Development of Ochratoxin A during Robusta (*Coffea canephora*) Coffee Cherry Drying

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The occurrence and formation of ochratoxin A (OTA) in Robusta coffee was studied for three consecutive seasons under tropical conditions in Thailand. Sun drying of coffee cherries consistently led to OTA formation in the pulp and parchment (husks) of the cherries. In replicated trials, dried coffee beans (green coffee) were shown to contain on average OTA concentrations that were ∼1% of those found in husks. OTA contamination of green coffee depended on cherry maturity, with green cherries being the least, and overripe cherries the most susceptible. Defects, and in particular the inclusion of husks, are the most important source of OTA contamination. OTA contamination occurred independently of whether cherries were placed on concrete, on bamboo tables, or on the ground. The study suggests that better raw material quality, an appropriate drying and dehulling procedure combined with a reduction of green coffee defects can effectively contribute to the reduction of OTA in green coffee.

Keywords: *Coffee; Coffea canephora; ochratoxin A; drying; postharvest; mold*

INTRODUCTION

Ochratoxin A (OTA), a nephrotoxic and nephrocarcinogenic mycotoxin in animal experiments, is potentially being produced by several fungal species from the *Aspergillus* genus and by *Penicillium verrucosum* at a minimum a_w of 0.85 (Moss, 1996). It is most commonly found in cereals and cereal products, but a wide range of other commodities including green coffee have been reported as containing the toxin (Pohland et al., 1992). Most of the OTA present in green coffee beans is eliminated during roasting and soluble coffee manufacturing (Blanc et al., 1998). This explains why a survey conducted by Pittet et al. (1996) on 101 commercial pure soluble coffee products gave only trace amounts of OTA (mean 1.1 μg/kg).

OTA contamination of green coffee might be reduced by understanding the stage and conditions under which it is produced during green coffee production and handling. However, very little is known about the origin of OTA contamination in green coffee. Recent results on the impact of industrial Robusta green coffee storage on OTA formation under tropical conditions in Thailand gave no evidence for the production of OTA upon storage (Bucheli et al., 1998). Clearly, OTA was already present before storage, pointing to the possibility that harvesting and postharvest handling of coffee cherries could be the critical steps leading to OTA contamination.

Preliminary screening of coffee materials from nine farms in Thailand undertaken in 1996 revealed that OTA was produced on coffee cherries during sun drying. This initiated a series of experiments aimed at determining the fate of OTA under different conditions and steps of postharvest handling. Trials were frequently carried out without knowing whether OTA would be present, or formed during the experiments. Here we present field and experimental data to support the hypothesis that the application of coffee cherry sun drying under tropical conditions can induce OTA formation on coffee cherries. The study describes the effect of weather conditions, cherry maturity, and drying conditions on the kinetics of cherry drying and the formation of OTA on coffee cherries and green coffee.

MATERIALS AND METHODS

Plant Material. The coffee cherries used in this study were all Robusta (*Coffea canephora* var. *robusta*). For the screening trial carried out in Feb 1996, coffee cherries and green coffee were sampled by Nestlé Thailand at nine different farms in the Chumphon area in the south of Thailand. All drying trials carried out at the Nestlé Coffee Buying Station (Sawi, Thailand) in 1997 and 1998 were performed with coffee cherries produced on three different farms. Cherry ripening stages were defined as nonripe (green), ripe (red), and overripe (dried on the tree).

Coffee Cherry Drying Experiments. The effect of the drying method (ground, concrete, and bamboo table) was tested in early 1997 at the Nestlé Coffee Buying Station, and on three different farms each using one of the drying methods. Drying experiments under optimal local conditions at the Nestlé Coffee Buying Station were repeated six times covering the main coffee harvesting period (Dec 1997 through Feb 1998). For each of the six trials, 150 kg of mostly ripe cherries were collected from two farms, located ∼50 km from each other. The mixed material (300 kg) was dried separately on concrete (4 m^2) in 3 × 100 kg lots by spreading the coffee cherries in 2−3 cm thick layers, avoiding any rain during the drying period. Nonripe, ripe, and overripe coffee cherries were used in four other experiments carried out in Feb 1998 for testing the effect of cherry maturity on OTA formation. For each maturity stage, 200 kg of coffee cherries from one farm were mixed, divided into lots of 50 kg, and treated as follows, avoiding any interference with rain: (1) drying under optimal local conditions, (2) storage in sealed plastic bags (from day 0 to day 5 of

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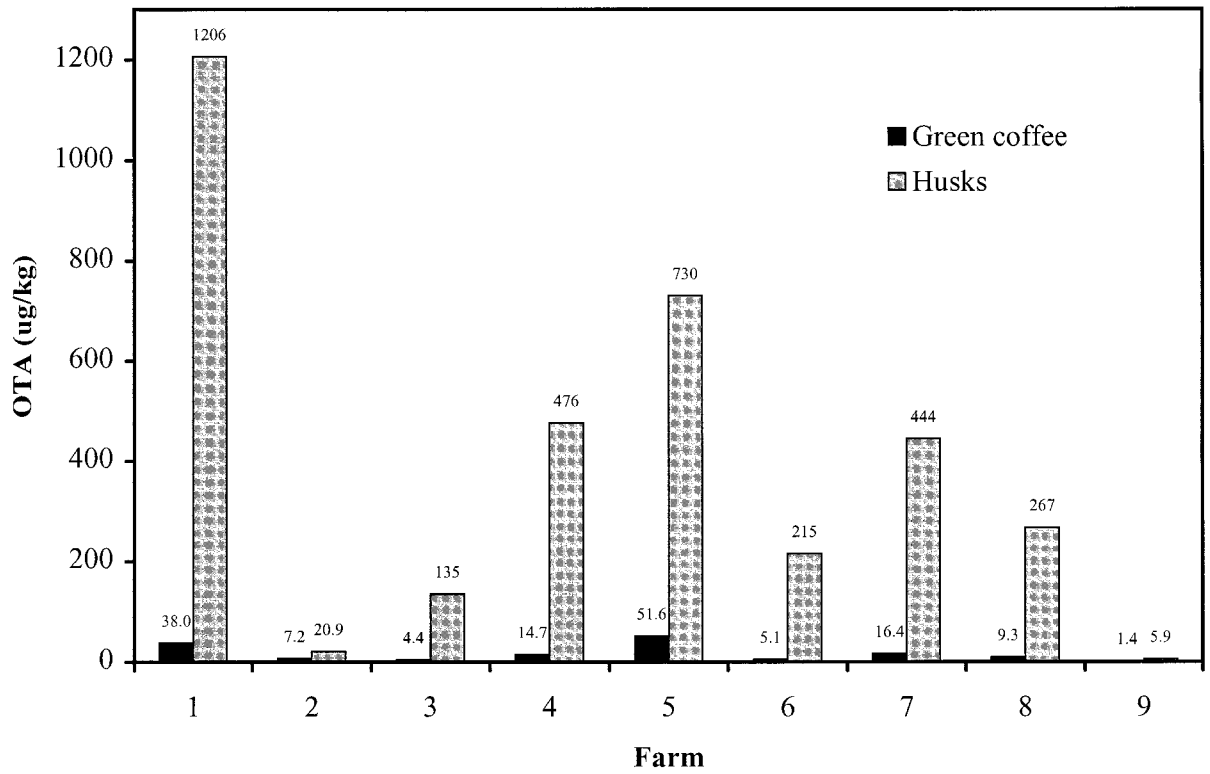

Figure 1. OTA concentration in the green coffee and husk fractions of dried cherries from farms in southern Thailand.

drying) followed by optimal drying, (3) rewetting of cherries with water between day 5 and day 10 (3 times per day) followed by optimal drying, and (4) soaking the cherries in sodium hypochlorite (NaOCl) at day 0 before optimal drying.

Sample Preparation. Coffee samples were kept frozen at −18 °C at Nestlé Coffee Buying Station (Sawi, Thailand) before being air-freighted in dry ice in sealed plastic bags to Nestlé Research Center Lausanne, Switzerland. Samples produced in 1996 and 1997 were freeze-dried, and if necessary oven dried at 70 °C, before being dehulled and separated into green coffee and husks (dried pulp and parchment). In 1998, 5 kg of dried cherries of each replicated trial were dehulled by cracking the fruits with a mortar, and separating husks from green coffee by shaking the material on a sieve. Dried cherries, husks, and green coffee were milled in a mill operating with a sharp steel disk (Perten Laboratory Mill 3303).

OTA Analysis. Each sample was analyzed for OTA according to the method described by Pittet et al. (1996) and currently used by the industry (van der Stegen et al., 1997). The finely ground test portion was blended for 3 min with methanol/3% sodium hydrogen carbonate (50:50) and filtered. An aliquot of 4 mL filtrate was diluted to 100 mL with phosphate-buffered saline (PBS) and applied to an OchraTest immunoaffinity column containing a monoclonal antibody specific for OTA (Vicam Inc., Watertown, MA). After washing with 10 mL of distilled water, the OTA was eluted with 4 mL of methanol and quantitated by reversed-phase HPLC with fluorescence detection. For samples showing OTA concentrations higher than 10 μg/kg, a confirmation of identity by methyl ester formation was carried out according to a procedure similar to that described by Nesheim et al. (1992).

Microbiological Analysis. Coffee samples of the 1998 drying experiments were stored under refrigeration before being analyzed at Nestlé R&D Center Singapore. Mold isolation was performed by weighing 9 g of the samples into 9 g of peptone diluent, and vortexing for 30 s. An aliquot of the resulting mixture, and subsequent dilutions, were surface plated on L5 agar. The plates were incubated at 30 °C for 3 days, after which any molds detected were counted (distinguished by their preliminary cultural characteristics), isolated, and subcultured on Czapek-Dox Agar supplemented with 5% yeast extract (CYA). The latter medium was also used for subsequent subcultures for identification purposes, and all identifications were made according to the classification scheme of Samson et al. (1995), and Raper and Fennel (1965).

Soluble Sugar Analysis. Ground coffee samples were extracted as described by Bucheli et al. (1998). Water-soluble extracts were separated in duplicate by high-performance anion exchange chromatography coupled to pulsed electrochemical detection (HPAE-PED) on an anion exchange column (CarboPac PA-100, 4 × 250 mm, Dionex, Sunnyvale, CA) by injecting 20 μL at a flow rate of 1 mL/min and application of a linear NaOH gradient (Bucheli et al., 1996).

RESULTS AND DISCUSSION

Farm Variability. The formation of OTA in coffee materials was investigated in 1996 by screening nine farms in the coffee producing area of Chumphon, Thailand. Nonripe and ripe, overripe, and dried coffee cherry samples were obtained from each farm. All samples were dried in the laboratory before being dehulled and separated into a green coffee and a husk fraction.

Nonripe and ripe cherries contained only trace amounts of OTA in the green coffee (range not detected to 0.6 μg/kg; mean 0.3 μg/kg) and husk fraction (range 0.2−0.9 μg/kg; mean 0.4 μg/kg). Overripe cherries tended to be more contaminated, although this was mostly due to one farm (31.8 and 38.6 μg/kg OTA for green coffee and husks, respectively). Average OTA was 3.3 μg/kg (range not detected to 31.8 μg/kg) for the green coffee fraction and 7.3 μg/kg (range 0.3−38.6 μg/kg) for the husk fraction. For cherries dried on the nine farms, the concentrations of OTA in the green coffee and husk fractions were very variable (Figure 1). They were consistently much higher in husks (range 5.9−1206 μg/kg; mean 389 μg/kg) than in the corresponding green coffee samples (range 1.4−51.6 μg/kg; mean 16.5 μg/kg). This was one of the first experimental indications that OTA is formed during sun drying in the coffee cherry pericarp (pulp and parchment), the part of the cherry which is removed as husk in the dehulling process.

Table 1. Effect of Coffee Drying Methodology (Ground, Concrete, Bamboo Table) on the OTA Contamination of the Green Coffee and Husk Fraction of Ripe Cherries during Sun Drying

		ground		concrete		bamboo table	
drying (days)	experiment[a]	green coffee	husk	green coffee	husk	green coffee	husk
0	1	nd[b]	nd	nd	0.3	nd	nd
	2	nd	0.2	nd	0.9	nd	0.5
	3	nd	3.4	nd	5.6		
5	1	1.1	19.1	0.4	29.9	nd	nd
	2	nd	3.0	0.2	2.9	1.6	34.1
	3	nd	41.3	0.2	220	0.2	10.1
10	1	0.4	5.8	0.3	15.8	nd	nd
	2	nd	1.5	nd	7.2	0.8	19.6
	3	0.2	11.7	0.8	124	0.2	0.9
15	1	0.2	15.6	nd	6.9	nd	nd
	2	0.5	3.3	nd	4.8	0.6	8.7
	3	nd	6.6	nd	31.9	0.6	2.4
20	1	-	-	nd	8.4	nd	0.3
	2	0.3	1.2				
	3	nd	11.6	0.2	16	nd	3.9
	av	0.2		0.2		0.3	

[a] Experiments 1, 2 and 3 were started on Jan 10, Jan 26, and Feb 8, 1997. [b] nd, not detected.

The green coffee produced on each of the nine farms all contained detectable levels of OTA, with a range of contamination of 0.4−19 μg/kg (mean 5.5 μg/kg). Similar levels of OTA in Thai green coffee (mean 7.3 μg/kg and 3.9 μg/kg, respectively) used for manufacturing trials and industrial storage in Thailand have been recently reported by Blanc et al. (1998) and Bucheli et al. (1998). Some of the nine farms were assessed again for OTA contamination during the 1997 harvesting season. The fact that the results were not consistent with the preceding season indicates that the formation of OTA on coffee cherries depends on multiple factors that might vary from farm to farm.

Effect of Drying Methodology on OTA Production. Preliminary results obtained in 1996 indicated that the formation of OTA was not affected by the sun drying methodology used. The kinetics of coffee cherry drying on concrete, most commonly used in Thailand, was compared in 1997 with drying on ground and bamboo table (Table 1). Ripe coffee cherries of good quality were used in these experiments, which were basically free of OTA (green coffee, not detected; mean for husks, 1.4 μg/kg) at the beginning of the drying process (day 0), confirming 1996 results. Within the first 5−10 days of drying, OTA content increased in most of the husk samples (Table 1). However, its evolution was quite irregular, largely resembling the observed variability between farms (Figure 1). Independent of the drying method used, green coffee had always at each drying stage very low OTA levels (average 0.2 μg/kg), close to the limit of detection (Table 1). Optimal raw material quality and careful drying were probably reasons for the consistent low contamination of green coffee in these experiments. The effect of ground, concrete, and bamboo table drying on green coffee OTA contamination was also assessed during the 1997 harvest on three farms, each applying one of the three drying methods. On average, similar results were obtained for green coffee issuing from concrete (4.3 μg/kg) and bamboo table drying (4.0 μg/kg), and lower values for ground drying (1.4 μg/kg). A similar level of green coffee OTA contamination was reported by Blanc et al. (1998) and Bucheli et al. (1998).

Table 2. Ochratoxin A Content (Micrograms per Kilogram) of Defected Green Coffee Samples Found in January 1997 in Different Deliveries of Green Coffee to Nestlé Coffee Buying Station (Sawi, Thailand)

	sample				
green coffee defect	1	2	3	4	av
broken beans	11.7	4.7	4.3	7.3	7.0
infested beans	15.7	8.2	9.4	5.3	9.7
black beans	0.7	1.4	1.2	5.2	2.1
partly black beans	8.1	2.9	3.5	1.9	4.1
husks (public dehuller)	319				
husks (factory)	35.6				

The described observations indicate that the three drying methods examined can all potentially lead to OTA contamination of green coffee. However, the results obtained from the farms demonstrate that the initial raw material quality, weather conditions during drying, drying management, presence of OTA-producing microorganisms, and local farm conditions undoubtedly play a more important role in OTA contamination of green coffee than the drying methodology used.

OTA Contamination in Defected Green Coffee. Storage trials carried out in Thailand (Bucheli et al., 1998) had indicated that OTA content of green coffee was linked to defect count. In this present report, OTA contamination of common green coffee defects was determined for green coffee delivered directly by farmers to Nestlé Coffee Buying Station, Sawi, Thailand (Table 2). Broken and infested beans, together with husks were the most important source of OTA contamination found in green coffee. The occurrence of 319 μg/kg of OTA in an aggregate husk sample of a public coffee dehuller demonstrated that husks are the richest source of OTA in green coffee.

Kinetics of OTA Formation during Cherry Drying. Comparison of drying methodology (Table 1) showed that OTA formation on coffee cherries occurs frequently in the initial stages of drying. To substantiate this information, the evolution of coffee cherry drying and its impact on OTA formation were studied in six replicated trials between Dec 1997 and Feb 1998, covering the whole coffee harvesting period in Thailand. The evolution of dry matter (DM) content of cherries is shown in Figure 2. Initial cherry DM varied between 37.1 and 40.6%. Within 10 days of sun drying, cherry DM content reached 79−88%. Drying was practically completed after 15 days. The daily duration of sunshine explained best the variability of the initial speed of drying observed between day 0 and 10 (data not shown), and not the ambient average temperature (26.4−27 °C) and relative humidity (77−82%) measured during each of the drying experiments.

Formation of OTA in coffee cherries took place during all six replicated drying experiments (Table 3). In most of the experiments, it was close to zero at the beginning of drying (day 0), confirming 1996 and 1997 observations (Figure 1, Table 1) that ripe cherries are usually not contaminated with OTA at harvest. The rapidity and extent with which OTA appeared on cherries during drying was very variable. In experiment no. 1, OTA increased rapidly within the first 5 days of drying, while it increased more steadily over the whole drying period in the other experiments. At the end of each drying experiment, a batch of 5 kg of dried cherries was separated into green coffee and husks and analyzed for OTA. Concentrations of OTA in green coffee were on average 1.1% (range 0.4−1.7%) of those found for husks

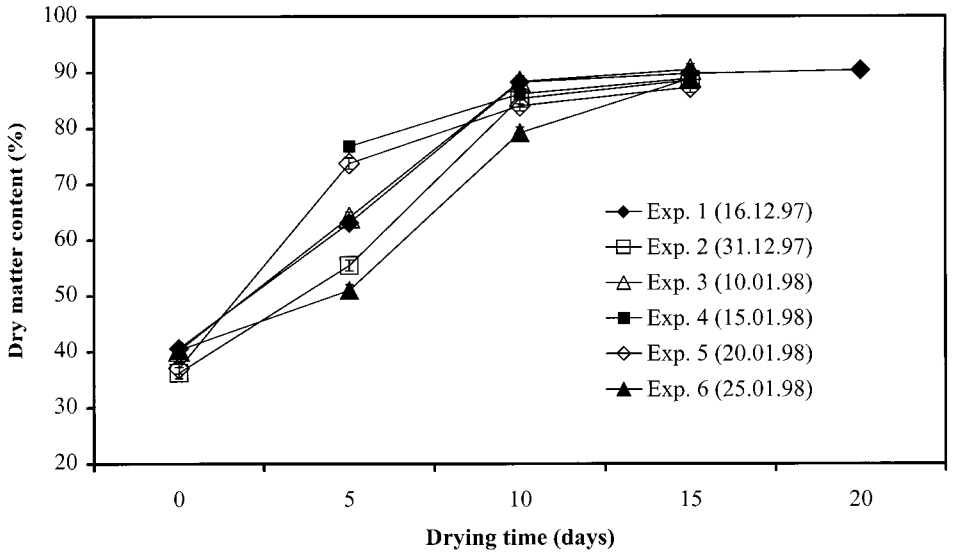

Figure 2. Evolution of dry matter content of cherries in six replicated sun drying experiments of Robusta coffee initiated during Dec 1997 and Jan 1998.

Table 3. Evolution of Ochratoxin A Content (Micrograms per Kilogram) in Replicated Coffee Cherry Drying Trials Carried out under Optimal Local Conditions between Dec 1997 and Feb 1998 in Thailand

		experiment[a]					
drying (days)		1	2	3	4	5	6
0	cherries	0.7 ± 0	0.4 ± 0.1	0.5 ± 0.3		3.9 ± 1.3	49.2 ± 80.4
5	cherries	436 ± 85	109 ± 69.2	9.3 ± 4.8	99.2 ± 7.1	22.4 ± 11.8	109 ± 100
10	cherries	256 ± 81	189 ± 113	61.8 ± 57	109 ± 80.5	67.8 ± 106	174 ± 37.5
15	cherries	353 ± 29	-	-	42.6 ± 10.9	161 ± 232	-
	husks	-	190 ± 62.6	87.8 ± 19.5	162 ± 22.6	77.3 ± 18.0	407 ± 278
	green coffee	-	2.5 ± 1.2	1.1 ± 0.5	2.8 ± 1.1	0.6 ± 0.3	1.7 ± 0.9
20	husks	677 ± 130					
	green coffee	6.4 ± 3.8					
green coffee/husks (%)		0.9	1.3	1.3	1.7	0.8	0.4

[a] Experiments 1−6 were started in chronological order on Dec 16 and 31, 1997, and on Jan 10, 15, 20, and 25, 1998. Results are expressed as the mean and standard deviation of three replications.

Table 4. Evolution of Ochratoxin A Content (Micrograms per Kilogram) in Coffee Cherries That Were Specifically Treated during the Drying Process in February 1998 in Thailand

		experiment 1[a]		experiment 2[b]		experiment 3[c]		experiment 4[d]	
drying (days)		unripe	ripe	unripe	ripe	unripe	ripe	unripe	ripe
0	cherries	17.0	2.8	1.5	1.0	0.6	0.5	1.1	0.9
5	cherries	0.5	2.0	nd[e]	6.3	nd	1.1	nd	1.7
10	cherries	0.7	231	0.3	5.0	2.2	52.0	0.2	0.8
15	cherries	1.0	2.3	1.0	15.5	3.3	49.3	0.2	0.5
20	cherries			0.3	12.5	0.6	158		
	husks	14.9	5.6					0.9	8.4
	green coffee	nd	0.3					0.1	0.2
25	cherries								
	husks			1.8	70.3	1.7	111		
	green coffee			0.1	1.3	0.2	1.1		

[a] Drying under optimal local conditions. [b] Storage in sealed plastic bags (day 0 to 5). [c] Rewetting (3 times per day) between day 5 and 10. [d] Soaking of cherries in hypochlorite (NaOCl) at day 0. [e] nd, not detected.

(Table 3). Together with the farm data obtained in 1996 on dried coffee cherries, which indicated an average green coffee concentration of ~4% of the concentration found in husks, the results presented here strongly suggest that OTA formation takes place in the pericarp (pulp and parchment) of coffee cherries during sun drying. Possibly, the principal sources of OTA contaminated green coffee are damaged and overripe cherries susceptible to microbial growth during sun drying, and also the dehulling process, a dusty procedure where OTA contaminated cherries come into direct contact with green coffee.

Effect of Cherry Maturity on OTA Contamination. Sampling of nine farms had indicated overripe cherries being more prone to OTA contamination. Drying under local optimal conditions, cherry rewetting, plastic bag storage, and hypochlorite treatment were therefore tested to see whether the use of unripe or ripe cherries would affect OTA formation on coffee cherries.

It can be seen in Table 4 that there was no evidence for generation of OTA on unripe cherries under any of the four drying conditions tested. Storage of unripe cherries for 5 days in tightly sealed plastic bags also gave no visual evidence for mold development, whereas ripe cherries were heavily infested by molds under similar conditions. Apart from cherry integrity and firmness, sugar availability in the pericarp might also affect microbial growth. Sugar analysis of unripe and

ripe coffee cherries showed that glucose and fructose concentrations were much lower in unripe cherries (data not shown), as found also by Rogers et al. (1999). Both sugars decreased during sun drying and were even completely depleted when drying was linked to conditions such as storage in plastic bags or rewetting.

For ripe cherries, initial storage for 5 days in sealed plastic bags led to OTA formation; however, OTA was formed only toward the end of drying and not during the period of bag storage (Table 4). Interestingly, rewetting between 5 and 10 days of drying promoted a strong OTA formation in cherries. Occasional rain being not uncommon in southern Thailand during the coffee harvesting season, rewetting must be seriously considered as a key factor promoting OTA formation on coffee cherries. In practice, it is still widely observed that many coffee farmers are not correctly protecting their drying coffee from rain. In all cases, treatment of green and ripe cherries with a hypochlorite solution before the initiation of sun drying prevented the development of significant amounts of OTA (Table 4). Similar observations were made in experiments with figs that had been treated before sun drying with sulfur-containing agents (Özay et al., 1995). Despite these encouraging results, more tests are necessary to verify whether surface sterilization or the use of antifungal agents would be a safe way to reduce the risk of OTA formation on coffee cherries.

Microbiological Analysis. Being able to isolate and identify the microorganisms producing OTA on coffee cherries could represent an important step forward toward understanding how to protect coffee cherries from the formation of OTA. Unfortunately, attempts undertaken in 1998 to isolate known potential ochratoxigenic molds, such as *Aspergillus ochraceus*, from fresh and drying coffee cherries were unsuccessful. Suppression of ochratoxigenic molds by yeasts, bacteria, or other molds (e.g., *Aspergillus niger*) during isolation and subculture, or the sample size as mentioned by Nesheim (1976) could be reasons for the nonisolation. *A. niger* was the most predominant mold found in all coffee cherry samples examined, and *Aspergillus brunneo-uniseriatus* was also often present. As a cause and effect relationship between the ochratoxigenic *A. ochraceus* and OTA in green coffee has not been demonstrated (Mantle, 1998), other microorganisms or molds might therefore be responsible. In that respect, it is interesting to note that Heenan et al. (1998) have recently shown that *A. niger* and *Aspergillus carbonarius* can produce OTA under in vitro conditions. However, this will have to be confirmed with further studies using a larger sampling size, and working on the actual coffee substrate.

Conclusions. This study provides data, previously not available, on the generation of OTA in Robusta green coffee. A series of field and experimental data collected in Thailand demonstrated that OTA is mainly produced during the coffee cherry drying. The presence of low-quality coffee materials such as damaged and overripe cherries is most likely one of the main parameters contributing to OTA development. Prevention and reduction of OTA in green coffee can be achieved by avoiding the use of damaged and overripe cherries, refraining from inappropriate cherry storage in plastic bags, optimization of drying conditions, and the reduction of green coffee defects. All these measures are part of coffee postharvest practices commonly known (Barel and Jacquet, 1994).

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