Lourenço, Alípio, & Venâncio, 2006; Serra, Mendonça, & Venâncio, 2006). OTA producing black aspergilli were isolated mainly in southern Portugal which has hot and dry summers (Serra, Abrunhosa, Kozakiewicz, & Venâncio, 2003), perhaps a factor in their frequent isolation (see below). OTA producing fungi were

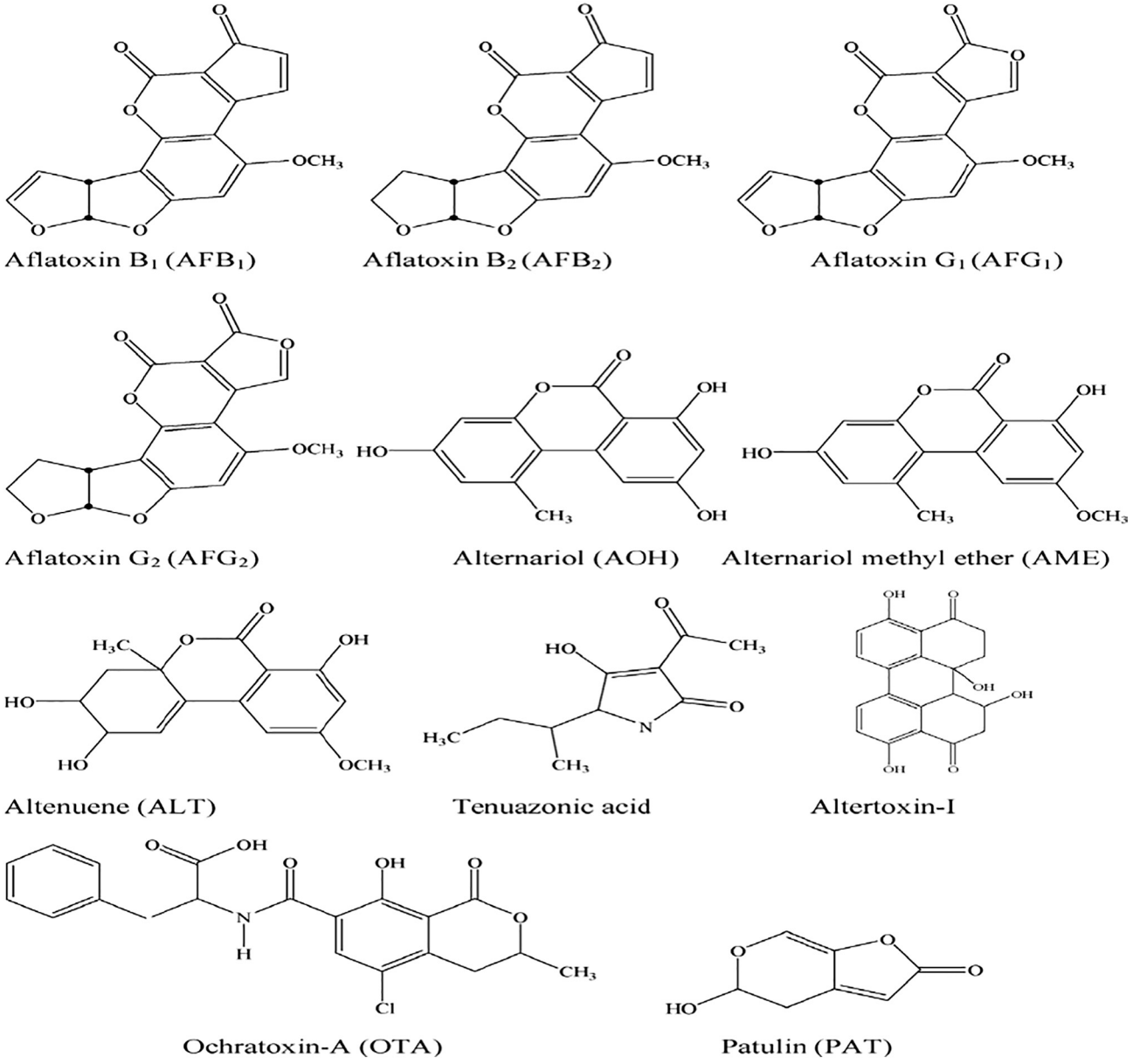

Fig. 1. Chemical structures of some well-known mycotoxins.

evaluated in the North, centre and South of Italy and *A. carbonarius* was found predominantly in the south, while *A. niger* was in the central and northern regions (Lucchetta et al., 2010). Predominantly *A. niger* aggregate strains were isolated from the vine environment in southern Italy, and ca. 50% produced OTA, mainly at low concentrations. *A. carbonarius* was identified at low frequency, with most isolates producing OTA at high concentrations (Oliveri, Bella, Tessitori, Catara, & Rosa, 2016). *A. tubinogensis* and *A japonicus* were isolated at low levels and OTA was detected in at least one strain of each, although *A. japonicus* is not considered an OTA producer (Perrone, Logrieco, & Frisvad, 2017). In Greece, 128 strains of *A. carbonarius*, *A. tubingensis*, *A. japonicus* and *A. ibericus* were isolated, with 4 *A. niger* aggregate strains. *A. carbonarius* and *A. tubingensis* were the main representative species. Of 44 *A. tubingensis,* 1 produced detectable OTA under the conditions employed. Most of the *A. carbonarius* produced OTA at high levels. OTA was not detected from *A. niger* aggregate and *A. iberius* (Kizis et al., 2014).

A. carbonarius was the most prevalent OTA fungus and was found in two regions in Spain (García-Cela, Crespo-Sempere, Gil-Serna, Porqueres, & Marin, 2015). *A. tubingensis* and *A. niger* were also isolated and at least some strains of all the species produced detectable OTA. Garcia-Cela, Crespo-Semprre, Ramos, Sanchis, and Marin (2014) described the ecophysiological properties of *A. carbonarius, A. tubingensis* and *A. niger* from Spanish vineyards. OTA-producing black aspergilli were isolated from southern Hungary (Varga, Kiss, Mátrai, & Téren, 2005) and Mikusová, Ritieni, Santini, Juhasová, and Srobárová (2010) detected numerous OTA producing aspergilli from Slovakian grapes. (Further relevant information on producing fungi can be found in Section 2.2 below.)

Zhang et al. (2016) discovered the novel OTA-producing fungi, *Talaromyces rugulosus, Penicillium commune, Penicillium rubens* and the uniseriate species, *Aspergillus aculeatus* from grapes of three organic vineyards in China, in a unique report from this country. However, Perrone et al. (2017) questioned the data and recommended criteria to establish the correct assessments. The current authors suggest it would be difficult for many ordinary laboratories to undertake the characterization criteria due to their complexity (see General discussion).

Similarly, reports of OTA production by *A. tubingensis* were not

Table 2
Occurrence of ochratoxin A in wine from Spain, France and Italy predominantly from Stratakou and van der Fels-Klerx (2010), except where there are individual citations in the table.

Country	Year	Colour	No. of samples	Max (μg L^{-1})	Mean (μg L^{-1})
France north	1997–99	Red	68	0.78	0.061
France south	1997–99	Red	40	0.47	0.07
France	1998	"wine"	29	0.19	0.038
	1998–99	"wine"	104	1.64	0.05
	2000–6	White	7	0.54	0.072
		Rosé	3	0.54	0.223
		Red	20	3.24	0.912
	2006–9 (Remiro, Irigoyen, González-Peñas, Lizarraga, & López de Cerain, 2013)	Red	12	0.088	0.039
	Purchased 2009 (Quintela, Villarán, Armentia, & Elejalde, 2012	Red	14	0.237 (of the 13 positives)	0.058 (of the 13 positives)
Italy	1998	Red	18	3.17	0.66
	1999	Red	115	15.61	2.1
		White	21	8.86	0.57
		Rosé	4	0.26	0.13
	1997–1998	Red	38	7.63	1.21
		White	9	0.97	0.16
		Rosé	8	1.15	0.63
	2005–12 (Giovannoli, Passini, Nardo, Anfossi, & Baggiani, 2014)	Red	17	0.941[a] (0.886[b])	0.364[a] (0.336[b])
	2005–8 (Remiro et al., 2013)	Red	12	0.286	0.054
	Purchased 2009 (Quintela et al., 2012)	Red	8	0.353	0.144
	(Prelle, Spadaro, Denca, Garibaldi, & Gullino, 2013)	Red	30	2.69 from the 2 positive samples.	2.34 from the 2 positive samples
North Italy	1997–99	Red	8	0.54	0.102
South Italy	1997–99	Red	43	2.55	0.193
		Red	20	3.31	1.153
	1999	Red	60		0.4
	1992–99	Red	184	15.6	1.565
	1999	White	20	8.864	0.596
		Rosé	4	0.283	0.122
		Red	27	7.63	0.131
		red home made	11	4.42	1.185
		Rosé	6	1.15	0.804
		Rosé home made	2	0.64	0.525
		White	7	0.06	0.045
		White home made	2	0.97	0.535
	1998–99	Red	96	3.177	0.419
	2000–6	"wine"	80	2.9	
		"wine"	150	5.2	
		Red	23	1.34	0.348
		Red	6	2.933	1.802
		Rosé	2	1.348	1.348
		White	18	0.456	0.264
		White	10	0.289	0.144
		White	27	0.02	0.015
		Red	36	0.1	0.041
	1999–2006	"wine"	1166	7.5	0.28
	2004–06	"Wine"	10	1.7	
	2006–2010	White	204	1.36	0.086
		Red	1002	2.63	0.121
Spain	1998	Red	14	0.193	0.133
		White	6	0.192	0.192
	2006–2009 (Remiro et al., 2013)	Red	12	0.104	0.033
	2004–2008 (Remiro, González-peñas, Lizarraga, López, & Cerain, 2012)	Red	51	0.070	0.011
	Purchased 2009 (Quintela et al., 2012)	Red	2	0.138	0.101

[a] Molecularly imprinted solid phase extraction
[b] Immune affinity column extraction.

confirmed (Storari et al., 2012; Storari, Bigler, Gessler, & Broggini, 2012) and should perhaps be analysed as discussed in Perrone et al. (2017) before being accepted as OTA producers. The presence of metabolites with retention times similar to the OTA signal in the *A. tubingensis* extracts or background noise from the growth media may be reasons for the misinterpretation of the chromatograms obtained by HPLC-FLD.

The role of OTA producing penicillia in contaminating wine is of interest, although isolation from grapes is considered infrequent. However, Mikusová et al. (2010) isolated OTA-producing *P. verrucosum* from Slovakian grapes. Battilani, Giorni, and Pietri (2001) and Rousseaux et al. (2014) reported OTA-producing *Penicillium* species from grapes in northern Italy and France, suggesting they could be involved in OTA contamination. The production of OTA from Chinese penicillia (Zhang et al., 2016) requires confirmation (Perrone et al., 2017). In general, isolating OTA fungi from grapes should not exclude penicillia.

2.1. Environmental sources of ochratoxin A producing fungi

The primary sources of *A. carbonarius* and *A. niger* in 6 Australian vineyards were soil and vine remnants on soil. They were isolated occasionally from fallen dried berries, dead canes, vine bark, dried bunch stems and dead cover crop trash, but were seldom from leaves (green and/or senescent), tendrils and green cover crop plants (Hocking et al., 2007). *A. carbonarius* conidia increased in air samples closer to the soil where a severe dust storm resulted in an increased of the fungus on grape bunches in Australia (Leong et al., 2006b; Leong, Hocking, Pitt et al., 2006) indicating how OTA could arise. Weeds were an important inoculum for *A. niger* in Argentinean vineyards (Chulze, Magnoli, & Dalcero, 2006). Increased concentrations of *A. carbonarius* occurred in soil (a) 0–1 cm below the surface, compared to deeper soil, and (b) directly beneath vines, compared to the inter-row area (Hocking et al., 2007).

2.2. How ochratoxin A occurs in wine

The period between early veraison and harvest is critical for OTA contamination because abiotic and/or biotic damaged of berries allow access to ochratoxigenic fungi if present. Rot and OTA production commenced after veraison and increased with berry damage and ripeness. Damage to grapes can be caused by insects, fungal pathogens, excessive irrigation and rain damage (Leong et al., 2007). OTA is produced primarily when *A. carbonarius* infects berries before harvest (Mateo et al., 2007; Pitt, 1997) and the mycotoxin can be detected in grapes at the beginning of ripening and during harvest. OTA detected in grapes was from infection of berries by *A. carbonarius* and is concentrated in shrivelled and discoloured berries in Australia. Detection is also associated with strains from *Aspergillus* section *Nigri* being present on grapes (Blesa, Soriano, Moltó, & Mañes, 2006). In addition, grape variety affects spoilage and growth of ochratoxigenic fungi in grapes (Blesa et al., 2006; Varga & Kozakiewicz, 2006).

Grapes and derived products, such as juices and wines, are often contaminated with OTA (Anli & Bayram, 2009). Interestingly, grapes from southern regions had a higher incidence of OTA reaching 2 mg L^{-1} and a positive correlation was found between the number of black aspergilli found in grapes from Spanish vineyards and high temperatures (Bellí et al., 2005; Bellí, Marín, Coronas, Sanchis, & Ramos, 2007). Isolation of aspergilli in cool and wet climates, such as parts of France, appears dependent on vintage (Bejaoui, Mathieu, Taillandier, & Lebrihi, 2006; Diguta, Vincent, Guilloux-Benatier, Alexandre, & Rousseaux, 2011; Guérin, Guyot, & Vincent, 2007).

Black aspergilli more prevalent in the south of Spain (García-Cela et al., 2015). These are resistant to the hot and dry environments, and the presence of melanin in the fungal cells protects the fungi from UV irradiation (Hocking et al., 2007). Differences in growth boundaries from geographical origin in Spain were found within *A. niger* aggregate isolates. Conversely, *A. carbonarius* from the hotter and drier region grew and produced OTA at lower aw than other species. Low genetic diversity in *A. carbonarius* was observed and so intraspecific variability did not correlate with the geographical origin of the isolates nor their ability to produce OTA (Garcia-Cela et al., 2014). OTA-producing black aspergilli were isolated only from southern Hungary compared to the remainder of the country (Varga et al., 2005). Higher geographical representation of *A. carbonarius* was in southern Greece whereas *A. tubingensis* was predominant in northern regions (Kizis et al., 2014).

2.3. Presence of ochratoxin A in wine

OTA is the only mycotoxin with legal limits in the European Union for wine and is 2 μg kg^{-1} (EC, 2005). Data concerning the occurrence in wines are provided from various countries (Blesa et al., 2006) and are also demonstrated in Table 2. In general, there is an increase in OTA in wines originating from southern Europe with warmer climates which influence fungal and OTA contamination. The presence of OTA was highest in wines from Italy, Spain and Greece where trends were assessed in the years 1995–1999, 2000–2006 and 2007–2010 (Stratakou & van der Fels-Klerx, 2010). The EU statutory limits in 2005 for table wines, and the publication of a code of good practices (http://www.ochra-wine.com; http://www.oiv.int/; 11/07/17) provided a stimulus for maintaining low concentrations.

The OTA concentration from 2000 to 2006 exceeded rarely the EU maximum allowed level (MAL) which was established subsequent to 2006. Espejo and Armada (2009) stated that OTA in wines produced in Europe varied between 0.01 and 3.4 l μg L^{-1}. Furthermore, a survey on 1470 wine samples demonstrated a mean OTA concentration of 0.36 ng g^{-1}, although an extremely high concentration of 15.6 μg kg^{-1} was reported in red wine from southern Europe (Miraglia & Brera, 2002).

Italian wines produced from 2000 to 2006 contained between 0.009 and 2.947 μg L^{-1} OTA. Also, the wines from 2006 to 2008 showed higher OTA incidences but lower mean and maximum concentrations compared to pre-2004 ones (Spadaro, Lorè, Garibaldi, & Gullino, 2010). The highest incidence and concentration was in red wines (78.4% and 7.63 ng mL^{-1}), followed by rosé, sweet and white, which is a different sequence to Spanish wines (see below). Sweet wine from Sicily is manufactured from grapes grown in the typical warmer climates and which have higher sugar content. Also, the over-ripening and the drying process of the grapes before fermentation conditions are responsible for the particular susceptibility of these to contamination by mycotoxin producing fungi. OTA and ochratoxin B were detected in 96.6% and 83.3% of the samples. The results indicated very low concentrations of OTA (Di Stefano et al., 2015), although the frequency was high.

Significant differences in OTA content in Rioja Alavesa Spanish wine from different regions revealed that lower rainfall and higher temperature regions produced highest OTA. No significant differences were found between types of wine in Quintela, Villarán, Armentia, and Elejalde (2011). The highest concentration was 15.25 μg L^{-1} in sweet wine, probably due to the production methods. The highest level was 4.5 μg ml^{-1} in table wine (Mateo et al., 2007; Stratakou & van der Fels-Klerx, 2010). Finally, OTA positive musts were only detected in southern Spanish vineyards compared to northern ones (García-Cela et al., 2015).

There are few data concerning French wine which is surprising considering their importance; however, 8 contaminated musts were found from 11 samples of French grapes with concentrations of OTA ranging from 0.010 to 0.461 μg L^{-1} (Sage, Krivobok, Delbos, Seigle-Murandi, & Creppy, 2002). French wines had lower maximum OTA concentrations than Italian and Spanish (Stratakou & van der Fels-Klerx, 2010) and OTA was detected in 29 wines (0.01–0.27 μg L^{-1}), although 0.78 μg L^{-1} was detected in a red wine exported to Germany (Mateo et al., 2007).

Greek wines from 1999 to 2006 had 69% positive samples but with low OTA concentrations: one sample contained the EU permitted maximum level and 91% had < 1.0 μg L^{-1}. The higher concentrations were in wines from the south, and in sweet wines (Labrinea, Natskoulis, Spiropoulos, Magan, & Tassou, 2011): red wines had high levels (Soufleros, Tricard, & Bouloumpasi, 2003). Very high median and mean values of 0.466 and 0.833 μg L^{-1} were obtained for six table wines which were similar to values previously reported for sweet wines (Burdaspal & Legarda, 2007).

Significant differences were observed in OTA content of Portuguese grapes between 2002 and 2003 which may have been related to temperature, although numbers of OTA producing fungi were similar between 2001 and 2003 (Serra et al., 2006; Serra et al., 2006). Sixty four Portuguese wines were negative for OTA, and it was present in 5 of 37 (13.5%) other samples. Furthermore, OTA was detectable in 69 (20.3%) of 340 Portuguese wines. Sixty seven wines had OTA levels below 0.5 μg L^{-1} and one had a value of 2.1 μg L^{-1} (Ratola, Martins, & Alves, 2004, 2005). The occurrence of OTA in Madeira wines was very low (Fernandes, Barros, & Câmara, 2013). All samples of Turkish wines had

detectable OTA and particularly high levels were in wines from 1998 to 2003, with a maximum of 7.96 μg L^{-1} in 1998. Values were slightly above 2 μg L^{-1} before 1998 and between 1 and 2 μg L^{-1} after 2003 (Altiokka, Can, Atkoşar, & Aboul-Enein, 2009).

Wines from North America had lower OTA levels than those from Europe and can be considered safe (Siantar et al., 2003; Soleas, Yan, & Goldberg, 2001). Australian wines were low in OTA and do not pose a serious problem (Leong et al., 2006a; Leong et al., 2006b). Brazil, Argentina and Chile wines were better quality than European (Chulze et al., 2006; Terra, Prado, Pereira, Ematné, & Batista, 2013) in this respect.

Wines from China were low in OTA and considered safe (Wu, Tan, Wang, & Xu, 2011; Zhong et al., 2014). The mycotoxin was detected in 24 local South Africa samples where a "noble" (rot) wine had the highest level of 2.67 μg L^{-1}. There was a high incidence of contamination in red wines from northern Africa (Anli & Bayram, 2009). All Croatian wine samples from the Adriatic coast were OTA-positive, while white wines from the north were uncontaminated (Varga & Kozakiewicz, 2006). Organic wines were low in OTA levels (Comuzzo, Rauhut, Werner, Lagazio, & Zironi, 2013) which may relate to improved management in the sorting of grapes and optimisation of vine growth.

In general, the occurrence data on OTA from 2006 onwards indicates the concentrations are decreasing in red wine with time which may relate to better agricultural practices because of the EU regulations and/or the effects of climate change (see later). The more recent conditions may be less suitable for the growth of ochratoxigenic fungi and OTA production. There have been reports of higher amounts of AF in some European crops because of the higher temperatures which may eventually replace OTA as the main problem (Battilani et al., 2016; Paterson & Lima, 2017).

2.4. Impact of climate

The temperature range resulting in high OTA levels on grapes varies with fungal species: *A. niger* aggregate strains and individual *A. carbonarius* have optima of 30–35 °C and 25–30 °C respectively (Bellí, Marın, Sanchis, & Ramos, 2004; Bellí, Ramos, Sanchis, & Marín, 2007). Furthermore, the effect of temperature is most important at the moment of infestation (Bellí et al., 2007; Clouvel et al., 2008). Twenty one degrees Celsius appeared the lower limit below which fungal growth and OTA production were insufficient to result in critical levels of OTA in wine during the susceptible berry period (Clouvel et al., 2008; Paterson & Lima, 2011a). High temperatures and relative humidity at the time of penetration of grapes by the fungi result in higher OTA content. Rainfall increases OTA by elevating relative humidity and causing damage to the grapes; berry splitting and high fungal colonization results if rain falls within the month before harvest, although high temperature must also occur (Stratakou & van der Fels-Klerx, 2010). The concentration of OTA in wine detected at 30 °C was higher than at 20 °C in most cases (Anli & Bayram, 2009). The highest relative humidity (100%) led to maximum amounts of OTA, while no significant differences were found between 90% and 80%. In summary, the conditions which favour OTA in wine are high temperatures reaching 30 °C and increased humidity, although dry condition cause problems occasionally: Lower than 21 °C is safer, with the effect of moisture being more ambiguous (Paterson & Lima, 2011a). However, high levels of OTA can result at lower temperatures related to the vines and grapes being infected with fungi with lower optimal temperatures for production and growth (Bellí et al., 2004; Bellí et al., 2007). Also, the effect of humidity can allow more OTA even at lower temperatures (Paterson & Lima, 2011a): OTA production is increased at temperate temperature (ca. 20 °C) and 0.96–0.98 a_w (Hocking et al., 2007; Medina et al., 2005; Medina, Jiménez, Mateo, & Magan, 2007).

Samples from 11 vineyards from 4 winemaking regions in the North and South of Portugal were assessed for OTA (Serra et al., 2006; Serra et al., 2006). Significant differences were observed in OTA from grapes between 2002 and 2003 which may have been related to temperature. Small increases in temperature bring the grapes into the ranges where critical heat levels will be reached as demonstrated for French and Spanish wines (Paterson & Lima, 2010a).

2.5. Wine colour

The occurrence of OTA is higher normally in red and sweet than white wines. (Sweet wines are consumed at lower rates in general and so are less significant in terms of risk.) Generally, white wines had lower OTA than rosé, which had lower concentrations than red indicating a relationship between maceration and the dissolution of OTA in the grape must: an increase of OTA of 20% during the maceration process has been reported (Fernandes, Ratola, Cerdeira, Alves, & Venâncio, 2007). The production of ethanol during these initial stages may increase the solubility of OTA, contributing to higher OTA (Fernandes et al., 2007). Red wines were considered the second most prominent source of OTA intake at a rate of 15% contamination and OTA exposure could be doubled by moderate red wine consumption (Zimmerli & Dick, 1996).

Levels of OTA as high as 15.6 μg kg^{-1} have been reported from wines in Southern Europe, with red frequently more contaminated than white from the same wine-growing region (Majerus, Hain, & Kölb, 2000). The maximum levels of OTA were 15.6, 8.86 and 6.32 μg kg^{-1} for red, white and rosé wines respectively (Miraglia & Brera, 2002). Pena et al. (2010) found OTA in nine (26%) and three (12%) red and white wine samples respectively, where one red and white wine sample had contamination levels at 1.23 and 2.4 μg L^{-1} respectively. Hence, in this case the white wine had the higher concentration.

2.6. Fortified and overripe/dehydrated grape wines

It is necessary to differentiate between wines of (a) standard and (b) high alcohol concentrations such as (a) table wines (e.g. Chablis, Rioja) and (b) fortified wines (e.g. Port, Vermouth), respectively when considering mycotoxin contamination. Also, wines from overripe and dehydrated grapes (EC, 1999) require consideration (Valero, Marín, Ramos, & Sanchis, 2008). Only wines at < 15% alcohol are subject to the EU regulation limiting OTA of 2.0 μg kg^{-1} wine (EC, 2005, 2006). Fortified wines are those where ethanol is added to above 15%. OIV did not differentiate between table and fortified wines when they recommended a maximum level of 2.0 μg OTA L^{-1} wine, unlike the situation for the EU regulation (EC, 2006).

A. carbonarius is probably responsible for OTA in liqueur wines in part because it is very invasive even without skin damage (Samson et al., 2007). Also, many sweet wines are produced after sun drying of grapes where they are subjected to climatic conditions favouring aspergilli (Ruíz Bejarano, Rodríguez, & Garcia, 2010). Sweet wine production, involving partial dehydration of grapes in the sun may allow fungal growth and higher OTA (Magan & Aldred, 2005). For example, OTA in sweet wines produced in south Europe from 2006 to 2010 exceeded by far the EU MAL (Espejo & Armada, 2009; Murillo-Arbizu, Amézqueta, González-Peñas, & de Cerain, 2010). Ratola, Abade, Simões, Venâncio, and Alves (2005) stated that OTA levels were below 2 μg L^{-1} in port and a Portuguese white-liqueur wine was naturally contaminated at 1.59 μg L^{-1} (Ratola et al., 2006).

Sweet wine had the highest percentage of samples that were positive for OTA (72%). Mean OTA values of 4.47 μg L^{-1} were detected in "special" Spanish wines, with an extreme of 15.25 μg L^{-1}, where 0.97 to 7.30 μg L^{-1} in Spanish sweet wines were also reported (Stratakou & van der Fels-Klerx, 2010). Other surveys indicated contaminated samples were at 51.5%. Median and mean values of 466 and 833 ng L^{-1} were obtained for a set of six wines and such levels were similar to values previously reported for sweet wines (Burdaspal & Legarda, 2007).

2.7. Ochratoxin A reduction by vineyard management

2.7.1. General

The occurrence of OTA in wine may be decreased by ca. 80% using effective vineyard management (Gambuti et al., 2005) and considerable control can be achieved by reducing damage to grapes (Cozzi, Pascale, Perrone, Visconti, & Logrieco, 2006). Efficient bunch aeration is useful for controlling fungal infection which is achieved by optimizing cultivation systems, resulting in lower temperatures and relative humidity microclimates in the vineyard. Also, *A. carbonarius* will infect the berries more readily if they are grown close to the soil and the risk of OTA in grapes can be minimised by careful visual inspection to avoid damaged and discoloured berries (Visconti, Perrone, Cozzi, & Solfrizzo, 2008). Reducing berry drop and discarding rotten bunches away from the vines may reduce the incidence of *A. carbonarius* in vineyard soil (Hocking et al., 2007). Finally, pressing of grapes and clarification steps can also remove a significant proportion of OTA during vinification (Hocking et al., 2007). Grapes require being stored for < 8 h in part because it prevents ochratoxigenic penicillia growing in stored grapes (Blesa et al., 2006).

2.7.2. Fungicides/insecticides

The highest OTA levels occur in vineyards where pest and disease control is insufficient and, for example, the greatest *Aspergillus* levels are observed where the moth *Lobesia* was uncontrolled (Varga & Kozakiewicz, 2006). The OTA reducing effects of fungicides depend on the type/specificity and timing of application: Cyprodinil and Cyprodinil/Fludioxonil are the most effective compounds for *A. carbonarius* and OTA reduction (Medina et al., 2007; Medina, Mateo, Valle-Algarra, Mateo, & J. M., 2007; Stratakou & van der Fels-Klerx, 2010). Combinations of Euparen (a sulphamide) and Mycodifol, or Captan were found effective against black aspergilli colonising grapes. Azoxystrobin (a strobilurin derivative) or Dinocap (a dinitrophenyl derivative), in combination with sulphur, effectively decreased OTA in wines. The fungicides Switch, Scala (containing the pyrimidine fungicide pyrimethanil) and Mikal (containing fosetyl-Al and the dicarboximide folpel) were found the most effective for reducing fungal colonization and OTA content (Battilani, Giorni, & Pietri, 2003). Furthermore, Lufox (a carbamate insecticide containing luferunon and fenoxycarb), Decis (a pyrethroid insecticide containing delthametrin) and *Bacillus thuringiensis* (Bt) were successful in lowering OTA content of wines (Bae, Fleet, & Heard, 2004; Battilani et al., 2003). The use of fungicides must be considered carefully because some (e.g. carbendazim) reduce fungi but stimulate OTA production (Amézqueta et al., 2009). Finally, the use of pesticides in the vineyard may result in mutation of the fungal population (Paterson & Lima, 2015) as some pesticides are mutagenic. The fungi may be unrepresentative of the wild type fungi and which requires consideration by researchers in the field.

2.8. Post-harvest factors affecting ochratoxin A

Rapid transport to the winery, cool storage of harvested grapes and sanitary wineries minimize the potential for postharvest OTA contamination (Visconti et al., 2008). Mechanical harvesting may permit a high amount of rotten bunches into the wine-making process, increasing OTA contamination. Delayed harvest increases the risk of OTA contamination (Bellí et al., 2004; Bellí et al., 2007; Gambuti et al., 2005).

2.9. Methods for reducing ochratoxin A

Yeast strains that display enhanced binding of OTA may be used for fermentation or added as dead cells (La Penna, Nesci, & Etcheverry, 2004; Petruzzi et al., 2014) where yeast hulls added to Shiraz wine yielded a 43% reduction in OTA by binding (Leong et al., 2006a; Leong et al., 2006b). However, the potential of OTA being added to wine by the addition of contaminated yeast must be avoided (Gottschalk, Biermaier, Gross, Schwaiger, & Gareis, 2016). Epiphytic yeast were used successfully in vitro to inhibit *A. carbonarius* and *A. niger* on wounded grapes (Bleve, Grieco, Cozzi, Logrieco, & Visconti, 2006). Furthermore, the addition of bacteria and adsorbents can reduce the OTA content of wine (Varga & Kozakiewicz, 2006) and OTA biodegradation by a *Pediococcus parvulus* strain is a potential treatment (Abrunhosa et al., 2014).

Bentonite decreased OTA by 67% (Stratakou & van der Fels-Klerx, 2010). Wine fining agents such as potassium caseinate or activated carbon have shown reductions of 82% but may damage wine quality (Abrunhosa, Santos, & Venâncio, 2006). Some (a) bacteria and (b) fungi are able to degrade OTA in vitro by > 95% where some also demonstrated detoxifying properties in vivo (Fuchs et al., 2008). Carboxypeptidase A degrades OTA and the use of atoxigenic *A. niger* strains as sources of the enzyme has been suggested; a carboxypeptidase present in *Phaffia rhodozyma* can degrade OTA by 90%. Other *A. niger* enzymes can degrade OTA efficiently such as a crude lipase preparation, and a metalloenzyme (Abrunhosa et al., 2006; Abrunhosa & Venâncio, 2007).

2.9.1. Reduction of ochratoxin A during vinification

Grape washing, fermentation and pressing can reduce OTA levels: reduction of OTA concentration amounted to 47–52% and 53–70% during red and white grape must fermentation respectively (Amézqueta et al., 2009), in part because OTA binds to grape proteins and yeast cell walls during fermentation (Bejaoui, Mathieu, Taillandier, & Lebrihi, 2004). Also, a decrease of 17% in OTA was observed in bottles of 'Negroamaro' wine after 12 months of storage (Grazioli, Fumi, & Silva, 2006) which may be explained by OTA being dissociated partially at the normal pH of wine causing the compound to have a negative pH and allowing it to combine with positively charged surfaces, hence reducing the free concentration in the stored wine (Anli & Bayram, 2009). Solid–liquid separations after pressing and fermentation are responsible for removal of OTA (Cecchini, Morassut, Garcia Moruno, & Di Stefano, 2006) from the metabolism of lactic acid bacteria (the malolactate fermentation) (Fernandes et al., 2007) and/or adsorption onto bacterial cell walls.

2.10. Growth and ochratoxin A production in vitro

Growth of *A. carbonarius* is favoured by high a_w (e.g. 0.98) and temperatures, whilst OTA production is increased at temperate temperature (ca. 20 °C) and 0.96–0.98 a_w (Hocking et al., 2007; Medina et al., 2007; Medina et al., 2007). *A. carbonarius* and *A. niger* grew optimally at 30 and 35 °C respectively, whereas optimum a_w for growth was 0.97–0.99 for both species (Anli & Bayram, 2009; Paterson & Lima, 2011). The optimum water activity quoted for toxin production was between 0.95 and 0.995. Warmer, followed by cooler temperatures will encourage high initial growth and OTA levels. Most *A. carbonarius* strains do not grow below 15 °C and the optimum a_w for growth varies from 0.930 to 0.987, with the widest a_w tolerance at 25–30 °C. A great deal of strain variation has been demonstrated by Garcia, Ramos, Sanchis, and Marín (2011) who employed 25 °C as the generalised optimal growth temperature. *A. carbonarius* strains produced the highest OTA levels at 15 and 20 °C compared to 20–25 °C for *A. niger*-aggregate strains. Maximum OTA production was at (a) 5 and (b) 7–13 days for (a) *A. carbonarius* and (b) *A. niger* respectively (Battilani, Magan, & Logrieco, 2006).

The survival of *A. carbonarius* conidia and subsequent growth was prolonged at low temperatures and a_w below 0.6 (Leong et al., 2006a; Leong et al., 2006b). Two Greek *A. carbonarius* isolates grew optimally at 30–35 °C and 0.96 a_w, although maximum OTA production occurred under suboptimal growth conditions (15–20 °C and 0.93–0.96 a_w). Growth was observed exclusively at 0.85 a_w and 25 °C where the fungus failed to grow at other temperatures at this a_w. Maximum OTA production was detected after 25 days of incubation at 20 °C and 0.96 a_w.

3. Aflatoxins and aflatoxigenic fungi

Aflatoxins (AF) are the most important mycotoxins with AFB1 being the highest carcinogenic natural compound known. They caused the toxicity of animals feeds containing contaminated peanut meal leading to the death of 100,000 turkeys from acute liver necrosis and are structurally related difuranocoumarins. The mycotoxins exhibit hepatocarcinogenicity and hepatotoxicity: acute aflatoxicosis occurs when moderate to high levels are consumed. The disease symptoms may include haemorrhage, acute liver damage, oedema, alternation of digestion, absorption and/or metabolism of nutrients and may result in death, often attributable in tropical and subtropical regions of the world. The IARC has classified AF as group I carcinogens.

Over 100 countries restrict the content of AF in food and feed (Varga et al., 2015), although wine does not have regulations relating to AF. These mycotoxins are produced predominantly by *A. flavus* and *Aspergillus parasiticus* (Paterson et al., 2014; Paterson & Lima, 2010a; Paterson & Lima, 2010b). The presence of AF in wine has not been studied systematically. Interestingly, AFB2 was reported in 87.5% of Spanish wine samples and a Valdepeñas wine contained as much as 25.73 μg L^{-1} AFB2, where many other samples were of similar values (Pérez-Ortega, Gilbert-López, García-Reyes, Ramos-Martos, & Molina-Diaz, 2012). This concentration is too high to be reasonable and the current authors assume it is a misprint. Nine (30%) sweet Sicilian wines contained at least one with quantifiable AF with concentrations up to 0.068 μg L^{-1} and 13% contained quantifiable AFB1 (Di Stefano et al., 2015). AFB1 contamination of musts was reported, with 40% of samples containing AF in a range from 0.01–0.46 μg L^{-1} (El Khoury, Rizk, Lteif, Azouri, & Delia, 2009). Serra, Braga, and Venâncio (2005) stated that AF had not been reported in wine, and Stratakou and van der Fels-Klerx (2010) erroneously cite Serra et al. (2005) as stating that AF in "grapes" was demonstrated as "low" whereas there are no data on this topic in Serra et al. (2005). However, *A. flavus* represented the third most frequent fungus isolated from grapes (Serra et al., 2005) and *A. parasiticus* has been isolated from grapes (Rousseaux et al., 2014). Overall, more work is required on AF contamination of wine.

4. Patulin and producing fungi

Patulin (PAT) is a water soluble lactone produced via the polyketide metabolic pathway by many species of fungi (e.g. those within *Penicillium*, *Aspergillus* and *Byssochlamys*). The mycotoxin is most often associated with *Penicillium expansum* and is found frequently in apple products. It was tested unsuccessfully as a cure for the common cold and was found too toxic to humans and animals. PAT mainly induces gastrointestinal disorders including ulceration, distension and bleeding. The compound provokes congestion and oedema of pulmonary, hepatic and gastrointestinal blood vessels and tissues. Subcutaneous injection of PAT produced local sarcomas in rats and is classified in group 3 as not classifiable as to its carcinogenicity to human by IARC (Varga et al., 2015).

PAT can be found in moldy grapes at the maturation stages. *Penicillium* spp. do not infect the berries before harvest (Serra et al., 2005) and the producing fungi might be from other genera. However, *P. expansum* can be present in stored grapes (Snowdon, 1990) and PAT could be produced: *P. expansum* is found frequently in botrytized grapes (Morales-Valle, Silva, Paterson, Venâncio, & Lima, 2011). PAT is present in grapes, grape juice and grape must (Bragulat, Abarca, & Cabañes, 2008), although the occurrence in wine is low because it is well-known to be degraded partially by the fermentation process (Moss & Long, 2002). The mycotoxin was detected in a rapid fermentation wine produced in Germany (Majerus, Hain, & Kölb, 2008) where it was reduced from 157 μg L^{-1} to 55.5 μg L^{-1} in 4 days. As part of the experimental procedure, 37 μg L^{-1} of pure patulin was added before fermentation to ensure that the compound was present. Díaz, Yañez, and Latorre (2011) state that the average reductions in fermented must were 67.3 to 83.3%.

5. *Alternaria* toxins and fungi

Alternaria spp. are ubiquitous plant pathogens that may invade fruit (Asam, Konitzer, & Rychlik, 2010) and were the most frequent fungi from grapes from Uruguay (Garmendia & Vero, 2016). *Alternaria alternata* has been found as the dominant fungus on grapes in Argentina and many strains produced mycotoxins in vitro (Prendes, Merín, Andreoni, Ramirez, & Morata de Ambrosini, 2015). *Alternaria* produce the phytotoxin tentoxin and the mycotoxins alternariol (AOH), alternariol methyl ether (AME), altenuene and tenuazonic acid. AOH and AME were (a) mutagenic and clastogenic in in vitro systems and (b) carcinogenic to rats fed contaminated feed (Pavón, González, & Martín, 2015; Prendes et al., 2015). There is no evidence of uptake from feeds into animals of *Alternaria* mycotoxins which questions whether they are true mycotoxins, although they are frequently referred to as such in the scientific literature.

Stable isotope dilution assays indicated AOH and AME in wine at high levels (Asam, Konitzer, Schieberle, & Rychlik, 2009). Furthermore, the capability of *B. cinerea* to produce AOH prompted a study on these compounds in wine (Asam et al., 2010) as this fungus is crucial to noble rots and grape diseases. AOH and AME were detected in four of six samples (AOH: 1.2–4.9 μg kg^{-1}; AME: 0.13–0.25 μg kg^{-1}) (Asam et al., 2009). The "special wines" contained AOH (4/6 samples; 1.2–4.9 μg kg^{-1}) and AME (4/6 samples; 0.1–0.3 μg kg^{-1}), but the values did not exceed those in wines in general (Asam et al., 2010). In addition, AME was significant particularly in grape juice and red wines. Red grape juice and wine contained significant amounts of AOH: Whereas red grape juice was contaminated with a maximum of 1 μg kg^{-1}, values ranged up to a maximum of 7.5 μg kg^{-1} in red wine, although median values were much lower. The maximum value of AOH in a white Riesling was 7.6 μg kg^{-1}, thus exceeding the maximum value of red wine. White wine contained lower median values of AOH (1.1 μg kg^{-1}) than red wine (4.5 μg kg^{-1}).

6. Fumonisins and fungi

Fumonisins (FUM) are nonaketide derived mycotoxins which can be classified into four groups structurally. They are similar to sphinganine and disrupt the biosynthesis of sphingolipids by inhibition of ceramide synthase. Ingestion is associated with several fatal diseases of animals. They are the possible aetiological agents of oesophageal cancer in several countries such as China and South Africa (Marasas, 2001). Fumonisin B1 is considered as possibly carcinogenic to humans (group 2B) by IARC. *A. niger* was considered to the fungus responsible for production of FUM in wine (Logrieco et al., 2009). However, Mikusová et al. (2010) isolated FUM-producing fusaria from grapes. The first reported natural occurrences of FUMB2 in the grape-wine chain, at levels of 0.01 and 0.4 μg L^{-1}, were in two samples of must (Logrieco, Ferracane, Visconti, & Ritieni, 2010). Thirty-one strains belonging to four *Aspergillus* species isolated from grape were evaluated for FUMB2 in vitro: Four of eight *A. niger* strains produced detectable FUMB2 (29–293 mg g^{-1}). Mogensen, Frisvad, & Thrane (2010) demonstrated that *A. niger* isolated from raisins produced FUMB2 and FUMB4 when cultured on grapes or raisins. Mogensen, Larsen, & Nielsen (2010) demonstrated the frequent occurrence of these mycotoxins in wine but in low concentrations.

Fusarium verticillioides has been detected from rotting grapes (Lorenzini & Zapparoli, 2015) and is a well-known fumonisin producer. Several other species of *Fusarium* can produce fumonisins, e.g. *F. oxysporum* produces only the C-series. *A. niger* and *A. welwitschiae* is considered to produce mainly FUM B2 and B4. Some *Tolypocladium* species may produce FUM B1 and B4.

7. Mycotoxins detected in wine by development of multi-mycotoxin methods

There have been various method development papers often using spiked samples. Some interesting results from these types of papers include reports of potential mycotoxins such as mycophenolic acid in "moldy" wine (Sulyok, Krska, & Schuhmacher, 2007); Tamura, Takahashi, Uyama, and Mochizuki (2012) analysed for patulin, deoxynivalenol, aflatoxins B1, B2, G1, G2, M1, T-2 toxin, HT-2 toxin, zearalenone, fumonisins B1, B2, B3, and ochratoxin A and detected only FUM and OTA in some samples. Al-Taher et al. (2013) found T-2 toxin in many samples of wine, which is interesting and requires further study.

8. Climate change, wine, mycotoxins and models

8.1. General

The influence of climate change on the production of wine may be profound (van Leeuwen and Darriet, 2016). Correspondingly, the production of mycotoxins on grapes will be highly effected. Will it be possible to produce wine (or the same wine) in (a) southern Europe and (b) parts of Australia and USA, as temperatures become increasingly high? High temperature will make growing the crop impossible in some areas and the mycotoxin issue will become irrelevant (Paterson & Lima, 2012; Paterson, et al., 2013, 2014, 2015). This scenario is likely to occur in large areas of land normally suitable for grape production (Paterson & Lima, 2011b). There will be an intermediate region where growing grapes is possible, but subjected to greater stress from sub-optimal climatic conditions, poor soil, insects, and pest and disease microorganisms, making the process less profitable or unprofitable. Here, mycotoxins will become a particularly serious problem as the toxigenic fungi will be able to invade the crop more readily. Modelling studies (see later) require to consider the effect of climate change on crops as a priority, in addition to the effect on mycotoxin contamination.

García-Cela et al. (2015) concluded that climate change could promote better adapted species such as *A. niger* in the south of Spain and that climate change may see an increase of FUM in grapes with decreasing OTA. Climatic change resulting in drier and hotter climatic scenarios indicates *A. tubingensis* and *A. niger* may be more prevalent over *A. carbonarius*, since they are better adapted to extreme high temperature and drier conditions (Garcia-Cela et al., 2014). More dangerous mycotoxins will probably become prevalent at 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; Paterson & Lima, 2017). Temperature and rainfall projections indicate that black aspergilli on grapes grown in the alpine region of Italy will probably increase in the future (Storari et al., 2012; Storari et al., 2012).

Another scenario is that grapes will be grown in new land resulting in low levels of mycotoxins because of the "Parasites Lost phenomenon", where new growth crops often have fewer pests and diseases (Paterson & Lima, 2010a, Paterson & Lima, 2010b; Paterson et al., 2014; Paterson & Lima, 2015). However, an opposing factor may be a reduction of natural competing organisms which act as a form of biological control against the toxigenic fungi. The production of wine may become optimal in regions located towards the Poles as part of the general "movement of crops to the Poles" (Paterson & Lima, 2010a, Paterson & Lima, 2010b; Paterson, Kumar, Shabani, & Lima, 2017; Pritchard, 2011). Overall, it is necessary to consider how climate change will impact on viniculture as this will in turn affect mycotoxin contamination.

8.2. Climate change and viniculture

Wine grapes are symbolic of a wide variety of crops where geographic shifts in response to climate change will occur (Paterson et al., 2017; Paterson & Lima, 2017). Investment in new varieties with similar flavors but with changed climate tolerances may be important (van Leeuwen & Darriet, 2016). Hannah et al. (2013) predicted that the area suitable for viticulture will decrease from between 19 and 73% in major wine producing regions by 2050. Climate change may cause establishment of vineyards at higher elevations and latitudes in areas such as western North America. An increase in disease and potential mycotoxin contamination can be expected as the climate becomes less suitable for vines (Paterson & Lima, 2010a; Paterson & Lima, 2011).

Most of the world's highest quality wine-producing regions experienced warming trends from 1950 to 1999 (Jones, White, Cooper, & Storchmann, 2005) which had a significant role in quality variations. Global wine producing regions predict an average warming of 2 °C in the next 50 years making high quality wine more difficult in high quality regions (van Leeuwen & Darriet, 2016). In other, currently less suitable regions, more optimal climatic regimes for the production of varietals may occur. Spatial modelling research has indicated potential geographical shifts and/or expansion of vinicultural regions, with parts of southern Europe becoming too hot to produce high-quality wines and northern regions becoming viable once again (Jones et al., 2005).

Climate change impacts are likely region-specific. Modifications in cool climate regions (i.e., the Mosel Valley, Alsace, Champagne, and the Rhine Valley) to warmer climates, could lead to more consistent vintage quality and possibly ripening of warmer climate varieties. Regions, with warmer growing seasons currently (i.e., southern California, southern Portugal, the Barossa Valley, and the Hunter Valley) may become too warm for the existing varieties and hot climate maturity regions may become overly hot to produce high-quality wines: Mycotoxins will likely be affected (Paterson & Lima, 2011). Winter temperature changes would also affect viticulture by making regions that experience hard winter freezes (e.g., the Mosel Valley, Alsace, and Washington) less prone to vine damage, while other regions (e.g., California and Australia) would have such mild winters that latent bud hardening may not be achieved and cold-limited pests may increase in number or severity. Mycotoxigenic fungi could also survive these warmer temperatures to a greater extent than occurs currently throughout the production system for wine and will tend to lead to higher, and more dangerous mycotoxin concentrations (Paterson & Lima, 2017). It may be possible the AF could begin to increase in wine under climate change.

White, Diffenbaugh, Pal, and Giorgi (2006) estimate that potential premium wine grape production area in the conterminous United States may decline by 81% by the late 21st century and increases in heat accumulation will cause a move to warmer climate varieties and/or lower-quality wines. Frost constraints will be reduced, and increases in the frequency of extremely hot days (> 35 °C) in the growing season may eliminate wine grape production in many areas. Furthermore, projected climate changes shift premium wine grape production to high humidity/precipitation regions of the Pacific Northwest and New England. High humidity is associated with higher risk of quality-reducing factors such as various forms of rot and powdery mildew, and higher frequency of raindrop impacts on leaves increasing fungal dispersal. This is likely to have an increased risk from growth of mycotoxigenic fungi and mycotoxin production. Potentially, wine regions within the Mediterranean basin today (e.g. Provence, the Languedoc, Côtes Rhône Méridionales) will change the most over time: Moriondo et al. (2013) report that increases of climate change in Europe would result in new areas on the northern fringes becoming viable, changes in varietal suitability in existing regions, and southern regions becoming too hot to produce high-quality wines.

Climate change may challenge the Portuguese wine-making sector, with the requirement for adaptation/mitigation strategies to ensure its

future sustainability (Fraga et al., 2015). Jones et al. (2005) considers that southern Portugal may become too hot for existing varieties to grow: Hannah et al. (2013) mention the predicted dramatic decrease by 2050 in suitable areas in the Iberian Peninsula and changes due to elevated CO_2 may further enhance this effect. The model suggested a decrease in suitable areas within some regions, although the majority of the present vineyards remained apt until 2050. The Tóth and Végvári (2015) predictions show a lower degree of area contraction than that of Hannah et al. (2013), where a decrease in area suitable for viticulture of 25 to 73% in major wine-producing regions was predicted by 2050. This value for the most important European vine-producing countries amounted to 2–48% in Tóth and Végvári (2015) (Moriondo et al., 2013).

Fraga et al. (2015) also project that climate change may significantly increase wine yield in the Douro Valley, Portugal, together with predicted increases under future climate conditions in Italy. However, larger grape yields are generally associated with higher economic risk and poorer wine quality. Possible adaption procedures include: (a) increasing the altitude of vineyards, (b) growing varieties more suited to future climate conditions, (c) adequate irrigation and (d) employing new agricultural practices (Santos, Malheiro, Karremann, & Pinto, 2011).

8.3. Modelling climate change and mycotoxin contamination

How are climate change effects on mycotoxins determined? This can be by experienced scientists (a) interpreting existing data (Medina, Akbar, Baazeem, Rodriguez, & Magan, 2017; Paterson et al., 2014; Paterson & Lima, 2010a; Paterson & Lima, 2010b; Paterson & Lima, 2011; Paterson & Lima, 2017) and/or (b) constructing models. A good knowledge of (a) fungal and crop physiology, (b) fungal and crop genetics and (c) what is predicted for climate change, can permit well-formed assessments.

Weather data are the main inputs in all modelling approaches, supported by cropping data (Battilani, 2016). Indeed, efforts to model the risk of mycotoxin contamination in crops are limited, perhaps relating to concerns regarding the modelling science. Empirical and mechanistic models are used to predict mycotoxin contamination in crops and are often linked and used to combine risk indices with contamination in the field (Battilani, 2016). The results of model analysis must always be interpreted carefully as plausible futures; they should not be considered precise predictions of the future (Liu & Battilani, 2016). Similarly, the more narrative-led papers are also describing plausible futures and are not precise predictions of the future. Each approach has merits.

A priority is to develop accurate models for the effect of climate change on (a) the ability to grow vines (Jones et al., 2005) and (b) mycotoxins in wine. A major effect of climate change will be on the host without considering pathogens as a healthy crop will be less susceptible to disease than one that is stressed because of climate change. There is no point in modelling mycotoxin contamination at high temperatures if the crop would not survive these temperatures (Paterson & Lima, 2011).

8.3.1. Non-wine systems

Modelling papers regarding mycotoxin contamination of crops in relation to climate change are but few. Battilani et al. (2016) undertook modelling studies of AF on maize and wheat in Europe and very high increases in AF was predicted in maize at 2° C after 100 years. Changes at 5° C indicated that AF in some areas began to decrease, particularly in the Iberian Peninsula, which may relate to the conditions being too severe even for aflatoxigenic fungi. Paterson & Lima, 2010a; Paterson & Lima, 2010b predicted this phenomenon and suggested that thermophilic fungi may succeed (Paterson & Lima, 2017).

The AF in maize data can be compared to that for the effect of climate change on the ability to grow maize, which may have relevance to mycotoxins in wine and ability to grow vines. Shaw and Osborne (2011) indicated how the ability to grow maize would change in 2050 and the data for Europe was considered further by Paterson and Lima (2012). It appeared that large parts of Europe would not be able to grow maize in regions indicted to have high risk of AF after 100 years by Battilani et al. (2016), perhaps relating to the different models employed. Ramirez-cabral, Kumar, and Shabani (2017) indicate that the most obvious change in Europe was an increase from mere suitability to optimal growth in large areas, including England, France, Germany, Denmark, the Netherlands, Poland, Slovakia and the Czech Republic. The model did not taken into account the high risk of AF contamination which would be detrimental to the economic and safe production of the crop (Battilani et al., 2016).

The only country with a decrease was Spain after 100 years using the MIROC analysis and this was from (a) areas where large populations of maize can persist to (b) marginal conditions for growth (Ramirez-cabral et al., 2017). Knox, Daccache, Hess, and Haro (2016) indicated a very high decrease in yield of maize up to the 2080′s in southern Europe (including Spain) in a Meta study of climate change effects. The large decreases in general would tend to discourage farmers producing maize in these regions, to the extent that they would cease, meaning AF would be irrelevant. Equivalent data for mycotoxins in grapes are required. In general, data on the effect of climate change on growth of vines and grapes are required when considering mycotoxin contamination of the crop.

Temperatures were projected as too high in Spain (18.2–38.2 °C) for *Alternaria* spp. to grow using models, relevant to mycotoxins produced by these fungi on tomatoes. The temperatures become closer to the optimal temperature (14.2–28.4 °C) in Poland for *Alternaria* spp. Hence, the situation in Poland in 2081–2100 is projected to be similar to the situation in Spain in 1981–2000 (Perre, Jacxsens, Liu, Devlieghere, & Meulenaer, 2015). It is possible that equivalent situations will occur for the mycotoxins in wine scenario.

Contamination of winter wheat with DON was modelled to increase in most of north west Europe (van der Fels-Klerx, Asselt, Madsen, & Olesen, 2013; van der Fels-Klerx, Olesen, Madsen, & Goedhart, 2012). Even more severe impacts were predicted for spring wheat. However, variations between runs and between regions were large, and in some grids, even a decrease in DON was predicted. A similar study for the Netherlands also predicted high spatial and annual variability of DON contamination in wheat with future climate, and again the wine/mycotoxins system can be compared.

8.3.2. Wine system

Even fewer models relate to mycotoxins in wine with Battilani and Marco (2015) being an exception, in this case for OTA and using the “mechanistic” approach. The importance of grape variety needs further consideration in this model as it will affect OTA contamination of grapes. One fungal taxon should only be considered normally as there appeared two in the report with different optimal growth temperatures (Battilani & Marco, 2015), although the authors adjusted for this factor. OTA producing black aspergilli have different optimal growth temperatures (see above and Garcia-Cela et al., 2014). Importantly, the model needs validation by real observations, especially of OTA contamination in the field. Two main research gaps in mycotoxin modelling studies have been identified related to the (a) (limited) number of existing quantitative models taking into account climate change and (b) validation being in limited (Liu & Battilani, 2016). Encouragingly, the output index of the Battilani and Marco (2015) model (i.e. OTA-grapes predictions/output), showed relevant differences between low (i.e. Emilai Romagna) and high (i.e. Apulia) risk OTA areas. The purpose of Ioannidis, Kogkaki, Natskoulis, Nychas, and Panagou (2015) was to develop a modelling approach to quantify the effect of temperature, water activity (aw) and sodium metabisulphite on the growth and OTA production of *A. carbonarius* in a growth medium. Optimum values for growth were from 30 to 35° C and 0.96 aw, while for OTA production they were 20° C and 0.98 aw. The temperature is high compared to that

quoted for this species of 30° C (Anli & Bayram, 2009; Paterson & Lima, 2011).

9. General discussion

Perrone et al. (2017) suggest using MS and NMR for the identification of OTA from fungi not normally associated with producing the mycotoxin as a general criterion. Reports of OTA production by *A. tubingensis* have been questioned (Storari et al., 2012; Storari et al., 2012) and should perhaps also be subjected to these procedures before decisions are made. In the current authors' opinions, few laboratories which require to determine the OTA capabilities of isolated fungi could afford this equipment and the technical support required. In addition, the methods can be questioned, even for strains from recognized producing species. The high majority of the papers reviewed herein do not employ MS or NMR for OTA analysis and some strains do not appear to produce the expected mycotoxin. Does this represent a false negative due to the analytical equipment? Can false positives arise from some strains within recognized producer species? An objective approach is required whereby the results of various levels of analytical sophistication (e.g. TLC, HPLC, multi-mycotoxin analysis, MS, NMR) should be compared for accuracy. A cost/benefit analysis could then be undertaken to determine the most suitable method for the standard laboratory. There is a difference between what is (a) feasible and (b) practical. This implies that laboratories without the most expensive equipment could contribute in this field of work within the limitations of the equipment employed.

An equivalent situation arises with fungal identifications. Few laboratories have the taxonomic knowledge or facilities to undertake identifications required by some expert fungal taxonomists. There may be a requirement for a second level identification at a level below the species. This has been discussed more generally by some of the current authors (Paterson, Venâncio, & Lima, 2004) (Paterson, Venâncio, & Lima., 2006) where a more fundamental structure of the conidiophores is recommended to be described, enabling a nonspecific identification, such as sub-genus *Penicillium* within the penicillia. Hence, a level of identification would be obtained less likely to be incorrect, thereby reducing confusion between, for example, species and mycotoxin production. Full identifications could be undertaken by expert laboratories at a later date. These points would enable laboratories with less (a) sophisticated equipment and/or (b) high level taxonomic expertise to participate in a satisfactory manner.

A discussion of the issues surrounding isolating fungi from coffee are presented in (Paterson et al., 2014) which apply to the current review on wine and can be referred to. Isolation methods may not give an accurate representation of the most important mycotoxigenic fungi in the wine related substrates. Some fungi are highly sporing, biasing the results of the isolation methods and endophytic fungi may not be isolated efficiently (if they exist in the wine system). Also, mutagens could alter the fungi that are isolated so that morphological or mycotoxin changes occur (Paterson & Lima, 2015) and, for example, this could occur through the application of pesticides in vineyards.

10. Conclusions

There has been considerable success in decreasing OTA in wine since EU regulations were introduced. Southern Europe produced wine with the highest levels of OTA and red wines generally have most OTA. However, threats on the horizon include other mycotoxins and climate change. Many studies report factors influencing the presence of OTA in wine, but there is little concerning AF, PAT, AOH, AME and FUM and this situation requires addressing. More research on AF in wine requires undertaking as this is the ultimate carcinogenic natural compound and is considered generally the most dangerous mycotoxin. It is possible the other mycotoxins will become more frequent as climate change progresses. Better correlations between mycotoxigenic fungi isolated from vineyards and the occurrence of mycotoxins in must are required. Practical (a) identification schemes for the fungi and (b) analytical methods for mycotoxins from the fungi are required. More models are desirable for how climate change affects mycotoxins in wine, complimented with astute interpretation of existing data to enable a useful dialog. Modelling the effect of climate change on the growth of grapes is a priority. Climate change and the effect on mycotoxins in wine will become increasingly important.

Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of the UID/BIO/04469/2013 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684) and the BioTecNorte operation (NORTE-01-0145-FEDER-000004). These were funded by the European Regional Development Fund through the Norte2020—Programa Operacional Regional do Norte.

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