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Aspergillus carbonarius in syrah grapes grown in three wine-growing regions of Brazil

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Abstract

Contamination by ochratoxin A (OTA) in agricultural products grown in regions with different climate conditions is difficult to predict since this contamination depends on a complex interaction of factors. Thus, the aim of this study was to use a mathematical model to predict OTA synthesis by *Aspergillus carbonarius* in grape *(Vitis vinifera)* of the Syrah variety grown in three wine-growing regions, considering the mean monthly temperature of the environment and water activity and pH of grape. Results indicate that the greatest risks of contamination by the toxin are in the months when the temperature is below 19.0°C and these months are just before grape harvest. However, with the predicted increase in temperature, these conditions change, i.e., they are no longer favorable to OTA synthesis, thus reducing the risks of the presence of the toxin in cultivated grapes. For the tropical region, the current environmental temperature and water availability to grape are not favorable to OTA synthesis, showing that even if *A. carbonarius* is present in the crop, it does not encounter suitable conditions of environmental temperature, water activity, and grape pH to synthesize OTA.

Introduction

Ochratoxin A (OTA) is a secondary metabolite synthesized by some species of filamentous fungi and is able to produce toxic effects in animals and humans, depending on the levels of consumption. One of the most publicized reports of OTA toxicity to humans was the Balkan Endemic Nephropathy (BEN), in which various cases of urinary tract cancer were associated with the presence of the toxin in food (Ringot *et al*., 2006; Marroquín-Cardona *et al*., 2014).

Contamination by OTA in agricultural products is difficult to foresee since this contamination depends on a complex interaction of factors, such as temperature and humidity of the environment, type of food, water activity and pH of the food, and storage conditions and duration (Pose *et al.*, 2010; Garcia *et al.*, 2011a; Paterson and Lima, 2011). Nevertheless, evaluating the effect of each one of these factors, the interaction among them, and the use of predictive models to estimate synthesis of the toxin by the fungus is fundamental for ensuring consumption of food safe for humans and animals (Schaafsma and Hooker, 2007; Khalesia and Khatib, 2011; Garcia *et al*., 2011b).

Predictive models derived from quantitative studies under experimental conditions allow prediction of the influence of each factor on the growth or production of a determined metabolite from the microorganism in question (Nakashima *et al*., 2000). The advantages of predictive models in food microbiology are numerous and include description of microorganism behavior under different physical-chemical conditions, prediction of microbiological safety of a product, assistance in analysis of hazards and critical points of control, and assistance in decision-making and risk analysis.

Thus, the aim of this study was to use the predictive model obtained by Passamani *et al.* (2014) in estimating ochratoxin A (OTA) synthesis by the fungus *Aspergillus carbonarius* in Syrah grapes grown in three wine-growing regions of Brazil. An additional aim was to evaluate the influence of temperature increase in two scenarios proposed by the fifth report of the Intergovernmental Panel on Climate Change (Intergovernmental Panel on Climate Change, 2014).

Materials and Methods

To predict OTA synthesis by *A. carbonarius* in wine-producing grapes *(Vittis vinifera)* of the Syrah variety in three wine-growing regions of

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Table 1. Predictive model and the coefficient of determination (R^2) based on variables obtained by Passamani *et al* (2014).

Fungus	Predictive Model	R^2
A. carbonarius (µg/g)	$+ 1.27 + 4.17 X1 - 44.27 X2 - 2.57 X3 - 0.09 X1 X2 - 3.91 X1 X3 + 48.77 X2 X3 + 1.56 X1^2 + 0.07 X2^2 + 1.28 X3^2$	0.827

In which: X1 is the mean monthly temperature of the region; X2 is the water activity of the substrate (Syrah grape), and X3 is the pH of the substrate (Syrah grape).

Brazil (Region 1 – State of São Paulo/SP, Region 2 – State of Minas Gerais/MG, Region 3 – State of Pernambuco/PE), the predictive model obtained in a study undertaken by Passamani *et al*. (2014) was used, which relates temperature of the environment, water activity of the substrate, and pH of the substrate.

Table 1 presents the predictive model and the coefficient of determination (R^2) for production of OTA by *A. carbonarius*. The mean monthly temperatures of the environment were obtained from the site of the National Meteorological Institute of Brazil (National Institute of Meteorology, 2015). Water activity (a_w) of grape was determined using an AquaLab CX-2 (Decagon Devices, Inc., Pullman, WA), and grape pH was measured through a digital pH meter (Digimed, Digicrom Analitica Ltda, São Paulo, Brazil). The OTA concentration was obtained from the equation described in Table 1, in which X1 was substituted by the value of the mean monthly temperature of the growing region, X2 was substituted by the value of water activity (0.99), and X3 was substituted by the pH of the substrate (3.5), i.e., the mean value found for the variety of grape analyzed.

The value of 1°C (optimistic scenario) and 3.7°C (worst-case scenario) were added to the mean values of current temperature; the values of the scenarios were obtained from the average of the sum of the temperature intervals found in the climate change projections presented in the fifth report of the Intergovernmental Panel on Climate Change (Intergovernmental Panel on Climate Change, 2014). The optimistic scenario predicts an increase from 0.3°C to 1.7°C over current mean temperature, and the worst-case scenario predicts an increase from 2.6°C to 4.8°C.

Results

Applying the mathematical model obtained by Passamani *et al.* (2014), the results show that there is a variation in OTA concentration in regions 1(SP) and 2 (MG), depending on the month (Table 2 and Table 3). Temperature is the variable that most influences OTA synthesis, i.e., the greater the temperature, the lower the synthesis of the toxin by the fungus *Aspergillus carbonarius* in the grape substrate. That means that the months in which the mean temperature is below 19°C are those that exhibit the highest values of OTA concentration obtained through use of the mathematical model.

In region 2 (MG), it can be observed that the concentration of OTA is also favored by lower temperatures (<19.5°C), which are found from April to October. The highest OTA concentration estimated was for July, the month that precedes grape harvest in this region. As the mean temperature of regions 1(SP) and 2 (MG) exhibited similar values, the same behavior was observed for region 1 (SP), where the highest value of OTA was also found for July. Grape harvest in this region is also scheduled to begin in late July to early August. This shows that if the fungus *Aspergillus carbonarius* is present in the growing location of these grapes, the temperature conditions of the environment and the water availability and pH conditions of the substrate (grape) can favor the fungus and synthesize ochratoxin A.

This may be considered a risk factor for OTA contamination in the wines prepared from the grapes produced in these regions, since values above 2 µg/L are not in conformity with Brazilian legislation for wine, grape juices, and derivatives (Brazilian Ministry of Health, 2011). However, considering the projections of an increase in environmental temperature, the conditions for OTA synthesis become unfavorable because the increase in temperature would negatively influence this synthesis. Nevertheless, for there to be OTA synthesis, the fungus must be present in the region and find conditions for colonizing the grapes. Yet, according to the results obtained by Passamani *et al.* (unpublished data), no *A. carbonarius* fungus was isolated on Syrah grapes grown in the 2011 crop year in region 2 (MG) and in the 2011 and 2012 crop years in region 1 (SP).

In relation to region 3 (PE), applying the predictive model and using the mean monthly temperatures and the projections of increase in temperature, positive values for OTA concentration were not found. That means that current environmental conditions of temperature and water activity and pH of the grape

Table 2. Values of variables: ambient temperature (X1), grape water activity (X2) and pH of the grapes (X3) used to determine the production of ochratoxin A (OTA) by the fungus *Aspergillus carbonarius*, through the use of the model obtained by Passamani *et al* (2014) for region 1 (SP).

	X1 (T°C)	X2 (aw)	X3 (pH)	OTA (T° atual)	OTA (+1°C)	OTA (+3.7°C)
January	20,65	0,99	3,5	-7,36*	-13,84*	-31,3*
February	22,30	0,99	3,5	-18,06*	-24,54*	-42,05*
March	21,28	0,99	3,5	-11,44*	-17,93*	-35,43*
April	20,13	0,99	3,5	-3,99*	-10,47*	-27,98*
May	15,57	0,99	3,5	25,57	19,09	1,58
June	15,93	0,99	3,5	23,24	16,75	-0,74*
July	15,63	0,99	3,5	25,18	18,70	-1,19*
August	15,92	0,99	3,5	23,30	16,82	-0,68*
September	18,69	0,99	3,5	5,34	-1,13*	-18,64*
October	21,88	0,99	3,5	-15,33*	-21,82*	-39,32*
November	20,97	0,99	3,5	-9,43*	-15,92*	-33,42*
December	21,02	0,99	3,5	-9,76*	-16,24*	-33,75*

The concentration of ochratoxin A (OTA) has been obtained by applying the mathematical equation: + 1,27 + 4,17 X1 – 44,27 X2 – 2,57 X3 – 0,09 X1 X2 – 3,91 X1 X3 + 48,77 X2 X3 + 1,56 X12 + 0,07 X22 + 1,28 X32, where X1 is the monthly average temperature of the wine region, X2 is the grape water activity and X3 is the pH of the grape.

* The values found indicate that in these conditions *Aspergillus carbonarius* will not synthesize ochratoxin A.

are not favorable to OTA synthesis by *A. carbonarius.* Thus, even if the fungus is present in grapes of this region, it does not find favorable conditions for synthesis of the toxin, which ensures the production of the tropical wines prepared in this region.

Discussion

The climate conditions of the wine-growing region can have an influence both on colonization of the fungus and on OTA synthesis in grapes. Increase in temperature and rainfall can have an impact on the presence and behavior of the fungus (Paterson and Lima, 2010). According to Tirado *et al.* (2010), to estimate and predict the impact of climate change on OTA contamination in grapes and their derivatives, such as wine, it is necessary to develop models capable of predicting mycotoxin levels, relating them to climate parameters.

In recent decades, much has been said about climate changes that are occurring on our planet and how these changes can compromise not only the quantity, but also the quality of foods produced, especially in relation to food safety. The Fifth Assessment Report of the Intergovernmental Panel on Climate Change shows intensification of climate changes, with an increase in mean global temperature. The alert was made by the scientists of the Intergovernmental Panel on Climate Change based on review of thousands of studies undertaken in the last five years. The document presents the scientific basis for identification of global climate change; four different scenarios that may occur by the year 2100 were simulated, which are called "Representative Concentration Pathways (RCPs)".

The most optimistic scenario predicts that the increase in world temperature may range from 0.3°C to 1.7°C in the period from 2010 to 2100. The worst-case scenario, in which emissions continue to grow at an accelerated rate, predicts that Earth's surface may warm from 2.6°C to 4.8°C throughout this century. Paterson and Lima (2010) believe that climate changes will have a considerable impact on the presence and production of these toxic metabolites by some filamentous fungi, but not many studies have yet been undertaken. In Brazil, there are few studies on the influence of environmental temperature of wine-growing regions and water availability in the substrate (grape) in OTA synthesis by the main ochratoxigenic contaminant species of grapes, *Aspergillus carbonarius.*

It has been observed that the wines produced in some wine-growing regions considered as traditional and located in temperate zones have exhibited OTA values above the limit established by legislation of the European community. In other words, the

Table 3. Values of variables: ambient temperature (X1), grape water activity (X2) and pH of the grapes (X3) used to determine the production of ochratoxin A (OTA) by the fungus *Aspergillus carbonarius*, through the use of the model obtained by Passamani *et al* (2014) for region 2 (MG)

	X1 (T°C)	X2 (aw)	X3 (pH)	OTA (T° actual)	OTA (+1°C)	OTA (+3.7°C)
January	20,59	0,99	3,50	-6,97*	-13,45*	-30,96*
February	21,90	0,99	3,50	-15,46*	-21,95*	-39,45*
March	21,13	0,99	3,50	-10,44*	-16,95*	-34,46*
April	20,04	0,99	3,50	-3,37*	-9,89*	-27,39*
May	15,70	0,99	3,50	24,76	18,24	0,74
June	16,15	0,99	3,50	21,84	15,33	2,17
July	14,76	0,99	3,50	30,86	24,34	6,83
August	15,26	0,99	3,50	27,61	21,10	3,59
September	18,33	0,99	3,50	7,71	1,19	-16,31*
October	21,29	0,99	3,50	-11,51*	-17,99*	-35,50*
November	21,15	0,99	3,50	-10,60*	-17,08*	-34,59*
December	22,44	0,99	3,50	-18,93*	-25,45*	-42,96*

The concentration of ochratoxin A (OTA) has been obtained by applying the mathematical equation: + 1,27 + 4,17 X1 – 44,27 X2 – 2,57 X3 – 0,09 X1 X2 – 3,91 X1 X3 + 48,77 X2 X3 + 1,56 X12 + 0,07 X22 + 1,28 X32, where X1 is the monthly average temperature of the wine region, X2 is the grape water activity and X3 is the pH of the grape.

* The values found indicate that in these conditions *Aspergillus carbonarius* will not synthesize ochratoxin A.

increase in temperature for these regions has become a problem for contamination by this toxin, which is considered to be nephrotoxic and with a cumulative effect (International Agency for Research on Cancer, 1993). Paterson and Lima (2010) conclude that the biggest risk with respect to mycotoxins from climate change will be found in developed countries with temperate climates.

Predictive microbiology can be considered a tool based on mathematical models to predict microbial growth and inactivation in foods. However, there are currently few models that predict mycotoxin levels (Schaafsma and Hooker, 2007; Van der Fels-Klerx and Booij, 2010; Garcia *et al*., 2011b). Marin *et al.* (2009) developed a model to predict the growth and synthesis of OTA by an isolate of *A. carbonarius* in accordance with water availability and storage temperature of pistachio. These authors showed that accumulation of OTA was mainly associated with temperature, with an increase from 15-20°C. In accordance with these results, significant amounts of OTA in natural substances can be produced in only 5 days at 0.97 of aw and temperatures of 15-20°C.

The use of the predictive model obtained by Passamani *et al.* (2014) is the first attempt at quantifying OTA based on environmental temperature, water availability, and pH of the substrate, factors that interfere in the growth and synthesis of the toxin by fungal species. These studies using predictive modeling represent an addition essential for food quality and safety, assisting in the adoption of preventive measures against mycotoxin contamination. In addition, the use of these models assists in analysis of risk of the presence of this toxin in the substrate of grapes from wine-growing regions with different climate conditions. According Magan *et al.* (2011), food security has become a very important issue worldwide and the potential effects of climate change on yields and quality of food crops, including mycotoxins, is now receiving scientific attention, especially from a risk analysis perspective.

Conclusion

For the three wine-growing regions in which the mathematical model was applied, the climate changes foreseen both in the worst-case scenario and the optimistic scenario show that even if the *Aspergillus* carbonarius fungus is present in the region of Syrah grape cultivation, it does not encounter temperature conditions to synthesize OTA, which ensures the consumption of wines produced in these regions. It is as if this region of Brazil were already experiencing that foreseen by the Intergovernmental Panel on Climate Change.

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