Enhancement of Coffee Quality through the Prevention of Mould Formation

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Final Technical Report

Enhancement of Coffee Quality through the Prevention of Mould Formation

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It was implemented under the supervision of the International Coffee Organization, and executed on behalf of the above by the Food Quality and Standards Service of the Food and Agriculture Organization of the United Nations.

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Acknowledgements

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However, we would like to thank all of our counterparts in participating countries, the various national and international project consultants and colleagues in the coffee industry for their hard work, patience and resilience over the past few years – the project would not have been completed without them.

FAO, Rome, July 2006

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Abbreviations and Acronyms

AEKI	Indonesian Coffee Exporter's Association
AGMARK	Agriculture Produce (Grading & Marketing) Act (India)
AGNS	Food Quality and Standards Service, FAO (after 1st January 2006)
ANECAFÉ	Asociacion Nacional de Exportadores de Café (Ecuador)
APEDA	Agricultural and Processes Food Products Export Development Authority (India)
ASIC	Association Scientifique Internationale du Café
A_w	Water activity
BIS	Bureau of Indian Standards
CBB	Coffee Berry Borer
CBD	Coffee Berry Disease
CBI	Coffee Board of India
CBK	Coffee Board of Kenya
CCFAC	Codex Committee of Food Additives and Contaminants
CCP(s)	Critical Control Point(s)
CENICAFÉ	Centro Nacional de Investigaciones de Café (Colombia)
cfu	Colony Forming Units
CI(s)	Collaborating Institution(s)
CIRAD	Centre de coopération Internationale en Recherche Agronomique pour le Développement (France)
CMB	Coffee Marketing Board (Uganda)
CNRA	Centre National de Recherche Agronomique (Côte d'Ivoire)
CORI	Coffee Research Institute (Uganda)
CRF	Coffee Research Foundation (Kenya)
CTO	Chief Technical Officer
db	Dry Basis (when referring to moisture content)
DTU	Danish Technological University
EAFCA	East African Fine Coffees Association
EC	European Commission
ECF	European Coffee Federation
EDABO	Portuguese acronym for DEWOB - Direct Evaporation of Water in Oil Bath

EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (Brazil)
ERH	Equilibrium Relative Humidity
ESNS	Food Quality and Standards Service, FAO (prior to 1st January 2006)
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FAQ	Fair Average Quality (unsorted, hulled green coffee)
FOB	Free On Board
FUNARBE	Fundação Arthur Bernardes (Brazil)
FY	Financial Year
GAP	Good Agricultural Practice
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practice
GPS	Global Positioning System
HACCP	Hazards Analysis and Critical Control Points
HPLC	High Pressure Liquid Chromatography
IACO	InterAfrican Coffee Organization
ICA	International Coffee Agreement
ICO	International Coffee Organization
ICCRI	Indonesian Coffee Cocoa Research Institute
ICT	Information and Communications Technology
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IPSM	Integrated Phytosanitary Management
ISIC	Institute for Scientific Information on Coffee
ISO	International Standards Organization
ITAL	Instituto de Tecnologia de Alimentos (Brazil)
ITIPAT	l'Institut pour la Transformation et l'Industrialisation des Produits Agricoles Tropicaux (Côte d'Ivoire)
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
KCC	Kenya Coffee College
KCTA	Kenya Coffee Traders Association
KEBS	Kenya Bureau of Standards
KEPHIS	Kenya Plant Health Inspectorate Service
KIRDI	Kenya Industrial Research and Development Institute

LAB	Lactic Acid Bacteria
LACQSA	Laboratório de Controle de Qualidade de Segurança Alimentar (Brazil)
LCs	Local Councils
LGA	Local Government Act
LoA	Letter of Agreement
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries (Uganda)
MAPA	Ministério da Agricultura, Pecuária e Abastecimento (Brazil)
m.c.	Moisture Content
MRS	De Man, Rogosa and Sharpe (medium)
MOB	Portuguese acronym for Official Brazilian Method (for measurement of moisture content in coffee)
MoU	Memorandum of Understanding
NAADS	National Agricultural Advisory Services
NARO	National Agricultural Research Organization (Uganda)
NGO(s)	Non Governmental Organization(s)
NMR	Nuclear Magnetic Resonance
NUCAFE	National Union of Coffee Agribusiness and Farm Enterprises (Uganda)
OTA	Ochratoxin A
PEA	Project Executing Agency (FAO)
PFA	Prevention of Food Adulteration Act (India)
PMA	Plan for Modernization of Agriculture
ppb	Parts Per Billion
PROMECAFÉ	Programa Cooperativo Regional para el Desarrollo Tecnológico de la Caficultura en Centroamérica, Panamá, República Dominicana y Jamaica
RDC(s)	Resident District Commissioner(s)
RH	Relative Humidity
s.d.	Standard Deviation
SB	Soft Bean
SB	Supervisory Body (ICO)
SPS	Sanitary and Phytosanitary Measures
TCP	Technical Cooperation Programme (FAO)
TLC	Thin Layer Chromatography
ToT	Training of Trainers'

UCDA	Uganda Coffee Development Authority
UCTF	Uganda Coffee Trade Federation
UFLA	Universidade Federal de Lavras (Brazil)
UFV	Universidade Federal de Viçosa (Brazil)
UN	United Nations
UNBS	Uganda National Bureau of Standards
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
UV	Ultra Violet
wb	Wet Basis (when referring to moisture content), also termed 'as is'
WCC	World Coffee Conference
WTO	World Trade Organization
YES	Yeast Extract Sucrose (medium)

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Project Summary

Project Title:	Enhancement of Coffee Quality through the Prevention of Mould Formation
Project Descriptor:	CFC/ICO/06 and GCP/INT/743/CFC
Project Executing Agency (PEA):	Food Quality and Standards Service, Food and Agriculture Organization (FAO)
Location:	Brazil, Colombia, Côte d'Ivoire, India, Indonesia, Kenya, Uganda, CIRAD (Montpellier), University of Surrey (UK) Ecuador (Project CFC/ICO/25FT)
Starting Date:	13th September 2000 (Disbursement of Authorised Allocation by CFC)
Completion Date:	October 31st 2005 (CFC Funds)[1] October 31st 2005 (Dutch Govt. Funds)[2]
Total Project Cost:	US$6,242,000
CFC Financing (Grant):	US$2,526,000 (under CFC/ICO/02), and US$60,000 (under CFC/ICO/25FT)
Co-financing (Grant):	European coffee industry (ISIC) - US$367,000 Dutch Government - US$1,500,000
Counterpart Contributions:	CIRAD - US$200,000 EMBRAPA (Brazil), Cenicafé (Colombia), CNRA (Côte d'Ivoire), CBI (India), ICCRI (Indonesia), CRF (Kenya), UCDA (Uganda) US$227,000 each (US$1,589,000 total)

[1] CFC funds available for disbursement until 31st May 2006.
[2] Dutch Govt. funds available for disbursement until 31st March 2006.

Part A

Introduction to Project Approaches and Objectives

Sorting fresh cherries, Indonesia

Part A
Introduction to Project Approach and Objectives

1.1 Background

Coffee growing and trade have exceptional importance in the economies of many countries, which are largely dependent upon this commodity for their export earnings, and thus for their social and economic development.

During the late 1990s, several reports on the occurrence of ochratoxin A (OTA) in coffee samples from different origins raised concerns among consumer representatives and national food safety authorities about the health risks to coffee consumers. The reports also indicated that neither roasting nor extraction could completely eliminate the toxin.

These findings led the European Union (EU) authorities in Brussels to consider including coffee among those agricultural commodities for which maximum limits for OTA would be set. Coffee producers, processors and distributors were also concerned about the possible negative consequences of this contamination on the coffee industry and the potentially disruptive impact of maximum OTA limits on international trade. The Codex Committee on Food Additives and Contaminants (CCFAC) emphasised the importance of a risk-based approach to preventing OTA contamination at all stages of the coffee chain through application of good hygiene practices.

An initial response to this problem came from the European Coffee Federation which commissioned a '*Pilot Study on the Prevention of Mould Formation in Coffee*' in 1997. Several coffee producing countries, through the International Coffee Organization (ICO) and the Common Fund for Commodities (CFC), requested the Food and Agriculture Organization of the United Nations' (FAO) assistance in developing and implementing a project to deal comprehensively with the issue of preventing mould growth and OTA contamination in coffee.

The project, '*Enhancement of Coffee Quality through the Prevention of Mould Formation*' became operational in 2000, and activities commenced in 2001. The project, with a total budget of US$6,242,000, was supported by funding from the Common Fund for Commodities (US$2,586,000), the Government of the Netherlands (US$1,500,000) and the Institute for Scientific Information on Coffee (US$367,000), as well as in-kind contributions from project counterpart institutions (totalling US$1,589,000).

FAO was given the responsibility for executing the project and ICO was named as the Supervisory Body. The producing countries directly involved in the project were Brazil, Colombia, Côte d'Ivoire, India, Indonesia, Kenya and Uganda covering all major coffee producing regions. Numerous other coffee-producing countries were also involved in training activities to varying degrees during the course of the project.

1.2 Objectives

The stated overall objective of this project was:

> '...to enhance the quality of coffee thereby impacting positively on the earnings of producers; and to improve production volumes of good quality coffee in producing nations. The improved quality would minimise potential health risk to consumers.'

The principle focus of the project was to prevent disruption in the coffee trade through effective management of OTA contamination of coffee. The main elements of an overall strategy for managing risks of OTA contamination include:

- Improving practices in all aspects of production and handling, based on a sound understanding of where the most important problems lie, so as to minimise contamination;
- Establishing and enforcing appropriate regulations at national and international levels;
- Monitoring of OTA contamination in coffee.

The project therefore sought to enable coffee-producing countries to develop and implement national programmes for the prevention/reduction of mould contamination in coffee that cover the above-listed elements. The specific objectives of the project were:

- Increasing awareness of the need to prevent mould contamination in coffee among decision-makers within the coffee sector;
- Achieving a better understanding of the mechanisms of mould formation and OTA production in green coffee and of the factors affecting them;
- Developing the necessary tools to support effective management of food safety hazards in coffee;
- Building the capacity of operators at all stages of the coffee chain to implement good practices;
- Enabling policy-makers in the producing countries to participate effectively in international deliberations on food safety measures relevant to the coffee sector;
- Strengthening the ability of the main coffee institutions in the producing countries to provide technical support on food hygiene issues related to all aspects of the coffee sector.

1.3 Approach

Achieving the project's objectives required a range of activities that were well coordinated, given the interlinkages among several of them, and also carefully timed in relation to the coffee seasons in each of the participating countries.

Activities were planned and implemented with the full participation of the collaborating national institutions who were responsible for the day-to-day supervision and management of project work at national level.

Situation assessment: The starting phase of the project focused on assessment of coffee production and handling practices in the seven core participating countries so as to inform the planning of training to be provided under the project on hygiene in the coffee chain, and to support planning of field trials and surveys such that they addressed the most important questions related to OTA in coffee.

Within each country, broad stakeholder participation from the public and private sectors was encouraged to facilitate collaboration and consensus among all key partners in implementing national programmes for OTA prevention and quality improvement in coffee.

Field trials: Field trials were designed to investigate the effect of selected processing and production factors on growth of OTA-producers and accumulation of OTA. Many of the trials aimed at developing guidance for improving existing practices in coffee-growing regions. In other cases, the trials examined the feasibility of transferring technologies from one region to another.

Training in good hygiene practices along the coffee chain: Training of Trainers' (ToT) courses on food hygiene principles and their application to coffee handling were the key element of the project strategy for reducing mould contamination by improving handling practices by all operators along the chain. The groups of trainers thus formed at national level were responsible for the development of training and communication programmes to reach all stakeholders. The project also provided guidance and financial support to initiating implementation of national programmes.

Guidelines for reducing mould contamination: An important activity within the project was the drafting of '*Guidelines for the Prevention of Mould Formation in Coffee*' (see Part D). Findings from the field assessments and field trials informed the drafting of these guidelines. They are seen as an important tool for promoting Good Hygiene Practices in all coffee producing countries.

Capacity building at collaborating coffee institutes: A range of activities for building the capacities of collaborating institutions to effectively handle food safety and hygiene issues affecting the coffee sector. These included:

- Direct training on a number of food hygiene issues relevant to the coffee sector;
- Support for strengthening capacities to develop and implement successful national training and communication programmes on coffee safety and quality;
- Training in OTA analysis and provision of equipment for carrying out this work;
- Training in mycological analysis and support for improving facilities for this work.

Socio-economic studies/market chain surveys: The project recognised the importance of ensuring the proposed improvements to practice or to technologies used are feasible in the context in which they are expected to be applied. This required the conduct of targeted studies to assess the feasibility of programmes or policies under consideration by the collaborating institutions to promote mould prevention and quality improvement.

1.4 Explanation of Report Structure

Part C of this report, immediately following the Executive Summary, discusses the field trials that were conducted under the project. This part of the report is sub-divided into twelve Sections. Each of the first eleven of these Sections deal with a group of related experimental trials. The first page or two of each of these Sections explains the reasons for which the trials were conducted and then summarises the main findings and relevance of these. For most readers this will be a sufficient overview of the field trials. The remainder of each of the sections provides a more detailed technical discussion of the trial results.

Section 12 outlines the overall conclusions of all of the trials and indicates areas where further work could contribute to a clearer understanding of OTA accumulation in coffee.

Where it was considered useful, further information related to the trials is provided in the Annexes, all of which are available on the CD-Rom included with this report.

Part D of the report contains the '*Guidelines for the Prevention of Mould Formation in Coffee*' which have been informed by the findings of the experimental work discussed in Part C.

Part E presents an overview of the socio-economic studies and market chain surveys conducted during the project, highlighting their relevance to the process of planning national programmes for mould prevention and coffee quality improvement. Full reports of the studies and surveys that were commissioned under the project and discussed in this part of the report are also provided as Annexes on the enclosed CD-Rom.

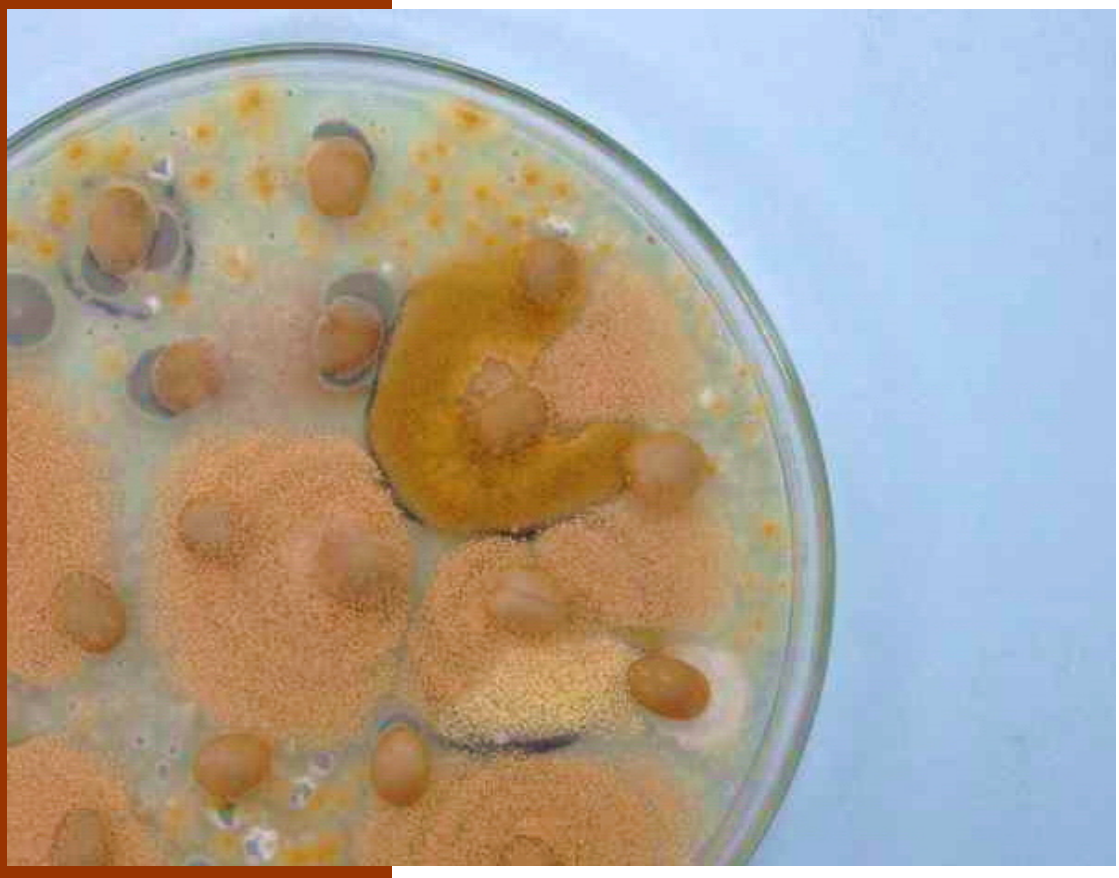
Part F reports on the capacity building and training activities carried out under the project covering food hygiene, OTA analysis and mycological analysis.

Part G shows how the training and capacity building discussed in Part F, and the studies and surveys outlined in Part E, should be utilised by the concerned national institutions to achieve improved management of hygiene and quality in the coffee chain. It also outlines the main findings of reviews of national systems of coffee quality and safety control that were conducted in three of the participating countries providing recommendations for improving these systems.

Part H states the overall project conclusions and recommendations.

In addition to this Final Technical report, the Project Executing Agency has also prepared a Final Management report. A copy of this report is also included on the enclosed CD-Rom.

Part B Executive Summary



Colonies of *Aspergillus ochraceus* and other *Aspergillus* spp. cultured in a petri dish

Part B
Executive Summary

1.1 Background

Coffee production has exceptional importance in the economies of many sub-tropical countries, some of which are dependent upon trade in this commodity for their export earnings. Over 90% of global coffee production takes place in the South, and it is estimated to directly involve over 25 million families worldwide.

In the late 1990s reports emerged concerning the occurrence of a mycotoxin, ochratoxin A (OTA), in coffee from various origins. This raised concern amongst consumer representatives and national food safety authorities over the potential health implications of drinking coffee, and among coffee-producers, processors and distributors over the possible negative consequences of OTA contamination in coffee on trade.

This situation led a number of coffee-producing countries to request the Food and Agriculture Organization of the United Nations' (FAO) assistance in developing and implementing a 'global' project to address prevention of mould growth and OTA contamination in coffee.

The resulting US$6.2m global project ('*Enhancement of Coffee Quality through the Prevention of Mould Formation*'), supervised by the International Coffee Organization (ICO) and funded by the Common Fund for Commodities (CFC), the Government of the Netherlands and the coffee industry, was implemented between 2000 and 2005.

The project focused on building the capacity of coffee producing countries to develop and implement national programmes for the prevention/reduction of mould contamination in coffee through field investigations and training in relevant disciplines. Seven major coffee-producing countries were directly involved in the project: Brazil, Colombia, Côte d'Ivoire, India, Indonesia, Kenya and Uganda, covering all major coffee-producing regions, and commercially traded varieties.

1.2 Field Trials

The basic requirements for OTA contamination of coffee are known and were known before the start of this project: there must be an active population of OTA-producers and adequate time at a water activity (A_w) level that permits the accumulation of OTA.

The aim of the field trials carried out under the project was to better characterise the conditions that lead to OTA contamination of coffee so that acceptable process controls could be more clearly defined and points of greatest risk along the coffee chain identified.

These trials contributed both to an improved understanding of mould and OTA contamination of coffee, and were also an essential input to developing science-based

recommendations on measures for improving coffee hygiene that are commensurate with the food safety risks involved through all stages of coffee production, handling and processing. Conclusions from the field trials are summarised in Part C, Section 12 of this report, and are not further synthesised in this Executive Summary.

However, it should be noted that some issues require further attention to improve risk-based approaches to preventing OTA contamination of coffee. National bodies or, where possible, collaborative action by groups of producer countries, need to prioritise and act to tackle the outstanding issues in continuing the process that the project initiated.

Findings from field trials under the project point to the following two issues as priority areas for investigation:

- Improving our understanding of the accumulation of OTA in coffee beans during primary production, and
- The association of certain coffee bean defects with OTA contamination.

1.2.1 OTA contamination of coffee during primary production

No correlation of OTA-producing fungi or general mycological load to any horticultural practice could be discerned by surveys undertaken under the project. However, given the finding that OTA contamination in the field is much more common than previously thought, it could be important to design further surveys targeting this aspect.

Aspergillus ochraceus is not uniformly distributed throughout coffee production areas, and there is evidence of greater activity in certain regions. However, there must be some uncertainty about apparent regional distribution patterns, not least because of the question of stability of such patterns from season to season.

Experiments carried out under the project showed that exposure of coffee flowers to spores of *A. ochraceus*, leads to bean infection. Even though this does not amount to proof that the observed field infection of coffee seeds is established via this route, the hypothesis merits further consideration.

Niger group aspergilli are commonly found in stem tissue, fresh beans and fruit. Infection by this group is almost universal in samples of robusta coffee, whether wet- or dry-processed and commonly reaches 100% infection during drying. Most niger group aspergilli in robusta coffee are *A. niger*, *sensu strictu*, and very few isolates of this species produce OTA. It may be that the ubiquity of this organism overcomes the rareness of its ability to produce OTA giving it a more important role in OTA contamination of coffee than previously thought. This needs to be better understood.

Other findings from the mycological surveys that could be of relevance to the question of OTA accumulation in coffee pre- and post harvest are:

- The finding of high levels of infection of *A. carbonarius*, a strong OTA-producing species of the niger group, in a few samples in certain regions. A systematic and rigorous survey to better understand the distribution of this species could be useful.

- The observation by some project collaborators that much of the ochre aspergilli that was isolated from beans proved to be the non-toxigenic species *A. melleus*, normally considered to be a fairly strict soil organism. Competition between the non-toxigenic *A. melleus* and the toxigenic *A. ochraceus* could influence OTA accumulation.

1.2.2 Defects and OTA contamination

Surveys carried out under the project documented instances where most of the OTA in the lot could be attributed to certain defect categories. This association, however, seems to be strictly related to the existence of certain conditions during processing of the coffee.

More work on this is urgently required as there are important implications for risk management measures at national and international levels. A combination of upstream surveys and investigation of OTA development in specific defects under defined situations is required to inform rational decisions about managing food safety risks associated with coffee defects.

1.3 Guidelines for the Prevention of Mould Contamination

The project developed '*Guidelines for the Prevention of Mould Formation in Coffee*' based on assessments of the coffee chain in several producing countries, expert opinion of associated risks of mould growth and mycotoxin contamination at the various stages of the chain as well as on the findings from experimental trials noted above. The guidelines are detailed in Part D of this report.

The guidelines are not intended for the direct use by every stakeholder, rather they aim to provide national authorities with concrete guidance for developing national guidelines or codes of practice specifically tuned to their respective sector given the diversity of practice in each producing country.

These guidelines, and any national guidelines or codes of practice that derive from them, will form the basis of national programmes for the reduction of OTA contamination in coffee. Implementation of national guidelines that promote modern systems of food safety management as opposed to simply informing on good and bad practice will require effective, carefully designed, and well thought out programmes of training by technical support institutions.

1.4 Training in Good Hygiene Practices along the Coffee Chain

The situation at the start of the project was a general lack of awareness of about food hygiene among professionals in the main technical institutes supporting the coffee sector. In most countries the coffee sector has evolved quite separately from the rest of the food sector and the coffee sector institutions were largely uninformed about the handling of food safety issues at national and international levels.

Training of Trainers' (ToT) courses on food hygiene principles and their application to coffee handling were a key element of the project strategy for reducing mould contamination by improving handling practices by all operators along the chain. Training involved over thirty coffee-producing countries, covering a total of over 90% of global coffee exports. Formal and informal feedback at the end of the Training of Trainers' (ToT) courses confirmed that the participants learned a lot from the courses which was directly useful in the execution of their duties.

Project countries have all reported on follow-up training activities. The nature of the follow-up activities depended very much on the existing mechanisms for training and information dissemination at each of the collaborating centres. Project funding was used in the printing of brochures and posters, targeting mainly small holder farmers, to convey simple messages about recommended improvements. However, and importantly, the absence of price incentives in much of the mainstream coffee market hinders uptake of good practices.

More elaborated guidance to several key stakeholders is still required. Support in the design of suitable quality and safety assurance programmes, and training to small-scale operators to apply them, should be provided by relevant institutions. This is not a simple job, but it is the next step that should be taken within coffee-producing countries.

1.5 CD-Rom Based Coffee Hygiene Resource Package

A CD-Rom based coffee hygiene resource tool was developed under the project to assist coffee institutes develop appropriate hygiene programmes. The tri-lingual CD-Rom (English, French and Spanish) has been widely distributed to concerned institutions in all coffee-producing countries and is also available from the project website (www.coffee-ota.org).

The guidance that it provides on training programme development will help training institutes take adequate consideration of factors influencing the 'coffee system' in planning and delivering training.

The CD-Rom will also guide trainers to redefine training objectives and training course content in relation to the new skills and approaches required for modern food safety and quality management in the sector.

1.6 Capacity for Mycological Analysis

At the start of the project, collaborating countries had varying technical capacity in the area of mycological analysis. This ranged from no research experience and no laboratory facilities, to scattered university and governmental facilities through to equipped and experienced, publicly funded, coffee research institutes working in the area of coffee mycology.

The project successfully upgraded the capacity of collaborating institutes to carry out the mycological work essential for completing the field activities. This capacity building included formal training, informal one-on-one training by the international

mycological consultant, as well as financial support for modifications to working areas and provision of materials and equipment.

This enhanced capacity will ensure strengthened scientific support to coffee sectors. A handbook of mycological methods and checklists of materials and equipment required for mycological work are available to other countries interested in strengthening their mycological capacity as a means of delivering concrete guidance on quality assurance and hygiene controls to their coffee sectors.

1.7 Capacity for OTA analysis

The project focussed considerable attention and resources on building capacity at project centres for OTA analysis in coffee. Capacity building activities included provision of equipment and materials, regional and national training courses, study tours to well established laboratories working in OTA analysis, and participation of all collaborators in a series of proficiency testing rounds.

OTA analysis laboratories at all collaborating institutes are now functional utilising official methods of OTA analysis based on TLC and HPLC techniques. Proficiency testing under the project has demonstrated a growing competence among the participating laboratories.

The project emphasised the need for a system of laboratory management that allows accurate results to be reliably obtained and that promotes international acceptance of analytical results. OTA analysis data from monitoring programmes provide essential feedback on the efficacy of prevention measures, and will play a crucial role in deliberations on the need for an International Code of Practice for prevention of OTA contamination, and any future decisions on OTA limits in green coffee.

A model manual on quality assurance for OTA analysis of coffee was developed for the project by LACQSA, of the Ministry of Agriculture, Livestock and Supply in Brazil. The manual is available for use by laboratories in any coffee-producing country interested in improving their analytical services.

Finally, in order to ensure the sustainable impact of capacity building in OTA analysis budgetary provision must be made at the national level for maintenance of equipment and replacement of laboratory consumables. National laboratory staff must regularly undertake OTA analyses to maintain their expertise.

1.8 Participation in International Food Safety Decisions Concerning Coffee

The project has made key stakeholders in the coffee sector aware of the World Trade Organization (WTO) and its role in enforcing rules of international trade. Equally, project counterparts and collaborators are now aware of the importance of Codex Alimentarius texts, and on how to contribute to the formulation of national positions on Codex issues that are relevant to the coffee sector.

Ongoing discussions within Codex to decide upon the need for an international Code of Practice for the prevention of OTA contamination in coffee present an important

opportunity for coffee producing countries to influence the rules that will govern the sector.

ICO obtained observer status in Codex on 20th January 2006 (as a direct result of the awareness-raising activities carried out under this project) and is an ideal forum for developing common positions on issues affecting the coffee sector.

It is important that policy-makers in all coffee-producing countries follow developments on maximum limits being considered for contaminants and residues in coffee by WTO members, in particular the pending decision of the EU on maximum OTA limits for green coffee.

1.9 Improving the Regulatory and Policy Frameworks for Control of Coffee Quality and Safety

Market liberalization, which took place in many coffee-producing in the early 1990s, brought about a profound change in the running of the coffee sector. Many in the coffee sector view with great suspicion any form of control which is considered as being antithetical to principles of market liberalization.

This project has played an important role in ensuring that decision-makers within the coffee sector understand that food safety regulation is not a departure from free market principles but rather a necessary complement to free trade if public health is to be protected.

The project has emphasised non-regulatory measures to promote Good Hygiene Practices, but some investigations undertaken during the project have shown the need for clear regulations and the means for their enforcement. Reviews of national systems for the control of coffee quality and safety under the project revealed many weaknesses in the institutional and legal frameworks that underpin such control. These will need to be addressed, with broad stakeholder input, if national authorities intend to improve and enforce relevant regulations.

In several countries policies and programmes are under consideration or have been recently adopted to support improvement of various aspects of the performance of the national coffee sector, including coffee quality and safety improvement. The project has emphasised the need for better information on the functioning of the sector to support policy and programme development. Market chain surveys carried out under the project provide such information which should be reviewed by collaborating institutes to improve the focus of their technical support to the sector.

Targeted studies were carried out in some of the project countries to assess the feasibility of proposed 'mould prevention and quality improvement' programmes and specific recommendations made to the national authorities in the reports of these studies. The studies show that there are many common issues to be considered by coffee authorities in different countries for example, work in both Uganda and Indonesia points to the importance of well-functioning farmers' groups in achieving sustainable programmes. Guidance from the feasibility studies should be of general interest among producing countries.

Part C

Review of Research Activities

Drying trial, Côte d'Ivoire

Section 1
Distribution of Fungi in Coffee Production Systems

1.1 Introduction

Information on the distribution of fungi in coffee production systems grew very naturally out of a need to better understand what farmers did and where an ochratoxin A (OTA) risk comes from as 'natural occurrence of toxigenic fungi' and 'farm conditions' interact.

It is known that a small minority of coffee lots (perhaps between 5-10% of commercial samples, though this is by no means a definitive figure) have levels of OTA contamination that cause concern. This begs the question of whether this reflects:

- The distribution pattern of some fairly rare OTA-producing species, or
- The distribution of certain 'poor' practices, or
- Some interaction of two essential preconditions, viz. the presence of a toxigen and conditions for its development.

In other words, it is important to understand both the occurrence and the non-occurrence of OTA in coffee.

If a particular practice strongly favoured the development of a particular fungus, one might, quite reasonably, expect the distribution of this fungus to correspond to the practice. To document this, one would have to be in a position to sample the preferred niche (or niches) accurately and efficiently. Distribution within the ecosystem must be known; if you are surveying rabbits, you would not sample treetops, but you would sample treetops if you were studying squirrels.

At the earliest stages of the project a concerted effort was initiated to gather detailed information on coffee production. The output of the surveys was a combination of i) detailed information on individual farms from which a regional picture of what farmers did and why emerged, and ii) samples from the farms, so that the two could be linked. The samples were of two kinds: coffee itself and coffee ecosystem samples such as soil, air, and swabs from processing equipment.

Once the niche is known where a species is found (if it is present at all), it is sampled according to a pattern chosen to represent the study area of interest. The occurrence is either positive (which gives a quantitative measure of presence), or negative (not found). Negative results require two qualifiers: the detection limit (or the level at which the method applied is capable of reporting a species), and that the sampling conducted represents the study area fairly. The practical issues relating to the realisation of such a study are discussed in more detail below.

To make comparisons between regions, it is imperative that the scope of sampling and analysis programmes match. In other words, that the sampling completed is equally representative and extensive, and that the conventions of analysis are also comparable. Because of the possible political and financial impact of a contention

that coffee from some particular place is more at risk of OTA contamination, such a contention must be based on only the best and most extensive factual basis.

Under this project, considering the uneven capacities and degrees of success in implementing a demanding field programme between the different collaborating groups, it must be noted that the distribution data obtained is not fully comparable between collaborators. The data therefore must be interpreted conservatively because of the ease with which analytical and/or sampling artefacts[1] can emerge, many of which are quite hidden, especially to the non-specialist.

1.2 Findings and Application

1.2.1 Distribution of OTA-producing fungi

The results fall into three related areas: geographical distribution, local distribution, and niche preference. Each of these is dealt with in turn below:

A. Geographical distribution:

On average there is a field infection rate by *Aspergillus ochraceus* of about 0.1 or 0.2% of beans. These field infections can be active in terms of OTA production, and the beans of freshly harvested, oven-dried cherries commonly contain trace levels of OTA, and contamination levels of up to 20ppb have been recorded. It does not follow that a higher OTA level is the result of a higher infection frequency, rather that the fungus is more active where it has become established in the seed.

Occurrence at frequencies of this magnitude makes mapping a distribution problematic since viable counting methods can, at best, produce a detection limit only approaching these levels. The methods employed here had a detection limit of 1 to 1.4% so a group of negative samples could represent actual absence or presence below detection. By considering the results from the regional sampling programmes collectively, one can infer that A. ochraceus is widely distributed but not necessarily uniformly so. Locally samples have been reported with up to about 15% infection rates of this fungus.

There is evidence that one of the study regions under the project supports a particularly well-distributed, active population of *A. ochraceus*, and another study region where this species is very difficult to isolate. However, given the lack of certainty of data of this nature for the reasons discussed below, the apparent patterns need to be further investigated to determine whether they are indeed real and the extent to which any pattern might be expected to be stable over time. Therefore, conclusions cannot be drawn at this point.

The differences in the overall fungal communities associated with coffee plants from different continents are similar in extent to that of the difference between samples of different regions of the same country. Quantitatively there is considerable variation between samples, but examples of high and low microbial load are to be found in all regions.

[1] An artefact is an anomalous result caused by a property, or the poor application, of a method.

East African project collaborators observed that much of the ochre aspergilli that was isolated from beans proved to be the non-toxigenic species *A. melleus*, normally considered to be a fairly strict soil organism.

Only a very few fungal species are to be found in almost *any* set of fresh (and the related) dry coffee: *Candida edax, Fusarium stilboides*, and, specifically in robusta, *Aspergillus niger* complex.

Several additional species are to be found in all regions: *Cryptococcus albibus*, *Aureobasidium pullulans*, *Penicillium brevicompactum*, *Cladosporium* spp., and in arabica, *A. niger* complex.

Other fungi are associated with specific stages of coffee production: *Kloekera apiculata* with fermentation, and *Wallemia sebi*, *Eurotium repens* and other species of *Eurotium* with the stored product.

B. Local distribution:

The patch size of the distribution of the less common fungi, such as the ochre aspergilli, is apparently small, being less than 50m diameter. However, the analysis is invariably near to the detection limit, limiting the confidence in these studies.

There is a lack of correlation between the presence of ochre aspergilli in one niche and the others, except that, unlike niger aspergilli, usually it is isolated in more than one soil sample from a farm, if it is isolated at all.

Some species, particularly *A. niger* in robusta coffee, *Candida edax* and *F. stilboides* are qualitatively universal. Quantitatively they show local differences within farms or blocks of farms at a scale similar to the 50m diameter alluded to above. It may be this is characteristic of the type of distribution of plant-associated fungi within the plant host range.

There is a degree of season-to-season persistence of the ochre aspergilli, but absolute confidence in the outcome of sampling between years is not justified.

No correlation of OTA-producing fungi or general mycological load to any horticultural or processing practice could be discerned from the survey data. It should be emphasised that this does not mean that correlations do not exist but that they could not be identified given the design of the survey.

Given the finding that infection in the field is much more commonplace than previously thought, it could be important to reconsider the investigation of possible correlations between selected production factors and contamination with OTA-producers or OTA.

Sometimes intra-regional sampling would indicate that a particular fungus, not isolated in sampling from other regions, was common and therefore apparently characteristic of that region. For example, *A. japonicus* was frequently isolated in samples around Air Kubang, Southern Sumatra but not in other districts, nor in Java. It is not clear whether this kind of trend is a seasonal quirk, or consistently related to some regional factor.

C. Niche preference:

There is no correlation in the relative numbers of yeasts and fungi across the identifiable parts of the fruit: external ('x') community, mesocarp ('m') community, and bean internal ('i') community. NB - the external community does not predict the internal (bean) one.

In coffee production systems, *A. ochraceus* is most commonly found on and in coffee cherry and beans, and in coffee rhizosphere soil. It has not been isolated, unlike *A. niger*, *sensu stricto*, from coffee stem tissue, and is uncommon in air sampling of orchards during harvest period. Air sampling was not carried out in other seasons so no conclusions can be drawn about the effect of the season on the microflora present in the orchard air. Reflecting it's relatively high frequency in dry and drying coffee, *A. ochraceus* is often recovered in air sampling in storage, curing and drying facilities.

Yeasts and yeast-like species dominate the fruit surfaces and the mesocarp niche, though *Cladosporium* can be important on surfaces and *Cladosporium, Fusarium stilboides* and *Penicillium brevicompactum* can be important in the mesocarp niche.

The coffee fruit associated fungal species show preference to one or two of the identified niches (i, x, and m), but have been isolated from all parts of the fruit. Through processing, as new niches are generated, rare or absent (i.e. below detection limit) species arise and displace some of the fresh fruit species.

The coffee rhizosphere community is distinct from local non-coffee soils, and is more likely to contain ochre aspergilli.

1.2.2 Farm audit and monitoring

From the experience of the surveys conducted under the project a protocol for conducting farm audits can be recommended. To evaluate a site for the presence of OTA-producers, samples from soil, air and coffee should be taken[2]:

- Remove soil from around the fibrous coffee roots that you will find just below the humus layer (~3cm) from within about 10cm from the stem at five trees scattered around about 1ha. Mix, dilute to 10^{-3} and plate out in replicate: $2x10^{-1}$; $5x\ 10^{-2}$ and $3x10^{-3}$;
- Deploy air exposure plates around the drying yard;
- Analyse 100 fresh beans from 100 cherries taken from a composite kilo, sampled as for the soil;
- If available, do the same for dried cherry, parchment or green coffee, depending on what is available. This sample should be stable and can be retained for analysis if necessary, depending on the result of the other analyses. Always check this coffee for moisture (or A_w) to ensure the sample is dry enough for stability.

In order to establish a monitoring programme, the objective must first be clearly enunciated. For example, the objective may merely be information gathering to inform policy development. If this is the case, the power of the sampling plan must

[2] Further information on these methodologies can be found in the project 'Handbook of Mycological Methods', available from www.coffee-ota.org, and included in the enclosed CD-Rom as Annex F.2.

be evaluated so that the data derived from it is not represented as meaning more than it does.

A more specific objective, such as early warning of OTA from a locale known to have had an OTA problem, would require periodic sweeps of sampling applied uniformly across the target region. In either case, more work is required on the consistency with which the same return from a sample site may be expected. This can be assessed with repeated sampling from a selection of sites representing the two possible cases: 'ochre aspergilli free' and 'ochre aspergilli present'.

1.2.3 Further research

Outside of the auspices of an international project, it would be useful to implement regional studies to answer the fundamental question of whether locales differ in occurrence of OTA-producers due to inherent or random factors, or whether it can be traced to some specific processing or horticultural practices.

Further work, using carefully prescribed sampling and analysis procedures, to examine the distribution of *Aspergillus carbonarius* would be very useful - the observed abrupt occurrence of *Aspergillus carbonarius* at a high frequency, but in only very few samples, continues to be a puzzle.

Additional work in East Africa on the occurrence and role of the non-toxigenic *A. melleus* and how it interacts with *A. ochraceus* could provide the means for a competitive exclusion technology to act against the toxigen.

1.3 Additional Notes

There is a degree of similarity between coffee production systems around the world enforced by the cultural requirements of the two commercial species of *Coffea*. Both are tropical hardwood forest under-story shrubs or small trees but *Coffea arabica* (arabica coffee) is a highland species, and *Coffea canephora* (robusta coffee) can thrive at low elevations. Both require 1200mm of annual rainfall or more and both are adapted to a dry season/wet season pattern.

These generalities, based on conditions where the respective species evolved, has become far more complicated as man has spread the plants around the sub-tropical and tropical world and tailored cultivation methods to economic necessities. Nevertheless, the primary factor in determining what fungi occur in these agri-ecosystems is the plant itself, and we see remarkable similarities between regions, given the same coffee species.

Drawing conclusions about distribution of micro organisms is fraught with difficulties. Among the practical problems are those associated with the method of analysis used. During the course of the project, viable counting was the only plausible approach for locating and identifying fungi. The lack of any selective media for fungi means that sample size is restricted by the density of the 'background' community. Therefore, the sample must be diluted extensively to make recruitment possible, while at the same time diluting out the less common species, thus making

detection limits high for the less common species. Viable counting methods are somewhat imprecise and high dilution rates make them more so.

Another problem in interpreting data from viable counting of filamentous fungi is that colonies are counted and the nature of the propagules that generated the colony is not known. In dilution plating it may be a piece of vegetative hyphae, a spore or a clump of spores. With direct plating of surface-sterilized beans, a tiny or large degree of infection produces the same result - one colony. This means the method is insensitive to the difference between frequency and biomass. Early attempts at using emergence rate as a measure of biomass at the time of analysis were only partially successful.

Lastly, identification of hyphomycetes is difficult and requires a lot of time, experience and expertise. Unlike yeast and bacterial taxonomy, based in large measure on objective chemical tests, fungi are identified based primarily on morphological criteria. Only through experience are the nuances of this sort of observation conquered.

Adding to the confusion, there is often a lack of consensus among specialists - as is the case with the genus *Cladosporium* or in parts of the niger group in the genus *Aspergillus*. Often the accepted system needs revision, as with the ochre group, also in *Aspergillus*. A great deal of effort was invested in the early stages of the study to be as sure as possible of the identification of the common fungi associated with coffee, and some of the findings have led to a revision of the taxa involved as well as additions of new species to the literature.

Few of the workers in the project were specialists so conventions of naming were adopted. 'Niger aspergilli' and 'ochre aspergilli' are the terms used for the black and yellow aspergilli respectively, in recognition of the fact that attempts to report species would lead to misrepresentation of the facts. *A. carbonarius* can be identified with confidence by the tutored non-specialist as long as a compound microscope is used, but this extra effort was only sporadically made by the project counterparts and this species is identified only erratically in the work.

Turning to the theoretically based issues of distribution studies, a major problem is that the distribution of organisms is not uniform, whether the organism is sessile or motile. The pattern established can be described as 'patchy', where the density is relatively high over one area and low or zero over other areas. The size of patches, distribution patterns within such patches, their regularity and the degree to which the distribution is affected by other factors (such as soil type, presence of a particular plant etc.) are largely unknown for the fungi.

To draw a distribution map, non-presence is equally as important as presence. Leaving aside the possibility of laboratory contamination, presence as a qualitative fact is solid. Non-presence relies on good sampling, efficient recruitment onto growth medium and the detection limit. If the appropriate niche is sampled and the media well established non-presence means presence below some threshold and when the population density equals or approaches this threshold, occurrence will be random since every sample will contain the organism but only some will actually get plated out. Though a rare organism may be present below detection limit and therefore the analysis is technically erroneous, it ***may*** be effectively absent because it isn't

numerous enough to express the problem associated with it. On the other hand it is important to note that certain conditions could favour survival of 'rare' organisms and suppress previously dominant communities.

The persistence of the presence of a micro organism at a location is another question. Efforts were made to re-sample but these were too irregular to be of much value in answering the question well. 'Typical' species are, of course, persistent and rarer species likewise seem to be persistent within the uncertainty of local sampling.

The uncertainty of this conclusion is distributed between the presence/absence and the distribution pattern of rare organisms. Ochre aspergilli were often isolated from the same farm on consecutive years, but a negative result could be due to inadequate sampling of a rare species as much as its absence.

1.4 Experimental Design

Details of the mycological methods applied for the enumeration and isolation of fungi from coffee production systems can be found in the project's '*Mycological Handbook*', a document developed specifically for mycological investigations in coffee.[3]

Early work established that not every lot of coffee had the biological potential to produce OTA, no matter what abuse it may be exposed to.

A two-step programme of extensive farm surveys, a combination of farmer interview and microbiological audit, followed by intensive sampling on selected farms found to contain fungi of interest was undertaken. The survey was guided by a questionnaire which was customised in consultation with local coffee experts, and based on a generic questionnaire previously developed in the pilot project. This questionnaire is included as Annex C.1 as an Excel file 'Survey questionnaire.xls' on the enclosed CD-Rom.

The various project collaborators carried out the survey to varying degrees of success, according to capacity and, in some cases, security related issues[4]. The first (extensive) farm survey provided the data for conclusions relating to geographical, regional and niche distribution.

These surveys, in most cases, could not be conducted using an optimised sampling pattern as one might, for example, impose in a survey of a forest. First, the distribution of farms is irregular, so a superimposed geometrical pattern would not necessarily correspond to coffee farms. Second, extensionists are used to working within local geo-political boundaries (viz. village, district, sub-district, etc.). Finally, it is often necessary to arrange appointments with farmers, and this is easier to do with farmers known to the extension service, which often means areas within the study area not being sufficiently represented in the sampling.

[3] This can be downloaded from www.coffee-ota.org, and is also included on the enclosed CD-Rom as Annex F.2.

[4] The socio-political unrest in Côte d'Ivoire that began in September 2002, and continued at varying levels of intensity through to the end of the project in 2005, restricted access by project staff to some coffee growing areas in the country. Similarly, some coffee growing areas of Colombia could not be included in the survey work due to the security risk to national project staff.

The (intensive) follow-up survey provided data relating to small-scale distribution as well as niche preference. Sampling was conducted within a single block on large farms, on single farms, or, in regions of very small farms, on two or three adjacent farms. The sampling points were taken in an estimated square form with sampling stations roughly 50m apart.

Conclusions on niche preference are based on specific types of sampling across different sites though the intensity of sampling varies depending on the importance ascribed to the niche. The fruit niches of external, internal and mesocarp have been extensively sampled, with samples running into the thousands. Hundreds of soil and air samples have also been analysed, but surfaces of processing facilities, internal stem fungi and insects have been sampled less thoroughly. Nevertheless, the total sampling of all of these niches under the project has been considerable.

1.5 Experimental Results and Discussion

A collation of the relative distribution of two important species of ochre aspergilli, *A. ochraceus* and *A. melleus*, found in coffee is presented in Table 1.1. The significance of this is that the latter species is not an OTA producer but is indistinguishable from *A. ochraceus*, which is the major OTA producer in coffee. A proportion of isolates described on the enumeration plates as 'ochre aspergilli' are removed for further taxonomic work as a matter of routine to assess the actual taxonomic status of the samples. Previous to these findings, *A. melleus* had been taken as largely a soil organism, but we see in East Africa that it is commonly isolated from coffee.

Table 1.1: Proportion of ochre aspergilli that proved to be either *A. ochraceus* or *A. melleus* when diagnosed from different origins. The niches of origin are also reported.

i = internal (bean); x= external (fruit); m = mesocarp; y = external (green coffee); n = processing by-product; s = soil; a = air

	A. ochraceus / *A. melleus*	Niche		
		i	x, y, m, n	s, a
K + U	5 / 14	16	1	2
B	22 / 0	14	2	6
I	10 / 4	11	1	2

Although it was not an objective of the study to systematically characterise the fungal communities in coffee production systems, a great deal of careful taxonomic work was completed as of necessity.

All records reported here have been identified by Dr. Jens Frisvad of the Danish Technological University (DTU), Lyngby, Denmark or Dr. J. M. Frank, mycological consultant to the project. The list of species that has been found on or in coffee is very long (over 650 records), and can be found in the Excel file 'Geographical Distribution of Fungi in Coffee.xls' on the enclosed CD-Rom as Annex C.2.

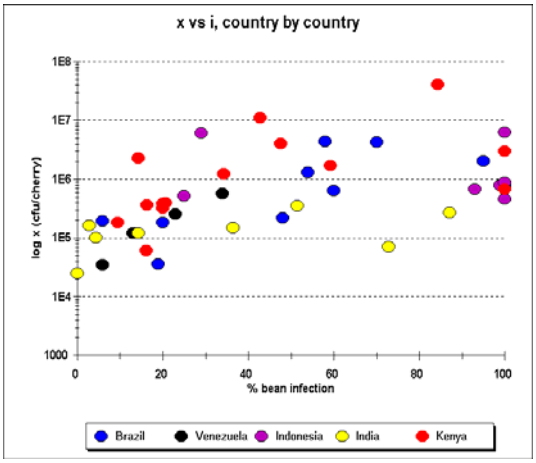
In addition, metabolite studies were conducted at the DTU on many ochre and niger isolates using YES medium (yeast extract sucrose) and high performance liquid chromatography (HPLC) detected with ultraviolet (UV) diode array spectroscopy. These results are provided for reference as Annex C.3 on the enclosed CD-Rom (as

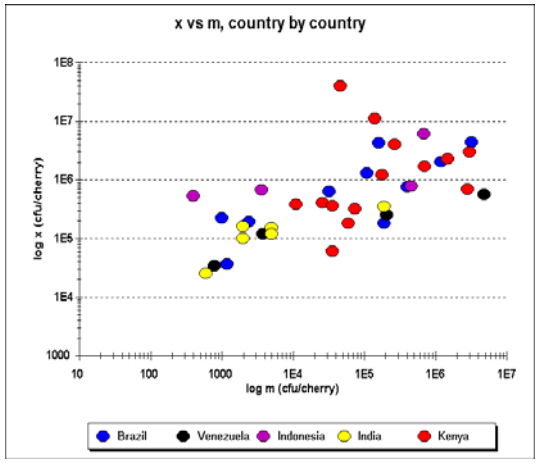
file 'Ochre niger Metabolites.xls'), and to document the distribution of the chemo-types. No pattern at this sub-specific level has emerged but this information has clarified the secondary metabolite production patterns of several of these taxa and thereby clarified their taxonomic position. Some of the other (non-OTA family) products are also biologically active in mammalian systems.

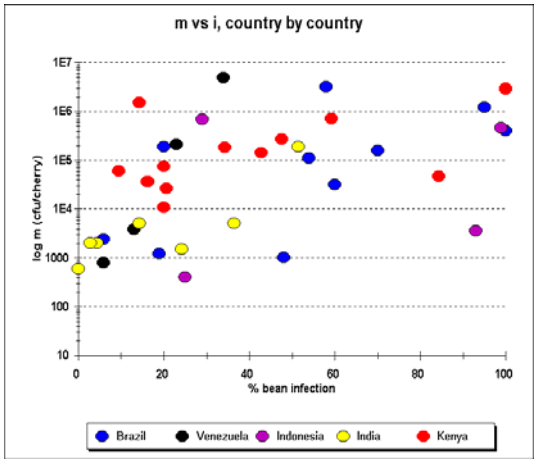
An extensive evaluation of *Penicillium brevicompactum* isolates (common in coffee) has confirmed that this relative (both are slow-growing terverticillate species in the old section *Asymetrica*) of *P. verrucosum*, which is a strong OTA-producer, does not produce OTA.

There is no overall relation in the mycological load between the three fruit niches as the graphical collation in Figures 1.1 to 1.3 shows. Looking at the data, country by country, a semblance of a relationship emerges, especially at lower bean infection rates except in the 'm' vs. 'i' comparison. That there isn't some relation between these adjacent tissues is a piece of evidence that suggests that for the most part the species adapted to the mesocarp are not so adapted to bean infection.

Figures 1.1, 1.2 & 1.3: The relationships between the mycological composition of the three fruit niches in numerical terms expressed using scatter plots. The data from each country of the study is distinguished by colour. i = internal (bean); x = external (fruit); m = mesocarp.







1.6 Distribution of Fungi

Data on the occurrence of fungi in the project countries can be garnered from the spreadsheets mentioned above. Specific sampling experiments were conducted to try to elucidate fungal distribution in farms so as to improve sampling and aid interpretation of information about the occurrence of OTA. Of course, the presence of OTA requires the former, or current, presence of an OTA producer.

Some effort was made in understanding the effect of microclimates in the coffee plantation on fungal distribution. As an example of these inquiries, where coffee is grown in full sun using the self-shading of row planting, often some rows are exposed on one side and the fruit shows weathering and sun-scorch on that side. The data in Table 1.2 indicate that the mesocarp flora is reduced in cherries from the weathered side of the tree. Although it appears that *Fusarium* copes much better than yeasts under these conditions, in absolute terms it does not occur in higher numbers; the yeasts are reduced drastically. A numerical increase in internal infection is recorded but is unlikely to be significant. Here there is no indication that weathered fruit is prone to invasion from ochre aspergilli but it is unclear whether this is merely due to their local absence.

Table 1.2: A comparison between cherries collected from the north-facing side of a row (sun) and the south facing side (shade). No ochre aspergilli were isolated.

	x (cfu/ch)	m (cfu/ch)	i %	1st Dominant	2nd Dominant
i			6%	0.05 *F. stilboides*	0.02 *P. brevicom.*
x	$1.9x10^5$			0.70 *C. edax*	0.30 *Fus. + Clado.*
m		$2.4x10^3$		0.80 *F. stilboides*	0.21 *C. edax*
IT (shade) i			20%	0.20 *F. stilboides*	0.09 *C. edax* + ?
x	$1.8x10^5$			0.77 *C. edax*	0.15 *F. stilboides*
m		$1.9x10^5$		0.85 *C. edax*	

Table 1.3 reports an early intra-farm distribution study conducted in Brazil. Even *Fusarium*, the most common coffee-related species which have a definite association with coffee, is not evenly distributed.

It is very unlikely that this is attributable to micro-climatic factors, and probably should be interpreted as an inherent characteristic of the interaction between plant and associated fungi.

Table 1.3: Distribution of fungi associated with ripe coffee within one block. Sites, samples taken from 5 adjacent trees, were located in a square pattern about 70m between. After 4 days drying, site A1 coffee i-rate was 16%. Samples of green and overripe fruit from site A1 both showed 63% i-rates. Diversity was low, but an unidentified fungus was prominent.

Site	x (cfu/cherry)	i total	*Fusarium* i per 70 bns
A1	$3.6x10^4$	19 %	8
A2	$1.0x10^5$	36 %	19
A3	$8.8x10^4$	74 %	49
A4	$1.6x10^5$	21 %	7

Using GPS to locate the sampling sites, similar studies were conducted in Uganda and Côte d'Ivoire. The frequency of ochre aspergilli in the fresh cherry from the stations at Divo and Bingerville were possibly a little higher than in other surveys, but not dramatically so. Ochre aspergilli were isolated from the fresh beans of 4 of the 14 sites, with rates ranging from 1 to 5%.

Figures 1.4,1.5 & 1.6: The relative positions of sampling stations for the intensive sampling protocol as measured by GPS. The orange markers show where ochre aspergilli were isolated from the fresh cherry. The accompanying numbers represent the percent infection of niger aspergilli, ochre aspergilli and *Fusarium*, respectively. Site B at Divo lies immediately south by south-east of a mature block of cocoa.

Figure 1.4

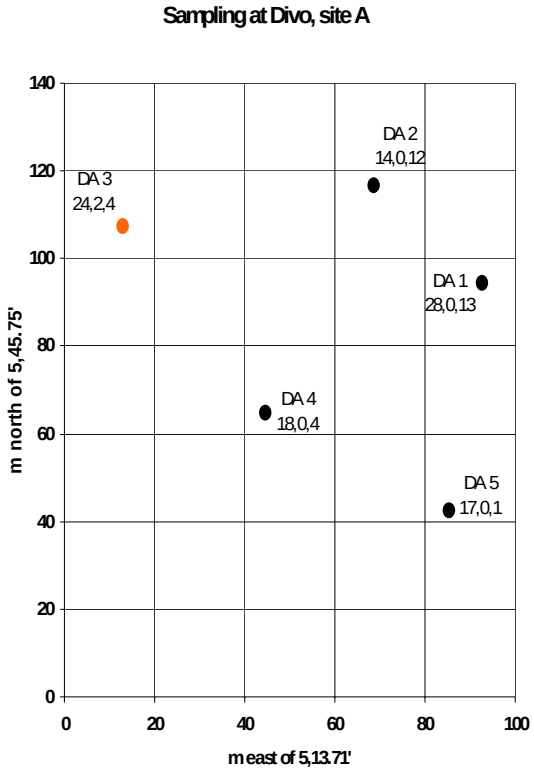


Figure 1.5

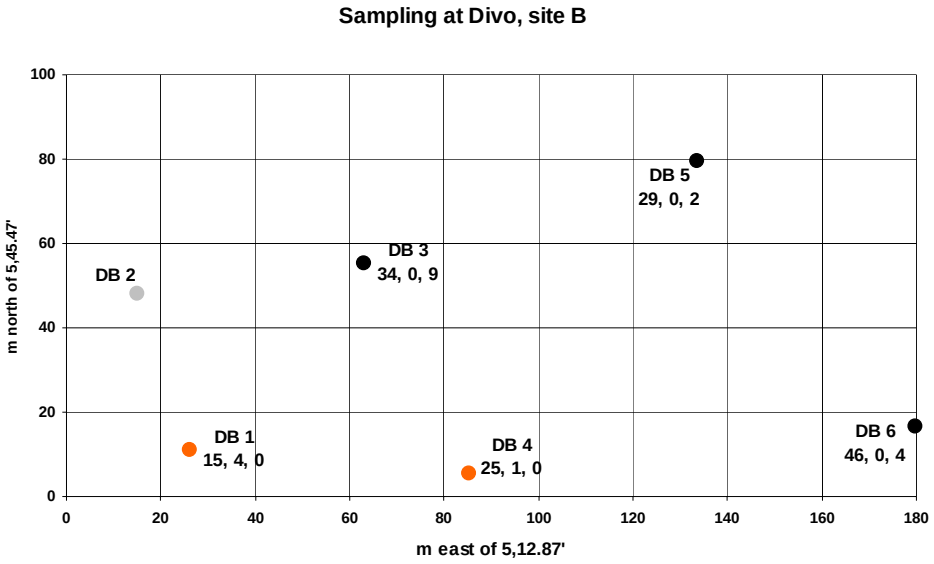
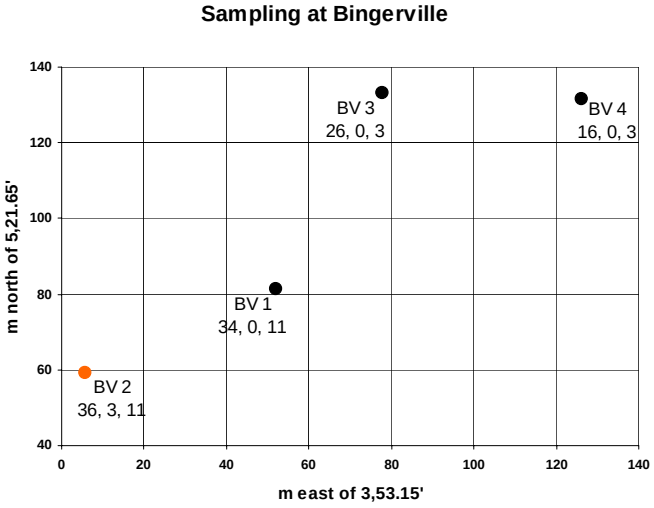


Figure 1.6



The second Divo site shows the greatest variation between sites of niger aspergilli but this is quite modest deviating only ±15% from the mean of 30%. In fact, the three sites taken together show a distinct uniformity with means falling between 20 and 30% infection rate.

These sites were also sampled for endophytic fungi (second internodes), air and soil. Endophytes were isolated from all sites. Divo A varied from 33 to 75% of internodes, all described as 'other aspergilli' aside from two *Fusarium* isolates. Divo B showed niger aspergilli from four of five samples varying from 75-90% of internodes, with the 'other *Aspergillus*' dominant. Bingerville was relatively low at 8 to 20% of other aspergilli.

From the air, niger aspergilli were most commonly isolated. The density was comparable at both Divo A and Bingerville at from 5 x 10^{-2} to about 2 x 10^{-1}

cfu/cm²/h.[5] Ochre aspergilli were isolated at 4 of the 9 sites sampled ranging from about 9 x 10^{-3} to the detection limit, 2.9 x 10^{-3} cfu/cm²/h. These stations were Divo A 1 and 4 and Bingerville stations 2 and 3. For reference the cocoa block was sampled and this was comparable to the coffee at 9.4 x 10^{-2} cfu/cm²/h for niger, and 7.0 x 10^{-3} cfu/cm²/h for ochre.

Soil samples from near to the stem (S_0) and between the trees (S_2) at the site were taken for comparison. Niger aspergilli were uniformly distributed in these samples at about 10^5/g at S_0 and a little lower at S_2. No ochre aspergilli were isolated from the S_2 samples. It was, however, isolated from the S_0 samples from Divo A1, A3, A4 and B1 and in Bingerville from station 1. Niger aspergilli were not as commonly isolated from soil and orchard air in other regions.

Table 1.4: Synopsis of the intensive sampling in Masaka, Uganda expressed qualitatively as presence or absence above detection limits.

	Niger aspergilli					Ochre aspergilli				
	Soil	Stem	Bean	x + m	Dry	Soil	Stem	Bean	x + m	Dry
(+) ve	14	11	21	35	48	11	0	3	0	2
(-) ve	36	14	35	13	4	40	25	53	47	50
n	50	25	56	48	52	51	25	56	47	52

Table 1.4 gives a synopsis of occurrence of ochre and niger aspergilli in a more extensive study conducted in Masaka, Uganda. The quantitative data from which this table is drawn follows in Tables 1.5 and 1.6.

Niger aspergilli are better distributed in stem tissue, fresh beans and fruit than in soil, and are almost invariably found infecting the dry robusta beans. Ochre aspergilli could only be said to be common in soil, and were not recruited in any of the 25 stem samples nor in any of the 47 dry hull (x + m) samples.

[5] Note that 1cm² is approximately the cross-sectional area of a coffee fruit, so this unit approximates to the settling rate onto a cherry.

Table 1.5: Farms from the northeast Masaka districts that were intensively sampled.

'<' = below detection limit; '>>' = too numerous to count; blank cells = no determination.
Bold red farm codes indicate farms from which ochre aspergilli had been isolated from dried beans previously.

	A. niger complex					Ochre group					
	Soil	Stem	Fresh	Dry		Soil	Stem	Fresh	Dry		Total
			i	x + m	i			i	x + m	i	i
Farm/Site	x10^3 cfu/g	% (n=5)	%	x10^4/ch	%	x10^3 cfu/g	% (n=5)	%	x10^4 /ch	%	%
MA 3A	<	<	<	2	31	<	<	<	<	<	67
B	2.8	20	<	45	100	<	<	<	<	<	100
C	<	20	2	19	10	<	<	<	<	<	12
D	<	<	2	<	45	<	<	<	<	<	69
E		<			2	<	<			<	10
MA 5A	<		2	2	27	<		<	<	<	55
B	<		<	<	94	<		<	<	<	96
C	<		<	44	43	<		<	<	<	59
D	<		12	1	16	<		<	<	<	65
MA 9A	<	<		<	78	<	<		<	<	78
B	<	<	<	3	43	<	<	<	<	<	61
C	0.3		<	2	57	<		<		<	78
D		<	<	<	20		<	<		<	24
MB 1A	<	<	<		20	<	<	<	<	<	27
B	<	<	<		<	0.7	<	<	<	<	10
C	<		3	3	18	2.3		<	<	<	25
D	<		<			137.2		<			
E	<		<			9.7		<			
MB 3A	<	<	2	53	88	<	<	<	<	<	96
B	<		<	3	29	<		<	<	<	31
C	<	<	2	100	29	<	<	<	<	59	75
D	<	<	4	>>	92	<	<	<	<	<	98
E	2.3	<	4	<	29	<	<	<	<	<	67
MB 9A	<		<			<		<			
B			<	1	14			<	<	<	31
C	<		<			<		9			
D	0.3	<	6	<	10	7.5	<	<	<	<	18
E	0.2			<	6	17.3			<	<	19

Preliminary studies had indicated that there was a lower frequency of ochre aspergilli in the northeast of the study district than in the southwest. The subsequent detailed sampling did not confirm this: there was little ochre aspergilli isolated from either area. However, there was a distinct clustering of positive soil samples. In contrast, niger aspergilli are sparsely and uniformly distributed throughout the region in the soil samples, rarely more than one positive sample per farm.

By this data, niger aspergilli frequently occur in and on the fruit whereas ochre aspergilli are restricted to the bean. A proportion of samples show levels higher than $5x10^5$/cherry (to uncountable), and these are likely to represent samples that had some fruits with superficial development of sporing structures.

Most of the farms' dried coffee are consistently either highly infected or lightly so. MA3, MB3 and MC8 are exceptions, but most groups of samples from single farms fall either between 70 and 100% infected at dryness, or less than 40%. It is important to note that these samples were dried side-by-side at the laboratory a considerable distance from the field site. This fact requires that the basis for these rates is carried in the fruit/bean unit (since this is cherry drying), not with the environ of drying.

Table 1.6: Farms from the southwest Masaka districts that were intensively sampled.

'<' = below detection limit; '>>' = too numerous to count; blank cells = no determination.
Bold/red farm codes indicate farms from which ochre aspergilli had been isolated from dried beans previously.

	A. niger complex					Ochre group					
	Soil	Stem	Fresh	Dry		Soil	Stem	Fresh	Dry		Total
			i	x + m	i			i	x + m	i	i
Farm/Site	$x10^3$ cfu/g	% (n=5)	%	$x10^4$/ch	%	$x10^3$ cfu/g	% (n=5)	%	$x10^4$ /ch	%	%
MC 1A	10.8		<	21	77	<		<	<	<	90
B	8.3	40	<	4	51	<	<	<	<	<	69
C	8.0		<	18	94	<		<	<	<	94
D	<		<	<	71	<		<	<	2	100
E			<	9	16			<	<	<	69
MC 3A	<	<	76	>>	100	<	<	2	<	<	100
B	<		<	>>	99	<		<	<	<	100
C	<		<	40	98	<		<	<	<	100
MC 8A	<	80	<	>>	74	0.5	<	<	<	<	82
B	1.2		3	2	16	4.5		3	<	<	24
C	<		<	3	41	7.0		<	<	<	55
D	<		<	<	<	0.8		<	<	<	10
E	2.5		<	3	37	0.7		<	<	<	100
MD 1A	<		<	3	10	<		<	<	<	23
B	15.0			1	8	<			<	<	35
C	<		<	1	<	<		<	<	<	2
D	<		4	4	<	<		<	<	<	37
E	<		2	3	86	<		<	<	<	92
MD 2A	<		55	<	6	<		<	<	<	22
B	<	40	33	2	6	<	<	<	<	<	10
C	<	80	59	<	14	<	<	<	<	<	20
D	<	100	62	<	2	<	<	<	<	<	16
E	<	100	67	<	10	<	<	<	<	<	14
MD 6A	11.3	40	<	>>	100	<	<	<	<	<	100
B	12.5	40	<	>>	98	<	<	<	<	<	100
C		40	84	44	98		<	<	<	<	98
D	2.3		4	70	90	<		<		<	92
E			<		98			<		<	98

The niger fresh infection rate does not predict the dry rate in any way. The x + m counts are only partially consistent with the associated bean infection rates in that the low infection rates are associated with low x + m counts.

Figure 1.7 below maps the relative positions of the farms included in the survey and Table 1.7 presents a synopsis of intensive sampling pertaining to the 17 farms from which ochre aspergilli were isolated. There is no apparent distribution pattern to the occurrence of ochre aspergilli in the dried product, and only a slight coincidence between its occurrence there and in the *beneficio* – thirteen farms contained one or the other, only two contained both.

Farms surveyed ranged from 0.7 to 115ha in size with a median of 15ha and with those farms containing ochre aspergilli spanning 0.7 to 68ha. In elevation they varied from 1100 to 1900m (with a median elevation of 1450m), with the 17 farms of the ochre positive sub-set ranging from 1250 to 1650m. Overall bean infection rates were low, though a few samples were highly infected.

The data shows a mean of 22% and a median of only 13% internal infection ('i'). The six farms with ochre bean infection were all close to the median varying between 10 and 27% overall infection.

Figure 1.7: Graphical elaboration of the relative positions of the farms surveyed in Caldas, Colombia.

Orange data points = farms with ochre group aspergilli from air in the beneficio; red ring = locations with ochre group aspergilli in the dried beans; black square = no dried bean analysis. The squares are 5km per side.

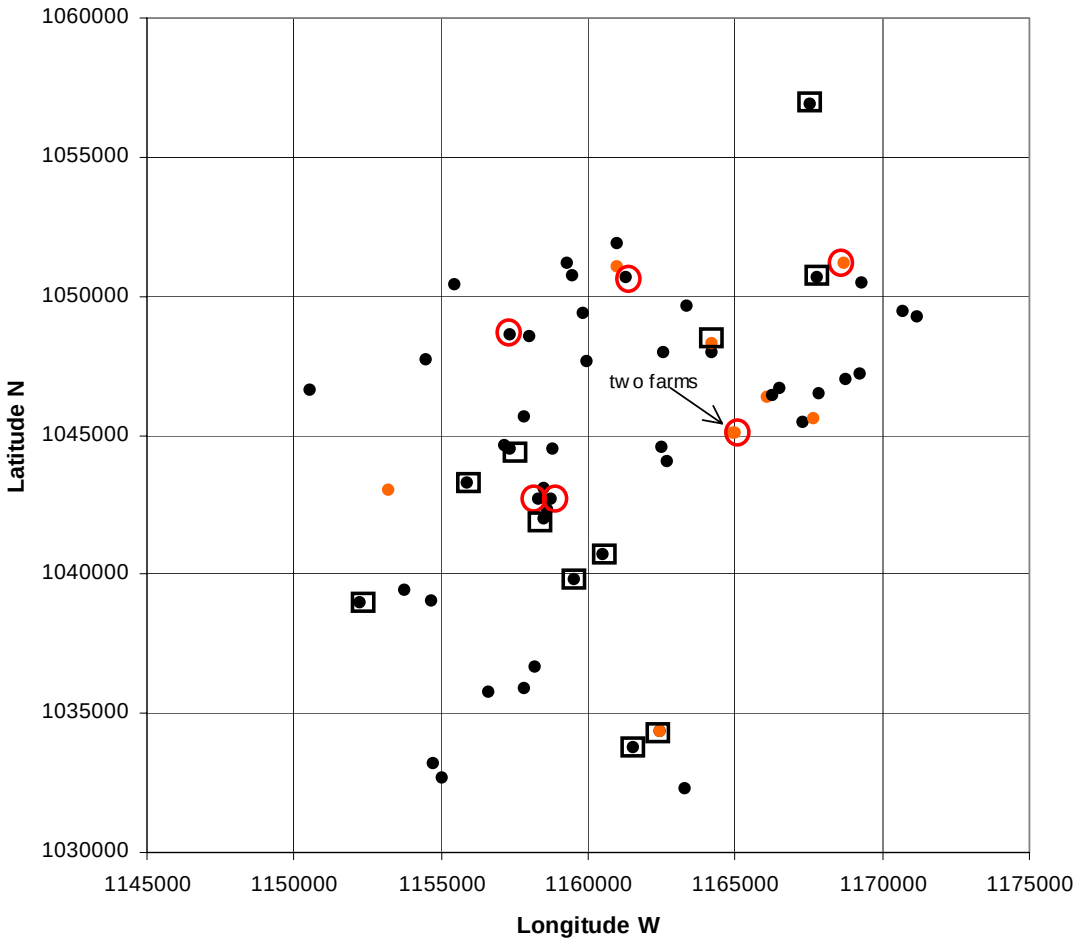


Table 1.7: Records of farms where ochre aspergilli were isolated.

Notes: wet methods: 1=fermentation; 2=mechanical. Drying methods: 1=sun; 2=sun+mechanical; 3=mechanical. Ochre group: 0=(-)'ve; 1=(+)'ve; n.d.=not determined; no entry=no equipment. totals: positives/total analyses made.

						Ochre Group						
Finca	Area (ha)	Altitude (m)	Wet Method	Drying Method	% Bean Infection	Bean	Soil	Orchard Air	*Beneficio* Air	Mech. Dryer Surface	Dry Yard Surface	Ferm. Tank
F22	0.70	1450	1	1	10.0	1	0	0	1		0	0
F15	1.00	1350	1	1	7.1	0	0	0	1		0	0
F57	1.89	1450	1	1	n.d.	n.d.	0	0	1		n.d.	n.d.
F47	1.91	1450	1	1	n.d.	n.d.	1	0	0		n.d.	n.d.
F2	4.80	1650	1	2	87.1	0	0	0	1	n.d.	0	0
F5	6.40	1398	1	1	41.4	0	0	1	0		0	0
F4	8.00	1387	1	1	27.1	1	0	0	0		0	n.d.
F13	9.90	1500	2	3	20.0	1	0	0	1	0	n.d.	0
F24	13.00	1450	1	2	n.d.	n.d.	0	0	0	n.d.	1	0
F18	15.00	1300	2	2	11.4	1	0	0	0	n.d.	0	0
F17	15.00	1350	2	2	14.3	1	0	0	0	1	0	0
F38	18.00	1250	2	2	4.3	0	0	0	1	n.d.	0	0
F23	19.50	1450	2	2	15.7	0	1	0	1	n.d.	0	0
F48	28.00	1500	1	1	1.4	0	0	0	1		0	0
F43	60.00	1364	2	2	14.3	0	0	0	0	1	n.d.	0
F26	64.00	1550	2	3	25.7	0	0	0	1	n.d.		n.d.
F56	68.00	1314	1	3	14.3	1	0	0	0	n.d.		0
Median	15.0	1450			13	Totals						
Max.	115.0	1900			99	6/48	2/59	1/58	9/57	2/17	1/18	0/49
Min.	0.70	1110			0							

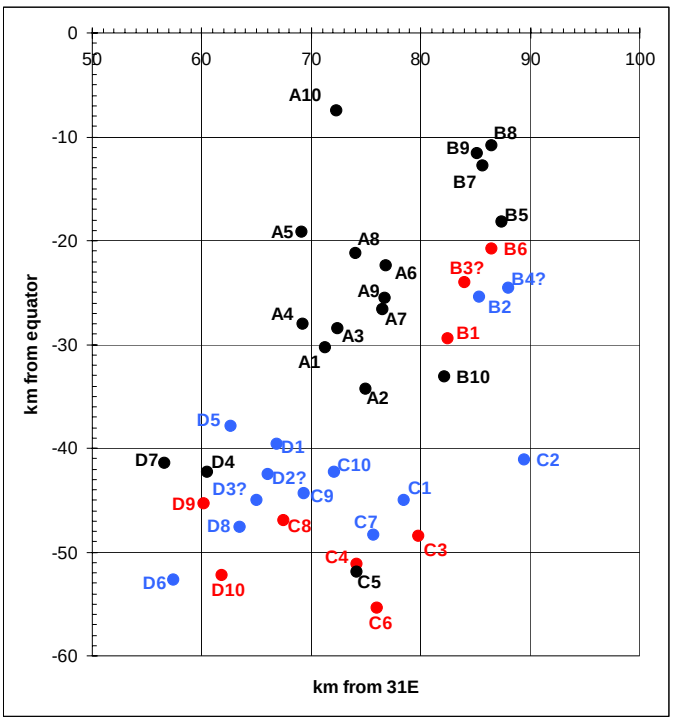
The most common source of ochre aspergilli was the air of the *beneficio* (9/57) and as an internal infection of the dried product (6/48). Of the 13 farms where ochre group aspergilli were isolated from beans and/or air of the *beneficio*, in only two were these fungi isolated in any other niche. Four farms were found to contain these fungi in other niches but not in the beans or beneficio air.

No ochre group aspergilli were isolated from any of the 49 fermentation tanks that were analysed, and rarely from the surfaces of mechanical dryers (2/17) with one of these two corresponding to ochre aspergilli presence in the beans. These OTA producers are, if anything, less common on drying yard surfaces (1/18) than on mechanical facilities, according to this data.

As is usual, even with the use of DC03, a semi-selective medium that allows larger samples to be made, ochre aspergilli are rarely isolated as orchard air spora (1/58). It appears that these fungi are also less common in the root rhizosphere here than in some regions, since only 2 of 59 of the soil samples contained them.

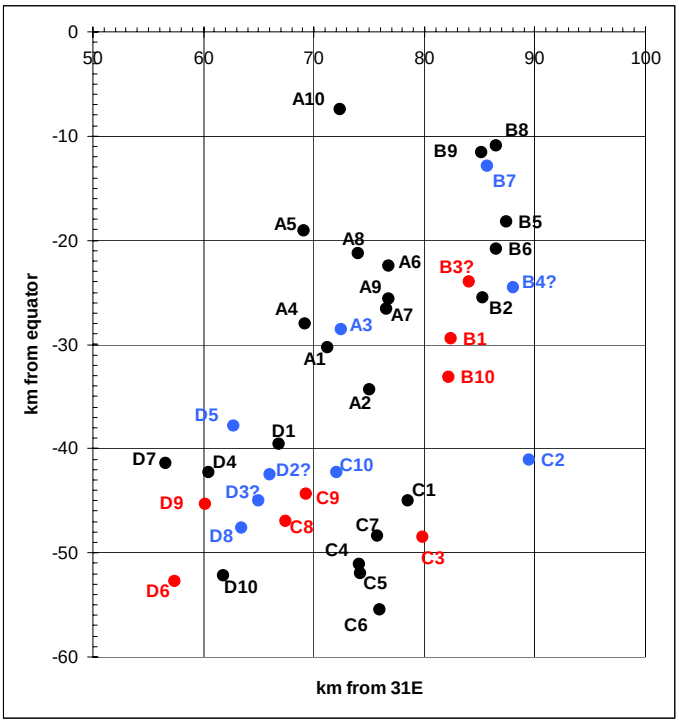
Taken together, this data is consistent with the picture we have built up that coffee is the most important source of ochre aspergilli in the coffee production chain. It is found in the environment where coffee is handled because spores are liberated in the handling of the coffee rather than ***because*** these locations are the source of contamination. Of course, cross-contamination of coffee in these locations is a potential problem that needs to be addressed by processors. The lack of coincidence between niches probably represents, and should probably be interpreted as, the sort of pattern to emerge from sampling populations at densities close to the detection limit. The pattern of occurrence then resembles a single distribution where chance becomes the only discernable factor.

Figure 1.8: Distribution of ochre group aspergilli in dried coffee in Masaka district, by four sub-districts. Black means none detected =<1/70; blue = 1-20%; red = >20%.



The regional data for Masaka, Uganda is presented in Figures 1.8 & 1.9. The northeast/southwest split in the occurrence has already been alluded to, and it applies to some extent to both the ochre aspergilli, which proved to be mainly *A. melleus*, a non-toxigenic relative of *A. ochraceus*, and *A. carbonarius*. It was discovered that the surface sterilization step was conducted inadequately in some of these analyses so the results are prone to some error and the true values are likely to be lower.

Figure 1.9: Distribution of *A. carbonarius* in dried coffee in Masaka district, by four sub-districts. Black means none detected =<1/70; blue = 1-20%; red = >20%.



With this data we see the interaction of niche preference, distribution and detection limit. To establish distribution, appropriate sampling must be devised, that is, not every niche is equally likely to contain the species of interest. In the data above, we see the presence of ochre aspergilli in several niches and a lack of correlation between these positive findings.

In the first place, the significance of the presence of the fungus in soil is different to its presence in fresh beans and these both to its presence in dried beans - and the frequency of its isolation varies between these niches. Perhaps the reservoir of this fungus is the soil, especially in association with coffee roots, but this is not a direct factor in bean infection since, if bean infection could be established by infection through the root, we would have detected the fungus in the stem.

Air samples, it has been emphasised, reflect the presence of sporing structures nearby, and typically this is in association with coffee handling. Since swabs of surfaces, especially in wet processing operations, rarely turn up ochre aspergilli, air-borne ochre aspergilli in processing and storage facilities reflect its presence in the coffee. Though much rarer, isolation of ochre aspergilli from orchard air may reflect its presence equally in the coffee, or the soil.

Section 2
Infection through the Flower

2.1 Introduction

It is very unlikely that there is a single route by which ochratoxigenic fungi become established in the coffee seed. Of course, one approach to preventing OTA formation is to prevent access of OTA-producers to the commodity in the first place. Ultimately this is not a practical solution. However, knowledge of when, where and how access can and can't occur is very valuable in focusing the development of risk-based prevention guidelines and their application.

The coffee plant flowers in 'flushes' that are induced by the onset of rains after a period of drought. Flower buds swell with the flowers, opening about nine days after initial rains, and persist for between five and seven days. Arabica expresses this control mechanism more strongly than robusta, but it is apparent in both species. There can be a series of flushes, especially where the rainy season begins with an erratic pattern, and even after this there can be 'running blossom'. Robusta breaks dormancy more readily, and even an unseasonable local shower can sometimes induce 'running blossom', which can be particularly troublesome as it can overlap the main harvest period.

There is no doubt at all that *Aspergillus ochraceus* can invade and exist in the seed during seed development since it is routinely isolated from the seed of freshly harvested coffee fruit. The project has also demonstrated that OTA can be produced before harvest, with numerous samples of oven-dried freshly harvested coffee containing OTA. Importantly there is no disease or defect associated with this coffee, so the clear inference is that *A. ochraceus* (or other OTA-producers) can reside in the bean without inducing a disease response by the host. This is characteristic of commensal fungi in higher plants, a kind of association that has proved to be remarkably commonplace.[1]

Although it is certain that these fungi can be introduced well before harvest and persist, the mode of introduction is not clear. Logically, access to the seed can only be gained through the vascular system, through the fruit tissue or through the flower. All three routes have been documented in better-studied mycotoxin-commodity systems, such as barley, maize and groundnuts.

Many analyses of coffee tissue of lateral stems under the project failed to produce a single isolate of *A. ochraceus*. *A. niger* has, however been isolated from this niche. *A. niger* is very rarely isolated from fresh arabica cherries, but can be isolated from the stem tissue, so it would seem better adapted to the stem than the seed, i.e. the reverse of *A. ochraceus*.[2]

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[1] For more general information about fungal interactions, including endophytism see: Hyde, K. D. (Ed). 1997. *Biodiversity of tropical microfungi*. Hong Kong University Press.

[2] See Tables 1.4, 1.5 and 1.6 in Part C Section 1 of this report, 'Distribution of Fungi in Coffee Production Systems'.

Coffee berry borer (CBB) has the potential introduce fungal propagules to the seed when it enters the coffee cherry, and scale insects and aphids can do the same to the vascular system of the plant.

Sucking insects were not studied under the project, but CBB damage can sometimes be associated with *A. ochraceus* and OTA. However, this cannot explain OTA and *A. ochraceus* in CBB-free beans and samples, so another mechanism must exist.

2.2 Findings and Application

There is good data deriving from the project's experiments that exposure of coffee flowers to spores of *Aspergillus ochraceus* leads to bean infection. The most probable route is through the pistil of the flower, although this was not proven by the experimental design employed, which is discussed in more detail below.

There is evidence that the ability to infect through the flower is held by some species and not others, and that the ability may be variable between coffee strains.

These results indicate that minimising exposure to ochratoxigenic fungi during brief and easily predicted periods of flowering is advised. This objective can never be completely realised, but there are clear practical measures to avoid excessive exposure as dealt with in the project's '*Guidelines for the Prevention of Mould Formation in Coffee*' (see Part D).

Infection through the flower is not the only route for bean infection, but its importance is implied by a tendency of those producer countries more prone to OTA contamination in the dry product to be those also more prone to OTA contamination in fresh cherries.

2.3 Further Research

It might be useful to investigate whether infection through the flower is accomplished by hyphal extension through the tissues of the pistil and ovule, which would also prove (or otherwise) that it is the flower stage that is involved. Perhaps of more practical significance would be assessments on:

- When the flower is most susceptible to infection, combined with comparison to fruit exposed during fruit development;
- What levels of spore load leads to increased infection;
- The role that flies and bees may play in the distribution of these spores.

The technique of infecting seeds at flowering should be used to investigate the production of OTA in the field, where many very useful experiments can be imagined. It is the one scenario where horticultural influences on OTA production could be studied, since the infection represents a means of reliably introducing the toxigenic principle to the experiment. Being able to produce coffee containing OTA-producers at a fair frequency would also make processing experiments reliably meaningful for the same reasons.

Finally, the capacity of *A. melleus* to infect the bean through the flower could be usefully investigated to see if this non-toxigenic species could displace the OTA-producing *A. ochraceus*.

2.4 Additional Notes

In coffee, the species that produce OTA are from two sections of the genus *Aspergillus*: *Nigri* and *Circumdati*. There is every likelihood these species have different ecological capacities. *A. niger* is by far the more common, although it is difficult to identify, *sensu strictu*, and there are undoubtedly many false reports in the literature. In food systems it is one of the most common of spoilage moulds and the fact that its spoilage activity, taken over the myriad products it commonly spoils, is ***not*** associated with OTA is one reason its importance as an OTA-producer in coffee is considered to be slight.

Another member of the *Nigri* section, *A. carbonarius*, is much less common but is undoubtedly a potential source of OTA in coffee. Based on current data, its distribution is irregular, but it has proven to be occasionally numerous. The physical conditions it requires for OTA production are also narrower than those of *A. ochraceus*. In laboratory tests in pure culture it ceased to produce below an A_w of about 0.92, and above a temperature of about 35°C.

A. ochraceus is often associated with seeds and soil (but not only seeds and soil), and is a relatively slow-growing species. There has been much confusion about the taxonomy of this section which has now been mostly clarified, in no small part through isolates from this project.

There are at least four species that can only be distinguished by specialists, three of which are OTA-producers[3]. The most serious confusion, from the standpoint of OTA production, is between these three producers and *A. melleus*, the non-producer.

A. melleus is generally reckoned to be a soil organism. However, a high frequency of *A. melleus* was found in East African coffee. It is an open question as to whether there is the potential to use this plant-adapted, *A. melleus* type to either displace or compete against *A. ochraceus*.

Infection through the flower, either by CBB or sucking insects, would present a scenario whereby coffee seeds with an existing internal infection are fed into to the processing chain. The fungal biomass that has developed in the seed and the physiological integrity of the seed provide the system on which the processing conditions will act.

This is quite distinct from the scenario where fungal propagules are introduced to the outside of parchment or fruit during processing. The general mycological and,

[3] The four species are *A. melleus*, *A. ochraceus*, *A. westerdijkiae* and *A. steynii*. For recent discussion on OTA producing species in the *Aspergillus* sections *Circumdati* and *Nigri*, see:

- Samson, R.A., Houbraken, J.A.M.P., Kuijpers, A.F.A, Frank, J.M., Frisvad, J.C. 2004. New ochratoxin A or slerotium producing species in *Aspergillus* section *Nigri*. *Studies in Mycology* 50:45-61.
- Frisvad, J.C., Frank, J.M., Houbraken, J.A.M.P., Kuijpers, A.F.A, Samson, R.A. 2004. New ochratoxin A producing species of *Aspergillus* section *Circumdati*. *Studies in Mycology* 50:23-43.

when above an A_w of about 0.97, bacteriological community, would be established over the surface of the coffee particle. The point here is that a previous internal infection would have an entirely different developmental dynamic than *de novo* contamination through processing and (possibly) during storage.

The experimental procedure described below was not designed to demonstrate that infection through the flower takes place in nature. Strictly speaking it can only conclusively demonstrate that spores applied to the open flower increase infection rates since it is conceivable that infection occurs after flowering by some completely different mechanism, as generally associated with plant pathology (e.g. sucking insects). This is a remote possibility in our view and a positive result, especially when one of two species known to infect coffee seeds produces no infection, strongly suggests this infection has occurred through the flower.

For purposes of prevention, it is enough to note the increase in infection caused by a high spore load during the flowering period.

2.5 Experimental Design

Pure cultures of selected isolates of *A. ochraceus*, *A. japonicus* and *A. carbonarius* were grown in Petri dishes and provided the source of spores for the experiment.

Image 2.1: Application of spores of selected isolates to arabica flowers as part of the inoculation experiment run in the 2003 season, Brazil.

Newly emerged flowers of arabica coffee were exposed to the spores of these several isolates by hand, using a small paintbrush. The cherries were analysed for the presence of fungi when ripe, following removal of mucilage and surface sterilisation of the beans with 1% hypochlorite for ten minutes and plating out on DG18.

In the second season the flowers were enclosed in paper sacks for one week after inoculation. Control samples were from flowers on other bearing stems that were brushed with a sterile paintbrush. It should be noted that 90% of the flowers in arabica coffee are already fertilized when the flower buds open. These experiments were conducted in the Sul do Minas region of Brazil over two consecutive seasons, 2003 and 2004.

2.6 Experimental Results and Discussion

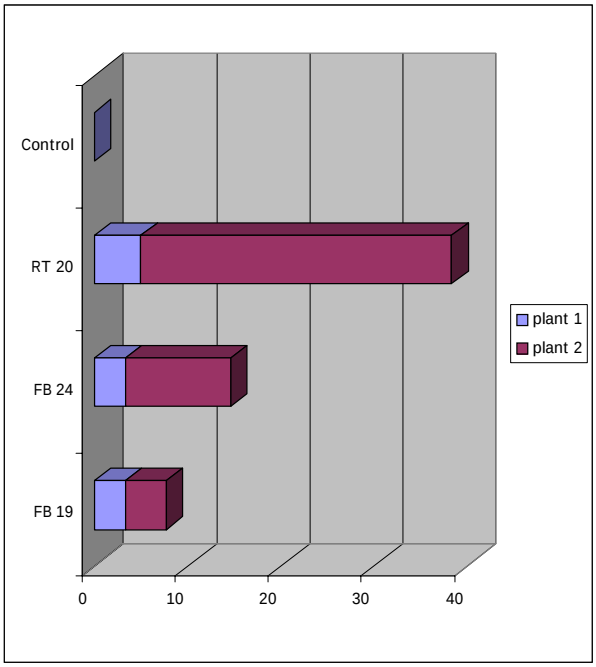
A marked increase in *A. ochraceus* infection rate over the control, which varied against isolate applied, was observed. No infection by *A. japonicus*, a member of the *Nigri* section also found in coffee, was recorded. This demonstrates that abundant spores during the flowering period leads to increased infection rates of the seed by *A. ochraceus.*

Table 2.1: Flower inoculation experiment run in the 2003 season. *A. japonicus*, a member of the niger section, showed no infection after its application.

Fungal strain	Ochre infection
Ochre 1	99
Ochre 2	86
Ochre 3	77
A. japonicus	14
Control	68

The treatments were applied stem-by-stem. The control treatment in 2003 was of untreated stems located on the same trees as the treated stems. Evidently, the infection efficiency was remarkably high in this case so the control was not adequately separated from the treated stems. The *A. japonicus* treatment was reportedly on other trees, so this produced a lower *A. ochraceus* infection than the control due to better physical separation from the source of the *A. ochraceus* spores.

Figure 2.1: Flower inoculation experiment run in the 2004 season. Spores of RT20, FB24 and FB19 (isolates of *A. ochraceus*) were applied with a brush to the flowers and the control was to use a sterile brush on the flowers. *A. carbonarius* was also applied but no infection was recorded.



In 2004 the separation was more effective, albeit against an apparently less efficient infection process. Of course, timing of the introduction of the spores could be a factor here but there is not sufficient information to say more than this. There was a nil infection rate of the control and 36 to 5% infection of treatments depending on the tree and fungal isolate applied.

Considering that the overall field infection of seeds by *A. ochraceus* is 0.1 to 0.2 %, and field samples have rarely been recorded as high as 5%, these figures represent a large augmentation in the expected occurrence of this fungus.

Section 3
Water Measurement in Coffee

3.1 Introduction

The measurement of water in commodities is a primary method of monitoring, controlling and predicting the stability and suitability of dried commodities.

In principle, growth of spoilage organisms in coffee can be controlled by temperature, oxygen restriction, pH, the addition of anti-microbials and water restriction. During processing, parameters such as pH, oxygen restriction and competition from benign organisms are called into play, but this is at stages when water cannot be restricted.

In practice, it is the restriction of water content to sufficiently low levels that is used to control growth of spoilage organisms in the later stages of processing and during all stages of transportation and storage. With this degree of importance, a firm understanding of the process of moisture measurement is required since it is the measurement of critical parameters that provides the possibility of control over a process.

There are two kinds of parameter that are measured: moisture content (m.c.), a descriptive measure, and water activity (A_w), a thermodynamic measure. The predictive power comes from A_w and is based on knowledge of the physiological capability of the most problematic group of spoilage organisms, the fungi. However, moisture content is more practical to measure, and it is this measurement that is most commonly used in commodity chains.

A_w/m.c. relationship is expressed graphically as a plot of the A_w against the moisture content of a series of samples, called the desorption (or absorption) isotherm. The relation is based on the physico-chemical interaction of water with the material comprising the commodity in question.

With seeds, in particular, this is complex due to the inhomogeneity of seed tissue layers which are also associated with the various storage and structural molecules that describe seeds and on the metabolic activity of what is a dormant but respiring biological entity.

Of course, in different commodities there will be different quantitative relationships between m.c. and A_w, and it is important to understand this is also true of different lots of the same commodity. Therefore, the studies to investigate the relationship between A_w and m.c. were motivated by this fact.

Formally, A_w is the ratio of the relative humidity of the air in a closed chamber that is in equilibrium with a sample, to the relative humidity of the air in a closed chamber hat is in equilibrium with pure water, at a given temperature. In other words it is the ratio of the relative humidity of air over a commodity to 100% humidity. In practical terms, A_w can only be measured as equilibrium relative humidity (ERH), and the

difficulty in getting a system to a state of equilibrium makes this measurement impractical as a routine tool, except perhaps *in situ* in a storage context.

Although there is no 'true' measure of water content in biological systems, due to the spectrum of binding energies with which water interacts with biological molecules, oven moisture content can be taken as the reference method[1].

Throughout the coffee-producing world, stakeholders have developed various empirical means of estimating when coffee has reached sufficient dryness, including for example, shaking the dry cherry or biting the green bean. However, these determinations are susceptible to error, and it was decided that more objective means of making this important assessment should be evaluated under the project.

Survey work completed under the project had shown that coffee was often stored on the farm and traded at moisture contents far wetter than recommended. This could be partly due to an inability of stakeholders to accurately assess moisture content.

Meters, which take measurements based on the electronic properties of commodities, have been used in the field for several decades to estimate m.c. but vary in their precision, accuracy and stability. They are also subject to errors caused by natural variation of the commodity, too infrequent calibration, and improper use. This required investigation.

Finally, moisture meters are generally out of the financial reach, or beyond the technical capacity, of many smallholder coffee producers. Therefore, another focus of this work was to evaluate other possible solutions to the problem of reliably estimating moisture content in the field, including the testing of low-cost moisture meters.[2]

3.2 Findings and Application

3.2.1 Desorption curves and implications for control of mould growth

Coffee has considerable lot-to-lot variation such that the confidence limits of the desorption isotherms that relate moisture content to the more meaningful measure of water activity for the four commercial coffee types (robusta parchment and cherry; arabica parchment and cherry) are relatively broad.

The **average** m.c. at which coffee can be said to be secure from OTA production is higher than previously recognised at between 17-20% m.c. (wb), depending on type.

However, and importantly, in order to account for the breadth of the confidence limits, the **target drying end-point for preventing growth of OTA producers should**

[1] See ISO 1446:2001 'Green coffee -- Determination of water content -- Basic reference method'.
[2] This work relates directly to Objective 2, Activity 2.1.1 of the project log frame, 'Selection and acquisition of optimal moisture measuring equipment for use by collaborating centres'. As well as procuring laboratory standard moisture meters for each collaborating institution for accurate field trial measurement, the project purchased and assessed low cost Chinese produced QCS-3Z and LDS-1D moisture meters. Locally produced moisture meters from Brazil (Gehaka brand) and Indonesia (Kako brand) were also evaluated.

be much lower than this. A moisture level of around 13% m.c. (wb) corresponds (at a confidence level of 0.98) to a A_w that does not support growth of *A. ochraceus*.

In storage of coffee the project recommends operating limits of 12.5% m.c. for cherry coffee, and 11.5% m.c. (wb) for parchment and green coffee.

In order to prevent **all** fungal growth (including non OTA-producers) the end points become 11.4% m.c. and 11.1% m.c, respectively (refer to Section 3.5.2 below for details).

3.2.2 Interaction between water and coffee tissues

Coffee beans have a marked distribution of hydrophilic tissue where most of the water resides in the partially dried state, other tissues being much drier. Loosely bound water is readily lost in the early stages of drying. Shrinkage - greater than the water-loss volume of the bean - accompanies the loss of intermediately bound water that is exhausted at an A_w of about 0.78.

Since changes occur in the coffee material during drying, rewetting is not a simple reversal of the drying process. NMR investigations suggested that the husk has a much reduced water binding capacity after drying and that re-wetting of cherry sees the added water go largely to the bean (refer to Section 3.5.1 below, and see Annex C.4 on the enclosed CD-Rom).

3.2.3 Use of moisture and water activity meters

The use of A_w rather than m.c. for monitoring in storage systems would give a more meaningful assessment of the stability of the coffee. Spear-type probes are now available with practical measuring protocols. However, these instruments are physically less robust, so a rigorous programme of validation must accompany a monitoring programme based on this equipment (refer to Section 3.5.5 for details).

Use of well-established (and relatively expensive) models of moisture meters by some stakeholders is common. However problems with the maintenance, use and calibration of these instruments were widely noted. The principles and practice of moisture measurement were therefore emphasised to the project collaborating institutes so that they could advise other stakeholders as required (refer to Section 3.5.3 below for further details).

The use of moisture meters, calibrated for green coffee, to measure the moisture content of monsooned coffee led to inaccuracies. Although monsooned coffee is exceptional, it demonstrates the principle that the physico-chemical properties of coffee can be modified according to its production history. In these cases the instrument must be calibrated for the specific type of coffee.

3.2.4 Performance of selected low-cost moisture meters

As noted above, two low-cost moisture meter models ('QCS-3Z' and 'LDS-1D') were sourced from a Shanghai-based manufacturer in 2003. The objective was to evaluate their accuracy and reliability at each of the seven collaborating centres, and to

ascertain whether there was a case to be made for their wider usage along the coffee chain.

Reports on the suitability of the Chinese produced moisture meters, based on testing carried out at the collaborators' laboratories, were mixed. Good calibration curves were obtained when moisture meter readings were plotted against oven moisture (r^2 0.97 – 0.99), but in some cases the internal calibration proved unreliable over the range of moisture values likely to be found in traded coffee (refer to Section 3.5.3 below for further details),

Further investigation of the meters is required before their widespread use can be recommended. This investigation must take into consideration the context in which the instrument would realistically be used.

There is evidence that if meters are to be widely introduced to coffee sectors, a very strong training programme and establishment of easily accessible centres for calibration would be an absolute requirement for such a programme to be successful.

3.2.5 Appropriate technologies for moisture measurement – the test weight method

Another (indirect) method of assessing coffee moisture content is to measure the weight of a constant volume of coffee, also known as the 'test weight method'.

The evaluation of this method showed it not to be an accurate method to assess moisture content. The bulk density of coffee depends, of course, on its m.c. but also on other factors which are linked with the physiology of the fruit, and which ultimately depend on varietal, location, agricultural practices employed, prevailing climate etc. Indeed, this method of estimating m.c. was shown under the project to be sensitive to both region and season, and shows considerable variation (see Section 3.5.4 for details).

In countries where there is traditional widespread use of the test weight method the coffee institutes have undertaken to improve the reliability of this method. They are doing this by reviewing the official recommendations on target test weights with different types of coffee in different regions.

Another method of moisture determination - the 'EDABO' method - has been successfully developed in Brazil and may be useful in some situations.

3.2.6 Putting moisture measurement into context

In summary, it is worth reiterating that the most effective way of preventing mould formation and OTA contamination in coffee is to ensure that a safe moisture content level is maintained, and achieved as quickly as possible. Moisture content is therefore an essential control parameter.

It must be underlined that field work and surveys carried out under the project provided limited evidence that observed high m.c. of coffee in local trading is caused by a lack of ability to measure m.c.

On the other hand, there was plenty of evidence that marketing policies strongly influence the practices of coffee handlers and traders. The problem of trade in high-moisture coffee must be well understood before any major commitment to meter dissemination is made.

Ultimately, however, the ability to accurately determine the moisture content of coffee is also of great economic importance to both farmers and small traders. Commonly, price discounts are applied by buyers when purchasing 'too wet' coffee. However, in cases where coffee is drier than the specified target level, no 'premium' is applied.

It should not be forgotten, therefore, that the ability of these stakeholders to accurately measure moisture content is also significant in relation to the income they receive.

3.3 Additional Notes

A_w measurement is not easily undertaken because of the requirement that the sample be in equilibrium in a closed system. As equilibration is asymptotic it is very difficult to be confident about, except through experience with the measuring system.

The method applied for A_w measurements in these studies required a minimum 15 hours equilibration period for coffee taken from processing. This arose from an early study where coffee was sacked overnight and re-spread in the morning and post sacking and pre-spreading measurements taken. Later measurements confirmed that 15-18 hour equilibration allowed a meaningful measurement to be made.

In storage facilities A_w measurement can be made immediately, allowing about 15 minutes for probe equilibration, since the interstitial air of the sacks or heaps is likely to be pre-equilibrated. However, the nature of the probe is such that it is susceptible to damage from heavy dust and, in particular, liquid water. If liquid water comes into contact with the measuring crystal the probe can be damaged beyond repair. Thus, if a cold probe is introduced to a warm moist sample, condensation can form and irreparably damage the probe.

Moisture content, though it appears simple in concept, is also not straightforward. It is not an absolute quantity. Rather, it is method-dependent because water is held by chemical bonds with a spectrum of energies from the very weak to very strong bonds. Thus the power of the drying method to disrupt the stronger bonds determines how much water is recovered. It is also an average quantity, as with the other parameters, since it measures a sample of many particles collectively.

The distribution of OTA is such that a very small proportion of beans are contaminated, but, since they can be individually heavily contaminated, the average value, as might emerge as an analytical result, can be high. Until more is known about the pattern of water distribution, particle by particle, the possibility exists that mycotoxin problems could primarily be a problem of particular, rather than average, conditions.

3.4 Experimental Design

Much of the data required to make a detailed evaluation of inter-lot variation of the A_w/m.c. relation (desorption [or absorption] isotherm) was acquired from other experiments in which the drying time-course was being documented. These data intensively characterise several different lots of coffee - both side-by-side and at different times during the season in all the collaborating institutions.

Other sources of data included the various surveys that the project conducted which yielded data on many coffee lots. In total, many thousands of determinations, a substantial proportion of which were completely independent, were recorded.

Evaluation of operational parameters of cheap moisture meters were conducted by several of the collaborators. The intention here was to calibrate the meters carefully and then compare their performance, against oven drying, in the field studies, as well as in repetitive evaluation of a few well controlled and characterised samples.

The nuclear magnetic resonance (NMR) work was completed in the physics department of the University of Surrey, U.K. using a non-commercial research imaging instrument with a resolution of 10u, and a Marran bench-top instrument for the acquisition of total NMR decay signal. Neither instrument could be cooled below about 30 to 35°C so this was the operating temperature for all acquisitions. The imaging system was controlled using a Unix work station.

For the imaging, the beans were pre-equilibrated over H_2SO_4 solutions then maintained in equilibrium in sealed NMR tubes containing some fresh A_w controlling solution below the acquisition path. The bean was positioned on a dry cotton plug which was found to contribute negligible interference or distortion. The images, collected at a pre-set relaxation time at three to five slices required 60 to 120 minutes each, depending on specifications. Many preliminary images were required to optimise the acquisition parameters.

For total NMR signal acquisition, both pre-equilibrated, as above, beans and beans taken from a drying time-course were measured. The bench top machines required 0.5 to 5 minutes per determination, depending on the water content.

3.5 Experimental Results and Discussion

3.5.1 Some basic phenomena in coffee

Controlling water availability is the most practical means available for the control of fungal growth in commodities. Its measurement is not a trivial exercise, and understanding its dynamic with biological and matrix systems is central to interpreting data where water is a variable.

Water activity (A_w), a measure of water availability, is a thermodynamic property. It is the relative humidity of air around a sample in a closed, equilibrated system.

Moisture content (m.c.) is a descriptive aspect of composition, the amount of water present as a proportion of the amount of dry mater present (dry basis, db) or of the

total fresh weight (wet basis, wb, also sometimes called 'as is'). There are several methods of measuring this, but most commonly a form of oven drying is used.

It is important to understand that there are no absolute values for these quantities and the determination is absolutely dependent on the method of measurement.

These two parameters are related through the chemical-physical properties of the system containing the water. Insofar as two systems are the same, the relationship between A_w and m.c. will be the same. This aspect is dealt with in more detail below.

The two molecular properties that govern the partial pressure of water vapour of the air in equilibrium with a solution, thus water availability, are (i) the solubility of the compound and, (ii) its ability to attract water, referred to as the 'activity coefficient'. In solid systems these properties combine and add to other physical interactions to comprise what is called 'matrix potential' and is the extent to which the material is hygroscopic. A heterogeneous system in equilibrium will have a uniform water activity, but the moisture content of the different elements of the system, if they could be known, would be different. The more hygroscopic portions (those with higher matrix binding) bind more water but exchange proportionately less yielding a lower partial pressure of water than another portion with the same water content but lower water binding capacity. In equilibrium, the hygroscopic portions would have the same A_w but a higher moisture content than the parts which are less hygroscopic.

NMR studies carried out under the project provided an understanding of the heterogeneity of the water contained within the coffee fruit and coffee bean and the distribution of the different 'types' of water (free, intermediate and tightly bound) in this complex system. This picture of water distribution in coffee at different stages of drying helps provide an appreciation of the complexity of coffee drying, and the difference between the behaviour of water in different types of coffee during drying and rewetting. Details of the findings of this investigation are presented in Annex C.4, which can be found on the enclosed CD-Rom.

3.5.2 Desorption isotherm for coffee (m.c. vs. A_w)

As discussed above, and in Annex C.4 in the enclosed CD-Rom, there will be one relationship between m.c. and A_w for a commodity to the extent that the commodity is absolutely uniform. But the real importance of knowing this relationship in detail is that A_w alone is predictive of microbial growth, ***not*** m.c.

However, m.c. is more easily and reliably measured in the field or production context than A_w. In other words, we get only data about m.c. and we need to convert it to A_w for it to be meaningful in predicting microbial performance.

Published work is based on individual coffee lots. This gives a smooth relationship with very good fit to one of the several published mathematical relationships. Our data utilises many individual lots as well as single points from a great many sources of coffee that were of different varieties, grown in different regions on three continents, and represented different processing histories. All these factors contribute to the scatter around the relationship that we report here.

With respect to the maximum recommended m.c., this guidance is based on the fact that *A. ochraceus*, the most important OTA producer, cannot grow at A_w levels below 0.78 . So the question becomes, at what m.c. can we be 95%, 99% or 99.9% sure that OTA producers do not grow?

Furthermore, according to the literature, OTA is not produced at a water activity below 0.80 providing a further margin of safety against an accumulation of OTA. It should be noted that some xerophiles can grow slowly at such levels of drought, so an A_w at this level would not necessarily prevent ***all*** microbial deterioration.

Four models relating A_w to m.c. were tested for conformance to our data sets, and it was found that the Henderson equation,

$$(aw = 1 - \exp(-A \times mc^B)$$

provided the best fit as measured by the regression coefficient r^2. Coffee in storage can take up water (as well as lose it, depending on humidity) so, in developing recommendations, due consideration must be made to account for this potential for change. Two of the regression curves are given below in Figures 3.1 and 3.2.

Table 3.1: m.c. of the four major coffee types, before de-husking. Quoted confidence limits reflect the fact that we are interested in error on only one side of the relation: not more than 0.78.

% Confidence	Cherry		Parchment	
	Arabica	Robusta	Arabica	Robusta
Mean	22.3	19.5	17.4	19.0
95	16.7	14.8	14.2	15.5
99	14.9	13.3	13.2	14.3
99.9	13.0	11.9	12.1	13.2

Figure 3.1: Regression curve fitted to the Henderson model for arabica parchment, showing the calculated confidence limits at 90, 98 and 99.8%.

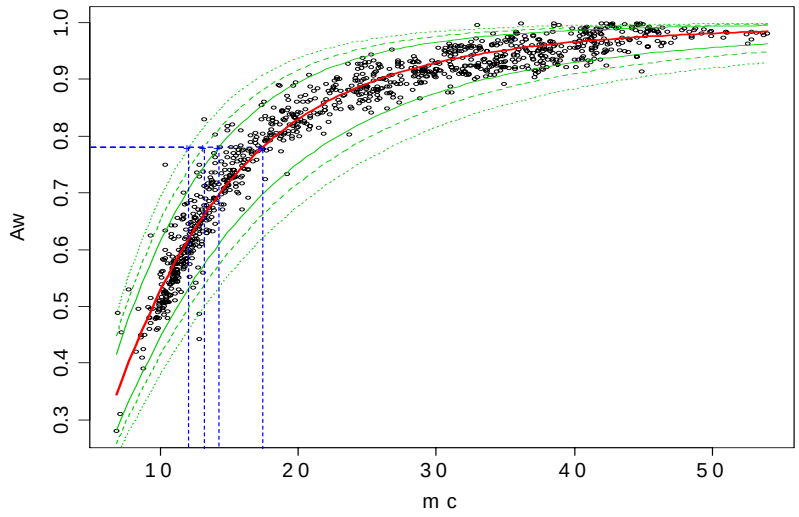
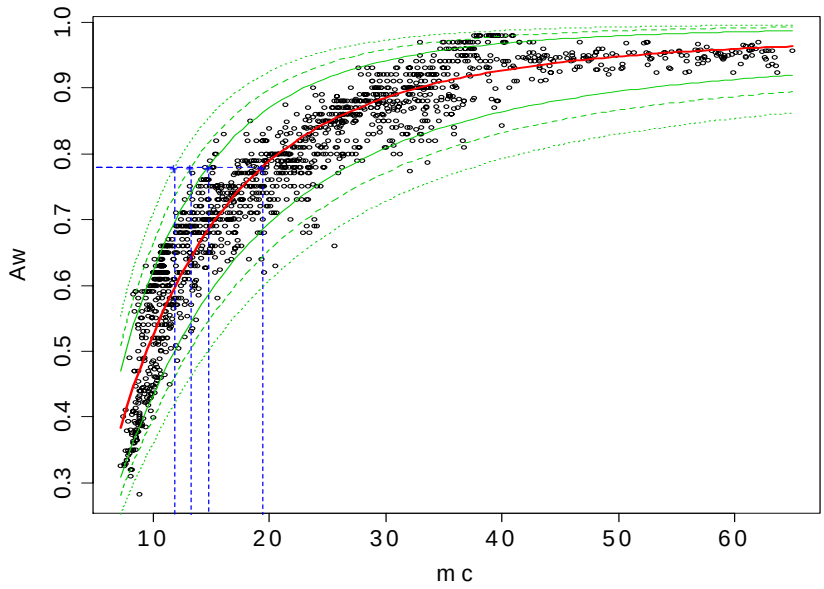


Figure 3.2: Regression curve fitted to the Henderson model for robusta cherry and showing the calculated confidence limits at 90, 98 and 99.8%



3.5.3 Measurement of m.c. using moisture meters

Moisture meters are by far the most common method for measuring m.c. in commodities. Moisture meters operate by measuring either the capacitance or the conductance of a sample of a specific size between two electrodes. Sometimes sample size is controlled by weight, sometimes by volume. In the first case, variations in density may cause and error. In the second case, variations in particle size, or the way the instrument is filled, could cause an error as the packing rate changes.

In principle, different coffees could also have different electronic interactions with water due to spatial or chemical differences. In the field, however, the most serious problem with the accuracy of these technologies is undoubtedly in equipment maintenance, use and calibration. Confusion about wet and dry basis m.c. calculations, and the method and use of calibration caused a remarkable number of problems, even in the collaborating research institutions participating in the project. Because such devices carry an especially high level of credence their unintentional, or intentional, misuse is especially significant.

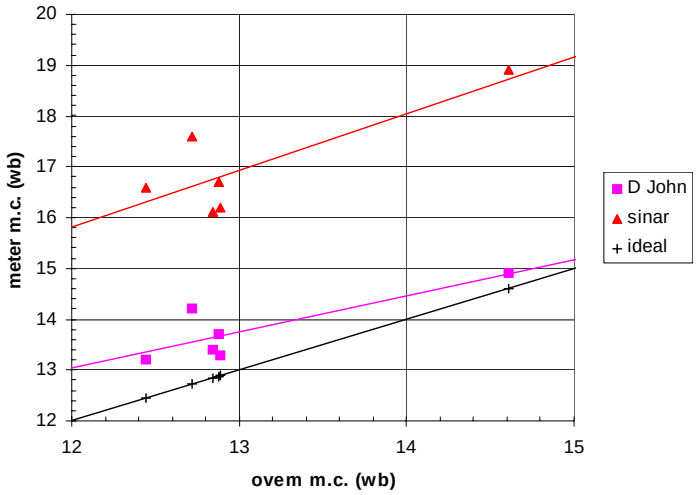
Monsooned coffee from India illustrates the potential inaccuracies caused by altered coffee properties. Monsooned coffee is a type of green coffee, produced from cherry that has been allowed to slowly re-hydrate in the high humidity of the Indian Malabar coast during the summer months, and then re-dried. The result is an especially bold bean of low density that is bleached white. In density it falls from around 1.2 sp.gr. of a normal bean, to 0.85 sp.gr. It is very soft to bite and easily bitten through, never mind dented, as in the traditional criteria for adequate dryness.

Several samples from different lots were taken and the m.c. measured using the oven method as well as a Sinar AP6060 and a Dickey John Multigrain moisture meter, both calibrated for green coffee (see Figure 3.3). Oven moisture content can be taken as the 'actual' moisture content.

The Sinar measured about 4% higher but in parallel, whereas the Dickey John was within 1% of the actual value between 18 and 12% m.c., but was not parallel, meaning that the extent of the error changes with the m.c.

Although monsooned coffee is exceptional, it demonstrates the principle that the physico-chemical properties of coffee can be modified according to its production history.

Figure 3.3: Oven moisture content vs. meter moisture content of several lots of monsooned coffee. The meters were calibrated for normal coffee. The sample with the highest moisture content is before polishing, the others are graded or un-graded, post polishing.



One possible mechanism here is that density affects the amount of dry matter in the sample chamber when the equipment uses volume to standardize sample size or less dry matter lies between the electrodes since the same amount of dry matter occupies more space. It may also be that different densities reflect different structural characteristics and these directly affect the electronic properties of the sample, which is what moisture meters actually measure.

The project's overall data suggests that the monsooned coffee's moisture content of 13% should correspond to a water activity level of approximately 0.64 rather than the 0.74 average that was actually obtained for this type of coffee. Evidently, monsooned coffee binds water less tightly than normal green coffee, the process makes it less hygroscopic meaning that there is greater water availability at a given m.c.. This suggests that the poor performance of two (high quality) moisture meters could be due to alterations in how water interacts with the coffee matrix, thus changing its electronic properties.

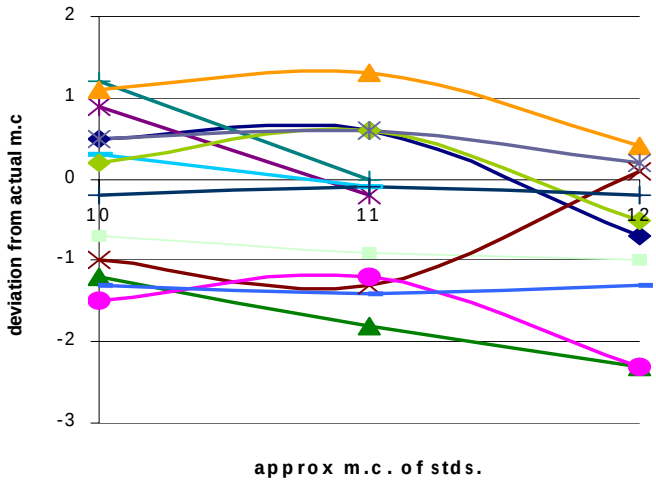
The fundamental characteristics by which equipment is evaluated is its accuracy (to what extent different samples produce a reading that is close to the respective actual value), precision (to what extent repeated measurements of the same sample produce the same result), and stability (how long the output is meaningful between calibrations and in different conditions).

Stability, a long-term characteristic, is difficult to evaluate effectively. One way to look at this is through the calibration records of an official calibration service such as the ISO approved service at the Indian Coffee Board. Figure 3.4 shows the evaluation

of several meters, all but two being Sinar moisture meters, received for annual calibration. Two meters are still well calibrated, but five have drifted off by between 1 and 2%, the remainder being out between nil and 1%. They are almost equally likely to drift up as down.

Ideally, the lines should be horizontal (that is, a constant error over a range of m.c.), since the calibration for these instruments can only be done as a one-point operation and falling or rising measurement indicates an in-built bias.

Figure 3.4: Performance on receipt of twelve moisture meters, ten of them Sinar moisture meters, sent for re-calibration. The x-axis is semi-arbitrary in that, for simplicity, only the approximate values of the standards are used in graphing and only the central range is shown.

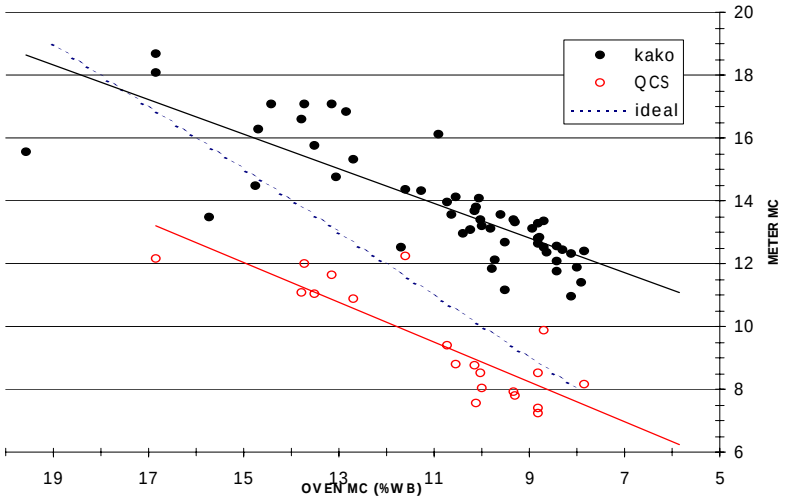


The collaborating centres were asked to test the relationship between the moisture readings given by the inexpensive moisture meters being tried under the project and moisture content obtained by the standard oven drying method. They all reported a strong linear relationship with very high values of the correlation coefficient. For example, with the instrument QCS-3Z the Colombian team reported r^2 values of over 0.97 for both parchment and green coffee and with the LSD-1D meter, values of 0.999.

However, when the internal instrument calibration was used for direct measurement the performance of the instruments was found to be unreliable in some cases.

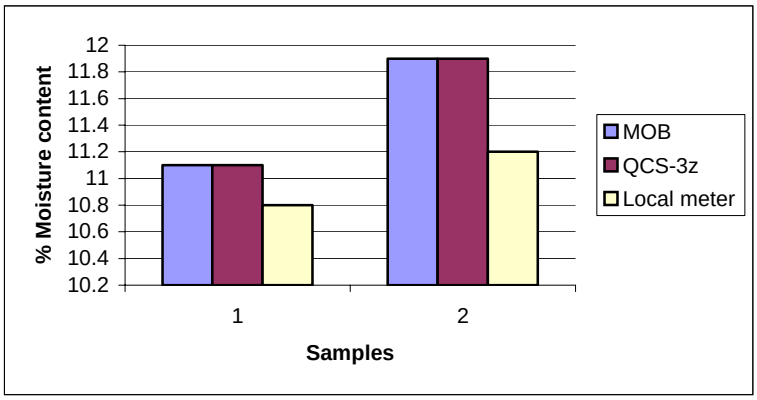
In Figure 3.5 two inexpensive meters, one of Indonesian design (Kako) and the other of Chinese origin are compared against oven dry weight determination. The fact that neither slope is parallel to the 'ideal' comparison (if the meter was in exact agreement with the oven) means that neither can be well calibrated, so both are accurate only around a narrow region. The QCS is only accurate around 7% m.c. and the Kako around 17% m.c.. However, personal experience with both of these meters indicated that they can produce better results than this graph would indicate.

Figure 3.5: Robusta cherry m.c. measurement comparing the performance of two inexpensive moisture meters with the reference method of oven dry weight determination.



In Brazil, however, in a smaller trial over a narrow moisture range (11-12%) the QCS-3Z was found to correspond well with the Official Brazilian Method (MOB)[3]. The QCS-3Z was found to be more accurate than another locally made moisture meter that is commonly used on bigger farms and cooperatives.

Figure 3.6: Results of moisture measurement in the 11-12% m.c. range using low cost equipment against official Brazilian oven test method.



In Figure 3.7 a protocol of measuring individual samples repeatedly shows that there is noticeable scatter. The average error from the actual value (oven dried) is 6.7% or 1% on a m.c. of 15%, but is only 1.1% error based on the cluster around the average of five determinations using the meter. In other words, the meter is more precise (repeatable) than it is accurate (representing the 'true' value).

[3] The Official Brazilian Method (MOB) is a gravimetric method, measuring weight loss of a sample of 50g, placed in an aluminium vessel in a drying oven at 105°C for 24 hrs.

Figure 3.7: Test of precision (repeatability) of the QCS Chinese 'scoop' moisture meter. Data from three machines is combined here because the results, after recalibration, were close, as can be seen. The red line gives the ideal fit.

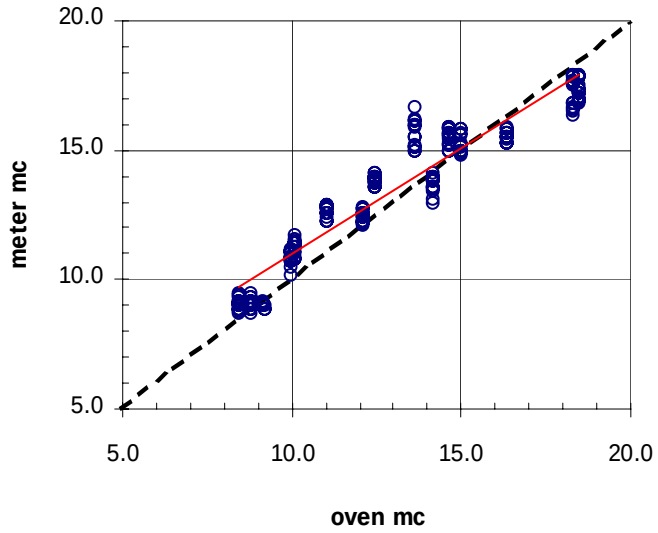
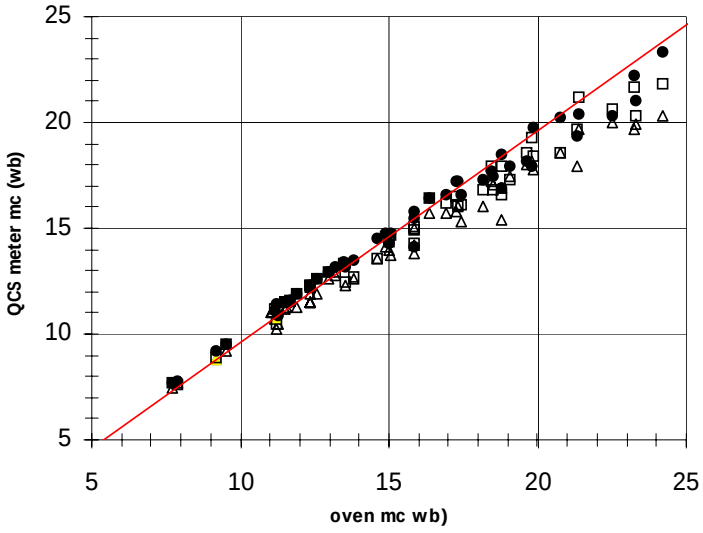


Figure 3.8 shows that the QCS meter gradually loses response and linearity between 15 and 20% m.c.. For use in this m.c. region the internal calibration would not be adequate, and ***to use the meter here would require a supplementary conversion chart***.

Figure 3.8: Extended oven/meter correlation showing the response range of the QCS Chinese 'scoop' meter.



If such low-cost meters are to be promoted for widespread field use, then it is important to assess the reliability and the stability of the instruments under realistic conditions.

Testing of the internal consistency and the stability of the instrument were only carried out under laboratory conditions. The performance of the instruments might be considerably different if subjected to rougher handling by farmers, small traders and extension agents. Sufficiently robust instruments are required for field use.

It should be noted that there were several reports from collaborators of having received faulty instruments, which might indicate a lack of quality control at the point of manufacture.

There are two points relating to any recommendation about the advisability of spending resources on the provision of moisture meters to producers and small traders. First, is their use really likely to give more accurate determination of water content in coffee in the early parts of the production chain than existing practices for its estimation? Second, will an improvement in the ability to estimate moisture content improve coffee safety and quality?

Biting and shaking are the most common methods used by farmers to judge whether coffee has reached a sufficiently dry state. There is no doubt that this approach has some efficacy even though the result can be affected factors unrelated to moisture content. A systematic assessment of the reliability and sensitivity of these empirical measures is warranted (refer to Section 3.5.5, below).

3.5.4 Appropriate technology for m.c. estimation

A. Test weight method:

Another technique, widely used in India, to determine when adequate dryness has been attained is referred to as the 'test weight' method. A standard volume (in India this is the 'forlit' = 40l) of coffee is weighed as the coffee approaches dryness and, when the prescribed weight is attained, the coffee is dry enough. The principle is based on the idea that as coffee dries it loses water, therefore becomes lighter and that this is a consistent relationship.

Of course, for an identified unit of dry mass this must be true, but, in practice, dry mass in a volume varies as the coffee shrinks, so the dry mass varies and the total mass of the volume varies as does density and packing rate.

The usefulness of this method was evaluated in two ways. First, drying trials that were being run under the project as part of the evaluation of drying surfaces were designed to incorporate parallel test weight determinations (alongside A_w, oven m.c., and meter m.c. readings). Second, it was possible to get data from well managed farms that had been using test weight, but had begun to use a m.c. meter and decided to use both methods until they felt they could rely on the meter alone. The data is graphically analysed below.

Note that the r^2 of these lines is very poor so they predict the relation between the measured parameters only poorly, and are included primarily as a reference to judge the degree of scatter. Nevertheless, the fact that the lines of different years have similar slopes suggest that over this narrow m.c. range, there is a consistent, if noisy, relationship between test-weight and m.c.

Perhaps the most clear-cut conclusion is that the relationship between test weight and m.c. can vary by year. In 1998 and 1999 a 17kg test weight fairly reliably assured that arabica parchment was below 11% m.c. In 2000, this guideline would yield coffee of 14 to 15 % m.c. (by extrapolation), and 16kg corresponded to the 11% target.

Robusta parchment from these years showed greater clustering, though the year 2001 is still separable from 1999 and 2000 (NB - robusta is harvested at the start of the year, so arabica year 2000 corresponds to robusta year 2001).

Collectively, this can be represented by the 'apparent density'. But which of these two parameters contributes most to the observed variation in the test-weight moisture content equivalence?

Figures 3.9 & 3.10: Arabica and robusta test weights and moisture contents (as measured by meter) for 1998-2000 from one estate in Coorg (Kadogu), Karnataka.

The number of data points is approximately equal for all years but may not appear so due to different frequency of over-lapping data. 'shan's' refers to data published by the Indian Coffee Board from Chickmagalur based on about 23 determinations from four estates, but with three out-lying points dropped. The lines are calculated omitting outliers in arabica 1998 and 2000, but the points are present on the graph.

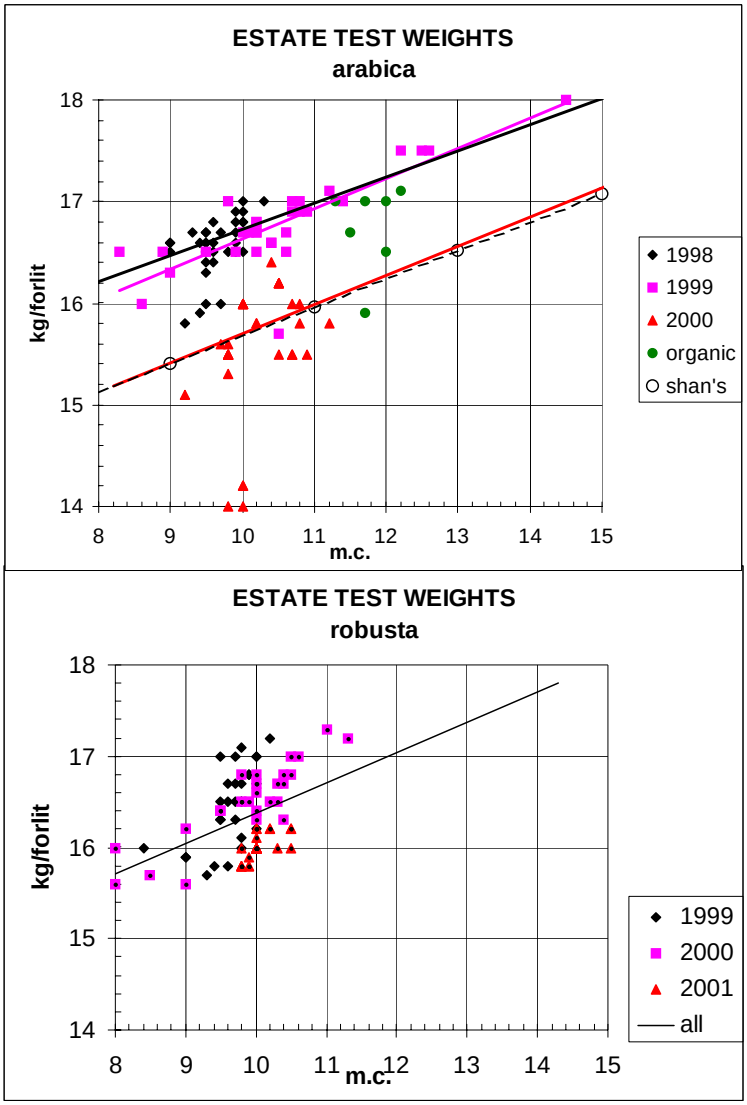


Figure 3.11, below, shows the scatter in apparent specific weight and Table 3.2 shows the breakdown of water and dry matter weights interpreted from this data.

The decline in apparent specific weight in samples of 14% m.c. to those at 9% is about 0.04. This implies a weight difference of 1.6kg on a constant 40l volume (the forlit

container) from an average forlit weight of 17.6kg at 14% m.c. Once the component weights of these two average samples are calculated, it can be seen that the change of weight from 14 to 9% is partly due to dry matter loss. This is a curious finding since it implies that the coffee dry matter is actually occupying more space as it dries through this region.

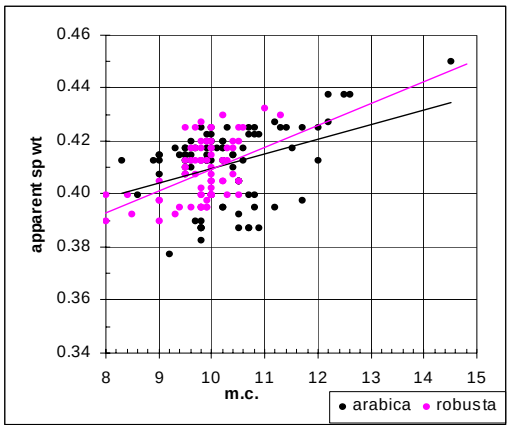
The fact that the coffee packs more loosely as it dries could be rationalized as an effect of the parch being strong enough to separate lighter coffee, but being compressed by heavier coffee. Significant error may well be invested here as well as it could be imagined that a high proportion of cracked parch or small beans could produce large packing errors.

Figure 3.12 deals with green coffee and shows that wt of 100 beans can vary much more than specific weight, thus bean size varies more than bean density. Density, itself, varies with a standard deviation of about 7% of the mean: the mean is 1.27 or 0.79ml per g of coffee. For a better feel of the impact of the variation in this data, consult table 4, which shows the values for the mean, standard deviation, 'edge' values and an outlier taken from this data set. It also applies the measured specific weight variation to a hypothetical 16kg forlit and shows that the actual volume occupied by 16kg of coffee can vary by almost 4l.

Based on other samples, apparent specific weight of green coffee was measured and comparison with actual specific weight shows that about 43 % of the apparent volume is empty space in green coffee and that this varies by at least 10%. If we apply the mean specific weight to the forlit data, we can estimate that parchment coffee is 65 to 70% empty space.

Figure 3.11 (below left): Apparent specific weight (effectively, g/ml) of all test weight data graphed against moisture content.

Figure 3.12 (below right): The actual specific weight of green coffee graphed against 100-bean weights. Data from the Indian Coffee Board - volume was measured by displacement of petroleum ether; all points are means of three determinations of each parameter. Samples are fully dried but vary in m.c.



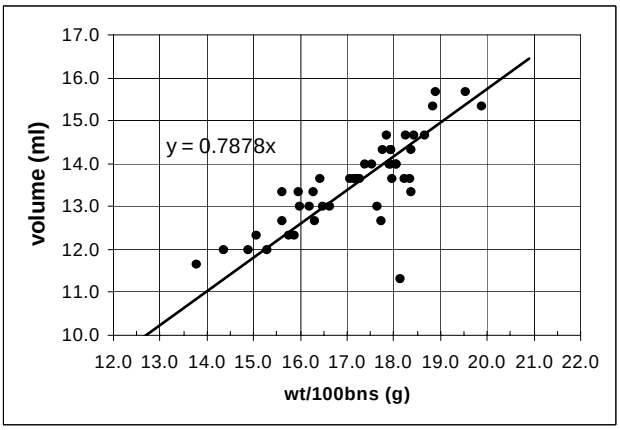


Table 3.2: Calculation table for water/weight relations based on the average of samples from three years of arabica and robusta parchment samples.

Apparent specific wt	m.c.	Total wt	Water wt	Dry mass	Δ water	Δ mass
.40	9%	16.0	1.44	14.6	-1.0	-0.5
.44	14%	17.6	2.46	15.1		

Table 3.3: Calculation table exampling the impact of variation in bean density on displacement volume, extrapolated to a 16kg forlit.

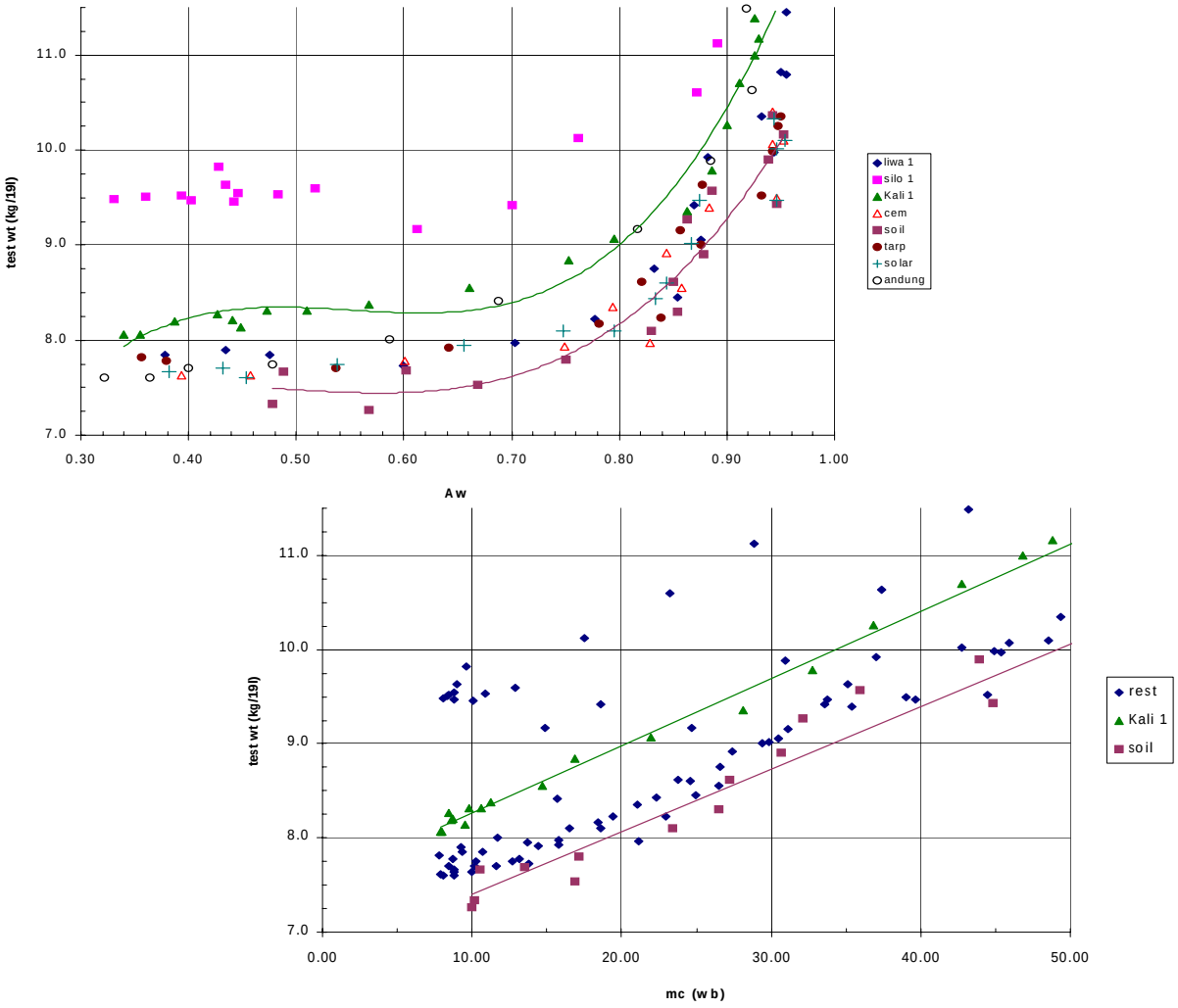
	ml/g	g/ml	Volume on 16kg
Outlier	0.62	1.61	9.9
Heavy edge	0.72	1.39	11.5
+ st dev	0.74	1.36	11.8
Mean	**0.79**	**1.27**	**12.6**
- st dev	0.85	1.18	13.6
Light edge	0.86	1.16	13.7

Coffee apparently has a significant inherent variation in density that may be great enough in magnitude to account for observed variation in test-weight estimation of moisture content. Indications are that climatic conditions play an important role in determining the properties of coffee in this respect.

The slope of the relationship of forlit weight to m.c. is always very shallow and this also contributes to the high error of the system. This is inescapable since the density of the coffee is almost 33% higher than that of the water, so as dry matter loading increases, due to shrinkage of the coffee as it dries, water loss is disproportionately masked. Only the space provided by the parch prevents this trend making the approach completely unworkable.

The drying trials give us the opportunity to assess the relationship of test weight with A_w as well as m.c., noting that A_w is the critical measure (Figures 3.13 and 3.14, overleaf).

Figure 3.13 and 3.14: Test weights from drying trials of robusta cherry from three regions of Indonesia against both A_w and m.c.. The four surfaces were all tested in Liwa. The graphs are rectified to the equivalent moisture for A_w/m.c. at A_w 0.80 according to this data set (= 20%). A_w fitted with 3rd order polynomials; m.c. by lines.



The regional differences in the weight m.c. relationship are evident: if we take an A_w of 0.60 as adequately dried (here that corresponds to 13% m.c.), 19l of dry cherry weighs about 9.25kg in Silo, 8.25kg in Kaliwining and 7.75kg in Liwa.

Note that weight changes very little against A_w below 0.70, the reason being that a very small loss of water causes a significant fall in A_w once the loosely-bound water is lost, and once added to packing error as discussed above. The late increase in weight, clearly seen in several of the data sets may be a real phenomenon as discussed above. Any differences caused by drying on different surfaces in Liwa are small.

At the very least this means that the test weight would have to be calibrated in each region. If the season-to-season differences shown in the Indian data are confirmed, this would infer an annual calibration in each region. Based on the findings of this work, the Indian Coffee Board has undertaken to revise its recommendations for target weights using the 'forlit'.

Another suggestion has been to adapt the test weight method by taking the endpoint of drying to be the stage at which the recorded weight change levels off, rather than the achievement of a pre-determined target weight.

An important point to remember in regard to this solution is that to err on the side of caution and to stipulate too rigorous an acceptability weight would mean that the farmer would be over-drying a lot of his product, thereby incurring a financial penalty as he is paid according to the weight of his coffee.

B. The EDABO method of moisture measurement:

Another method of moisture determination – known as the 'EDABO' method - has been successfully developed in Brazil, and may be useful in some situations.

The method is a variation on the Brown-Duvel distillation method, and involves measuring the direct evaporation of water (by weight loss) from a sample of green coffee heated in an oil bath. The equipment is robust, its components are readily accessible and relatively inexpensive, but the apparatus is not portable. Details of this method are included in Annex C.5 to this report.

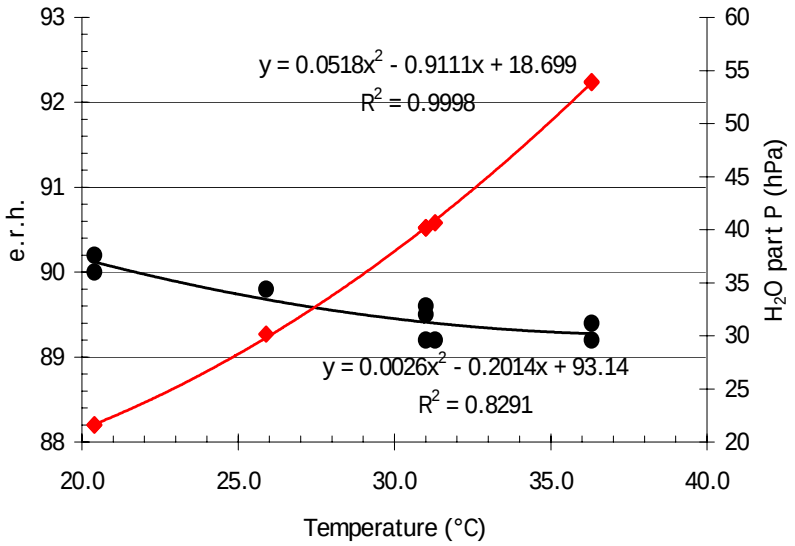
3.5.5 Characteristics of A_w measurement

Formally, A_w is the ratio of the relative humidity of the air in a closed chamber in equilibrium with a sample to the relative humidity of the air in a closed chamber in equilibrium with pure water, at a given temperature.

In other words it is the ratio of the relative humidity of air over a commodity to 100% humidity. Temperature determines the vapour/liquid equilibrium in any given system but, since it is two relative humidities that are used in the calculation, they both change in response to temperature at a similar (but not identical) rate so producing only a small error (see Figure 3.15).

The A_w remains the same within the rated precision of the instrument (±0.01 A_w or 1% ERH) between 20 and 37°C. Temperature disequilibrium or temperature differences between the probe and sample can produce large errors, especially problematic at high A_w.

Figure 3.15: Effect of temperature on A_w and partial pressure of water above rewetted arabica beans in a closed system. Repeated measurements allow assessment of instrument precision (repeatability).



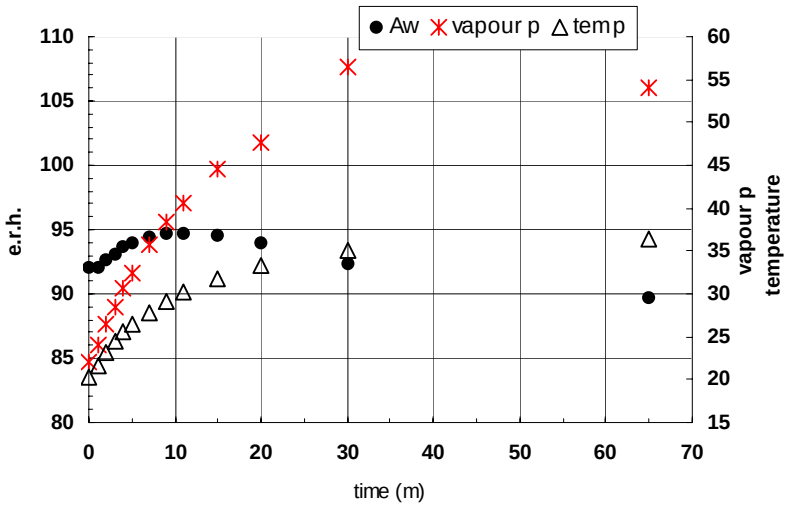
In practice, A_w is generally measured with a probe that measures conductivity or resistance of a hygroscopic crystal (traditionally LiCl), a property that changes with hydration level. The uniformity of the crystal ensures an accurate calibration. The crystal equilibrates with the air quickly, but an accurate measurement requires that the probe be in temperature equilibrium with the sample and that the sample is in equilibrium with the air around it in the closed measurement chamber.

The length of time required for these equilibrations varies with particle size (larger means longer) and the amount of water in the system (more water means a longer period is required). If the sample is in a state of disequilibrium, for example as it is being subjected to drying, an additional period is required for the particle to equilibrate within itself – the outer layer being dryer than the inner mass.

The main aspect that makes accurate A_w measurement difficult is the difficulty in reaching air/sample equilibrium. Temperature can be troublesome in the field but this is mainly a difficulty with temperature equilibrium since the main issue over normal temperature range is differences in temperature between the probe and system or temperature drift of the air which has a much lower heat content than the sample so changes more readily thus producing a disequilibrium.

Figure 3.16 demonstrates the effect of temperature disequilibrium on measurement. In fact, probably another hour would be required for the equilibrium to be fully established but this two hour period is small compared with that required to achieve moisture migration and equilibration in a sample such as coffee.

Figure 3.16: Equilibration time-course of a closed saturated KNO_3 solution system equilibrated at 20°C then moved to 36°C at t = 0. The changes are due to temperature re-equilibration.

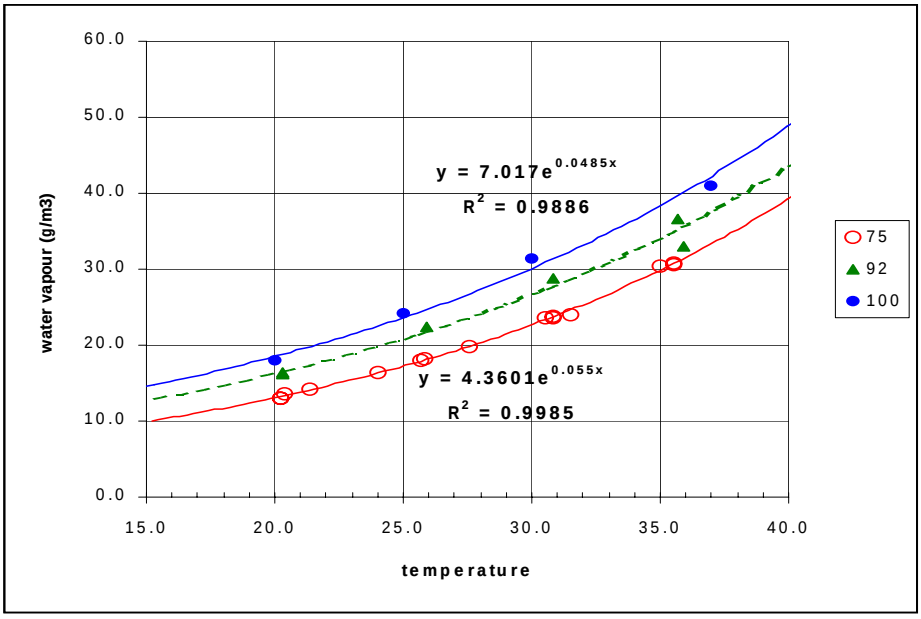


Water must migrate within the particles to reach internal equilibrium, then vaporize and condense to equilibrate with other particles and with the air of the closed measurement chamber. The rate of this migration is driven by gradients so the rate of redistribution falls as equilibration gets closer. This makes ascertaining whether equilibration has been reached, in real time, more or less impossible, especially so since the required period is partly a function of the degree of drying: wetter samples require more time. Experience has shown a 12-15 hour period is required for partly dried coffee in order to get a reliable determination.

The potential for evaporative loss or redistribution of water in a bi- or tri-phasic system is circumscribed by the ability of air to hold water. As discussed above, temperature is a key controlling parameter which is familiar to all. The absolute values of how much water is involved, as opposed to the relative measures that are conventionally used is helpful in understanding the magnitude of these reactions.

Figure 3.17 gives the temperature and humidity dependent water content of air. The blue line is water and so describes the solubility of water in air as affected by temperature.

Figure 3.17: Water content of air at different humidities and temperatures controlled by saturated salt solutions. The relation fits an exponential function.



3.5.6 Traditional methods of moisture estimation

The traditional means of assessing coffee for adequate dryness is by biting and/or shaking. If the bean is brittle, so that a strong bite on it does not result in an indentation, it is less than 12 or 13% m.c. However, it was observed that robusta is generally softer than arabica, and that some origins produce coffee that is very soft, in relative terms, but completely dry. However, this might not present a problem because the farmer concerned only deals with one kind and origin of coffee.

The shaking test is used on both cherry and parchment coffee. In both cases the dry bean in the husk or dry bean in the parch gives a higher pitched or more metallic sound when rattled (cherry), or rattled together (parchment). This phenomenon is due to the fact that the bean shrinks during drying leaving a space between the bean and the hull.

An attempt to measure the accuracy of traditional assessment was made as a part of a small farmer survey under the project, but this aspect of the survey was not sufficiently well planned and the findings are unreliable. The basic idea was to ask what level of dryness the farmer intended to dry to, then measure a sample of his coffee that he deemed dry using his method of assessment, and compare the result of the meter measurement with the intended m.c.

The samples that were judged to be 'dry' by the farmers using traditional methods turned out to have a wide range of moisture levels. However, the local team explained that it was likely that several of the farmers did not have fully dry coffee available (as often they sell quickly) but, feeling obliged to grant a request for a sample, would hand over whatever was available.

A more systematic consideration of these empirical methods is warranted. In the case of the 'biting' test, the Instrom Texture Testing machine could provide objective

evidence of whether biting - by a trained practitioner - might be expected to reliably determine a given point of dryness.

There is a real and valid question about ***why*** wet coffee is traded, but there is no clear evidence that this is due to an inability to distinguish slightly wet coffee from dry coffee. In most cases the farmer responds to market signals (within reasonable bounds).

Therefore, if the farmer is selling wet coffee, it is because the traders are happy to buy it, not because neither party knows that the coffee is too wet, had it been measured using other methods.

Section 4
Factors Affecting Drying Rate in Sun-drying

4.1 Introduction

Coffee is most commonly dried by spreading it in a thin layer under the sun. The exceptions to this are in certain origins with reliably rainy harvest periods that rely on mechanical drying, and large farms where the sheer volume of coffee makes the exclusive use of drying terraces impractical. But even where mechanical drying is conducted, sun drying is generally also necessary since (with the exception of certain designs of silo dryers), mechanical dryers require partially dried coffee with an input moisture content (m.c.) of between 35-40% (wet basis, wb).

The received wisdom that only sun drying can produce coffee of the best quality has less currency now, and better dryer design has encouraged their use over the past decade despite periods of low prices during this time.

Drying is a critical transitional period between the fully wet condition where toxigenic and spoilage moulds are controlled by hydrophilic organisms and seed physiology, and the fully dried state which prevents any further biological development taking place. The efficacy of sun drying is susceptible to poor weather, such as rain and dew, high humidity or clouds reducing solar energy. Obviously, these cannot be controlled. Management of drying is also affected by other processing activities such as harvesting or wet processing and, of course, financial constraints.

There are several parameters that affect, or may affect, drying rate and these may have a different impact and relative significance from climate to climate. So, aside from the management of drying within the production system, the impact of certain activities and parameters in different places requires evaluation.

In order to install a system to successfully control drying, two hurdles have to be overcome. First, a series of decisions have to be made as to what should be done, both in the normal course of events and also in exceptional circumstances, as far as can be anticipated, for best results. Second, actions have to be taken to ensure that these practices are actually and systematically applied. This may mean a regime of training, record keeping and monitoring.

This Section is about the first of these hurdles: what is the impact of certain common procedural alternatives on coffee drying. The activities and parameters that were evaluated include the relative efficacy of various drying surfaces and sun-based drying methods, loading rates and frequency of turning the coffee layer during the drying day, fruit maturity, and more basic studies on conditions of the layer of coffee as it dries. The determination of when drying is accomplished, and more fundamental studies concerning the association of water with coffee, are discussed elsewhere.

4.2 Findings and Application

4.2.1 Drying yard surfaces

None of the common drying surfaces produce consistently faster drying than the others (trial results are discussed in Section 4.5.2, below). This means that the selection of the surface should be based on considerations of practicality, given the nature of the climate along with financial considerations. Of major practical concern would be the prevalence of rainfall, which, if common, would require consideration of the ease with which the coffee can be protected and the drying yard drained and dried for a return to use.

The most critical set of factors regulating drying rate are meteorological and essentially not subject to control. However, optimising the location and surroundings can affect performance in so far as it selects the best available micro-climate for drying. The drying yard should be arranged so that it is exposed to the maximum amount of unimpeded sun and the prevailing wind.

One cabinet solar drier built in Uganda by the local project team indicated good drying behaviour, but was shown to be unfeasible for use by small farmers. The *parabolicos* drier, which has had a long application in Colombia, was found to require long drying times in almost all cases it was trialed. The poorer performance seemed to be linked to reduced airflow over the coffee.

4.2.2 Loading rate and stirring frequency

Loading rate of the drying yard is the major factor affecting the rate of drying. In practice the loading rate is dictated by the availability of space during harvest. With a knowledge of production levels and average drying rates, the required drying yard size can be estimated so to prevent over-loading. The fact that thicker layers significantly reduce drying rate means a feedback exists that intensifies the original problem of inadequate drying yard space if harvesting has not been completed. Parchment coffee is more susceptible to poor drying conditions because it is less protected.

There is some evidence that impervious drying surfaces (i.e. plastic sheets and tarpaulins) may exacerbate problems of using excessively thick drying layers due to the accumulation of condensate formed due to the temperature gradient across the coffee layer.

While stirring rate was not shown to influence drying rate, there is some evidence that stirring at least 4 times per day reduces the proportion of physical defects in comparison with stirring only once per day. It is possible that this effect could be attributable to more uniform drying. The uniformity of drying was not, however, measured in the trial in question.

Occurrence of OTA-producers and OTA contamination were not related to differences in drying behaviour observed. This suggests that, although adequate moisture is an essential condition for OTA production, other factors are involved in producing an unfavourable outcome. The results are discussed below in Section 4.5.3.

4.2.3 Split cherry drying

Splitting of coffee cherry is used in some countries as a technologically simple means to quicken drying. When split cherry drying was compared with whole cherry drying on the basis of equivalent weights of cherry per unit area (this means a much thicker layer of split cherry since this is a more bulky material) the former showed a reduction in drying time due to a higher maximum drying rate and the absence of a drying lag phase at the start of drying as was seen with whole cherry. When the two forms of cherry are spread on the basis of equivalent layer thickness the differences in drying behaviour are much more marked, with split cherry drying much faster than whole cherry.

There are indications that split cherries may be more prone to extra fungal contamination attributable to the destruction of physical barriers to fungal dispersal between fruit, and the ready availability of the sugars of the mesocarp to this inoculum. It is supposed that re-wetting could be especially serious. Under conditions of slow drying split cherry drying may be associated with a higher defect count as compared with parchment or cherry drying (split cherry drying is discussed in more detail in Section 4.5.4).

4.2.4 Overall fungal development in drying coffee

Fungal infection rates increase during drying, depending on the speciation in the initial community. Often the final infection rate is lower than intermediate rates, probably indicating mortality of some species in the conditions of the drying yard.

As a physiological class, the successful coffee seed inhabitants are mesophilic fungi that apparently have a facility for endophytism since they all have a well-documented ability as straight saprophytes. The period of most rapid increase in infection rate, which may be taken as an indication of an ability to grow (though not as a measure of actual growth), is a period of intermediate moisture content, which agrees well with what we know about the relative success of these species in a mixed community.

From these considerations, the best theoretical measure to predict OTA formation in drying coffee is the length of time during which the coffee is wet to the extent of A_w = 0.95 to 0.80. This period is related to drying rate, but this parameter did not predict outcomes in treatments under the project. This, we believe, is due to the balanced, multi-factorial system of coffee bean/fruit with active microbial and plant metabolism, in which moisture conditions usually function as a *pre-condition* rather than a forcing function.

4.3 Additional Notes

The strongest outcome of investigations on the factors influencing drying rates is that the single most important factor is prevailing weather during the period of drying.

Conceptually, the average climate of a region provides the background (or default) drying conditions, which can sometimes be exceeded, and can sometimes fall short.

This is not something that can be precisely measured, but there are other ways of seeing it in the field.

The drying time-course curve when plotted as m.c. (wb) has three identifiable phases. A flat lag accelerates to a linear period that eventually flattens as the coffee approaches dryness. With parchment, the initial lag is often all but absent and in very good drying conditions (parchment can dry in as quickly as 5 days, and cherry in as quickly as 8 days) the final flattening is minimised. The last phase is due both to the last water being held more tightly by the coffee, but also because the free energy drop from commodity to ambient, necessary for net water removal to occur, has diminished: the A_w of the coffee approaches the ambient A_w (i.e. the relative humidity, RH).

One aspect that is frequently overlooked is that moisture content is an average of a population of particles and little is known about the variation between particles. The distribution of mycotoxins is not one of uniform distribution over many particles. Rather, significant contamination is restricted to few seeds so it would be useful to know if water distribution in a population of seeds is of this form too.

4.4 Experimental Design

In designing experiments where samples represent points along a course of steady change, the experimentalist can take advantage of the fact that adjacent points support each other. This is calculated as a regression, represented by some mathematical form, and shares some properties with the mean of measurements of a stable situation, but is somewhat more difficult to handle. In effect, the measurements characterising the effect of a treatment are dependent, but they are not equivalent.

One of the major problems in designing experiments in this area of inquiry is that the conditions under which a given experiment is run will not be the same as subsequent runs – weather varies, especially when comparing experiments run on three different continents. Indeed, it turned out that it was this difference, rather than the treatments, that was the most prominent factor in the outcome of a drying run. In other words, there was an interaction between the conditions of drying and the treatments that could not be controlled. This has the advantage of exactly representing the real conditions of coffee production but it could, and in fact did, cause the relative results, the ranking of the treatments, not to be repeatable. It also prevents the results from different runs being pooled, since the fastest drying rates measured in poor drying conditions are frequently slower than the slowest rates measured in good drying conditions.

Early studies had shown that the amount of coffee in the tests was important because it was observed that small amounts of coffee dried unrealistically quickly. On the other hand, the larger the amount of coffee in the test, the poorer small sub-samples represented the whole so it was desirable to minimise the total amount for the veracity of mycological and OTA analyses.

The intention was to represent a part of coffee in a large mass of coffee, that is, in a lot that has no open sides, just more coffee around it. We arrived at a convention of

using 2m^2 of coffee enclosed in a wooden frame of that dimension and of close to the intended height of the layer. Because differences tended to be small, numbers of replicates were maximized in the side-by-side tests and runs repeated.

The drying time-courses, aside from testing drying yard management parameters, were also designed to test and compare moisture measurement and acquire data for desorption isotherms. Oven drying was used as the reference m.c. determination method supplemented by A_w determination measured below 0.95, after a 15 hour equilibration period to give the full picture of water status. Moisture meters were often used once 25% m.c. was attained. In some studies the weight of a constant volume (i.e. test weight) was measured.

Sampling from the frames described above was completed by combining small amounts of coffee removed from at least six positions around the frame. In addition, daily stirring comprised a part of all treatments so the sampling was randomised by this means as well.

4.5 Experimental Results and Discussion

4.5.1 General considerations in sun-drying

The purpose of drying yard operations is to remove water from coffee without compromising its quality, so as to stabilise the commodity. Coffee is not dried as individual particles but as particles in a layer. The thermodynamic driver for drying is the establishment of a moisture gradient between the layer and some 'sink'. It is in maintaining the steepness and persistence of this gradient that good drying is achieved.

4.5.1.1 Moisture and temperature gradients across coffee layers

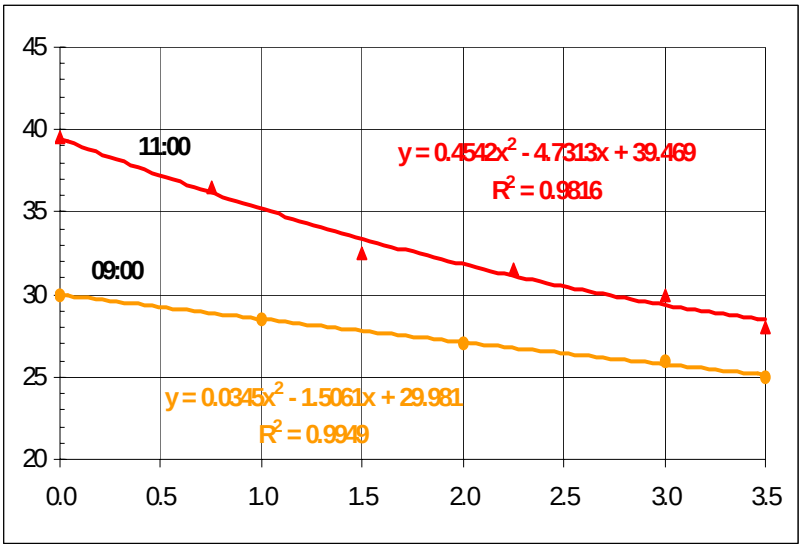
The area of the layer is an essential feature because it defines the surface area for absorption of solar energy, which is the energy input providing the heat of vaporization required to evaporate water, and the surface area for gas exchange. The thickness of the layer below this surface determines the total amount of water to be lost and the proportion of the total that is exposed to drying conditions (the uppermost part) relative that under condensation conditions (the lowermost part).

Cherry drying is more difficult than parchment drying not just because there is almost twice the amount of water to remove, but the bean shrinks away from the husk forming an air barrier between the bean and husk across which an internal gradient must form. At night this gradient would dissipate if the humidity was high, and perhaps even reverse if there was dew or condensation, and drying of the bean would be slow to begin each morning. This would be expected to exaggerate the differences in cherry drying times between regions with humid harvest periods and regions with arid harvest periods beyond any differences that might be noted in maximum drying rates.

In Figure 4.1 it can be seen that the difference between top and bottom of the layer doubled, in increasing by 5°C in two hours. The 10°C difference between the top and bottom of the layer will cause condensation of vapour evaporating from the upper

parts of the layer, diffusing in all directions and saturating the interstitial spaces. The vapour will migrate toward any vapour 'sink', preferentially along the steepest gradient. The coolness of the lower layers, acting like a cold-finger, competes with the air of the boundary layer. A non-porous limiting surface, such as plastic, would prevent this condensate from escaping and thus could cause an increase in m.c. in the lower reaches of the layer as the topmost dry.

Figure 4.1: Development of the temperature gradient of a bed of fairly wet parchment coffee from 09:00 to 11:00 in a parabolic dryer with a cement floor.



The maximum temperature experienced in a bed of drying coffee follows that of the air temperature so it peaks typically at about 14:00. What is perhaps more interesting is the progressive increase of the maximum temperature as the coffee dries. This shows the cooling effect of evaporation, of course diminishing as water loss rates fall with the increasing dryness of the coffee.

Figure 4.2: Temperature in a bed of robusta cherry at 08:00, 14:00 and 16:00 as drying progresses.

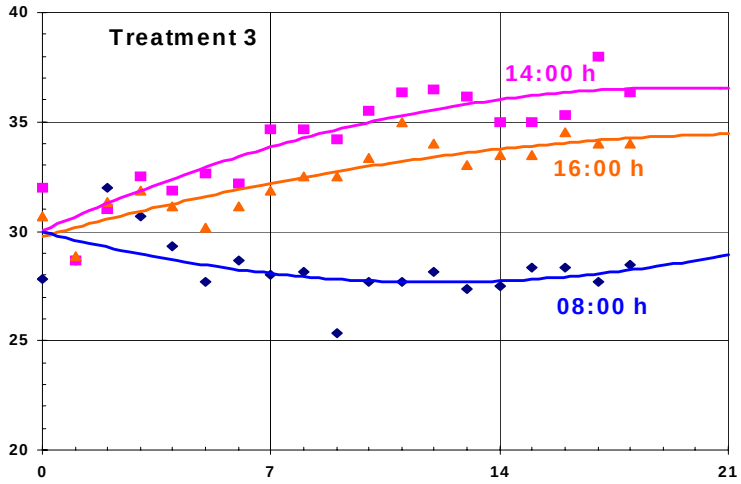
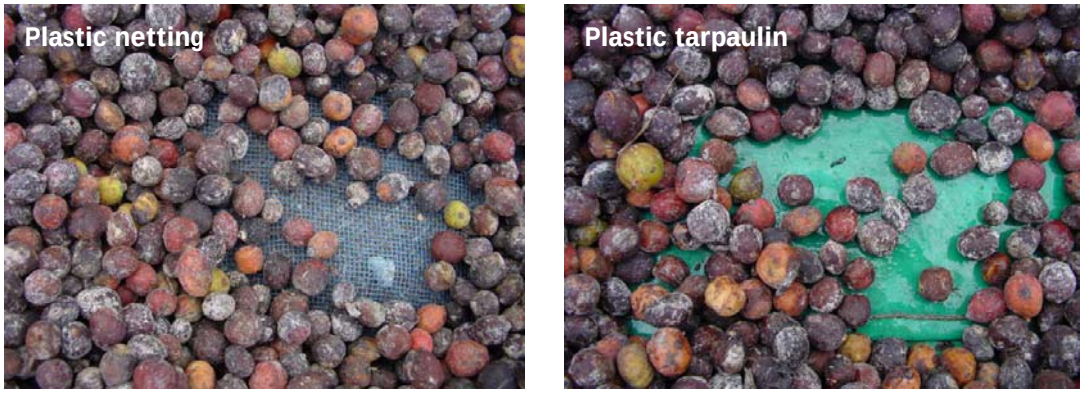


Image 4.1 shows that this is actually the case, there is condensation at the base of the layer and this water is undoubtedly condensate from water lost from the upper

layers. Often in comparing drying surfaces, it was noted that the coffee on tarpaulin showed superficial mould development though in the trials pictures below, both the porous and non-porous surfaces show this. Parchment coffee would be expected to be more susceptible to quality loss than cherry since the bean is less protected.

Image 4.1: Drying robusta cherry after five days on plastic tarpaulin and plastic netting. Note the condensation glistening on the plastic tarpaulin.



4.5.1.2 Controlling operations on the drying yard

Four potential parameters that must be controlled to ensure best practice are discussed: Size of drying yard; drying technology; loading rates; stirring or turning rates. Some comparisons of cherry drying were made between maturity groups. Other aspects such as routines for protection from rain and dew and protection of parchment from high sun was not systematically studied. Mechanical drying was not studied.

Determining the required drying yard area is informed by loading and drying rate studies. Determination of dryness is discussed elsewhere (see Part C, Section 3 on 'Water Measurement in Coffee'). Two models ('rules of thumb') are presented below to aid the assessment of drying yard area requirements.

First rule of thumb for drying yard area requirement: Expressed in various units, for cherry drying, is as follows:

$$\mathbf{S = 5 \times 10^{-4}\ Q.T}$$

where:

S = area required for drying in m^2 /1000 trees if
Q = annual average yield of cherry coffee, in liters/1000 trees

or

S = area required for drying in m^2 /ha if
Q = annual average yield of cherry coffee in litres/ha

or

$$\mathbf{S = 7.9 \times 10^{-4}\ Q.T}$$

where:

S = area required for drying in m^2/ha if
Q = annual average yield of cherry coffee in kg/ha

and

T = average drying time in days.

To accomplish only partial drying (from 60% moisture content to approximately 30% wb), in order to use complementary mechanical drying, the drying terrace area can be reduced to 33% of the original value.

Second rule of thumb for drying yard area requirement: The rationale here is that harvest rate approximates a bell-shaped curve that peaks at about 2% of the total harvest brought in per day. This notion is combined with an assumed residence time on the yard (i.e. time required to achieve dryness) of three weeks for cherry and two weeks for parchment coffee. It is also tied to a loading rate of 30kg/m^2. It should be pointed out that thickening of the layer during the later drying stages and use of methods such as conditioning bins or mechanical drying, and rapid harvesting methods such as stripping and especially mechanical harvesting, would throw off the estimate. The rule of thumb is best adapted for family farms.

$$S = Q \times (0.02 \times D)/L$$

where:

S = area required for drying in m^2/ha of plantation
Q = annual average yield of cherry coffee in kg/ha
D = average drying period (days), nominally 21d for cherry and 14d for parchment
L = loading rate on the yard, nominally taken to be 30kg/ m^2 cherry or 40kg/m^2 parchment.

The basis for this estimate is for a two or three month harvesting period with the most intense harvesting at mid-season. This is not valid for small farms where often the entire harvest is realised in two weeks or less, nor is it suitable for mechanical harvesting which also compresses the harvest period.

4.5.2 Comparison of drying surfaces in sun-drying and selected solar driers

One way to approach the question of the suitability of one surface over another is to look at it as a piece of equipment designed to aid the extraction of water from coffee.

This can be expressed as days required to achieve a dryness criterion (12% m.c. or A_w of 0.80 to mention two examples), maximum rate of drying (fall in m.c./d), or period residing at a moisture level giving an A_w between 0.95 and 0.80. For the purpose of general evaluation of the equipment, the maximum drying rate has been used.

Table 4.1 collates the statistical analysis of the effect of drying technology on maximum drying rates. As mentioned above, the largest factor is, in fact, the meteorological conditions during the period of drying, in part determined by location and, in regions with extended or multiple harvest seasons, time of year. The same comparisons in different runs do not tend to produce consistent relative efficiencies.

If the results in Table 4.1 can be generalised, it would be to say there is little to distinguish a cement surface, tarpaulin, table, soil or cow dung plaster surfaces in terms of drying rate over a wide range of conditions. The *parabolicos* solar dryer tended to reduce the drying rate in most of the cases where it was tested.

Table 4.1: Synopsis of statistical analyses of drying technology comparisons conducted during the 2004 harvest season.

First half year 2004		Second half year 2004	
Country	**Conclusions**	**Country**	**Conclusions**
Uganda Robusta Dry process	Tarpaulin > Ground = Cement	**Uganda** Robusta Dry process	Cement > Soil (Run 1) Cement < Soil (Run 2)
Kenya *Kirinyaga* Arabica Wet process	Mesh 1 = Mesh 2 = Sagging mesh > *Parabolicos* (Run 1) Mesh 1 = Mesh 2 > Sagging mesh > *Parabolicos* (Run 2)	**Uganda** Robusta Dry process	Cement = Tarpaulin (2 Runs)
Kenya *Kitale* Arabica Wet process	Mesh 1 = Mesh 2 = Sagging mesh > *Parabolicos*	**Indonesia** Arabica Wet process	Tarpaulin > *Parabolicos*
Côte d'Ivoire Robusta Dry process	Tarpaulin = Cement = Bamboo table = Ground	**Indonesia** Robusta Dry process	Tarpaulin = Cement > Solar dryer = Soil
India Robusta Dry process	Ground = Tarpaulin = Cow dung	**Brazil** Arabica *Decascado*	*Araponga:* Table = Cement (Run 1) Table > Cement (Run 2) *Ibituruna:* Table = Cement (2 Runs) *Coromandel:* Table > Cement (Run 1) Table = Cement (Run 2)
India 1 Robusta Dry process	Tarpaulin > Ground = Cow dung (Run1) Tarpaulin > Ground = Cow dung (Run 2)		
India 1 Robusta Wet process	Cement = Tarpaulin > Parabolicos		
India 2 Robusta Dry process	Tarpaulin = Ground = Cow dung		
India 2 Robusta Wet process	Cement = Tarpaulin = *Parabolicos*		
Colombia Arabica Dry process	Tarpaulin = *Parabolicos* = Table		
Colombia Arabica Wet process	Table = *Parabolicos*		

For background, an analysis is presented below pertaining to some of the Brazilian work noted in Table 4.1 above.

The idea of assessing the performance of drying equipment is usefully explored by evaluating efficiency, based on the ability of the equipment to convert energy into work. Evaporative loss is a measure of potential drying, or available input energy, integrating sunlight, temperature, air circulation, humidity and atmospheric pressure into one practical unit. The ratio of actual water loss from the coffee to potential water loss gives an estimate of how close the potential is realised or the efficiency by which potential drying is converted to actual drying.

Not all the data is as neat as that for the time-course of the second run in Coromandel, Brazil as shown in Figure 4.3, partly due to the labile nature of ratios when the denominator is near zero and when there is an increase in weight due to rainfall. The dataset contains days where no evaporation took place (so 'zero' was replaced by 0.1 in order to evaluate the efficiency ratio) and when the weight of the coffee increased, presumably due to rain. One explanation for the lability of the measure beyond the characteristics of ratios is that water loss is related to the evaporative demand in a discontinuous relation: such that different water contents have threshold energy requirements below which there is negligible water loss, and above which water is lost quite freely.

Figure 4.3: Drying efficiency of *descascado* table drying, expressed as the ratio of water loss from the commodity to evaporative loss, plotted against days drying and fitted with a 'power' function.

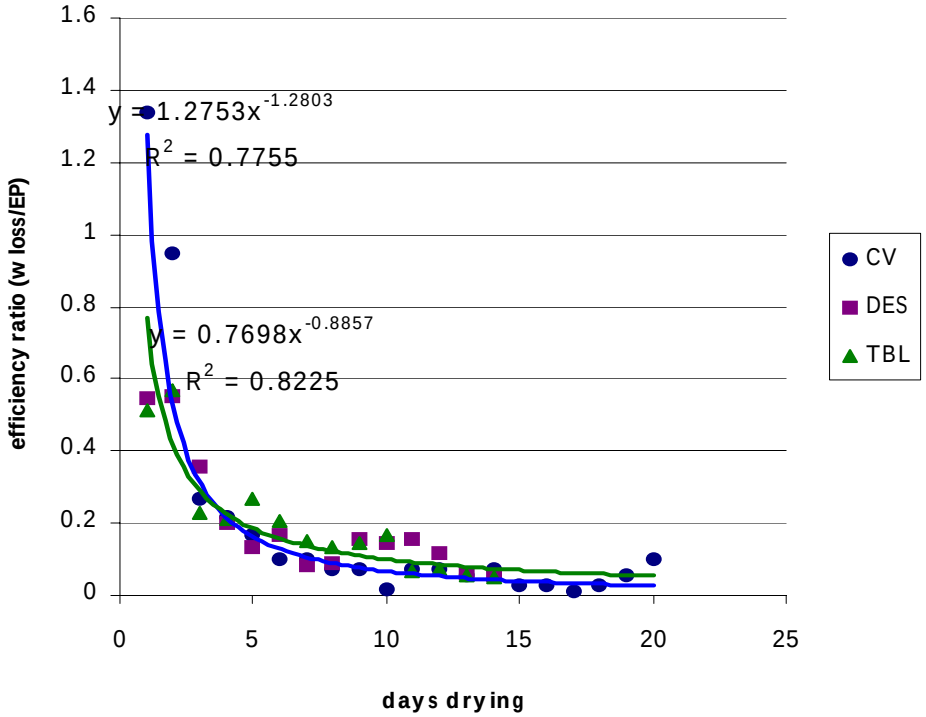
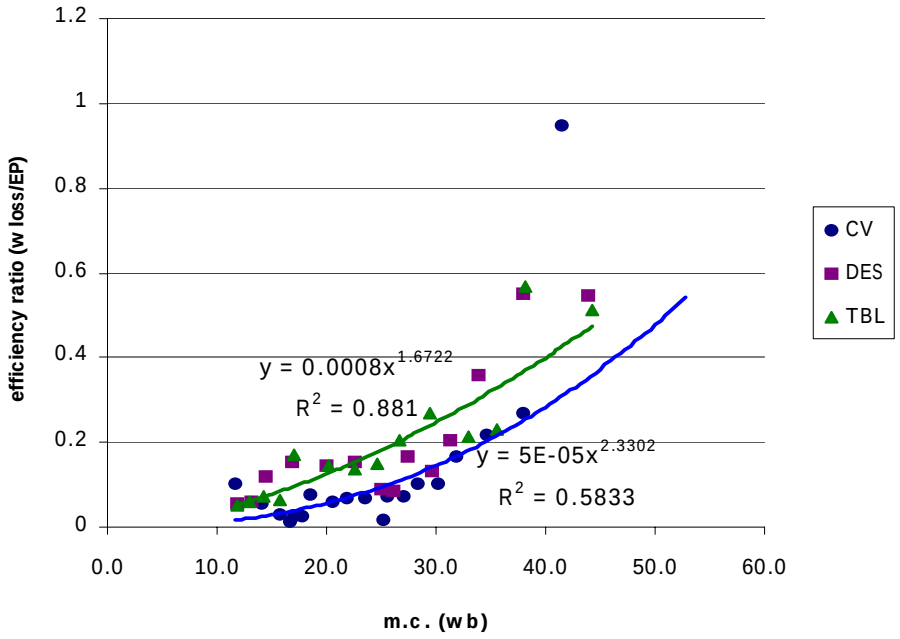


Figure 4.4: Drying efficiency of *descascado* table drying, expressed as the ratio of water loss from the commodity to evaporative loss, plotted against moisture content.



The three treatments are overlapping throughout the time-course, but the main feature is that efficiency falls approximating a power function. This implies that the difficulty in removing water from coffee rapidly increases with dryness or, theoretically, that the drying equipment works better with wetter coffee. The latter possibility is not plausible given the simplicity of the equipment (a surface) so a graph of the relation between efficiency and water content (Figure 4.4, above) gives this relationship.

Although the precision of the relation between the efficiency of water removal from coffee and its state of dryness is not good, it is clear that water is removed with considerably more difficulty as it dries.

The main difference between parchment (DES and TBL in the Figures above) and cherry drying is in the initial period where there is much more easily removed water in cherries. This is especially evident in Figure 4.4 where there is an indication that water removal from cherry is generally 10 to 15% more difficult to remove than from parchment. Most of the change in efficiency takes place in the first five days and falls slowly from about 20% efficiency at a m.c. (wb) of about 25%. There is no measurable difference between cement and table dryers.

Table 4.2: Synopsis of results from Araponga, Zona do Mata (ARA) and Coromandel, Cerrado (CORO) from drying of cherry coffee (CV) and depulped coffee (*descascado* = DES) on cement and tables. Two replicates in each of two runs. The cup grades were assigned numbers: only soft; soft, hard, rioy, rio were assigned numbers 5 to 1 since 'soft' is considered superior to hard and 'rio' is a defect.

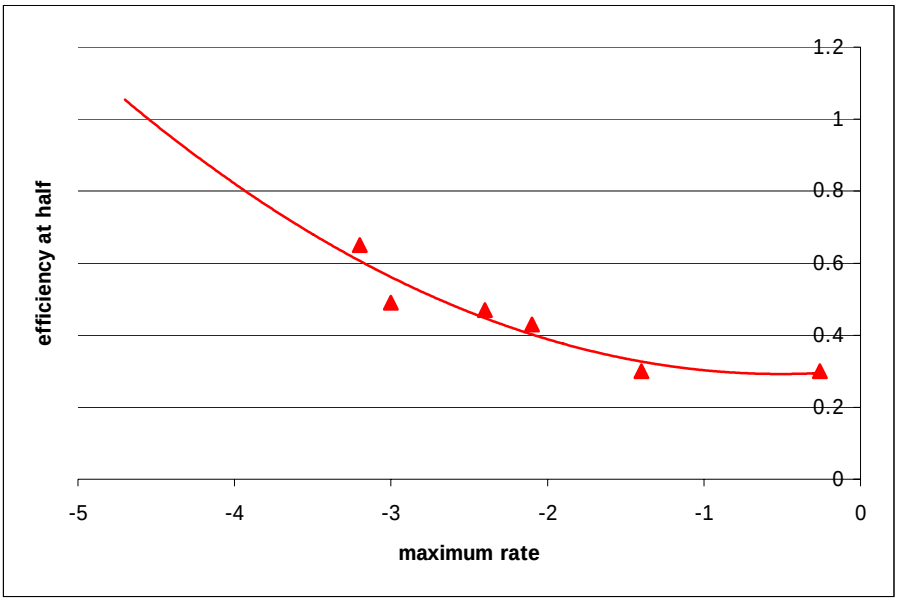
		Means	ARA	CORO
CV	**Cement**			
	Max rate	2.9	-3.2	-2.6
	Efficiency	0.48	0.65	0.30
	Drying time	20.0	20.0	20.0
	Cup	3.0	2.5	3.5
DES	**Cement**			
	Max rate	1.9	-1.4	-2.4
	Efficiency	0.38	0.30	0.47
	Drying time	20.4	26.3	14.5
	Cup	2.7	1.8	3.5
	Table			
	Max rate	2.6	-2.1	-3.0
	Efficiency	0.46	0.43	0.49
	Drying time	15.7	19.3	13.0
	Cup	3.7	3.5	3.8

NB - efficiency here is calculated from the mean of the two data points closest to half dryness (taken as (62%-12%)/2=25%; 12% + 25%=37%).

Figure 4.5 shows that there is a reasonable relation between the maximum drying rate and a measure of efficiency, which is the drying efficiency at half dryness as described in Table 4.2. This is confirmation that within the treatment variations, that is, sun drying of cherry and parchment dried on cement and tables, evaporation predicts most of water loss rate, regardless of the nature of the coffee and dryers. By extrapolation it predicts a maximum rate of water loss as 4.5%/day which has been exceeded in other trials but may pertain to the conditions of the Brazilian test.

Solar dryers: Three solar dryers were field-tested in the course of the project (see Image 4.2, below). The *parabolicos* is, in effect, a plastic greenhouse without ends and it increases the air temperature in the structure while inhibiting air circulation and protecting the coffee from rain. The lack of air circulation may have more than compensated for the increased water-holding capacity of the warmer air since it would rapidly become saturated without steady exchange. This was not solved by closing the ends and providing periodic modest fan-assisted air exchange.

Figure 4.5: The relation between the maximum drying rate and efficiency at ½ for the average values of the Araponga and Coromandel trials of 2004. Efficiency at ½ is explained above in Table 4.2.



The cabinet dryer was selected from several designs contributed by the Ugandan collaborators, all of which are pictured below. Its main design innovation is the combination of a chimney and a solar collector (seen leaning against the back of the cabinet in the picture) to increase the solar input beyond the area occupied by the coffee itself. The collector extends from the thin end of the wedge-shaped cabinet.

Despite the request of the central project team that solar driers tested by the project should be designed on the basis of direct feedback from farmers on what features (capacity, cost, mobility, etc.) were required by them, this was, unfortunately, not done. Evaluation of the feasibility of the solar designs later showed them to be unsuitable for use by smallholder coffee farmers. They are discussed here only for interest.

The ITIPAT is an earlier design featuring a tilting bed that can be adjusted to optimise the incident radiation angle and provide a chimney effect based on the heated air rising out of the top, drawing in fresh cool air through the screen at the bottom.

Image 4.2: Three solar dryer designs field-tested during the course of the project.

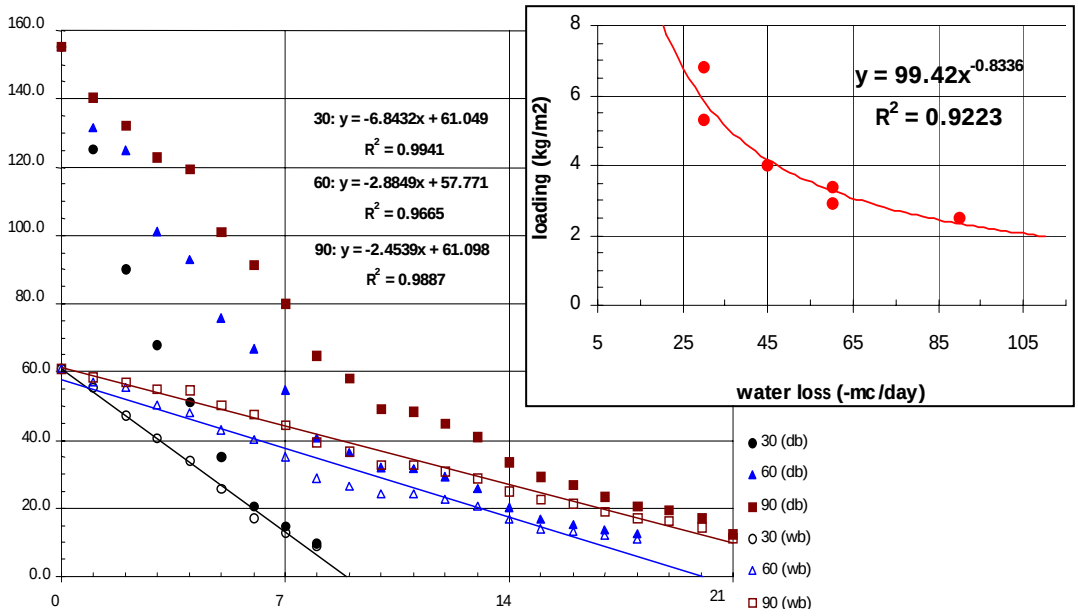
In Table 4.1, above, it can be seen that in 5 of 8 comparisons the *parabolicos* dried significantly more slowly than sun-drying, and in the other three comparisons was only equal.

There were encouraging results from the cabinet dryer in terms of drying rate. No control (sun-drying) was run side-by-side in these trials, but the mean of sun-drying during the same season was 4.5 (loss in m.c./d) at a loading of 30 kg/m^2, so the cabinet can dry about 40 kg/m^2 at that rate (see Figure 4.4) or about 30% more than sun drying.

Mycological analysis showed that niger aspergilli infect 100% of the beans in the loading at 45 kg/m^2 and up, similar to most dry Ugandan cherry coffee. There was little ochre aspergilli in the experiment, all in the second run, in the 30 and 45 kg/m^2, dry samples at a few %. No OTA analysis is available.

Note that the drying rates expressed on dry basis in the figures above are either linearly bimodal or geometric, in some form. This is an interesting observation because the relation of m.c. (db) to water loss (as opposed to days) during drying is also essentially linear while m.c. (wb) expressed against water loss is geometric demonstrating that water loss is more difficult as the coffee dries. The use of the wet basis expression of m.c. masks this important fact.

Figure 4.6: Drying time-course of cabinet dryer (Ugandan design) loaded at 30, 60 and 90kg cherries/m^2 expressed as loss of m.c. wet and dry basis. First run. Inset is a graph of drying rates (m.c. dry basis) of both runs fitted to a power function.



Condensation was commonly observed in the ITIPAT dryer. This is a very significant factor and may cause mycological problems even if drying rates of the coffee appear to be favourable.

4.5.3 Influence of loading rates and stirring frequencies on sun-drying

In the field, loading rate is measured by thickness and usually is subject to the immediate requirements of the producer given a fixed-size drying yard at his disposal.

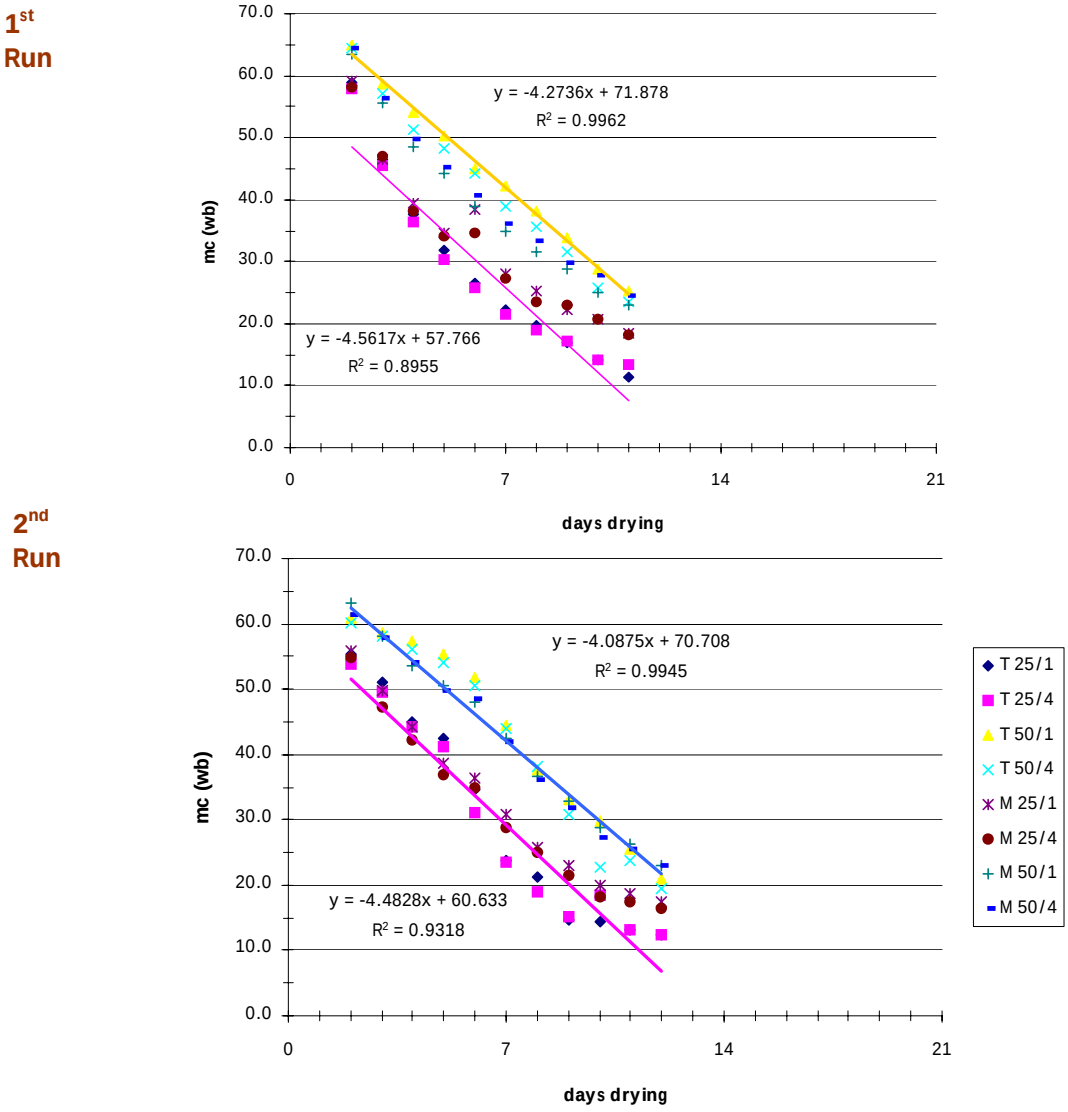
A layer of 4-5cm corresponds to 25kg/m^2, which is the recommended loading rate based on the balance between drying yard space and drying rate. One centimetre of coffee spread over 1m^2 amounts to about 5kg. The harvesting rate is often inadequately controlled and excessively thick layers are commonly observed in the field. Often the yard is adequate most of the time but is inadequate at peak times or during peak times in high yield years. Mechanical dryers and conditioning bins are two methods used to relieve pressure on drying yards in these situations.

Therefore, it is important to know what the impact of layer thickness on drying rate is but also if the usual means of increasing the drying rate, stirring, can be used to reduce the impact of thick layers of coffee. In Kenya, fermented parchment was dried on wire mesh or tarpaulin at either 25 or 50 kg/m^2 and stirred either once or four-times per day. Each 'experimental unit' comprised 2m^2, each treatment was replicated three times, and the experiment run twice.

Figure 4.7 shows that the treatments had surprisingly small effects on the drying rates over the first 11 days but there are big differences between the y-intercepts of the lines describing the drying time-course. The initial m.c. was actually very similar for all the treatments. The treatments that caused an extended the drying time appear

to have been wetter to begin with because they dried more slowly at the start before eventually drying at close to the same overall rate.

Figure 4.7: Drying time-course between Day 2 and 11 or 12 for arabica parchment drying on tarpaulin and wire mesh at 25 and 50 kg/m^2 and 1 and 4 stirrings per day. There were two runs with each treatment replicated three times.



But there is a second feature observable in the graphs. The treatments that were quicker to dry also have a lower r2 value, indicating a poorer fit to the linear regression. This is due to a brief period of fast drying between Day 3 and Day 8. This could be due to a period of very good drying conditions that had little beneficial effect on the thicker and less well stirred treatments but more likely, since it can be seen in both runs, it represents a subtle feature of parchment drying. Table 4.3 examines these transitory maximum rates since they may be more helpful in understanding the effect of the treatments on drying, *per se*.

Table 4.3 compares the treatments in terms of drying rates between Day 3 and Day 8 by sorting the data, first according to the outcome of the first run, then by second run and finally by overall means. The standard error of this data set is mostly low at well below 5% so the treatments are grouped around 10% intervals to give some idea of

how the treatments cluster according on how fast they can dry coffee. On tarpaulin the rate is high regardless of the stirring rate between one and four times daily. When the two runs are combined, all the other treatments form a continuum but it seems that tarpaulin is more affected by heavy loading rates than is wire mesh. Stirring between one and four times daily does not appear to affect these drying rates.

Table 4.3: A comparison of treatment drying efficacy as measured by drying rate between Day 3 and 8, a period that showed the fastest rates. Loading was 25 or 50 kg/m^2 and stirring was 1 or 4 times daily. Coffee was parchment and each treatment was replicated thrice in each of two runs.

Sorted by 1st run

			1st	2nd	Means
TARP	25	1	-5.30		
TARP	25	4	-5.18		
MESH	50	1	-4.77		
MESH	50	4	-4.57		
TARP	50	4	-4.27		
MESH	25	4	-4.24		
TARP	50	1	-4.08		
MESH	25	1	-3.91		

Sorted by 2nd run

TARP	25	4	-5.18	-6.44
TARP	25	1	-5.30	-6.30
MESH	25	1	-3.91	-4.65
MESH	25	4	-4.24	-4.38
TARP	50	1	-4.08	-4.19
MESH	50	4	-4.57	-4.16
MESH	50	1	-4.77	-4.09
TARP	50	4	-4.27	-3.98

Sorted by overall means

TARP	25	4	-5.18	-6.44	-5.81
TARP	25	1	-5.30	-6.30	-5.80
MESH	50	1	-4.77	-4.09	-4.43
MESH	50	4	-4.57	-4.16	-4.36
MESH	25	4	-4.24	-4.38	-4.31
MESH	25	1	-3.91	-4.65	-4.28
TARP	50	1	-4.08	-4.19	-4.13
TARP	50	4	-4.27	-3.98	-4.13

The mycological impact of the treatments is reported in Table 4.4. There was little in the way of OTA-producers detected in these studies. Six of seven positive samples were from the final, dry parchment. Half of these six samples had been dried on tarpaulin and half on wire mesh and the levels of occurrence were indistinguishable, lying between 1 and 3% infection rate. Four of the six positive final samples had been dried at the recommended optimal loading of 25 kg/m^2 and only one came from a 1 stirring per day treatment. In fact, neither of the 50 kg/m^2, 1 stirring/day treatments produced OTA-producers at detectable levels.

Fungal infection rates were uniform and low, and lower in the dry parchment than in the initial beans. In Run 1 the highest recorded final overall infection rate was 12%. The highest ochre infection rate in the study was on wire mesh at 50kg/m^2, stirred 4 times daily with 3% accounting for much of the total 8% infection of that treatment.

Run 2 showed higher overall infection rates attributable to higher yeast infection in all but one case, but 50% in the final product was not exceeded. Interestingly, the only sample containing more than 1% infection rate of niger aspergilli was in the 50kg/m^2, 1 stirring/d, wire mesh treatment of this run.

Table 4.4: Mean values for the frequency of infection of coffee beans by selected fungal taxa as influenced by load (25 or 50 kg/m^2); stirring frequency (1 or 4 /d) and drying surface (wire mesh or plastic tarpaulin).

Run 1

Surface	**Wire Mesh**				**Tarpaulin**			
Loading	**25 Kg/m^2**		**50 Kg/m^2**		**25 Kg/m^2**		**50 Kg/m^2**	
Stirring	**4/d**	**1/d**	**4/d**	**1/d**	**4/d**	**1/d**	**4/d**	**1/d**
Initial								
Total	10%	10%	17%	11%	18%	18%	39%	17%
Creamy yeasts	1%	0%	1%	0%	2%	1%	1%	0%
Black aspergilli	1%	0%	1%	0%	0%	0%	1%	0%
Yellow aspergilli	0%	0%	0%	0%	0%	0%	1%	0%
Penicillium spp.	0%	0%	0%	0%	0%	0%	0%	0%
Fusarium spp.	0%	1%	1%	1%	1%	1%	6%	1%
Cladosporium spp.	7%	8%	7%	9%	10%	14%	25%	11%
Final								
Total	4%	1%	8%	12%	11%	5%	9%	3%
Creamy yeasts	0%	0%	0%	0%	0%	0%	2%	2%
Black aspergilli	1%	0%	0%	3%	2%	2%	0%	0%
Yellow aspergilli	1%	0%	3%	0%	0%	1%	0%	0%
Penicillium spp.	0%	0%	0%	0%	0%	0%	0%	0%
Fusarium spp.	1%	0%	0%	4%	1%	0%	1%	0%
Cladosporium spp.	1%	0%	3%	3%	1%	1%	4%	1%

Table 4.4 contd.: Mean values for the frequency of infection of coffee beans by selected fungal taxa as influenced by load (25 or 50 kg/m^2); stirring frequency (1 or 4 /d) and drying surface (wire mesh or plastic tarpaulin).

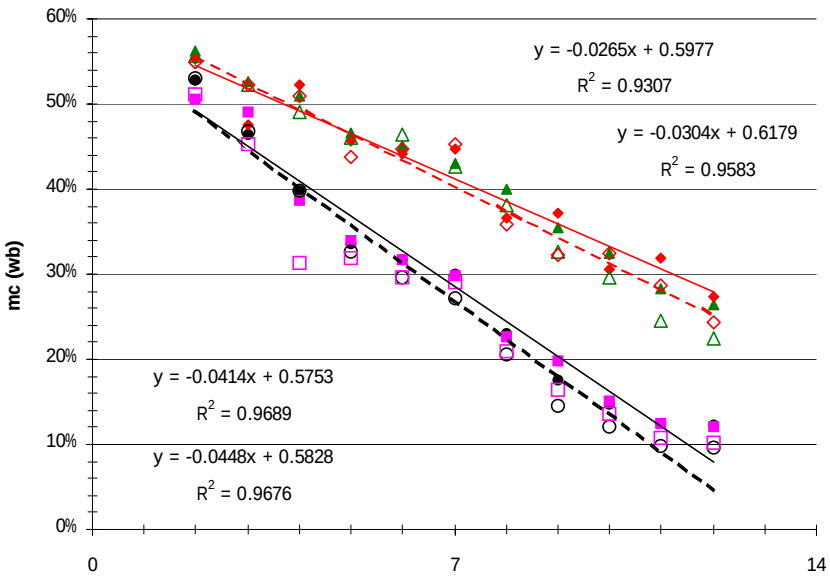
Run 2

Surface	Wire Mesh				Tarpaulin			
Loading	25 Kg/m^2		50 Kg/m^2		25 Kg/m^2		50 Kg/m^2	
Stirring	4/d	1/d	4/d	1/d	4/d	1/d	4/d	1/d
Initial								
Total	37%	51%	58%	73%	26%	35%	29%	29%
Creamy yeasts	7%	25%	34%	46%	5%	5%	13%	5%
Black aspergilli	0%	0%	0%	0%	0%	0%	0%	0%
Yellow aspergilli	0%	0%	0%	0%	0%	0%	0%	0%
Penicillium spp.	1%	2%	0%	1%	0%	0%	0%	1%
Fusarium spp.	10%	6%	9%	4%	1%	4%	4%	9%
Cladosporium spp.	14%	16%	10%	9%	16%	19%	7%	12%
Final								
Total	37%	9%	1%	46%	11%	2%	7%	23%
Creamy yeasts	30%	0%	0%	2%	2%	0%	0%	19%
Black aspergilli	0%	0%	0%	22%	0%	0%	0%	0%
Yellow aspergilli	2%	0%	0%	0%	2%	0%	1%	0%
Penicillium spp.	0%	2%	0%	12%	1%	0%	3%	0%
Fusarium spp.	1%	0%	0%	0%	1%	0%	0%	2%
Cladosporium spp.	1%	0%	1%	3%	5%	1%	0%	1%

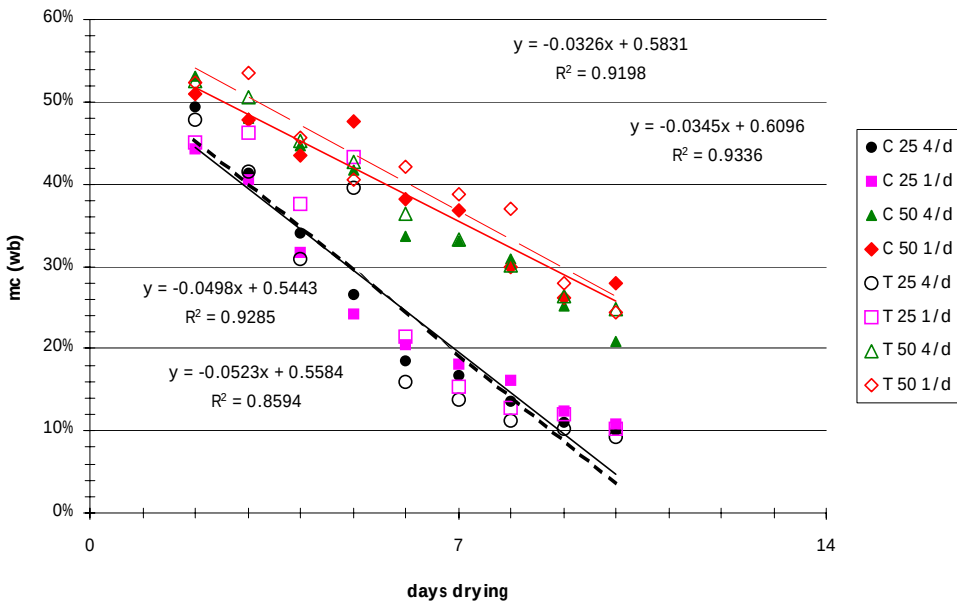
The same experimental design was applied to cherry coffee drying in Côte d'Ivoire. The time course of drying (Figure 4.8) shows some differences from the arabica parchment drying. The steeper section of the relationship is present but less obvious. This may explain why the drying rates (slopes) differ more than the y-intercepts in this data set. This could be interpreted as indicating that cherry coffee is more sensitive to loading and stirring within the range of conditions tested.

Figure 4.8: Drying time-course between Day 2 and 10 or 12 for robusta cherry drying on tarpaulin and cement at 25 and 50 kg/m^2 and 1 and 4 stirrings per day. Only the regressions for 25kg/4 stirrings and 50kg/1 stirring are shown as per the colour–open/closed legend scheme.

1st Run



2nd Run



As to the significance of stirring frequency, once again the treatments quite clearly cluster into the 25kg/m² and 50kg/m² treatments. In the first run the extremes produced a dry product after about 13.5 and 19.5 days while in the second run the comparable figures were 9 and 15.5 days. Even though the doubling of layer thickness has a profound effect on the drying time, variations in drying conditions can produce commensurate effects.

Within these there is overlap so once again there is no measurable effect of stirring on drying rate. In the second run there is some indication that tarpaulin makes a poor surface at the high loading rate but here cement, unlike wire mesh, may not provide enough of a contrast for this to be convincing.

Increased loading increases the amount of water per unit area, thus increasing the energy requirement. The maximum energy input (sun intensity + wind) is essentially fixed so this implies an increase in the time required. Also the balance of the layer is changed such that there is a greater mass that is cool, providing a larger 'sink' for condensation of water vapour within the layer. This may explain why the most water impervious surface (plastic tarpaulin) tended to perform worse with the thick layer.

Stirring is an input that should accelerate drying but could also affect the ability of fungi, with their mycelial habit, to grow from cherry to cherry. Regular mechanical disruption should reduce this since there is a time requirement involved in extension growth.

The initial fungal populations show that there is a significant contribution from *Penicillium* and 'other Aspergilli'. This latter designation includes *A. versicolor* which can easily be mistaken for a *Penicillium* spp. on DG18. *Fusarium* is not prominent. Yeasts and ochre aspergilli were perhaps more common infecting fresh beans then is usual at up to 35% and 83% respectively. The initial presence of ochre aspergilli is stable in these samples at 5 to 8% and corresponds to a relatively stable initial OTA contamination rate in the fresh coffee of about 4.2, 3.0 and 6.6ppb in the three blocks.

The final i-communities were dominated fairly uniformly by niger aspergilli with 'other aspergilli' making noticeable contributions in some cases. There is no pattern so the dynamics of ochre aspergilli is discussed below in conjunction with its product OTA.

Table 4.5: Initial mycological conditions of the beans in the fresh cherries from the three orchard blocks used in two runs of the drying yard control parameters study.

Blocks	1	2	3
Total	94%	98%	100%
Yeasts	35%	11%	9%
Black aspergilli	24%	43%	83%
Ochre aspergilli	5%	9%	8%
Flavi aspergilli	0%	0%	2%
Other aspergilli	1%	10%	2%
***Penicillium* spp.**	34%	37%	14%
***Fusarium* spp.**	2%	1%	2%

Since the only real differences in the drying rates caused by the treatments are between 25 and 50 kg/m² loading rates and between Run 1 and 2, it is here we would look for differences in fungal development and the other measured parameters. Other differences can only be considered to be fortuitous or the result of phenomena related to the treatments but not to drying itself.

The 25kg/m² treatments of the second run dried fastest, followed by the 25kg treatments of the first run which are nearly comparable to the 50kg/m² treatments of the second run and the 50kg treatments of the first run are significantly slower to dry. Numerical differences within these three bands can only be attributable to variability or other effects. Consistent differences between these bands can be attributed to the differences in drying rates if these differences exceed the expected variability.

Table 4.6 gives the full physical evaluation of the products of this experiment while Table 4.7 gives the ranking of the means of out-turn, sound beans and foxy beans, a defect often attributed to fermentation during processing. Out-turn is of particular importance to the farmer but is also influenced by pre-processing factors.

Table 4.6. Out-turn and defect proportion of the dry product of the stirring frequency/loading/surface comparison trial. Out-turn is the difference in weight before and after hulling.

Surface	Load kg/m²	Stir per day	Mean Defect Frequencies (%)					
			Out-turn	Sound	Foxy	Black	Husk	Broken
Cement	25	4	46.12	74.45	11.33	1.11	7.89	5.22
		1	50.12	61.36	26.65	1.68	6.64	3.66
	50	4	37.87	81.74	5.48	0.91	6.07	5.80
		1	43.63	70.39	18.73	2.03	6.09	2.76
Tarpaulin	25	4	44.76	73.07	13.07	1.56	9.87	2.44
		1	35.36	63.40	28.47	1.26	4.20	2.66
	50	4	40.78	74.19	14.66	1.56	7.53	2.06
		1	42.56	68.47	20.85	1.88	6.60	2.19

Treatments dried on cement occupy the first two places by all evaluations and the sound bean criterion gives almost the same result as the foxy bean criterion. Also striking is that the 25/1 treatment gives uniformly poor results except in terms of out-turn when dried on cement. It is difficult to understand how it is that the 25/1-cement treatment could produce the best out-turn and the 25/1-tarpaulin the worst. It is also surprising that there is not a good correspondence between out-turn and defect frequencies.

Table 4.7: Objective evaluation of processing quality assessed by the criteria: out-turn; sound beans; foxy beans.

Out-turn	% between	Sound beans	% between	Foxy beans	% between
C25/1	4.00%	**C50/4**	7.29%	**C50/4**	-5.85%
C25/4	1.36%	**C25/4**	0.26%	**C25/4**	-1.74%
T25/4	1.13%	**T50/4**	1.12%	**T25/4**	-1.59%
C50/1	1.08%	**T25/4**	2.68%	**T50/4**	-4.07%
T501	1.78%	**C50/1**	1.92%	**C50/1**	-2.12%
T50/4	2.91%	**T501**	5.06%	**T501**	-5.80%
C50/4	2.51%	**T25/1**	2.04%	**C25/1**	-1.82%
T25/1		**C25/1**		**T25/1**	
TOP-BTM	14.76%	**TOP-BTM**	20.38%	**TOP-BTM**	-22.99%

Based on proportion of sound beans and frequency of foxy beans, the best four treatments are the four stirrings per day treatments, irrespective of the loading or surface. As discussed above, this could not be attributed to drying rate since the frequency of stirring has little effect on drying rate; loading rate does but both loading rates, hence both slow and fast drying, are in the top group. Evidently there is something about stirring that reduces defects unrelated to any capacity of faster drying to do this. This might be related to more uniform drying in the case of more frequent stirring. Unlike the other defects, differences in the frequencies of sound and foxy beans between the best and worst are large at over 20%.

Cup evaluation provides criteria for further comparison. Coffees from different sources have inherent taste characteristics so each block of coffee must be referenced internally. Taste defects, however, can be attributed to coffee handling between harvesting and roasting. Only one of the five tasters detected any such defects and three of these were mouldy taste from Cement-50/4 (Block 1) and Tarp-25/1, Tarp-50/4 (Block 3). Cement-25/1 (Block 1) was ascribed a burned off-taste ('*brulé*').

Table 4.8 makes the comparison of the average cup evaluations of five tasters and as compared within each block, using theoretical best practice of 25kg/m^2 with 4 stirrings per day as the internal reference and Table 4.9 uses the treatment with the highest proportion of sound beans as the reference. Positive figures indicate that the comparator has more of the attribute than the reference. The recommended practice gives a cup of moderate bitterness and body and low astringency and acidity.

Acidity is not an important character in robusta cherry and 'astringency', as opposed to bitterness, is probably a term for the unpleasant taste associated with robusta coffee sometimes referred to as 'robust character'. Treatment Cement-50/4 shows superior body and moderate bitterness but is at the top for astringency and acidity. The fermentation of parchment coffee is said to develop acidity so perhaps this high value is an indication of fermentative conditions due to slow drying of cherry, or perhaps it is literally the accumulation of organic acids from fermentative micro-organisms.

Table 4.8: Cup evaluation of robusta cherry from three sources, dried in two runs. The scale seems to be out of 10 and the average for the reference coffee is given in red. recommended practice provides the reference value. C = cement; T = tarpaulin.

C 25/4 = Reference value

	Body	Astringency	Acidity	Bitterness
C 25/4	**4.4**	**4.3**	**4.1**	**5.3**
C 25/1	0.0	0.5	0.2	-0.1
C 50/4	0.6	1.3	1.1	0.0
C 50/1	0.9	0.9	0.3	0.5
T 25/4	-0.1	0.1	0.2	-0.5
T 25/1	-0.3	0.7	0.0	0.0
T 50/4	0.2	0.9	0.0	0.4
T 50/1	-0.1	0.1	0.1	-0.4
Samples below reference value	**3/7**	**0/7**	**0/7**	**3/7**

Table 4.9: Cup evaluation of robusta cherry from three sources, dried in two runs. The scale seems to be out of 10 and the average for the reference coffee is given in red. Best out-turn provides the reference value. C = cement; T = tarpaulin.

C 50/4 = Reference value

	Body	Astringency	Acidity	Bitterness
C 25/4	-0.6	-1.3	-1.1	0.0
C 25/1	-0.6	-0.8	-0.9	-0.1
C 50/4	**5.0**	**5.6**	**5.2**	**5.3**
C 50/1	0.3	-0.4	-0.9	0.5
T 25/4	-0.6	-1.2	-0.9	-0.5
T 25/1	-0.9	-0.6	-1.1	0.0
T 50/4	-0.4	-0.4	-1.1	0.4
T 50/1	-0.7	-1.2	-1.0	-0.4
Samples below reference value	**6/7**	**7/7**	**7/7**	**3/7**

Table 4.10 gives the final ochre aspergilli bean infection frequencies and the corresponding OTA content of the samples. Merely by inspection it can be seen that as the means of the replicates increase, so too does the variation. There are no statistical differences in this data set, however, the outcome is not meaningless.

The variation before the treatments, though high at a standard error of about 75% of the mean, is manageable. The dataset can be approached by applying the observed standard error in the largest replicated sampling, that of initial conditions, as a threshold. When the outcome of a treatment exceeds this, it can be accepted as an effect of the treatment and used as the basis for a hypothesis.

150% change from the initial condition can be taken as the threshold as this approximates or exceeds the degree of initial standard error in a comparison of two. Blocks 1 and 2 must exceed 8ppb and block 3 must exceed 14ppb. A value approaching 0 is taken as a reduction. Applying this to OTA, there are four increases in the 50kg treatments and 5 in the 25kg ones; 5 on cement and 4 on tarpaulin; 4 with

4 stirrings/day and 5 with 1 stirring/day. In fact, the distribution across the treatments is as even as it is possible to get. Looking at the source of the cherries as a treatment, however, a strong pattern emerges. There was a single large increase and one reduction across all the treatments applied to Block 3 cherries (note, however, that this is Run 2 which dried some 4 days quicker than Run 1); Block 1 cherries contain two increases in the eight treatments, both on cement with 1 stirring/day; Block 2 has three of four treatments on both cement and tarpaulin exceeding the threshold, thus six of eight treatments produced an increase.

Table 4.10: Percentage of beans infected with ochre aspergilli and contaminated with OTA in final samples [initial conditions = 7 and 8% infection and 3 and 6.6ppb OTA]. **Bold** figures are means; red are putative increases from initial conditions; green are reductions.

Final bean infection rate (%)				
Kg/m²	**25**		**50**	
Stirrings/day	**4**	**1**	**4**	**1**
Cement				
Block 1	0	1	1	3
Block 2	4	17	11	24
Block 3	0	7	0	0
Mean	**1**	**8**	**4**	**9**
Tarpaulin				
Block 1	31	7	1	9
Block 2	83	0	10	3
Block 3	0	0	1	0
Mean	**38**	**2**	**4**	**4**

Final OTA content (ppb)				
Kg/m²	**25**		**50**	
Stirrings/day	**4**	**1**	**4**	**1**
Cement				
Block 1	6.8	7.7	4.1	13.4
Block 2	17.3	46.5	22.1	2.2
Block 3	9.3	9.6	5	9.3
Mean	**11.1**	**21.3**	**10.4**	**8.3**
Tarpaulin				
Block 1	3	2.6	4.8	5.4
Block 2	6.6	16.4	18.4	14.2
Block 3	41.4	0.2	11.5	7
Mean	**17.0**	**6.4**	**11.6**	**8.9**

As discussed above, there are measurable differences in the drying periods produced by the treatments. These do not correspond to expected increases in OTA or ochre infection. The 50/1 treatment produced average ochre infection and the numerically lowest OTA levels whereas the reference treatment (25/4) produced some replicates that were genuinely high and an average that was numerically highest of all treatments. Using the criterion set out above it is impossible to distinguish any meaningful pattern of outcomes related to the drying data.

The experimental design limited the size of the treatments to make the sampling for OTA and fungi as representative a practical, in recognition of the problem of sampling in mycotoxin studies. The data indicate there is an increase in OTA during drying but this is not ascribable to the drying in that the reference method performed poorly. It can also be said that the reference method was not better than the treatments expected to give poor results. What cannot be established is whether it was worse.

One way to rationalize the overall performance of the experimental system is to suppose that the physical conditions during drying, over a fairly broad range, is not the most important feature determining success of OTA producers. In other words, the conditions suit development of OTA producers to some degree, but other factors arise to produce outcomes.

To use the current data as an example, different replicate experimental units produce 3 or 41ppb of OTA. The system itself is complex and greater water availability alone will never lead inexorably to toxin production. The lack of correspondence between increases in OTA and OTA-producers during the slow drying of the 50kg, once-stirred treatment, is consistent with fermentative conditions in thick layers. Sample by sample correlation of OTA with fermented off-tastes in the cup, as one might expect with strong fermentative conditions, does not, however, arise.

4.5.4 Split cherry drying

Differences in parchment and cherry drying rates are not great, though cherry drying times are longer because they contain more water and because the skin of the cherry initially prevents water loss. A low technology modification of cherry drying has been practiced by smallholders in Sumatra and Java for many years consisting of splitting the skin of the cherries with a rotary machine, often home-made, without separating the skins from the beans. This is functionally cherry drying because the seed is in contact with the fruit during drying.

We see these trends in this extensive dataset, and in addition we see that the split cherries dry very much as parchment coffee. In these trials, equivalent weights of split cherry and whole cherry were spread per unit area. Seeing that split cherry has a lower bulk density, this means that the split cherry drying layers were thicker. In these trials, with what can be considered a better than average drying period of about 13 days for cherry, the parchment and split cherries dry some 2 days more quickly.

In trials where smaller quantities of cherry were used and equivalent ***volumes*** of fresh cherry and split cherry were spread (with the same layer thickness), the split cherry dried in 4 days as compared with 10 days for the whole cherry. In cases where

'time' rather than 'drying yard space' is the critical factor, splitting the cherry can lead to substantial reductions in drying time.

Table 4.11: Drying rates (means of 4 replicates) calculated site by site for the four coffee processing intermediate types. P MET= parchment produced with a manufactured pulper; P WD = parchment produced with an artisan-built wooden pulper. A single line beneath treatments indicates no statistical difference.

	Whole Cherry	Split Cherry	Parchment (WD)	Parchment (MET)
Kaliwining	4.54	5.30	5.64	5.71
Silo	4.62	4.93[1]	5.50	5.66
Liwa	4.24	4.55	4.01	3.75

Drying rates for all treatments at Liwa was found to be significantly lower than at the other two trial sites ($p>0.975$). This reinforces the importance of local drying conditions on the performance of any drying system comparison. This slower drying seems to have led to a higher occurrence of defects in the spilt cherry coffee produced at Liwa.

Table 4.12: Comparison of defect counts in coffee dried as cherry, split cherry or parchment prepared with 2 different pulpers.

	Cherries			Split-cherries			Parchment WD			Parchment M		
	Kali	Silo	**Liwa**	Kali	Silo	**Liwa**	Kali	Silo	**Liwa**	Kali	Silo	**Liwa**
Rep1	38,2	49,3	**109,1**	47,2	49,9	**211,05**	56,7	44,6	**132**	57	39,7	**116,25**
Rep2	80	40,5	**124,25**	38,2	59	**154,75**	52,5	76,9	**110,25**	48,1	30,6	**92,4**
Rep3	42,3	55,3	**136,75**	55,3	51	**194,5**	58,1	41,1	**199,2**	48,9	46,9	**111,2**
Rep4	38,8	47,9	**78**	33	65,6	**203,9**	39,6	36,8	**50,65**	61,1	29,5	**116,35**
Mean	49,83	48,25	**112,03**	43,43	56,38	**191,05**	51,73	49,85	**123,03**	53,78	36,68	**109,05**

Overall, there is no statistically significant effect of drying treatment on defect count. However, when the trial locations are considered separately a difference emerges. At Liwa, where drying was found to be significantly slower, defect count was significantly higher for split cherry drying ($p>0.975$). There were no differences in defect count between the whole cherry and parchment treatments. At Silo and Kaliwining, there were no differences in defect count between any of the treatments.

During splitting of cherry there may be some degree of physical damage to the bean. It could be that such damage, under poor drying conditions, leads to increased defects or that for some reason there was more damage incurred in the Liwa trial. The details of the defects found were not specified.

[1] Even though there is no statistical difference in the drying rate, the split cherries dried 2 days faster due to the absence of the initial lag phase seen with whole cherry drying.

In terms of the impact of these treatments on the fungi, there is an indication that the split cherry might be more prone to infection from mesophilic fungi, in this case the potentially aflatoxigenic group of Flavi aspergilli. These are aggressive, fast-growing organisms with broad physiological capacity of temperature and drought tolerance. The cherry coffee, in general, developed less infection especially in the Silo trials which is perhaps surprising since robusta cherry routinely has rates of niger *Aspergillus* infection exceeding 85%. Clearly there was little success for the ochre group here.

Table 4.13: Comparison of the fungal infection rates of the more prominent groups after drying as cherry, split cherry or parchment prepared with two different pulpers.

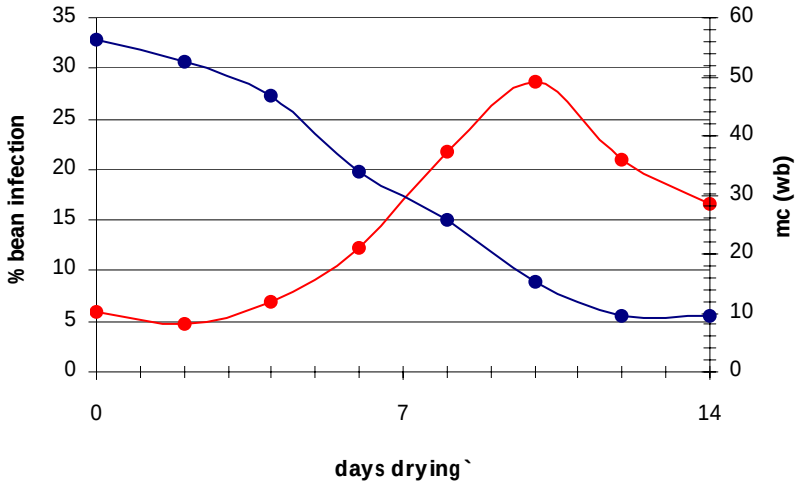
Liwa	Niger	Ochre	Flavi
Whole cherry	96	0	1
Split cherry	97	0	2
Parchment WD	73	0	1
Parchment M	79	0	1
Kaliwining			
Whole cherry	54	0	20
Split cherry	99	0	57
Parchment WD	78	2	19
Parchment M	89	0	14
Silo			
Whole cherry	32	4	11
Split cherry	99	0	60
Parchment WD	93	0	8
Parchment M	69	1	12

4.5.5 Fungal community dynamics during drying

Logistically it is very difficult to do studies to characterise the short-term changes in the fungal community during drying due to the shear quantity of sampling and analysis required. Two such studies are presented here. In the first (Figure 4.9) the community is small and, as typical of arabica parchment, there is little niger aspergilli. The second is the typical robusta cherry situation, dominated by niger aspergilli.

Bearing in mind that the mycological analysis does not measure growth *per se* but it may be taken as an overall measure of relative success in the conditions since it is clear the fungus is present in the trial. In other words, in conditions where infection increases, we are entitled to conclude the fungus is well suited in comparison to conditions where it does not. It is essential to understand that a cessation in the increase of infection does not imply a cessation of growth and growth rate is not necessarily proportional to infection rate or infection rate changes.

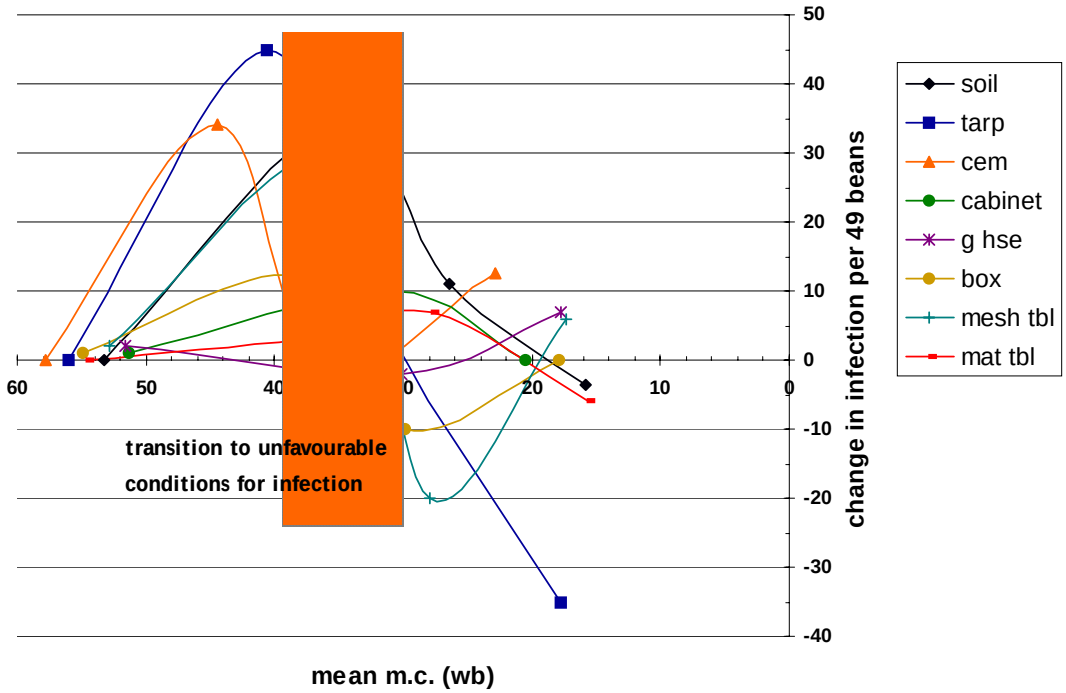
Figure 4.9: Time-course of total fungi and m.c. during arabica parchment drying in Indonesia.



Infection in the arabica parchment increases from about the 4th to the 10th day corresponding to m.c. of about 45% to below 20%. The reduction after 10 days may be real since such reductions have been noted in other studies. A reduction represents mortality, presumably of fungi that are sensitive to desiccation, displaced by better adapted species or the seed itself.

Figure 4.10 interprets the dynamics of niger section infection during robusta cherry drying on a variety of surfaces. Ochre group aspergilli were absent during this trial.

Figure 4.10: Population dynamics of *A. niger* complex during cherry coffee processing in Uganda utilizing several alternative technologies. 'Cabinet', 'greenhouse' and 'box' are types of solar dryers.



It can be seen that infection rates fall somewhere between 30 and 20% m.c., broadly in agreement with the arabica parchment results. In two cases, tarpaulin and soil, this is a numerical necessity since 100% infection was rapidly reached. The negative changes may represent death of the fungus but most are small, likely reflecting sampling and analysis error where the initial determination was somewhat high and the subsequent one (change in rate data is calculated from the difference between two determinations) is at or below the 'actual' value. Although this type of analysis suffers from a doubling of method error (two measures per determination), it is still difficult to rationalise the large negative values of the tarpaulin and mesh table without hypothesising mortality. The moisture conditions are not extreme so it would be difficult to rationalise mortality without suspecting an interaction with other fungi or the seed itself.

OTA is produced during growth, within certain physical conditions, by a fungus capable of producing it. The presence of OTA requires the presence (or previous presence) of a significant amount of OTA-producer biomass, an increase over time implies fungal growth roughly commensurate with the relative magnitude of the OTA change and a decrease implies active mechanisms of breakdown or chemical modification, which could include activity by the producer, or if attached to the plant, translocation.

Image 4.3 (and next page): Various sun drying systems. In the course of the project, many different surfaces and equipment for drying coffee in the sun were recorded. Each image is labelled separately.


Fish-farm nets


Cow-dung plaster
(with inset of
damaged surface)


Elbas


Carros


Soil


Cement


Tarpaulin


Mesh tables

Section 5
Delay Between Harvest and Processing

5.1 Introduction

During the second year of the project some startling OTA contamination data was obtained from dried cherry coffee taken from a drying trial at one of the collaborating centres. The results of the OTA analyses showed generally high levels of OTA contamination of the coffee bean throughout the drying course with an average OTA content of 46ppb (mean of 50 samples) and with a maximum value of 353ppb. On receipt of these results the central project team initiated an investigation to ensure that the experiment had been carried out well, and to try to understand what factors might lie behind these exceptional results.

It was discovered that there had been a delay between the harvesting of the cherries and the initiation of drying. It was estimated that the delay had been roughly 4 days. It was not possible to determine how the cherries had been stored or any other factors in their handling that could have led to the observed high level of OTA accumulation. The trial results were therefore disregarded as it was impossible to interpret the findings.

Later in the project, information derived from the farm surveys of several cherry coffee-producing countries revealed that keeping harvested cherries in sacks, heaps or thick unstirred layers for some days before spreading and drying is practiced by between 35 and 90% of smallholders, depending on the region.

This surprising finding, which was unexpected even by coffee sector institutions, along with the earlier data suggesting that delay before processing ***might be*** an important risk factor in OTA contamination, motivated a series of systematic investigations into how OTA producers and OTA production are affected by this practice. It was also considered important to monitor selected quality parameters to determine how they are affected by delay.

The aim of this work was to formulate improved guidance to farmers and processors on the maximum acceptable duration of any delay between the harvesting and the processing of coffee cherry.

Some farmers report that the motivation for delays before processing was to enhance drying. They generally claimed that the blackened cherries dry faster once spread. In the case of Balinese farmers, where cherries are kept in thick unstirred layers for five days or so before commencing drying, the claim was that this imparts a particular desirable quality to the product. It is also sometimes used as a way of managing an inadequate drying area or inadequately controlled harvesting rates. Fear of theft of ripe cherries from the trees is one reason for harvesting their cherry as quickly as possible even if they lack drying capacity. The period the cherries are held in sacks before spreading is typically between 4 and 7 days. Delays of lesser duration are also incurred in transferring the cherry from the point of harvest to the place of drying or processing.

Other reasons, peculiar to wet processing, include unforeseen circumstances such as break-downs, power failures, or the late delivery of cherries from the orchard preventing prompt processing. Low harvesting rates can also entail delays as an appropriate quantity of coffee is accumulated to supply the pulping station. Under these circumstances some processors are said to submerge coffee in water. The project team only became aware of this practice towards the end of the project and therefore included only two trials in one country to make a preliminary assessment of this practice.

The work reported in this Section relates to the systematic trials carried out in various project countries to investigate the impact of delays between harvesting and the onset of processing on OTA risk and on selected quality parameters.

5.2 Findings and Application

5.2.1 Relationship between delays in processing and OTA contamination

None of the systematic trials carried out in different project countries showed any relationship between delays before processing and OTA accumulation in coffee.

This could be due to the unpredictable presence or absence of OTA producers in the initial batch of cherry. In some cases there did seem to be taxa capable of OTA production but no OTA production. In other cases OTA was produced. In these, invariably, there were no statistical differences between treatments (delay or no delay) in these studies.

The extremely high variability and the practical limitations imposed on increasing replication within the trials reduced the power of the statistical analysis. However, even simple numerical analysis failed to show a pattern between holding cherries in sacks for varying periods and presence of OTA or OTA producers. The reasons for this are not clear, despite there being numerous possible explanations. The data are discussed in detail in Section 5.5.6, below.

5.2.2 Microbial development in cherries during delays before processing

Very quickly after detaching from the tree, coffee fruit begins to senesce and fermentation organisms, specialist saprophytes, multiply rapidly to high densities as long as there is abundant water in the system. In the period between harvest and the attainment of a stable moisture content, microbial activity (whether this is in traditional fermentations, in the drying fruit, in cherries held sacks or in heaps) is controlled primarily by available oxygen supply and time restriction through prompt drying.

Coffee held in sacks: When cherries are stored in sacks, the presence of air spaces between the cherries allows gas exchange. In this case respiration is very rapid and the 'fermentation' self-heats to around 45-48°C. There is also a marked drop in pH to below 3.5. Such values of temperature and pH could be expected to exert significant influence on microbial development (refer to Section 5.5.1, below, for details).

The qualitative development of the dominant micro-organisms (particularly bacteria and yeast) within the system is relatively consistent. However, the OTA-producing fungi represent an extremely minor component of the mould population present in the system and their qualitative development, and therefore the outcome in relation to OTA risk, was found to be highly inconsistent.

It should be noted that the dynamics of microbial development in heaps vs. sacks vs. thick layers may well be different, but these comparisons were not undertaken. For example, different 'conformations' of storage of fresh cherry might lead to different levels of aeration or different rates of heat dissipation which might affect the eventual course of microbial development in the coffee mass. Unfortunately no conclusions can be drawn since the trials did not consider the potential influence of these factors.

5.2.3 Effect of delays before processing on selected quality parameters

For most farmers, the return for dried product at the farm gate is determined by out-turn and by some sort of physical evaluation where the buyer assesses the quality for resale. Out-turn can be affected, especially over the longer delay periods, but this is not consistent. An increase in defect rates was also noted in some studies but, especially with robusta cherry, this was not invariably the case.

Cup quality is more important to arabica price than robusta, with the exception of actual defects in the cup. The combination of wet processing with delay in sacks produced some very poor results but, remarkably, some of the delayed arabica parchment coffee scored higher than the reference processes. However, if replications are compared, it becomes clear that although some instances of delay produce a good or even a superior product, other instances, following the same production parameters, produced a gravely inferior one. In other words, consistency – a hall mark of effective quality management – is compromised.

To summarise with respect to general quality issues, delay of processing in sacks, and probably heaps, is a less controlled process than is immediate cherry drying or immediate pulping for wet processing.

To build quality assurance into a production process, there must be practical means of control - the fermentation processes taking place in harvested coffee fruit are not controllable. The results of the studies are not one-sided enough to warrant an outright condemnation of the practice, but it is recommended that it be avoided in favour of processing that can be controlled to a greater extent.

5.2.4 Storage of cherries under water

As limited work in only one country was carried out to investigate the impact of this practice on the quality and safety of coffee, the findings can only be considered indicative.

Soaking effectively inhibits both yeast and mould development whereas sacking, cherry drying and traditional fermentation all produce a rapid increase in yeast numbers and a modest increase in mould numbers, excepting traditional fermentation where there is a fall in moulds.

Soaking may also slightly inhibit the growth of *Enterobacteriaceae* and lactic acid bacteria (LAB) in comparison with the other treatments, but this may be purely a consequence of the lower temperature ensured by the mass of water, considering that many LAB's have a temperature optimum above 40°C.

In mycological terms, dry parchment coffee prepared from cherry that was held under water for a period of 4 days was indistinguishable from parchment prepared by traditional fermentation without delay. There was no indication of any impact of a soaking step on OTA risk.

Physical quality assessment of the green bean showed a high proportion of bleached beans when a soaking period was included in the process. Deterioration of the 'cup' was not detectable with periods of soaking up to 4 days, however some off-flavours were noticeable on 6-day treatments.

After fermentation, mucilage removal from the 'soaked' treatment was difficult and a pink discolouration was associated with the hardened mucilage covering the parchment.

5.2.5 Summary of key issues related to delays in processing

Good hygiene practice requires that potential hazards be controlled. If the 'system' of cherries stored in sacks behaved predictably, we could have refined guidance on this question. However, as the trials showed the 'system' to be ***highly unpredictable*** the only advice is to follow established good hygiene practice and to avoid delays before processing.

In the case of dry processing, this means immediate, efficient, drying. In the case of wet processing this means expediting pulping and well-managed fermentation.

5.3 Additional Notes

It is important to understand what happens to coffee fruit immediately after harvest. The coffee fruit has no capacity for dormancy and begins to senesce immediately after detachment from the tree. This may be the main reason that tree-drying is distinct from terrace-drying, as discussed in Part C, Section 8 on 'Cherry Quality'. The microbial community, both in and on the fruit, previously in balanced co-existence with the plant tissue, begin to change to a saprophytic mode, and the species most capable of this ecological role begin to multiply rapidly. This initiates a rapid shift to microbial fermentation which becomes well established within approximately 24 hours after harvest.

If cherries are pulped and fermented as is traditional, either 'dry' or 'wet' (under water), the mucilage restricts oxygen ingress and microbial activity is oxygen limited and therefore slow – there is little or no elevation of temperature.

Cherries in a sack or heap, however, allow ready access of oxygen via the gaps between cherries and a vigorous fermentation becomes established. The fermentation is not oxygen controlled but due to the vigour of the respiration, microaerophilic hydrophiles such as lactic acid bacteria and species of *Bacillus* grow rapidly alongside

facultatively microaerophilic organisms such as yeast. If the coffee is immediately committed to sun-drying, superior aeration limits growth of the microaerophiles and water availability soon begins to control microbial development under good drying conditions, excluding the hydrophilic species, within a few days.

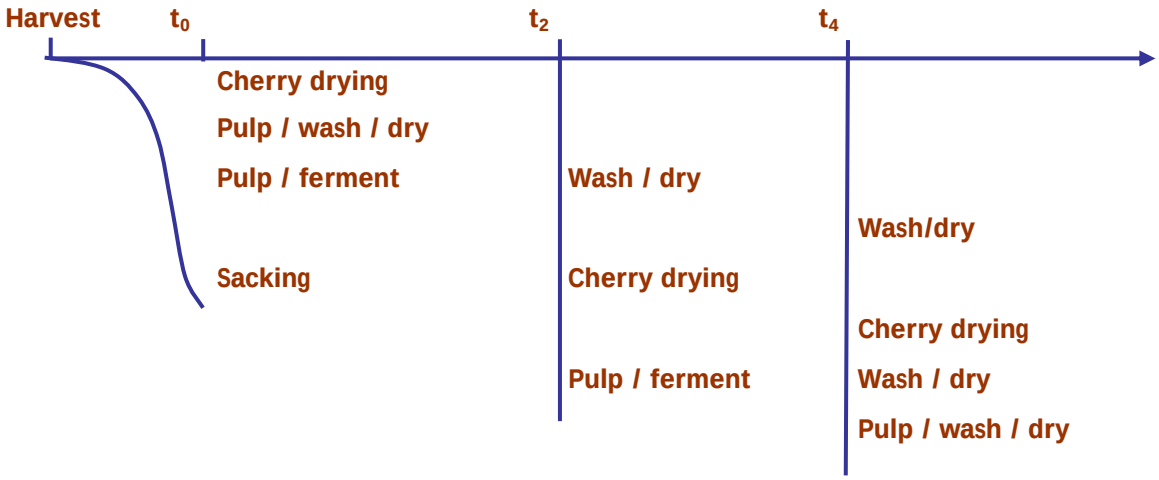
The micro organisms selected by the three sets of conditions so described, oxygen limited, nutrient/pH limited or water limited, though starting with the same initial conditions, develop very differently and can be expected to affect the coffee differently.

5.4 Experimental Design

The effects of delaying processing were investigated with several variations in experimental detail, but using a design that incorporated several common features. Analysis of these experiments focused largely on fungal development and OTA contamination, but in some cases bacterial analysis was also done.

- The treatments were all done side-by-side, and set up from one mixed lot of coffee;
- The sizes of the treatment lots were minimised within consideration of the coffee masses required to provide realistic drying time courses;
- Pre-drying delay periods were synchronised so direct comparisons between different fermentation conditions over the same length of time could be made;
- There were always direct comparisons to cherry and parchment processing available;
- Whenever possible, drying routines were manipulated to test the stability of the intermediate products. This was done by imposing slow drying conditions on some treatments by shading the coffee during the early stages of drying;
- In some cases storage experiments, utilising the output of these experiments, were also set up to test the stability of the final product. These are discussed in Part C, Section 10 on 'Storage and Conditioning'.

Figure 5.1: Schematic of the general design of delay of processing experiments. The exact timings and treatments were selected according to availability of manpower, climatic conditions and prevailing coffee production technology. When soaking delay by holding fresh cherries under water was investigated, this was run in parallel with the sacking treatments. In some cases, treatments were split and slow drying imposed on one of the sub-samples.



Pulp = pulping; Wash = washing; Ferment = traditional dry fermentation; Sacking = delayed in sacks; Dry = sun drying

5.5 Experimental Results and Discussion

5.5.1 Macroscopic and physico-chemical changes in cherry and parchment due to delayed processing

The coffee changes its appearance and some properties during retention in sacks (known as 'sacking') and under water (we refer to this as 'soaking') during post-harvest delay of processing.

Within 24 hours the temperature in sacks increases beyond 40°C and the pH falls to below 3.5. In traditional fermentation the temperature is never more than 2-3°C above the ambient temperature and the pH usually doesn't fall below 4.2. The temperature gradient to the sack surface is steep (see Figure 5.2) indicating that almost all the coffee is exposed to super-optimal temperatures for OTA production. Delay of processing under water, like traditional fermentation, does not produce a significant increase in temperature.

Figure 5.2: Temperatures from a central and a peripheral position (2-3cm from the surface) in a sack. '0' hours is upon insertion of the thermometers, but some hours after harvest.

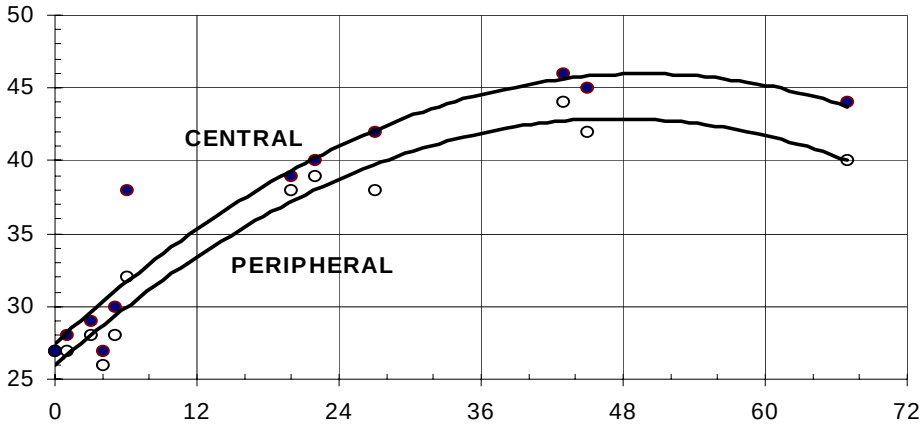
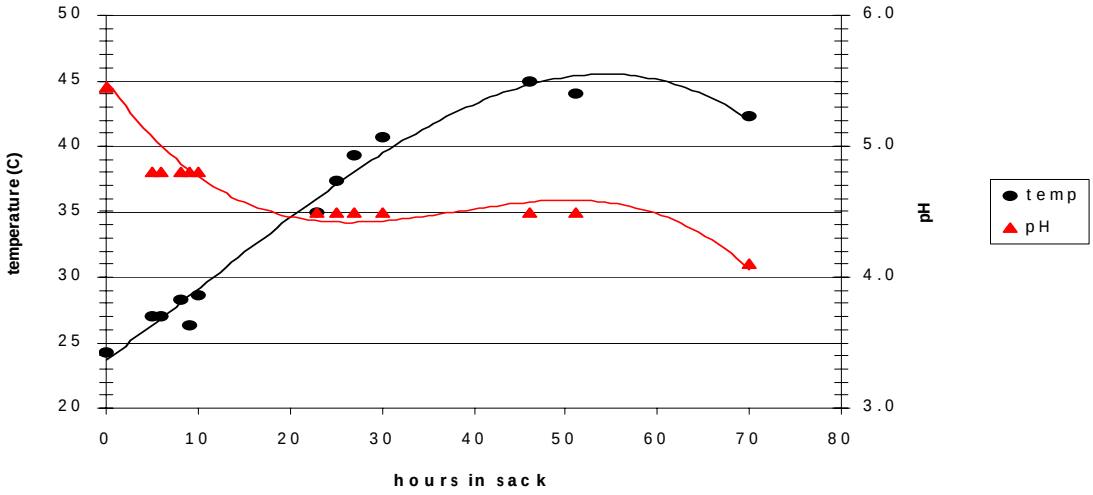


Figure 5.3: Temperature and pH of the mucilage in sacked cherries during the 'delay' period. Temperature can be taken as a measure of metabolic rate and acid production as a measure of fermentative activity.



There seems to be a maximum temperature attainable in sacks of 46-47°C, which is high enough to kill mycelia of some species of fungi and many environmental bacteria such as species of *Pseudomonas*. These temperatures would halt growth and toxin production of the OTA producing fungi, and could cause some mortality.

The pH profile, with its intermediate plateau, could indicate a microbial succession, such as occurs in Sauerkraut production, where more acid tolerant species replace the initial fast growing more modest acid producers. In fact this could also represent a transition to spoilage after about 48 hours.

Within 24 hours water is expressed from the mass of cherries and anomalous senescing reactions can be observed (Image 5.1). The mucilage becomes pulpy and soft and browning reactions become evident in a proportion of the mature and immature fruits.

During soaking, on the other hand, the mucilage of about half of the beans becomes pink and hardens to the point that it is impossible to remove, even after

fermentation. This proportion increases over time. The colour is reminiscent of Fusarium pigments but direct microscopic observation failed to confirm its presence. It could possibly be anthocyanins from the coffee skins hydrogen-binding to the pectins. The hardening of the pectin could be from cross-linking with multi-valent ions in the soaking water. The quality of the soaking water was not monitored or controlled in any way in this trial.

Image 5.1: Physical aspects of delaying processing. The cherries had been in the sacks 24h or an average of about 28h after harvest, a mixture of *amarillo* (yellow) and *rojo* (red) varieties. The soaking had been conducted for 40h followed by 24h fermentation.

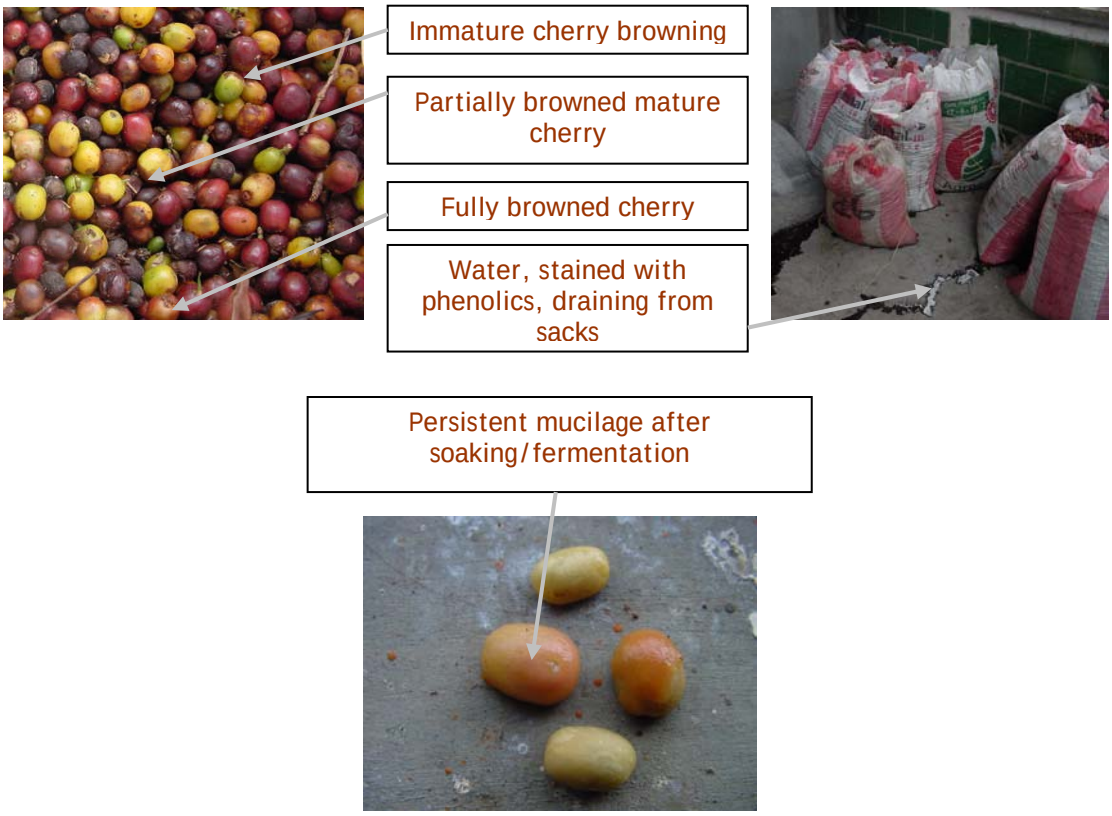


Image 5.2: Condition of the products of the soaking/sacking treatments after 96h, timed from delivery of harvested cherries to the processing facility.
From left to right: (i) Fermented for 2.5d, 1.5d drying; (ii) Sacked for 1.5d, fermented 1d, dried 1.5d; (iii) Soaked for 1.5d, fermented 1d, dried 1.5d; (iv) Cherry dried for 4d; (v) Cherry sacked for 1.5d, dried for 2.5d.

There are few differences in appearance between delayed and directly processed coffee after four days of processing, as Image 5.2 above shows, except in the soaking treatment. The brown colour of the parchment of this treatment is due to residual mucilage, which hardens during soaking and becomes impossible to remove. If *descascado* is being produced, this characteristic would have no impact.

The farmers' belief that drying is faster after sacking is ***not*** borne out by the evidence: the maximum drying rates of sacked cherries proved to be comparable to the maximum rates measured in the control treatment of fresh cherries. However, water (liquid) is lost during the period of sacking and there is a negligible lag phase before maximum drying rates are obtained compared to fresh cherries, so, while the time spent on the drying yard may be reduced, the period between harvest and full dryness is comparable.

5.5.2 Microscopic observations of coffee after delays in processing fresh cherry

Direct microscopic examination of some coffee after about 70 hours of delay in sacks was made. Adhering mucilage, parch tissue itself, silverskin and hand cut thin sections running longitudinally along a *broca* (Coffee berry borer (CBB)) gallery were made. The thin sections were as thin as two cell layers which made examination easy. A small spindle-shaped yeast with polar budding could readily be observed in the mucilage, but filamentous fungi were not seen. The cells of the mucilage if not actually intact at the time of examination had been until very recently, and the nuclei were still mostly intact and even the nucleoli had not lost their integrity.

No fungi were seen associated with the parch, which comprised what looked like a combination of rather short xylem elements running in flattened bundles in criss-cross orientations in two or three layers and bundles of xylem vessels. Although there was fungal mycelium in one such preparation, it appeared to be attached to other material, perhaps silverskin, and not closely associated with the parch. Silverskin from the centre cut was examined but showed nothing.

The broca gallery itself contained fungi as could be discerned with a dissecting microscope. The margins of the gallery had a greenish discoloration as is normal in *brocado* (beans with CBB damage). Although this colour is one associated with some common fungi, it is in fact a phenolic pigmentation produced by the plant tissue induced by physical damage. In the thin section, the pigmented cell layers could easily be discerned and the cells were clearly alive and functioning. Despite the proximity of fungi in the gallery, there was no evidence of fungal penetration of the grain. Such grains may still come up as having an internal infection (after hypochlorite sterilization) if an air-lock forms and prevents contact with the sterilent.

5.5.3 Impact of delay on general physical quality of bean

Data from the project indicates that delay of processing has a deleterious effect on the physical quality parameters in wet processing (Table 5.3, below), but a mixed outcome in cherry coffee processing. In cherry processing (Figure 5.1, above), there was a consistent reduction in the proportion of sound beans after a delay of processing, almost all of which is accounted for in the greater frequency of black beans in these treatments.

In Table 5.2, the same results are ranked by out-turn, % sound beans and % foxy beans, in turn (ranking by % black bean or % sound beans produces the same result). Within a fairly narrow range of values, two of the three top spots for out-turn are occupied by delayed cherry processing, which is also the case if ranked by foxy bean frequency.

Table 5.1: Out-turn and defect occurrence in the final product of robusta cherry exposed to a delay of processing. Cement (C), Tarpaulin (T), Bamboo table (B) after 0 and 4 days delay of processing.

	Out-turn	Sound	Foxy	Black	Husk	Broken
Cement 0	45.1%	68.8%	20.7%	1.2%	5.6%	3.8%
Tarpaulin 0	44.0%	67.7%	23.3%	2.6%	4.2%	2.1%
Bamboo 0	43.2%	71.9%	14.4%	1.9%	8.0%	3.8%
Cement 4	46.5%	64.6%	19.5%	6.0%	6.3%	3.6%
Tarpaulin 4	44.8%	61.4%	18.6%	8.4%	7.8%	3.8%
Bamboo 4	45.8%	63.1%	22.2%	5.1%	5.8%	3.8%

Immediate drying on bamboo tables gives the lowest out-turn but the highest proportion of sound beans, and there doesn't appear to be any relationship between these parameters. The variation in sound bean percentage between treatments is much greater than that of out-turn (max-min = 10.5% vs. 3.3%) and, together with the lack of correspondence between these parameters when ranked, it suggests that bean density is the principle factor in out-turn under these conditions.

Table 5.2: Rank of treatments descending according to out-turn and sound bean proportion and ascending according to foxy bean frequency.

Out-turn	Sound	Foxy
Treatment order	Treatment order	Treatment order
C 4	B 0	B 0
B 4	C 0	T 4
C 0	T 0	C 4
T 4	C 4	C 0
T 0	B 4	B 4
B 0	T 4	T 0

Foxy bean generation in cherry coffee processing, unlike black beans, is not apparently produced by the conditions of sacking. Foxy beans are variously attributed to over-drying, poor stirring during drying and over fermentation or fermentation with excess pulp in the mixture.

Black beans almost triple in frequency as a result of the delay in sacks treatment, here to about 6% on average. The black colouration can be restricted to the surface or through the tissues but is produced by the seed itself in response to injury. Fungal activity is usually said to cause the thorough blackening case and spotted or partial blackening but most cases of general but superficial blackening is attributed to the conversion of immature beans to black beans during drying at too high a temperature. Possibly this is a defect that could be generated in the sacks by the high temperatures noted to occur under these conditions.

In arabica parchment processing a delay in sacks beyond 40 hours tends to show a reduction in quality by all parameters except discoloration, roughly following the expected quality of processing pattern. Compared with robusta cherry, black bean rates are low. Vinegar beans are generally attributed to problems in fermentation, so it is perhaps expected that a delay in sacks could produce more of this defect. Discoloured beans, however, are less frequent in the delayed treatments than in the product of the reference processing protocols. When the defect categories that are causally unrelated to processing conditions, such as CBB, broken beans and husk fragments, are removed from the defect totals, the correspondence to expected processing quality apparently breaks. However, this anomaly is largely attributable to the formation of discoloured beans, so perhaps these too are produced either before harvest or during drying.

Table 5.3: Effect of delay of processing on physical quality of arabica parchment coffee. t1 and t2 are the reference process; 'Becolsub' is mechanical mucilage removal; all fermentation treatments were washed in channels; sun drying in ambient conditions; plus shade for t5 & t7. t1 = 0 delay, becolsub, sun; t2 = fermentation, sun; t3 = 40h delay, becolsub, sun; t4 = 40h delay, fermentation, sun; t5 = 40h delay, fermentation, slow drying t6 = 64h delay, becolsub, sun; t7 = 64h delay, becolsub, slow drying.

	Treatment	Naked	p wt/70	BB	Vin	Disclr	Tot proc D	Tot D
May Run	**t1-t2**	**2.0-1.7**	**104.0**	**0.2**	**1.2**	**6.3**	**7.7**	**16.8**
	Difference from reference mean							
	t3	0.8	0.5	0.1	0.8	-0.1	0.8	0.2
	t4	2.0	8.3	0.1	0.8	1.9	2.8	5.1
	t5	2.0	1.7	0.6	1.8	-3.8	-1.4	-1.8
	t6	5.9	4.8	0.7	2.3	-2.6	0.4	3.0
	t7	6.4	8.7	1.1	7.1	-2.3	5.9	5.8
Sept. Run	**t1-t2**	**6.8-4.4**	**100.4**	**0.3**	**2.2**	**2.3**	**4.7**	**16.3**
	Difference from reference mean							
	t3	3.1	-3.7	0.0	-0.8	0.7	-0.1	-3.1
	t4	6.0	-1.1	0.0	-0.4	1.9	1.5	-0.6
	t6	8.1	18.9	1.5	5.0	2.8	9.3	13.7

Naked = naked beans or '*pelado*'; p wt/70 = the weight of parchment required to get 70kg of green coffee; BB = black beans; Vin = vinegar beans; Disclr = discoloured beans; Tot proc D = percent of defects in the bulk that could have been affected by processing; Tot D = percent of total defects in bulk. t5 and t7 are not reported in the September run.

In the case of holding fresh cherry under water (soaking) prior to wet processing there was found to be a marked increase in the number of 'bleached' beans (22.7%) as compared with immediate wet processing (0.7%). Notably the coffee produced from the 'floats' also produced a high proportion of bleached beans (15.1%). The 'floats' are removed from the wet processing stream prior to pulping (separated on the basis of buoyancy in water) and then are processed as dry cherry.

5.5.4 Impact of delay on cup quality

The robusta cherry coffee described in Tables 5.1 and 5.2 above was cupped by six tasters. Three samples, all from the second run, were recorded as mouldy by one of the tasters and one, the bamboo/sacked treatment (4 days) was identified by two of the six tasters as mouldy. It is not clear whether a low frequency of occurrence reflects an irregular occurrence or a very mild degree of the mouldy taste.

Leaving aside taste defects, there is little evidence here that the pre-drying treatment has any pervasive effect on the taste characteristics of robusta cherry since differences between drying surfaces overlap differences between delay treatments (Table 5.4). Numerically, the delayed coffee has more body and acidity and less astringency and bitterness but the variation in outcome within the three surfaces

shows none of these can be taken as deterministic. Apparently four days in sacks does not disrupt the coffee processing enough to consistently shift the product into a distinguishable category based on these assessment criteria.

Table 5.4: Combined data from the two runs comparing the reference processing method of immediate drying on cement to each of the treatments. The means for immediate drying and 4 day sacking treatments are provided, scale out of 10.

Means of Two Runs		Flavour Evaluation			
Delay (Days)	Surface	Body	Astringency	Acidity	Bitterness
0	Cement (mean)	4.5	5.3	4.0	5.3
	Tarpaulin	0.1	-0.5	0.9	0.5
	Bamboo	0.2	-1.3	0.8	-0.3
4	Cement	0.6	-1.0	1.0	-0.6
	Tarpaulin	0.6	0.0	1.0	0.4
	Bamboo	0.0	-0.3	0.9	-0.3
0	ALL	4.6	4.7	4.6	5.4
4	ALL	4.8	4.4	4.9	5.2

Delay of processing probably affects subsequent wet processing cup quality, as measured by 'overall' assessment, more than cherry coffee production, at least for delays of more than 40 hours. Not surprisingly, however, the two sets of data we have on this are not entirely consistent. Generally each of the individual character attributes mirrors 'overall' quality aside from 'fragrance', which is slightly less susceptible to deterioration, so the discussion is based on this parameter.

The consistently poor quality of coffee from treatments 6 and 7 with their long (64 hour) delay is apparently attributable solely to the delay, since slow drying does not further reduce quality. Direct mechanical washing (t1) outperforms the 40 hour fermentation process by virtue of its greater consistency. One replicate of t2 in the May run produced poor quality coffee.

The 40 hour delay treatments (t3 and t4) produced coffee with a similar quality to the fermentation reference treatment (t2) and sometimes as good as the best. Again, consistency of outcome is not good with both of these treatments producing one replicate of very poor coffee in the September run. Strangely, t5, which is t4 with slow drying, was very good in May but was not evaluated in September. From this data it appears that once a 64 hour delay is reached, cup quality will be poor.

Table 5.5: Cupping evaluation of coffee samples from the coffee physically described above in Table 5.3. Twenty cups were tested for each of two replicates for each treatment.

t1 = 0 delay, becolsub, sun; t2 = fermentation, sun; t3 = 40h delay, becolsub, sun; t4 = 40h delay, fermentation, sun; t5 = 40h delay, fermentation, slow drying t6 = 64h delay, becolsub, sun; t7 = 64h delay, becolsub, slow drying.

May Run

	Fragrance	Aroma	Acidity	Bitterness	Body	Overall	Overall s.d.
t1	6.7	6.7	6.6	6.4	6.4	6.4	0.8
t1	6.6	6.2	5.8	5.8	5.9	5.7	1.5
t2	6.3	6.3	4.4	4.2	4.1	4.2	2.1
t2	6.5	6.3	6.5	6.6	6.3	6.5	0.5
t3	5.9	6.0	4.3	4.0	4.2	4.0	1.9
t3	5.6	6.0	5.7	5.9	6.0	5.9	1.7
t4	6.3	6.3	5.5	4.5	5.0	5.3	1.3
t4	6.4	6.2	6.2	6.7	6.3	6.3	0.6
t5	6.5	6.4	6.2	6.1	6.1	6.1	0.8
t5	6.7	6.2	6.3	6.5	6.3	6.2	1.3
t6	2.6	2.0	2.0	2.0	2.0	2.0	0.0
t6	5.2	3.9	3.9	3.9	3.2	3.0	1.8
t7	4.8	3.5	2.9	2.9	2.9	2.7	1.8
t7	4.2	1.9	2.8	2.3	2.2	2.2	1.2
September Run							
t1	6.2	6.4	6.8	6.8	6.8	6.6	0.6
t1	6.2	6.4	6.7	6.5	6.6	6.5	0.5
t2	5.0	5.2	5.9	4.6	5.2	5.2	2.4
t2	5.5	5.8	4.5	3.8	4.5	4.6	2.6
t3	6.1	6.5	6.0	6.4	6.6	6.2	1.6
t3	5.8	4.9	3.5	1.9	1.8	1.8	1.6
t4	6.4	5.1	5.5	5.0	5.8	5.3	2.3
t4	2.8	3.5	2.0	2.0	2.0	2.0	0.0
t5	no data	no data	no data	no data	no data	no data	no data
t5	no data	no data	no data	no data	no data	no data	no data
t6	3.4	2.6	2.6	1.8	2.6	1.8	0.4
t6	2.8	1.9	2.6	1.8	2.2	1.8	0.4
t7	no data	no data	no data	no data	no data	no data	no data
t7	no data	no data	no data	no data	no data	no data	no data

The raw data of this evaluation were noted to have a high degree of inconsistency in some samples. The pattern in September is, as one might predict (see column 'Overall s.d.' in Table 5.5 above), one of low variation in the best and worst treatments with high variation in the intermediate treatments, but this is not the case in May when quality was better generally.

In Table 5.6 the qualitative comments of the cuppers are evaluated. There is some tendency for biological defects to increase with delay but some of the non-delay treatments show considerable defect occurrence too. The chemical defects are most prominent in the intermediate treatments. Note that the May t5 replicates also have several defects. This is indicative of the variability in these samples with 7 and 12 'very good' cups along with 6 and 5 defect cups. September is more uniform than May. Poor reproducibility can be exampled comparing both the t3 and t4 replicates in May and the t3 and t4 in September. This is reminiscent of the data often acquired

from OTA analysis of such samples, and may reflect the irregularity of the distribution of badly affected beans. The sample size for cupping is 9g and for OTA 10 to 25g so a similar pattern would be expected if the distribution of the causative agents of the two is similar.

Once again there is little indication in this data that slow drying added to the delayed processing causes any particular problem.

Table 5.6: Tabulation of the tasters' qualitative comments from the cupping evaluation described quantitatively in Table 5.5. VG = very good; G = good; Microbial = defects including mouldy, sour, earthy, fermented, dirty; Chemical = defects including phenolic, metallic, woody, astringent.

t1 = 0 delay, becolsub, sun; t2 = fermentation, sun; t3 = 40h delay, becolsub, sun; t4 = 40h delay, fermentation, sun; t5 = 40h delay, fermentation, slow drying t6 = 64h delay, becolsub, sun; t7 = 64h delay, becolsub, slow drying.

May Run	VG	G	Microbial	Chemical	Stinker
t1	11	6	0	3	0
t1	7	7	2	3	0
t2	3	3	8	5	0
t2	9	11	0	0	0
t3	1	4	7	8	0
t3	9	5	3	2	0
t4	3	5	2	10	0
t4	6	13	0	1	0
t5	7	7	0	6	0
t5	12	3	1	4	0
t6	0	0	11	0	0
t6	0	4	14	2	0
t7	0	2	7	4	5
t7	0	1	13	1	0

May Run	VG	G	Microbial	Chemical	Stinker
t1	11	9	0	0	0
t1	9	11	0	0	0
t2	10	4	3	1	2
t2	10	0	0	10	0
t3	10	8	2	0	0
t3	1	0	14	5	0
t4	6	10	0	4	0
t4	0	0	20	0	0
t5	no data	no data	no data	no data	no data
t5	no data	no data	no data	no data	no data
t6	0	0	16	2	2
t6	0	0	15	5	0
t7	no data	no data	no data	no data	no data
t7	no data	no data	no data	no data	no data

The last set of cupping data following a delay by soaking in processing compared robusta and arabica, parchment and cherry in three iterations. The tasting methodology was less rigorous than above and the cup quality was not impressive with 'above average' the highest designation awarded. Arabica parchment in the first

iteration was poor (scored at 'below average') throughout in comparison to the cherry coffee and it became foul after 6 days delay.

Unexpectedly, the cherry coffee was 'above average' throughout - although a sour odour was noted in some of the delayed treatments, cupping results were uniformly 'above average'. In the other two iterations delays of less than 6 days did not reduce cup quality and in the second run robusta parchment prepared after a 6 days delay cupped as 'above average'.

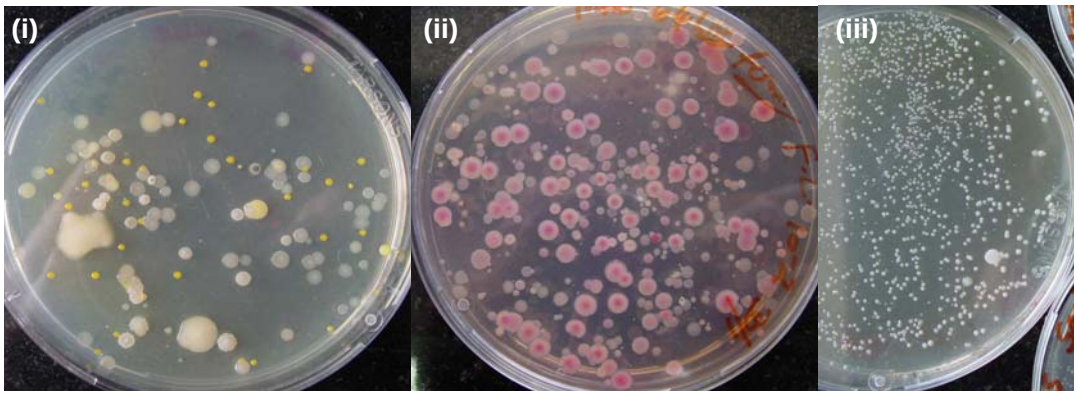
Table 5.7: Selected cup-tasting parameters describing samples from two field trials run in the 2003-4 season. Delay method was soaking only. Light grey shading identifies the reference methodologies, and dark grey the greatest deviation from reference. Physical quality was uniformly poor throughout. 0 = average or normal; -1 = below average; -2 = poor; 1 = fair; 2 = above average; 3 = good

Run	Treatment	Coffee type	m.c. (Sinar)	Odour	Flavour	After-taste	Quality rating
Delay of Processing							
1	Delay 0d	AC	12.9	0	1	1	2
1	Delay 0d	AP	12.9	0	-1	0	-1
1	Delay 2d	AC	13.7	Sourish	1	1	2
1	Delay 2d	AP	13.0	0	-1	0	-1
1	Delay 4d	AC	13.3	0	1	1	2
1	Delay 6d	AC	13.3	Sourish	1	1	2
1	Delay 6d	AP	12.5	Foul	-2	-2	-2
2	Delay 0d	RC	11.7	0	1	1	2
2	Delay 0d	RP	11.6	0	0	0	0
2	Delay 2d	RC	12.4	0	1	1	2
2	Delay 2d	RP	10.0	0	1	1	2
2	Delay 6d	RP	12.2	0	1	1	2
3	Delay 0d	RC	12.2	0	-1	-1	-1
3	Delay 0d	RP	9.8	0	0	0	0
3	Delay 2d	RC	11.2	0	0	0	0
3	Delay 2d	RP	11.2	0	0	0	0
3	Delay 4d	RC	11.8	0	1	1	2
3	Delay 6d	RC	12.0	0	-1	-1	-1
3	Delay 6d	RP	12.0	0	-1	-1	-1

5.5.5 Microbiology of the mucilage during delayed processing

Bacteriologists have developed a host of selective media that allow bacteria sharing certain characteristics to be counted with very low detection limits. However, complex samples from environmental sources, such as vegetable fermentations, can produce surprising outcomes and a good understanding of how the media work is required to interpret such data.

Image 5.3: From left to right: (i) Initial bacterial m-community, the yellow colonies are putative *Bacillus subtilis*; (ii) *Enterobacteriaceae* from traditional fermentation showing weak but clear lactose fermenters (the pink colonies); (iii) Lactic acid bacteria from the soaked treatment after fermentation, this the lowest population of the treatments.



For example, MacConkey agar selects *Enterobacteriaceae* based on their resistance to bile salts (middle plate in Image 5.3 above), and in medical applications can be incubated at 42 or 44°C to increase selectivity. The presence of lactose and a pH indicator also allows identification of bacteria that can produce acid from lactose, an important character of faecal coliforms and some other *Enterobacteriaceae*. However, several environmental members of this group cannot survive high temperatures, so some of the selectivity of the medium must be sacrificed in order to recruit these species. Lactose fermenting, bile tolerant isolates are putative *Enterobacteriaceae* (such as *Erwinia* spp.), though not all of these ferment lactose and there could be a high non-enteric background as well.

MRS is one of the media available for counting lactic acid bacteria (LAB) based on tolerance to high acetic acid/low pH and the ability to assimilate lactose in microaerophilic/high CO_2 conditions established with the use of a candle jar.

Table 5.8: Bacteriological analysis during the first 64 hours after harvest (measured from delivery to the processing facility) comparing traditional wet and dry processing to delayed processing by immersion in water ('soaking') or holding in woven poly sacks ('sack') for 40h before pulping and 24h fermentation. The experimental design compared four different fermentation conditions.

Robusta	Initial	40h sampling (mucilage of cherry)			Fermentation liquor from (64h)		
	Fresh (cfu/ch)	Soak (cfu/ch)	Sack (cfu/ch)	Drying yard (cfu/ch)	Traditional (cfu/ml)	Soaked (cfu/ml)	Sacked (cfu/ml)
Run 1							
Lactics (MRS)	$6.0x10^1$	$6.0x10^5$	$1.2x10^6$	$3.0x10^6$	$2.4x10^7$	$1.8x10^7$	$>10^7$
Enterics (mac +)		$8.0x10^4$	$7.6x10^4$	$<5x10^4$	$5.6x10^4$	$<5x10^4$	$<5x10^4$
Enterics (mac -)	$2.6x10^2$	$2.6x10^5$	$2.8x10^5$	$>10^6$	$2.6x10^5$	$>10^6$	$>10^6$
Yeasts (DG18)	$2.0x10^1$	$2.0x10^2$	$1.2x10^5$	$2.0x10^5$	$>10^6$	$>10^6$	$>10^6$
Run 2							
Lactics (MRS)	$6.0x10^1$	$3.8x10^6$	$2.6x10^5$	$1.2x10^4$	$1.0x10^7$	$9.0x10^6$	$8.0x10^6$
Yeasts (DG18)	$3.4x10^3$	$4.4x10^3$	$2.0x10^5$	$5.0x10^3$	$>5x10^5$	$2.8x10^5$	$1.9x10^5$

Table 5.8 shows that LAB increase by 5 to 6 orders of magnitude over 40 hours to about 10^6/ch. The low value in the drying cherry of the second run may be due to good drying conditions reducing water activity quickly below that which is required by these hydrophiles. The conditions in sacks and on the drying yard are more conducive to yeast growth than soaking. Otherwise soaking and sacking appear similar but distinguishable from the drying yard by supporting greater development of the lactose fermenting *Enterobacteriaceae* (mac+). These could include species of the genus *Erwinia* some of which characteristically produce potent exogenous pectinolytic enzymes. Notably the hardening of the mucilage that was observed in the soaked cherry (Section 5.5.1, above) suggested incomplete breakdown of pectins in the mucilage.

Of course, in traditional fermentation the 'fresh' microbial community is subjected to the fermentation conditions whereas with the delay treatment this community has be greatly altered by the conditions of the delay period, either sacked or soaked. The data describing 'fermentation liquor' represents a comparison of 64h of 'dry fermentation' with 40 hours of delay and 24 hours of dry fermentation. Surprisingly, it appears that the lactose fermenting *Enterobacteriaceae* of the delayed communities rapidly disappear, despite the fermented coffee fostering a substantial community of

the same category. This could mean that different species of Lac+ organisms have been selected in the sacks and soaking tubs than in the fermentation mass.

Table 5.9, below, tends to confirm that soaking inhibits the development of bacteria after harvest though the sampling was somewhat erratic.

Table 5.9: Bacteriological analysis of soaking delay of processing experiment. The soaked cherry was pulped and fermented, thus the 'soaked, fermented liquor' sampling. MacConkey agar was available only for the first run. Blank cells = not determined.

Arabica	Fresh (cfu/ch)	40h soaked (cfu/ch)	Soak water (cfu/ml)	Fermented liquor traditional (cfu/ml)	Soaked (cfu/ml)
Run 1					
Lactics (MRS)	$1.0x10^3$	$2.4x10^5$	$1.5x10^5$	$1.2x10^6$	
Enterics (mac +)	$<5x10^2$	$3.2x10^2$	$<5x10^3$	$<5x10^4$	
Enterics (mac -)	$<5x10^2$	$2.0x10^4$	$<5x10^3$	$<5x10^5$	
Yeasts (DG18)	$<5x10^2$	$<5x10^2$	$<5x10^2$	nd	
Run 2					
Lactics (MRS)	$3.0x10^3$	$1.6x10^5$	$3.2x10^5$	$>5x10^7$	$2.2x10^6$
Yeasts (DG18)	$<5x10^2$	$<5x10^2$	$<5x10^2$	nd	
Run 3					
Lactics (MRS)	$1.5x10^3$	$2.1x10^5$	$1.1x10^4$		$2.8x10^5$
Yeasts (DG18)	$2.5x10^3$	$<5x10^2$	$3.0x10^3$		$3.0x10^3$

Figure 5.4 (below) gives a graphic representation of microbial communities over 72 hours of processing in comparing cherry drying and traditional fermentation with sacking and soaking of cherries. The bacterial load of fresh cherries is low at about 1 x 10^4 / cherry, about 33% possibly *Bacillus subtilis*, but enteric bacteria and lactic acid bacteria are present only at less than 200/cherry together. Lactose fermenting *Enterobacteriaceae* were not observed initially and didn't arise in cherry drying, but became prominent in the soaking and sacking treatments during the first 40 hours.

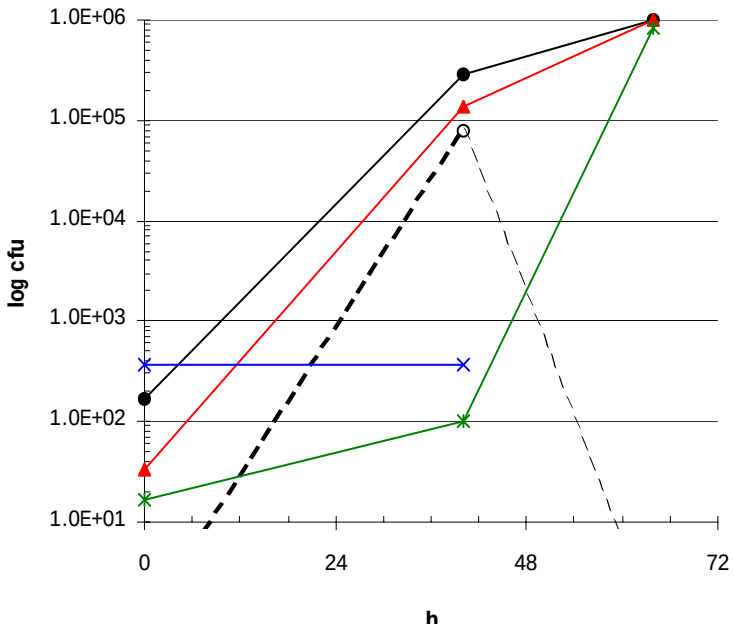
Here, however, their numbers collapsed during post pulping fermentation (after 40 hours in sacks or soaked) which suggests that the lactose fermenters in those treatments were different than those in traditional fermentation since lactose fermenters were prominent at the end of traditional fermentation proving it is not the fermentation conditions *per se* that prevents lac + *Enterobacteriaceae* growth.

Soaking effectively inhibits both yeast and mould development whereas sacking, cherry drying and traditional fermentation all produce a rapid increase in yeast

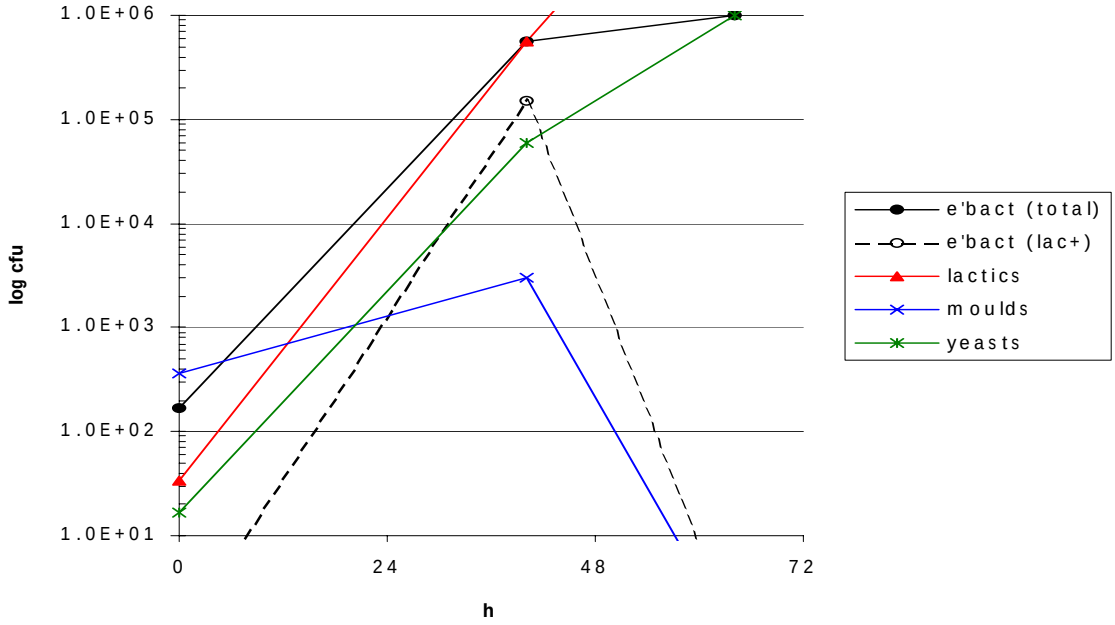
numbers and a modest increase in mould numbers, excepting traditional fermentation where there is a fall in moulds. Soaking may also slightly inhibit the growth of *Enterobacteriaceae* and LAB in comparison with the other treatments, which may be purely a consequence of the lower temperature ensured by the mass of water, considering many LAB's have a temperature optimum above 40°C. LAB increased at a similar rate in all other treatments.

Figure 5.4: Bacteriological and mycological analysis of sacking/soaking experiment. Delayed wet processing treatments are fermented at 40h.

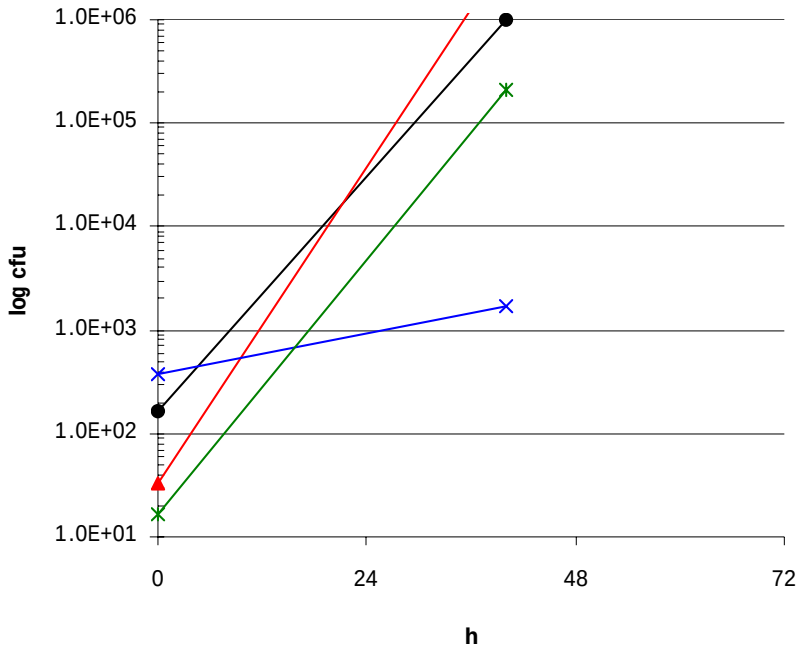
Soaking t course



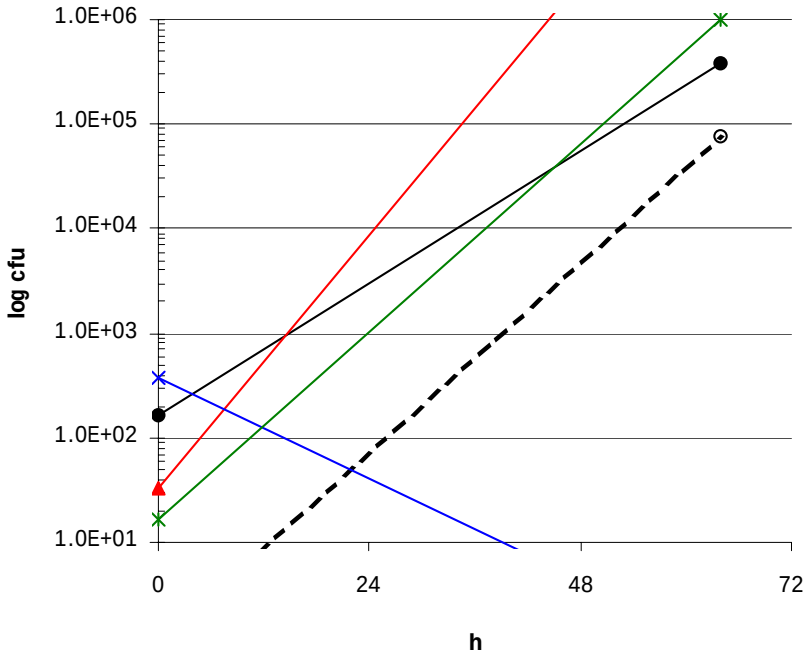
Sacking t course



Cherry drying t course



Fermentation t course



5.5.6 Mycology of the seed during delayed processing

Several iterations of trials involving delay of processing by holding cherries in sacks prior to wet and dry processing have been carried out in several project countries. ***In no single trial was there any evidence of a relationship between delay and either presence of ochre group fungi or OTA contamination.***

We have already seen that the ochre group fungi - the most important OTA-producers in coffee – are widely present in coffee but at extremely low levels. We have also seen that coffee microflora is complex and that, under a given set of conditions, patterns of growth of the dominant organisms (particularly yeast and

bacteria) are fairly predictable. However, the survival or growth of OTA-producing fungi is highly unpredictable. The evidence coming from the trials shows that the factors that were considered and controlled in the experiments are ***not*** determining, in predicting OTA risk. The design of the trials was based on the hypothesis that increasing the period of time during which the coffee was microbiologically unstable (at high moisture) before processing was an important factor influencing growth and OTA production by moulds capable of producing this mycotoxin. The results, however, show that the reality is much more complex and that ***under the conditions of the trials***, delay in processing did not lead to a pattern of microbiological development that increased the risk of OTA contamination even under poor drying conditions (in some treatments, slow drying of parchment or cherry was intentionally imposed by partially shading the coffee from the sun on the drying yard).

There is high variability in the 'frequency of infection' of ochre group fungi among replicate treatments and, where OTA was found, there is also high variability in levels of contamination found in replicate samples. This extremely large variation has meant that even fairly large apparent differences in the outcomes of some treatments fall short of statistical significance. However, even simple numerical analyses failed to reveal any pattern linking delay to increased OTA risk.

In some of the trials, there were isolated samples that showed substantial increases in ochre group fungal infection or in OTA levels, but these cases were equally likely to correspond to control treatments (those processed in the traditional way with no delay after harvest) or delayed treatments. Furthermore, the isolated occurrences of increased levels of OTA-producing fungi or of OTA in 'delayed' treatments occurred in all of the different processing scenarios (dry, wet processing with a traditional fermentation step, wet processing with mechanical mucilage removal).

Even in the absence of apparent trends in OTA risk due to delayed processing, the results can provide some useful insights if interpreted in light of experience with both the measurement and the biological systems involved and against clearly enunciated hypotheses.

The results of selected trials are discussed below. Interested readers are directed to Annex C.6 on the enclosed CD-Rom for a discussion of all of the delayed processing trials completed under the project.

One trial carried out in Côte d'Ivoire during 2003-2004 considered periods of delay of 0, 2, 4 and 6 days (fresh robusta cherry held in sacks) and two types of processing (wet processing and dry processing). Tables 5.10 and 5.11 present OTA contamination levels and average bean contamination frequencies of main fungal communities of the bean.

Table 5.10: Selected findings from mycological analysis of coffee before and after processing.

Run 1									
Delay	Process	Initial (% contamination)				Final (% contamination)			
		Total	Yeast	Black Asper.	Ochre Asper.	Total	Yeast	Black Asper.	Ochre Asper.
0	Wet	32	32	16	1	91	42	76	<
	Dry					94		88	<
2	Dry	74	74	38	1	99	1	99	<
4	Wet	91	91	50	<	100	72	99	<
	Dry					100	<	98	<
6	Dry	96	96	73	5	100	<	100	<
Run 2									
Delay	Process	Initial (% infection)				Final (% infection)			
		Total	Yeast	Black Asper.	Ochre Asper.	Total	Yeast	Black Asper.	Ochre Asper.
0	Wet	42	74	36	1	99	42	107	2
	Dry					96	100	51	3
2	Dry	90	81	53	1	100	83	95	5
4	Wet	98	96	86	3	100	<	103	2
	Dry					100	100	98	<
6	Dry	99	100	90	9	99	5	99	1

The coffee in the second run had a higher initial infection rate of yeast and niger aspergilli. Excepting the persistence of the yeasts in the beans of the '4-day delayed – dry processed' treatment and the persistence of yeasts in immediately dried cherry coffee, the initial high population of yeast in the second run did not significantly affect outcomes.

Similarly, with the niger aspergilli, outcomes are very comparable, except that the immediately dried cherry developed fewer infections during drying when the initial infection rate was higher. This could have been due to the exceptionally high yeast population.

Ochre aspergilli frequency was initially the same (0.5%) in both runs. In the second run the ochre aspergilli persisted through most treatments (all except 4-day delayed cherry processing) whereas it was not detected after drying in the first run. The actual differences are too small to be considered statistically significant but it is clear that in the second run, the fungus survived processing and it was this run where some probable increases in OTA were observed. It should be borne in mind, that infection rates with ochre group fungi were determined by viable colony counting. As pointed out earlier (see Part C, Section 1 on 'Distribution of Fungi in Coffee

Production Systems') this method does not give information on biomass or extent of infection. The data must therefore be interpreted with care.

During sacking, a numerical increase in ochre infection was recorded that corresponds to a period during which there is no increase in OTA. This could signify that there was little increase in biomass, whether or not there was an actual increase in infection, or that the fermentation conditions prevented ***net*** accumulation: possibly the observed super-optimal temperatures and undoubted very low O_2 levels prevented OTA production, or perhaps there was higher gross production but enzymatic or respiratory breakdown took place during this fermentation.

Table 5.11: OTA content of coffee before and after processing with different periods of delay.

		Run 1				Run 2			
Delay (days)	**Processing method**	**Initial OTA (ppb)**		**OTA in dry bean (ppb)**		**Initial OTA (ppb)**		**OTA in dry bean (ppb)**	
0	**Wet**	3.2	2.9	1.9	1.9	6.1	10.6	28.8	19.3
	Dry							16.3	18.7
2	**Dry**	6.1	4.8	2.3	5.4	14.4	7.9	7.9	10.6
4	**Wet**	3.0	2.4	5.9	5.6	10.9	10.9	28.8	30.6
	Dry			4.7	13.7			6.4	4.2
6	**Dry**	3.6	3.5	5.8	4.7	6.1	5.1	12.5	5.7

In the first run, there are no apparent differences among treatments in relation to OTA contamination levels. In other words, throughout delay and processing there is no increase in OTA levels. In the second run there are three treatments where there are apparent increases in OTA contamination although the variability is too high and replication is too low for statistical significance. These treatments correspond to wet processing with no delay, wet processing after a 4-day delay and dry processing with no delay. Clearly these data do not support the contention that delays before processing are an important factor contributing to increased levels of OTA contamination.

The difference between the outcomes of the two runs from the Côte d'Ivoire 2003-4 season might be explained by a difference in initial conditions. If we consider the initial levels of OTA contamination as an indicator of biomass of infecting ochre group fungi, then it is plausible that the coffee used in Run 2 had a better established ochre group community at the start allowing faster growth and OTA production when conditions become suitable.

At the start of the project there was a suggestion that wet processing – through the action of competing organisms – might offer some inherent protection against OTA contamination. This does not seem to be justified.

Similar delay of processing experiments were conducted in Meru and Thika, Kenya, although neither dry processing nor delay of processing are common in Kenya. The intention was test the impact of delay in different environments.

There was very little in the way of OTA-producers and no ochre aspergilli were detected in either of the two Meru trials. In the Thika trials, one sample that had experienced a 6-day delay before cherry drying, and another that had been fermented on the day of harvest had ochre aspergilli at or around the detection limit. Tables showing the means of infection rates of selected fungal taxa are contained in Annex C.6. The fungal infection data are broadly consistent with the picture of OTA content.

One of 24 samples from Meru showed OTA at quantifiable levels (0.1 ppb), this from four day delayed cherry coffee. From Thika, three samples contained enough OTA to quantify and all were less than 0.75 ppb. All four of these were from the cherry coffee treatments, one each from no delay, 4-day delay and 6-day delay treatments. The latter treatment is one of the two that also contained ochre aspergilli at detectable levels.

Other studies designed to investigate the impact of holding fresh cherries in sacks for periods between 0-6 days before wet and dry processing on microbiological and OTA contamination of the coffee bean, were carried out in Colombia, Côte d'Ivoire, India and Uganda. The results are discussed further in Annex C.6.

A second form of delay, holding under water (or 'soaking') was also studied but to a lesser extent. Two trials were carried out in India. The first compared the outcome of soaking followed by traditional wet processing to the outcome of traditional fermentation. The second compared soaking to sacking, fermentation and dry processing over two seasons. The summary of results from the first study is presented below.

Table 5.12: Mycological data from individual runs and the means of three runs of delayed arabica processing by soaking. All data is % internal bean infection.

'<' = not detected; 'floats' = floats coffee, was processed as cherry coffee; 'final' data is from the dried product; only the cupping for the first run was completed.

	Initial fresh			48 soaked			Fermented		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Total infection %	20	63	63	68	56	60	92	86	95
Ochre aspergilli	**<**	**<**	**<**	**<**	**<**	**<**	**<**	**<**	**<**
Niger aspergilli	<	<	<	<	<	<	20	2	<
Fusarium	<	<	<	<	<	<	<	<	<
Yeast	<	47	43	<	38	43	<	24	46
Cladosporium	3	1	8	24	2	<	4	10	<
Others	17	15	12	44	16	17	68	50	49
	Final parchment			**48 soaked**			**Floats**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Total infection %	100	28	40	100	40	48	100	96	98
Ochre aspergilli	**<**	**<**	**<**	**<**	**<**	**<**	**6**	**2**	**<**
Niger aspergilli	32	18	10	15	24	42	83	16	16
Fusarium	47	<	6	54	8	4	6	<	<
Yeast	11	4	18	<	4	2	<	78	80
Cladosporium	<	<	2	15	<	<	6	<	2
Others	11	6	4	15	4	<	<	<	<
Bleached	0.67			22.67			15.14		
Bean density	1.36			1.17			1.26		
Cup quality	FAQ+			FAQ			FAQ to FAQ-		

Means	**Initial**			**Final**		
	Fresh	**Soaked**	**Fermented**	**Parchment**	**Parchment soaked**	**Floats**
Total infection %	49	61	91	56	63	98
Ochre aspergilli	<	<	<	<	<	3
Niger aspergilli	<	<	7	20	27	38
Fusarium	<	<	<	18	22	2
Yeast	30	27	23	11	2	53
Cladosporium	4	9	5	1	5	3

The second and third runs share common initial mycological conditions while the first run shows a very low initial infection rate that includes no detectable yeast infection. Yeast fails to develop through any of the treatments in this run, except in the parchment during drying. In the other runs, yeast infection is steady through the wet portion of processing at around 40% but falls, as expected, during drying.

Ochre aspergilli are only detected here in the floats coffee of the first two runs at low levels. Niger aspergilli arise during drying, though in the yeast-less first run it emerges during fermentation but not soaking. It is not common for this group to be so well represented in arabica parchment. In the floats coffee, which was prepared as cherry coffee, either yeast dominates the flora or niger aspergilli do.

Fusarium, as with the Colombian studies, develops during drying, a period when it normally falls in infection rate. *Cladosporium* provides a steady background which could suggest a degree of lab contamination by this troublesome air-borne fungus, although there is no doubt that it occurs in coffee. During fermentation and some of the soaking replicates, miscellaneous moulds provide the largest collective component of infective flora. These largely disappear during drying.

Some OTA analysis was done on these samples, though without replication. Once again, none of the delay treatments showed any indication of promoting OTA production.

Section 6
Contact with Soil

6.1 Introduction

Coffee may come into direct contact with soil at two points in the production chain: before (or at) harvest, and during drying if a compacted earth drying terrace is used. The latter route is only relevant in cherry drying, because parchment is never dried on soil.

There are two factors controlling the potential development of quality or safety problems in this category of coffee:

1. Why did the coffee fall to the ground?

2. How long does the coffee stay on the soil?

What falls from the trees is a combination of diseased fruit, tree-dried fruit and possibly normal fruit that has been damaged by an animal or knocked off by workers harvesting or tending the trees. Arabica coffee falls more readily once dry, whereas robusta coffee does not normally abscise.

Diseased fruit has largely fallen before or during the first weeks of harvest and so may spend months on the ground. Fruit inadvertently knocked off during harvest or other horticultural activities may also stay for a long period, or may only be briefly in contact with the ground.

Cherry coffee that has been taken from the ground often has its own designation such as 'gleanings', '*café chão*' or '*varrição*'. However, coffee that has fallen and has lain on the ground for substantial periods can become mixed into main production stream cherry coffee depending on harvesting methods. One traditional method is to strip the coffee onto the ground before using a winnowing screen to separate coffee from soil, leaves and other extraneous material. Here any coffee that had previously fallen would be included into the main harvest, unless the orchard had been conscientiously cleaned beforehand, or picking tarpaulins were used.

Extensive soil sampling under the global project indicated that *Aspergillus ochraceus*, the most important of the OTA-producers, is more common in the soil of the coffee rhizosphere than the soil between coffee trees. *A. niger*-complex fungi can also be isolated but *A. carbonarius* has not been confirmed from soil. *A. melleus*, an OTA non-producer that is difficult to distinguish from *A. ochraceus* and which is also found in coffee, is generally found in soil, including soil beneath coffee trees.

The evidence of OTA-producers being widely isolated from orchard soil along with general concerns of contact of a food (i.e. coffee) with soil for long periods motivated investigation into the impact such contact may have on coffee safety. Coffee cannot be prevented from coming into contact with the micro organisms of the soil; coffee production, including processing, is an outdoor activity and contact of the fresh fruit with micro organisms of the soil during the seven to eleven months of fruit

development is frequent through rain splash, dust and insect activity. This continues during processing, which almost always includes at least an element of sun-drying.

The collection of fallen cherry after completion of harvest is an important integrated pest management (IPM) tool for the control of coffee berry borer (CBB), and should also be undertaken to minimize the spore load of OTA-producing fungi in the orchard. After collection this coffee is often marketed, at a very low price, to defray these costs, and anecdotal evidence suggests it may be mixed into main crop coffee in the trading chain.

Mechanical harvesters generate a lot of fallen cherry, and in areas where mechanical harvesting has become important between 15 and 20% of the total harvest will have been collected from the ground. It is given its own designation, and is known as *varrição*.

Coffee dried on soil drying yards is in a more controlled situation and the period of contact will typically persist for two to three weeks. Protection from rain is normally provided by a well-managed yard, at least after the first five days of drying. Nevertheless, dry cherries can be seen covered with soil, implying an episode of heavy rain during sun drying, and is fairly typical in some regions.

Some farmers claim faster drying on soil, but the main factor for selecting this form of drying terrace appears to be the additional capital, and maintenance costs, required by higher technology drying surfaces.

6.2 Findings and Application

6.2.1 Cherries in contact with the soil in the coffee plantation

The balance of evidence is that there is an enrichment of *Aspergillus ochraceus* in the rhizosphere soil of the coffee plant, and that infection of the bean over time can be affected by contact with this soil.

A long residence under trees leads to a gradual increase in bean infection by fungi which is partly eliminated if drying is completed on a yard. Two of four trials where coffee was placed under trees indicated there was an increase in infection rate by ochre aspergilli, and two did not. These did not confirm that the ochre aspergillli reported included *A. ochraceus*, and it should be noted that *A. melleus*, an OTA non-producer, is more common in soil than *A. ochraceus*. The results of these trials are discussed in Section 6.4.2, below.

The characteristics of the locales of these trials may explain some of the differences in results. The Brazilian locales, though not the driest of the Brazilian production regions, are drier than the Ugandan and Indian forest coffee systems. It is perfectly reasonable to assume the impact on coffee in long residence on the soil in a forest system, or one without a hard dry season, would be more severe than that of an open system which did have daily low humidity and rare rainfall during harvest.

A study on defects and OTA in coffee (see Part C, Section 9 of this report) shows that diseased beans are, at least in some situations, associated with increased risk of OTA

contamination. As mentioned above, disease is one reason for the early fall of cherries. This introduces another factor in evaluating risks associated with fallen cherries.

Coffee that has been in contact with orchard soil for more than a few days represents an OTA risk. The risk is greater In forest production systems and regions that have frequent rain during harvest any such coffee should be removed from the food chain The responsible authorities, with an understanding of the local situations and the nature of risks of OTA production must develop Codes of practice that are both practicable and adequately manage the risk.

6.2.2 Use of compacted-earth drying yards

There is no evidence from these trials that drying coffee on compacted soil yards presents an OTA risk, though the possibility that general quality may suffer from long drying periods on soil cannot be dismissed (drying trials are reported in Section 6.4.4, below). In trials where slow drying conditions were imposed, the microbiological data might indicate a slight nominal increase in ochre group infection rates when soil drying yards are used ('Hindered Drying' trials are reported in Part C, Section 7 of this report).

The implicit questions in investigating OTA risk of soil drying yards are related to the moulds found in the soil and their ability to grow through the cherry husk to colonize the bean in the time it takes the coffee to dry

In terms of the microflora on the drying yard, cement surfaces favour the development of a yeast-dominated community, whereas the soil under coffee of a compacted-earth drying yard tended to reflect the dominant fungi on the coffee, whether due to growth in the soil or collection of spores detached from the overlying coffee. In the case of robusta cherry this is *Aspergillus niger*-complex species (see Section 6.4.3, below). The mould contamination of the external cherry tissues were shown to be unrelated to the internal bean contamination.

The drying time on soil surfaces was compared with drying time on other surfaces through an extensive evaluation involving almost all collaborating countries. Differences in drying rates on different surfaces are generally not statistically significant, and the most important factor are the prevailing atmospheric conditions.

From the safety point of view, there is no evidence that well managed compacted soil yards pose additional risk, though it may be that they are more difficult to manage well.

6.3 Experimental Design

The design of experiments seeking to characterise such an ill-defined entity as 'fallen cherry' is difficult. What falls from the trees is a combination of diseased fruit, tree-dried fruit and, possibly, normal fruit that has been damaged by an animal or knocked off by workers tending the trees. In multi-pass harvesting systems fruit knocked down by workers would be expected to be more significant.

The fact that there should be an enrichment of diseased fruit in the fallen fruit class makes it particularly difficult to generate an adequate control treatment so the experimental designs used address particular circumstances rather than the actual processing stream represented by *café chão* or gleanings.

Given these constraints, the most useful comparison is between fruit harvested and dried on a drying yard, and fruit harvested and dried beneath trees. This at least represents a direct comparison between conditions on the terrace with conditions under trees of the orchard. Such an experiment would include an initial sample, a 24 hour sample to account for brief contact with orchard soil, and period samples to document the changes as the coffee moves toward dryness. The example of this design (run at Visçosa, Minas Gerais, Brazil) contained a second control of cherries on the soil, in the orchard but not under trees. This was to account for the differences in fungi in the coffee rhizosphere.

Other experimental designs were implemented by collaborators. One, for example, compared cherries placed under trees of farms which an earlier survey had indicated to be poor in ochre aspergilli in the soil, against farms that showed the common occurrence of these fungi in the survey. Another incorporated no comparison, instead '*boia*' (tree-dried cherry) was placed under trees and periodically sampled.

The following section also reports on studies where coffee is dried on compacted-earth terraces, probably the most common technology employed world-wide by smallholder farmers producing cherry coffee. These experiments were all drying time-course based, so comparisons with drying on cement and other surfaces are available.

6.4 Experimental Results and Discussion

6.4.1 Occurence of *A. ochraceus* in the soils of coffee plantations

Aspergillus ochraceus tends to be associated with the coffee rhizosphere though other members of the ochre group often appear in forest soils. The association is not so strong that isolation of this species is expected from any given sample but neither is it a surprise. Tables 6.1a to 6.1c below present results from extensive sampling and detailed analysis of soils from Indian coffee regions and gives a good idea of fungal communities in coffee plantation. Sampling in other countries, though in less taxonomic detail, has corroborated this general picture.

Note that most of the ochre group isolates did not prove to be *A. ochraceus* and that those that did were from samples taken in the rhizosphere area. *A. melleus*, an OTA non-producer normally taken to be a soil organism, is more common here than *A. ochraceus* to which it is very similar. *Penicillium brevicompactum*, also commonly isolated from coffee fruit and seed is readily found in coffee soils but apparently only in Chickmagalur of the Indian regions sampled.

Table 6.1a: Soil analysis from coffee plantations in Chickmagalur district, Karnataka state. Site 0 = at tree trunk; site 1 = at extent of foliage; site 2 = between trees; site 3 = on path or similar location. 'Ochre per group' = *A. ochraceus*/ochre grp total; nd = not detected.

Site and sample		t count cfu/g	Dominant taxonomy	% dom	Ochre group	Ochre per group	Other significant recorded taxa
MS							
Arabica	**0**	$1.9x10^5$	*Penicillium*	65	0.05	0.50	*P. manginii* *A. ochraceus* *A. melleus*
Arabica	**1**	$4.4x10^5$	*Penicillium*	>85	nd	nd	*P. manginii* *Phoma*
Arabica	**2**	$1.2x10^5$	*Cladosporium*	70	nd	nd	*P. brevicompactum* *Phoma*
Arabica	**3**	$6.2x10^5$	*Cladosporium*	>85	nd	nd	*P. decumbens*
Grevillea		$4.6x10^5$	*Cladosporium*	78	nd	nd	*P. sclerotiorum* *P. pulvillorum*
Grevillea		$3.6x10^5$	*Penicillium*	69	nd	nd	*P. manginii* *P. odoratum*
NK							
Arabica	**0**	$3.9x10^4$	*Penicillium*	50	nd	nd	*P. raciborskii* *Cladosporium* *Mucor*
Arabica	**1**	$3.4x10^5$	*Paecilomyces*	70	nd	nd	*Paec. lilacinas* *P. raciborskii* *Eurotium*
Arabica	**2**	$2.3x10^5$	*Mucor*	40	nd	nd	*Cladosporium* *P. brevicompactum* *Phoma*
Arabica	**3**	$5.0x10^4$	*Paecilomyces*	80	nd	nd	*Paec. lilacinas*

Table 6.1a contd.: Soil analysis from coffee plantations in Chickmagalur district, Karnataka state. Site 0 = at tree trunk; site 1 = at extent of foliage; site 2 = between trees; site 3 = on path or similar location. 'Ochre per group' = *A. ochraceus*/ochre grp total; nd = not detected.

Site and sample		t count cfu/g	Dominant taxonomy	% dom	Ochre group	Ochre per group	Other significant recorded taxa
CCRI							
Arabica	**0**	$3.0x10^5$	*Paecilomyces*	>85	0.04	nd	*P. velutinum* *Eurotium*
Arabica	**1**	$3.0x10^5$	*Penicillium*	80	0.03	nd	*Geotrichum* *P. velutinum* *A. melleus*
Arabica	**2**	$2.0x10^5$	*Paecilomyces*	85	nd	nd	*P. brevicompactum*
Ficus		$7.6x10^5$	*Cladosporium*	79	0.20	nd	*A. melleus* *P. raciborskii*
Coffea bengalensis		$1.0x10^5$	*Cladosporium*	50	0.10	nd	*A. melleus* *P. pulvillorum*
CCRI							
Robusta	**0**	$2.3x10^5$	*Penicillium*	50	0.04	nd	*P. raciborskii* *A. melleus* *P. brevicompactum*
Robusta	**1**	$2.0x10^3$	*Mucor*	50	0.14	nd	
Robusta	**3**	$4.9x10^5$	*Paecilomyces*	80	nd	nd	*Paec. lilacinas* *P. laverii*
Grevillea		$1.2x10^5$	*Aspergillus*	50	0.25	nd	*A. melleus* *P. pulvillorum*
Feral robusta		$5.5x10^5$	*Cladosporium*	67	0.02	nd	*A. melleus* *P. brevicompactum* *P. sclerotiorum*

Table 6.1b: Soil analysis from coffee plantations in Coorg district, Karnataka state. Site 0 = at tree trunk; site 1 = at extent of foliage; site 2 = between trees; site 3 = on path or similar location. 'Ochre per group' = A. ochraceus/ochre grp total; nd = not detected.

Site and sample		t count cfu/g	Dominant taxonomy	% dom	Ochre group	Ochre per group	Other significant recorded taxa
GR							
Robusta	**0**	$3.0x10^5$	White ?	0	nd	nd	*Geotrichum* *Paec.* *P. steckii*
	1	$5.5x10^5$	*Penicillium*	36	0.05	0.57	*P. mantanense* *P. chrysogenum*
	2	$2.2x10^5$	*Penicillium*	21	nd	nd	*Eupen. sumatrense* *Cylindrocarpon?*
	3	$5.0x10^5$	*Cladosporium*	62	nd	nd	*P. melinii*
CE							
Arabica	**0**	$1.4x10^5$	*Penicillium*	33	nd	nd	*P. chrysogenum* *Cladosporium*
	1	$4.0x10^6$	*Penicillium*	71	nd	nd	*P. crustosum* *Cladosporium* Yeast
	3	$7.0x10^4$	*Penicillium*	41	nd	nd	*P. anatolicum* *Cladosporium*

Table 6.1c: Soil analysis from coffee plantations in Wayanad district, Kerala state. Site 0 = at tree trunk; site 1 = at extent of foliage; site 2 = between trees; site 3 = on path or similar location. 'Ochre per group' = *A. ochraceus*/ochre grp total; nd = not detected.

Site and sample		t count cfu/g	Dominant taxonomy	% dom	Ochre group	Ochre per group	Other significant recorded taxa
MG							
Robusta	**0**	$1.2x10^6$	*Wallemia*	65	0.01	0.17	Yeast *Cladosporium* *P. sclerotiorum*
	1	$2.6x10^5$	*Cladosporium*	15	nd	nd	*P. pulviorum*
	3	$1.5x10^5$	*Penicillium*	46	nd	nd	*P. rigulosum*
CV							
Robusta	**0**	$6.0x10^5$	Yeast	19	nd	nd	*Eupen.shearii* *A. niger*
	1	$5.0x10^6$	?	?	nd	nd	*Eupen. shearii* *Botrytis?*
	2	$3.7x10^6$	*Wallemia*	55	nd	nd	*Eupen. Shearii* *A. niger*
	3	$1.9x10^5$	*Cladosporium*	43	nd	nd	*Trichoderma Eupen. shearii*
Forest							
Forest		$5.8x10^5$	*Penicillium*	72	nd	nd	*P. bravicompactum* *P. raciborskii*

6.4.2 Impact of prolonged cherry contact with coffee plantation soil

In Lavras, Minas Gerais, Brazil, *boia* was reportedly spread beneath the trees and only analysed upon removal from the ground, not after full drying on a terrace as well. No moisture data is available except a comment that after one week the A_w was about 0.80. This indicates that the coffee was not fully *boia* since boia typically reaches an A_w of 0.75 or less.

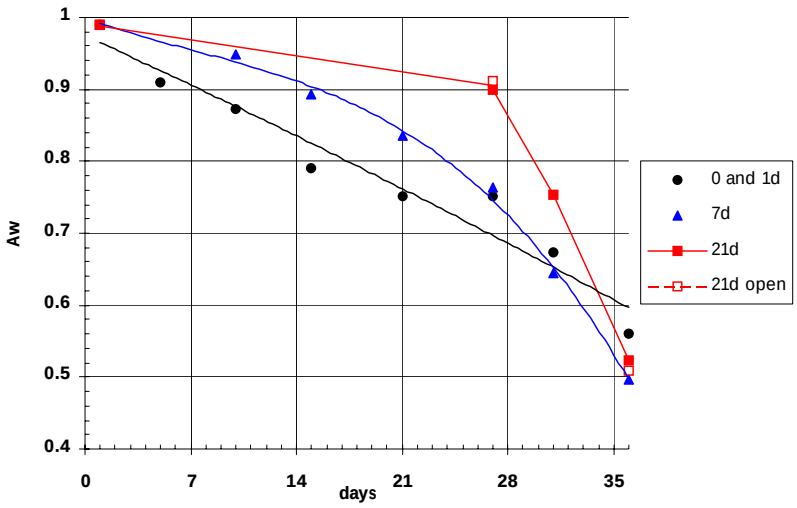
Table 6.2: Progressive changes in the infective fungi as boia lies beneath a hedge of coffee. The figures are means of three 60 bean samples, hence with a detection limit of about 0.6%.

Period of contact with soil (d)	i-analysis (%)				
	A. ochraceus	*A. niger*	*Fusarium*	*Cladosporium* spp.	*Penicillium*
0	12	<0.6	1	0.6	21
7	81	<0.6	0. 6	<0.6	46
14	14	6	4.6	18.6	20
21	18.6	9	15	18.6	36

It is difficult to give much credence to the reported infection rate of *A. ochraceus* after 1 week under the coffee plants of 81% as shown in Table 6.2 above, since the other three samples are consistent and indicate only the possibility of a small increase after three weeks (from 12% to 19%). The other taxa all show a similar possible modest increase. Changes of this magnitude are unlikely to be statistically significant.

The experiment conducted in Visçosa followed the prescribed protocol hence has drying data and the appropriate controls or reference samples to normal sun drying. The most curious feature of the drying time-course was that all the treatments required about the same length of time for drying, whether it was under coffee trees for three weeks or not at all. The maximum drying rate of the coffee left under trees, once dried on the terrace, far exceeded those of the immediately dried coffee. So although the overall drying time was very similar, the period spent at an A_w above 0.80, the OTA-production limit, was very much longer in the under tree treatments.

Figure 6.1: Drying time-course of *cereja* laid beneath coffee hedges (and between rows = '21d open') and moved to the drying terrace after 1, 7 or 21 days in Visçosa, Minas Gerais.



As the coffee lies on the ground there is a general increase in yeasts and moulds inhabiting the seed (Figure 6.2, below). In particular, yeasts move into this niche, from which they were absent in the fresh material, but the more mesophilic fungi did not make inroads. Drying kills a substantial proportion of this community, but a long residence on the soil appears to improve survival during terrace drying.

Figure 6.2: General mycological changes of the internal seed (i-community) community of ripe cherry coffee during residence on the ground ('fresh' when sampled) and after subsequent terrace drying of the corresponding samples (the bar graph).

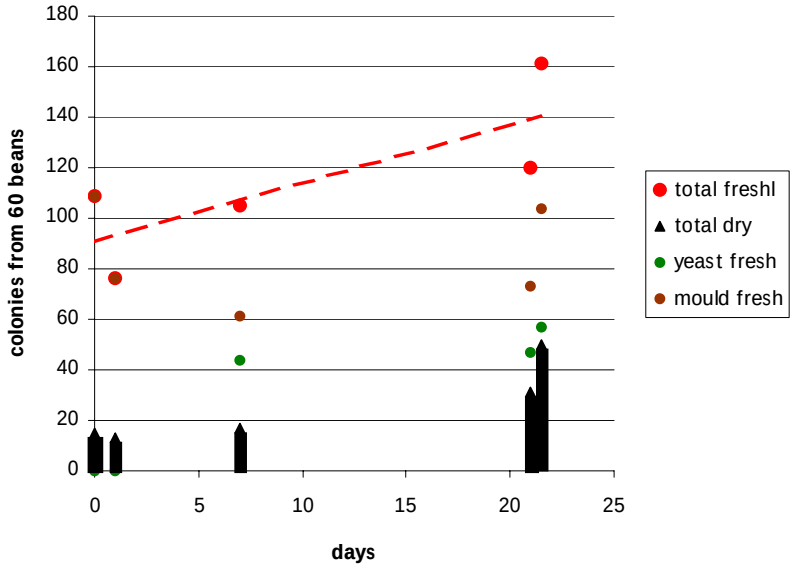


Table 6.3 documents the details of speciation and their changes in occurrence in both the x+m niche and the bean niche. There is no change in the speciation of the x+m niche (external and mesocarp-inhabiting fungi) while on the soil. There are a few minor changes whilst on the terrace. There is a general small increase, up to an order of magnitude in some cases, during residence on the soil, and a further greater increase on the terrace. The increases in filamentous fungi on the terrace may be spurious to the extent that the counts reflect merely spore production as the environment dries out and the fungi cease vegetative growth. Overall there is remarkably little difference between the fungi of this niche.

With respect to the i-community, it appears that yeasts displaced the fungi to some extent within one week during the period on the soil. However, the intermediate samples interpreted strictly as the time-course they represent are irregular and not completely coherent, so the detail is open to some doubt. The difference between initial and 1 day gives an indication of sampling/analysis error.

Penicillium and yeasts apparently failed to survive drying in detectable numbers and *Cladosporium* was substantially killed off. It may be that the colonies scored as 'other aspergilli' in the fresh beans after 0 and 1 day of soil contact were, in fact, *Penicillium*, rather than *Aspergillus* spp. – some can be confused by eye. If true, this would rectify the mysterious disappearance of 'other aspergilli' from around 60% and the sudden appearance of *Penicillium* at 100% contamination over six days.

The speciation of the i-community changed less than had the x+m community during drying. Importantly, potentially toxigenic taxa represented by the ochre and flavi

groups arise during drying, usually uncorrelated with their presence either in the corresponding fresh samples or the x+m community.

The exceptions are flavi group, which were present in the fresh material after 0 and 1 days of soil contact, and were also present in the corresponding dry beans. Equally the ochre group were in turn numerous in the x+m community after drying, but without contact with the soil (0d), and were present in the corresponding i-community. Clearly contact with soil did not increase ochre aspergilli recruitment in the bean.

The frequencies of these fungi, though low, are more than enough to result in significant toxin accumulation (both OTA and aflatoxin) if growth had been significant. To draw conclusions about this, OTA analysis would be required.

Table 6.3: Mycological analysis of *cereja* introduced to the ground beneath coffee trees with varying residence times from 0 to 21d and the terrace-dried result. A treatment where the coffee was spread between rows was indistinguishable from the main treatment so this is not reported here. 'Other aspergilli' does not include niger aspergilli, none of which were isolated in this study.

Fresh

	x+m - community				i - community (% contamination)			
Days on ground	0	1	7	21	0	1	7	21
Yeast	7.9E+06	7.3E+04	4.7E+07	1.1E+07			73	78
Ochre grp								
Flavi grp					8	7		2
Other aspergilli					60	65		
Penicillium **spp.**	2.2E+04	2.4E+03	2.3E+05	3.5E+05	22	2	100	23
Fusarium **spp.**	1.7E+04	5.2E+03	1.2E+05	4.1E+05	37	7	2	70
Cladosporium **spp.**	1.2E+04	2.5E+03	3.2E+05	1.2E+05	55	47		27
Alternaria **spp.**								
Total	**7.9E+06**	**8.4E+04**	**4.8E+07**	**1.2E+07**				
Moulds	**5.0E+04**	**1.0E+04**	**6.7E+05**	**8.8E+05**				
Yeasts	**7.9E+06**	**7.3E+04**	**4.7E+07**	**1.1E+07**				

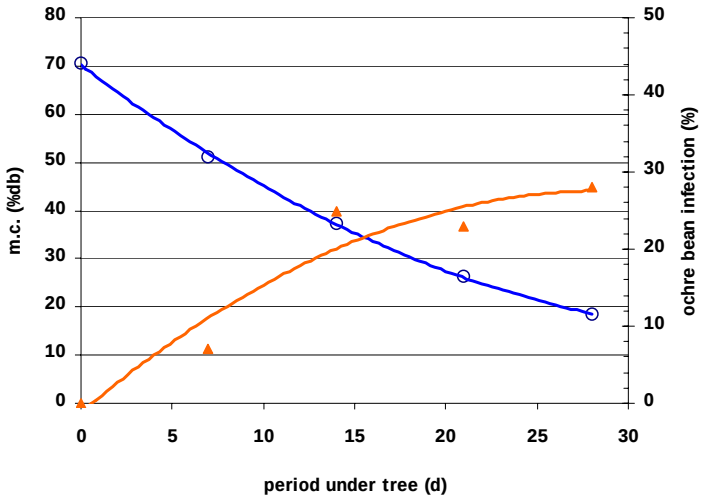
Post Drying

	x+m - community				i - community (% contamination)			
Days on ground	0	1	7	21	0	1	7	21
Yeast	1.9E+07	3.2E+06	2.6E+07	4.9E+08				
Ochre grp	5.0E+05				2	5		2
Flavi grp	2.0E+05					2	2	3
Other aspergilli	7.0E+05	4.0E+04		4.0E+06	3	3	2	15
Penicillium **spp.**	4.0E+05	2.6E+05	2.8E+06	4.4E+07				
Fusarium **spp.**	1.9E+06	3.0E+05	4.9E+06	1.0E+07	17	12	25	28
Cladosporium **spp.**		1.0E+04	2.0E+05	3.0E+06	3			3
Alternaria **spp.**	2.0E+07							
Total	**4.3E+07**	**3.8E+06**	**3.4E+07**	**5.5E+08**				
Moulds	**2.4E+07**	**6.1E+05**	**7.9E+06**	**6.1E+07**				
Yeasts	**1.9E+07**	**3.2E+06**	**2.6E+07**	**4.9E+08**				

Contrary to the Brazilian studies, two trials (one in Uganda, the other India), produced high ochre aspergilli infection rates in beans of cherries that had been spread under trees as ripe fruit and allowed to dry. Unfortunately there were no controls in these trials, so we do not know what the outcome would have been if the same coffee was allowed to dry slowly in unstirred layers on cement.

The conditions of the usual Indian plantation of heavy shade and heavy daily dews suggest, *a priori*, that India is one of the least favourable origins for producing gleanings of anything like acceptable quality. Examination of gleanings from three sources confirmed that ochre aspergilli could be found consistently at a high frequency of 30% of beans amongst a diverse community. A test was set up where ripe cherries were spread beneath trees and the moisture and mycological relations monitored (Figure 6.3).

Figure 6.3: Drying rate and change in ochre aspergilli bean infection rate of arabica gleanings placed under coffee trees as ripe cherries.



Apparently the combination of slow drying, lack of stirring and orchard soil contact can produce an unusually high ochre aspergilli bean infection rate of 20 to 30%. OTA accumulation would be expected to the extent that the increase in infection rate correlated to growth.

The Ugandan trial (Table 6.4) similarly lacked a control, though there is some basis of comparison between farms previously not showing the presence of ochre aspergilli and those from which these fungi had been isolated in the previous survey. Farms that had lacked OTA-producers in a previous survey provided the coffee. The samples were picked after 3 weeks of drying and i-analysis was completed. There is no indication of the degree of dryness reached after this period.

The levels of niger aspergilli reported here are quite normal for robusta cherry. It is also normal that there is little or no bean infection by this group of species at harvest, and that bean infection arises within days of drying commencing.

Levels of ochre aspergilli are much higher than normally observed and there is no effect of whether the farm where the coffee was exposed to the soil was categorized as high or low ochre aspergilli incidence. There is a consistent difference between the

Penicillium infection rates in this respect, however, with higher infection rates in the ochre-present sites.

These results suggest that drying any coffee under the trees of any farm results in gross ochre aspergilli contamination of the beans – a proposition not generally supported elsewhere.

Table 6.4: Bean infection rates of coffee cherries dried under coffee trees. Farms MB10, MA8 and MD2 provided the fresh cherries and had been assessed to have low ochre aspergilli frequency.

Sample	Niger aspergilli		Ochre aspergilli		*Penicillium*	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Ochre-'free' sites						
MD2	0	89	0	29	0	17
MA8	0	95	0	49	0	11
MB10	3	100	0	100	9	23
Ochre-present sites						
MA1	3	98	0	43	17	100
MD5	0	66	0	80	3	83
MB7	6	98	9	97	9	63

The most complete study of the four indicates that ochre aspergilli are, typically, in the harvested fruit and potentially increase during drying and that residence on the soil did not facilitate additional infection. The second study where there was some kind of a control treatment may have shown an increase in ochre aspergilli infection with soil residency, but the magnitude of the difference is too small to be statistically demonstrable. However the two studies in India and Uganda suggest that residence on the orchard soild for 2-3 weeks is a major risk factor for OTA.

Better controlled trials combined with monitoring data of 'gleanings' or '*variçao*' coffee would provide a clearer assessment of risks associated with cherries harvested from the orchard soil.

6.4.3 Soil drying yards: mycological considerations

Of the soil microbes, bacteria are almost all hydrophilic so could grow on coffee only early on in the drying process. Soil yeasts, too, seem to be largely hydrophilic, but there are numerous soil fungi capable of growth in dry conditions. It should be noted that soil fungal populations are much higher and more diverse where there is settled plant cover. For example, even arable fields have a paucity of fungi in comparison to grassland soils.

In the comparison of the two most common drying surfaces, cement and soil, both harbour fungi. The more important question is ***what*** fungi and ***what capacity*** they might have to out compete the fungi already numerous on the surface of the cherry

or parchment, and grow through the husk or parch to colonize the bean in the time it takes the coffee to dry.

Table 6.5 presents some analyses of swabs taken from cement drying yards beneath drying coffee in India and Brazil. Yeasts and *Fusarium* dominate these surfaces with the white yeast dominated by *Candida edax* and the red yeast probably species of *Rhodotorula*. The *Fusarium* is largely *F. stilboides*. Unlike the Brazilian samples, some niger group aspergilli (and other aspergilli), which have been isolated more often in India than Brazil, could be isolated from the drying surface but only actually under drying coffee.

Table 6.5: Fungi from swabs of drying yard surfaces of sites in Coorg district (GR, CE) and Minas Gerais, Brazil (A, C) expressed as colony forming units (cfu) per cm^2. All cement surfaces, except C which is brick tile. nd = not detected.

Farm	GR		CE		A	C (Brick tile)	
Taxon	**Under parchment**	**Under cherry**	**Under parchment**	**Under cherry**	**Cherry**	**(2day) Cherry**	**(15 day) Cherry**
White yeasts	$3.3x10^4$	$6.4x10^4$	$2.4x10^4$	$5.1x10^3$	$>10^6$	$5.1x10^5$	$9x10^5$
Pink yeasts	$8.8x10^3$	$2.0x10^2$	$1.2x10^4$	$1.2x10^3$			
Fusarium	$2.4x10^3$	nd	nd	nd	2^{nd} dom	2^{nd} dom	2^{nd} dom
Niger grp	nd	$3.6x10^3$	nd	$2.9x10^3$			
Mucoraceus	nd	nd	nd	$1.0x10^2$			
Cladosporium	$3.0x10^2$	nd	$6.0x10^2$	nd		3^{rd} dom	3^{rd} dom
A. versicolor?	nd	$3.8x10^3$	nd	nd			
Others	$4.0x10^2$	$1.0x10^3$	$3.0x10^2$	nd			
Total counts	$4.5x10^4$	$7.3x10^4$	$3.7x10^4$	$9.3x10^3$	$>10^6$	$5.7x10^5$	$1x10^6$
% Yeasts	75	88	97	68	?	90	90

Cladosporium, on the other hand, was recovered from the surface only in association with parchments in India, but it is common under Brazilian cherry. *Cladosporium* is common if not ubiquitous in the external flora of coffee cherries.

Table 6.6 below shows that there is no quantitative correspondence between the air spora, surface community and bean infection of the coffee on the surface in the two Indian farms. Most of the air spora sampled on a drying yard comes from the coffee external community but includes ambient sources as well.

Table 6.6: A collation of quantitative results relating to the fungi in three niches associated with two sites on two drying yards in Coorg district, India. The air plates were 5 minute air exposure plates.

	CE		GR	
	Parchment	**Cherry**	**Parchment**	**Cherry**
Air spora (raw counts)	64	57	25	119
Patio surface (cfu/cm^2)	$3.7x10^4$	$9.3x10^3$	$4.5x10^4$	$7.3x10^4$
Bean i (% contamination)	6	9	24	81

In the Lampung highlands of Indonesia robusta cherry coffee can require up to six weeks to dry with a maximum period of almost four weeks in the lowlands (at about 600m). The fungal soil community under the drying coffee is denser and qualitatively distinct from the soil of the open yard (Table 6.7) and although it is difficult to compare the cfu/cm^2 of the cement and brick yards, to cfu/g of the soil yards, it is clear that the surface millimetre of the soil yard would hold a population similar in extent to the cement yard.

Yeasts are less important in soil than cement where they dominate and the dominant fungal species, *A. niger*-complex, is one that is clearly associated with robusta cherry rather than soil. The coffee layer may alter the habitat of open soil so that *A. niger* finds it more amenable and yeasts less so or the high *Aspergillus* numbers may only reflect spores dislodged from the coffee rather than significant growth in the soil. At least temporarily, there is an enhancement of fungal numbers (as cfu) under the coffee as well as the shift in speciation. Swabs of cow-dung plaster yards, a surface only found in parts of India, had higher levels of yeast than cement with a second dominant of *Fusarium* (data not shown).

Table 6.7: Analysis of soil from compacted earth robusta cherry drying yards in Lampung province, Indonesia. (ll) = Lampung lowlands; (lh) = Lampung highlands

Farm	Sample	Total cfu/g	Dominant taxon	%	2nd dominant taxon	%
JL (ll)	Under coffee	$4.0x10^4$	Niger grp	0.88	*Penicillium*	0.09
SM (ll)	Under coffee	$4.9x10^5$	Niger grp	0.44	*Penicillium*	0.44
WR (ll)	Under coffee	$5.9x10^5$	Niger grp	0.94	*Penicillium*	0.04
MJ (lh)	Under coffee	$5.9x10^5$	Niger grp	0.81	*Rhodotorula*	0.08
MJ (lh)	Open yard	$8.5x10^3$	?	0.71	Niger grp	0.24

6.4.4 Studies of the performance of soil drying yards

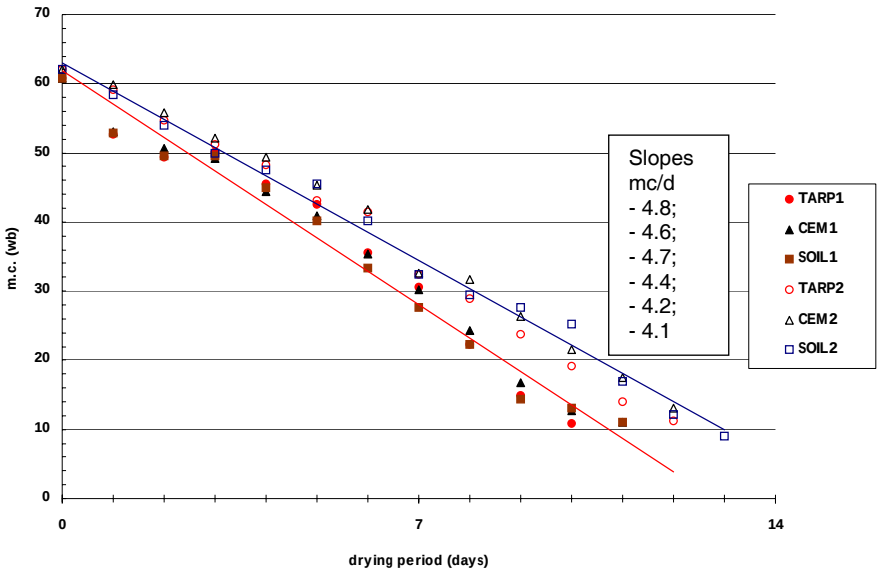
Extensive statistical evaluation of sun drying on various surfaces in almost all collaborating countries was completed. Differences in drying rates on different surfaces are generally not statistically significant, and the most important factor are the prevailing atmospheric conditions. It is more difficult to demonstrate whether or not the occasional statistical differences that have arisen can be explained by genuine performance differences that arise only under certain drying conditions. In other words, are certain drying technologies better suited to certain climatic zones or prevailing conditions than others?

The relevant point here is that differences in sun-drying time-courses in side-by-side comparisons of different surfaces are minor, so any observed differences in the mycological outcome is not attributable, in the main, to rates and periods of drying. In comparisons to other surfaces, soil has certain characteristics such as high porosity, high matrix potential (especially in clay soils), loss of integrity in rainy conditions, and a high microbial population.

From the mycological and OTA perspective, the drying studies were designed as an in/out query: i.e. what goes into treatments is measured and what comes out from each treatment can be compared.

Fast drying rates minimize any potential differences in the drying performance of surfaces and the time-course becomes linear as this instance in Uganda (Figure 6.4). The only slight difference is between the two runs.

Figure 6.4: Sun-drying time-course comparing rates on plastic tarpaulin, cement and compacted soil in two separate runs in Uganda. Drying was slightly slower in the second run.



The general picture we have of fungal population dynamics is that the initial loading of niger complex in fresh beans is low, often negligible and *Fusarium* is often the dominant fungus at this stage. Once drying is underway, *Fusarium* dies, often disappearing, and niger aspergilli increase rapidly reaching 85 to 100% infection of beans by the time dryness is reached. The increase in niger aspergilli is typically over

the first 3 to 4 days. Introduction to the seed by external contamination is not a plausible explanation considering how consistent and rapid this pattern is on all surfaces. It is quite clear that for this important fungus (in robusta cherry) it is in the fruit at harvest, and rapidly grows into the bean from this platform. This is apparently quite small in biomass terms since it sometimes is below detection limit in the analysis of the pulp.

There are no differences in development of the niger or flavi aspergilli between Run 1 and 2, nor between the three surfaces tested here. Ochre aspergilli did not appear in the analysis.

Table 6.8: Internal analysis of beans before and after sun-drying on cement, plastic tarpaulin and compacted soil.

	Initial conditions	Outcome (dry)		
		Cement	Tarpaulin	Soil
Run 1				
Total	35	90	88	100
Yeasts	0	0	0	0
Black aspergilli	0	83	89	100
Ochre aspergilli	0	0	0	0
Flavi aspergilli	0	23	13	16
Other aspergilli	0	0	0	0
***Penicillium* spp.**	4	3	0	0
***Fusarium* spp.**	17	1	0	0
Run 2				
Total	17	100	87	95
Yeasts	0	0	0	0
Black aspergilli	1	100	86	93
Ochre aspergilli	0	0	0	0
Flavi aspergilli	1	0	2	2
Other aspergilli	1	0	0	0
***Penicillium* spp.**	0	0	0	0
***Fusarium* spp.**	13	1	15	4

A second study was conducted in Côte d'Ivoire where drying on soil is also common. The initial conditions are reported as 100% infection by both niger aspergilli and yeast along with about 1% of ochre aspergilli infection.

This high yeast infection in cherry coffee could mean there was a technical problem with the surface sterilization step of the analysis procedure. Ochre aspergilli was isolated from all the final products at about 5% except one replicate dried on tarpaulin (Table 6.9). Results for yeast and niger aspergilli infection were uniformly high, except in the case of one replicate each.

In this case the drying rate as measured by the period of time spent in the A_w range considered to be most advantageous to OTA-producing species and other mesophiles did differ between the surfaces tested. Coffee drying on soil had the most suitable moisture content for growth of the key fungal species under competitive circumstances (i.e. subject to the activities of other fungi and the seed) for at least two days longer than soil and one day longer than tarpaulin. There was no discernable effect on infection rate.

Growth can take place without new infection, and changes in OTA give a better idea of growth. Increases in OTA would require growth, though it is true that the quantitative relationship between growth and OTA production is dependent on a complex of interrelated growth conditions which may even include loss of toxin through metabolic activity of the microbes and seed of the mixed community.

Table 6.10 details the results of the OTA analysis. If there were any significant changes, it was in the tarpaulin and bamboo table treatments, both of which showed a numerical increase of about 100% above the initial content, and 350% more than the other two treatments, both of which showed a numerical fall in OTA.

Combining the drying data in the interpretation, the treatments that spent the longest and shortest period of time in the moisture range where OTA could be produced were the two that showed a numerical decrease (though one had no statistical difference). This leaves two avenues of interpretation open: either we must conclude that all the apparent changes are attributable to experimental error and there are no contraindications of drying on soil for OTA contamination in this experiment or that there are conditions not adequately characterised by A_w measurements that support OTA production on tarpaulins and bamboo tables.

Based on observed variation in these experiments it is most likely that there are no differences in the treatments measured as OTA content, similar to the mycological parameter. Another parameter such as cup quality might produce a different result, though robusta cup quality appears to be a more stable quantity than that of arabica.

Table 6.9: Summary of the mycological data from the drying trials run at Bingerville. The initial conditions are reported as ochre = 1%; niger and yeast = 100%. The table reports means of two replicates (at 2m2 per each replicate) and where replicates disagree, both are reported.

Treatment	Ochre (i%)	Niger (i%)	Yeast (i%)	Residence in A_w 0.95 - 0.80 (days)
Cement	3	>85	>85	5.2d
Bamboo	5	27 /90	38 / 76	5.9d
Tarpaulin	6 / 48	>90	>80	6.5d
Soil	5	>85	>85	7.8d

Table 6.10: OTA levels before and after drying on four different surfaces. Each final product is represented by four replicates, the initial sample by three.

Initial	Cement	Bamboo	Tarpaulin	Soil
4.6 ppb	2.8 ppb	9.8 ppb	9.6 ppb	2.8 ppb

Section 7
Impact of Hindered Drying

7.1 Introduction

A common problem reported in the farmer surveys from several origins was the efficient drying of their coffee. Partially dried coffee should be the most critical condition for loss of product quality.

Hydrophilic organisms cannot grow in the partially dried commodity. This gives mesophilic fungi, including OTA-producers, the chance to develop without competition from the hydrophiles.

This has long been identified as an important area for investigation. An answer was required on the point where inclement weather during the harvest season ceases to be a mere inconvenience, and instead becomes a safety issue.

In the context of developing a Hazards Analysis and Critical Control Points (HACCP) based plan for coffee processing, it is clear that the drying step requires special attention and that risks of OTA associated with this step have to be defined as precisely as possible.

Indeed, this is a common problem from a farmers' perspective. There is a known potential to spoil coffee, and thus a definite potential functionality as a prevention tool. Clearly this was an important area to characterise as thoroughly as possible.

Reinforcing the importance of this step of processing, the drying trials demonstrated that local weather conditions are the single most important factor controlling drying rate and period. The difference in climate between coffee production regions translates into differences in average drying rates and periods, as well as the way in which drying is delayed, whether the delay is due to high humidity, or because of periods of rain between relatively arid periods.

There are many variations in practice observed in drying yard management, some of which have been discussed elsewhere. In this Section we look at the impact of conditions that produce a slow drying rate, and consequently a prolonged period of drying.

7.2 Findings and Application

7.2.1 Impact of slow drying on OTA risk in dry-processed robusta

Typically the 'hindered drying' treatments extended the drying period by 6-7 days in comparison to the 'normal drying' treatments. In general the hindered drying treatments reached dryness in about 21 days with a residence period within the A_w range 0.95-0.8 of about 6 days.

In the robusta trials, ochre aspergilli were present at about the detection limit, and did not increase in any of the treatments. These moulds were more frequently isolated from the 'hindered-drying on soil' treatment, but this outcome was highly irregular as shown by the very large standard errors, and is not statistically significant.

There was a consistent presence of OTA in the initial cherries and in all of the treatment samples taken during the trial. Presence of OTA means the presence of OTA-producers, and the hindered drying treatment, designed to encourage their growth, did not cause an increase in OTA. In other words, OTA-producers were there but drying over three weeks on soil or cement did not cause an increase in OTA content.

There was little evidence of the conditions imposed within the trials facilitating an increase in either the infection rate, or the biomass of ochre aspergilli. Drying within 21 days, including less than 6 days between A_w 0.95 and 0.80, from an initial condition of less than 5ppb of OTA, did not cause increased OTA accumulation.

7.2.2 Impact of slow drying on OTA risk in arabica coffee

In one highly replicated arabica processing trial there was a low but not uncommon occurrence of ***internal*** bean infection by ochre group fungi. There is no pattern of ochre presence related to rapidity of drying: ochre positive samples were evenly divided between 'normal' and 'slow' drying treatments. Interestingly, of the seven samples that were positive for ***external*** contamination by ochre group fungi, only one was from the normally dried material, compared to six in the slowly dried coffee. This may indicate that these fungi require extra time to sporulate, or to produce a significant layer of superficial growth on the drying yard.

In another highly replicated arabica trial, there were relatively few samples shown to be positive for ochre group fungi and all at the limit of detection. Occurrence of neither internal nor external bean infection was related to the speed of drying.

The findings do not provide evidence of increased OTA risk associated with periods of slow drying over the range of experimental conditions used in this trial. However, the presence of OTA producers was irregular in these trials, and therefore conclusions about the effect of slower drying on OTA production ***cannot*** be confidently drawn.

7.2.3 Impact of slow drying on arabica coffee quality

With arabica coffee, in conditions where hindered drying extended the drying period to more than 24 days, an increase in *Fusarium* infection was observed. Although there were no quantitative differences in the external yeast population of parchment from processes using fermentation and mechanical washing, the latter generally had higher infection rates of moulds, a difference intensified by slow drying.

Slow drying increased the rates of some physical defects and reduced out-turn, both having significant economic consequences for the producer. There is also some evidence that slow drying had an influence on the cup quality.

7.2.4 Overall comments on impact of slow drying on coffee processing

There is still a major gap in understanding what combination of conditions are required to lead to a significant OTA accumulation. The maximum rate of net OTA production we have measured *in situ* is approximately 2µg/kg/d (see Annex C.7 on the enclosed CD-Rom). This implies that once established and in the production phase, OTA can accumulate very fast; a week at that rate would see OTA increase by 14ppb. In the robusta trial, where there is evidence, both from viable counts and from OTA analysis, of an active population of OTA-producers at the start of the experiment, there was no significant increase in OTA accumulation during the trial and the isolated nominal increases that were seen were equally associated with hindered and normal drying conditions.

The difficulty in interpreting these findings may lie partly in an inability to measure the biomass and growth of producing organisms. Equally, an explanation may lie in the required conditions for OTA production being much narrower than we imagined. This would mean that the presence of OTA-producers under moisture conditions that are suitable for their growth for adequate periods of time only results in the growth and OTA production by these fungi in cases where certain other conditions pertain. The pattern of OTA development in this and other trials suggests that the required conditions frequently are not met.

This position can be rationalized by assuming the coffee seed to be stable with its associated fungi and its high content of phenolic and alkaloid compounds and paucity of readily available carbohydrate (since most is in the form of a branched poly-mannan) making it an unsuitable substrate for many organisms.

It would only be possible to define a critical limit for residence within the 'OTA-production window' if all other conditions required for OTA production were present. As this seems not to have been the case, and as we do not know what the 'required conditions' are, we cannot use this approach to generate evidence on the effect of slow drying on OTA risk. The best advice therefore remains to dry as quickly as possible to avoid contamination as we are not able to control the 'other required conditions' that are still unknown.

Quality parameters were not evaluated in the robusta trial and therefore no conclusions about contraindications from the quality perspective can be drawn.

7.3 Additional Notes

As has been discussed elsewhere, the occurrence of mycotoxins requires the presence of a producer, under suitable conditions, for a sufficient period of time.

There is now clear evidence that regions vary in the occurrence of key microbial species associated with coffee production and in the horticultural practices followed. We have presented evidence that the variation in drying conditions present the most significant differences between, and within, regions over a harvest season.

The system, taken as a whole, is remarkably fragile in terms of directing a set of initial conditions to a given outcome. Reproducibility is not an inherent property of this system. This makes any experimental design difficult so the general approach, as

in other areas, was to expose the coffee to a gradient of conditions that began with the recommended practice and ended with something that would be expected to consistently cause significant problems.

Of course, even with this approach there can be no assurance that OTA-producers will be present, so often the interpretation must be purely based on fungal activity of other mesophiles – never absolutely satisfactory. In other cases, there was direct evidence of the presence of ochre aspergilli but, despite the presence of what we would predict to be favourable conditions for their development, we recorded little increase and sometimes even a decrease. We never found the conditions that would reliably produce unsafe coffee.

Even when deliberately producing conditions that should favour mesophile success, we noted their frequent failure. This could be due to the existence of (probably several) alternative communities or successions (through processing as conditions change), with no single one being dominant.

The above factors lie behind the variety of approaches employed in our investigations.

7.4 Experimental Design

Several experimental designs were devised to examine the impact of hindered drying, based on one of two basic approaches. All used shading nets to reduce exposure to the sun (see Image 7.1).

The first basic design sought to compare hindered drying at the start of drying, when the coffee is at a very high water activity (A_w), with the same delay applied to the middle and end of drying, once the coffee had reached an intermediate A_w. This gives four treatments: normal sun-drying; 4 days of good drying followed by hindered drying; 6 days of hindered drying followed by good drying; and uniform hindered drying. The idea was to compare two time-courses with comparable total inhibitions, one being exerted during the period when the coffee is above an A_w of 0.95, and the other exerted after it has dried below that level.

Image 7.1: An example of the arrangement of shade cloth to inhibit drying on mesh tables.

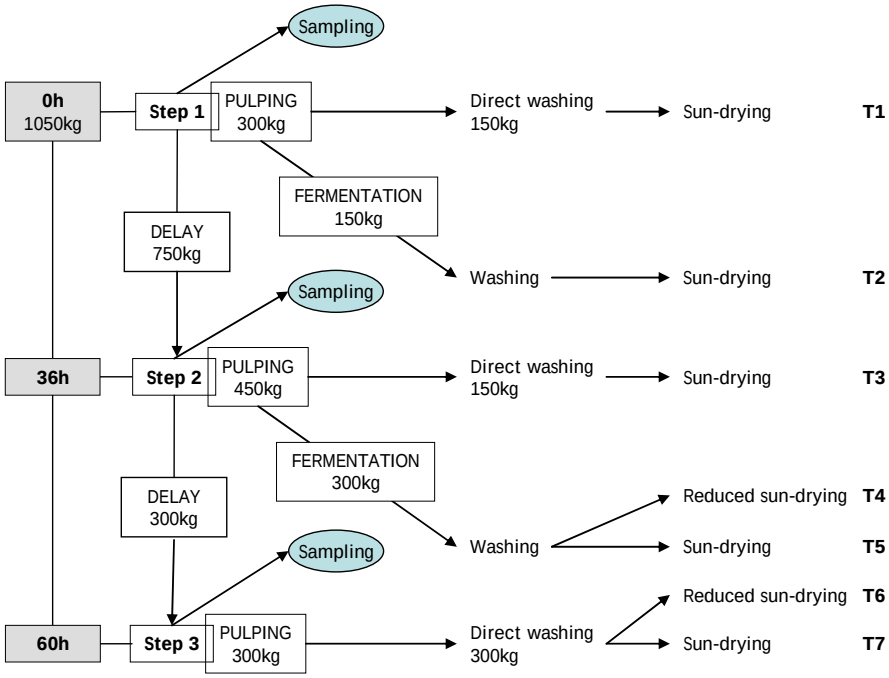
The second basic design applied a continuous lowered rate of drying to coffee that differed in other processing particulars. In other words, this design added the effect of hindered drying to some other processing variable.

Specifically, the following were compared: different drying surfaces; different post-harvest delay periods; different processing protocols; and mechanical mucilage removal was compared to traditional fermentative mucilage removal. The rationale here was that the outcome of hindered drying is contingent on the condition of the coffee exposed to the conditions.

The processing issue tested in the fermentative vs. mechanical mucilage removal comparison was whether the build-up of fermentative micro organisms in and on the coffee that takes place during fermentation confers any advantage (or disadvantage) to the stability of the commodity through subsequent processing. Drying was hindered to give scope for problems to arise, a necessary element in a stability test, but also to provide a control of the potential of the initial conditions to produce OTA contamination.

The variation on this, where more complicated and (it was hoped), more severe processing alternatives would generate more extreme initial conditions in the coffee to be dried, is outlined in Figure 7.1. This design treats delay in sacks and traditional fermentation equally as fermentative delays to drying. It was designed to permit the direct comparison of equal delays spent in alternative regimes divided between these two forms of delay. The shaded drying extended the 'bad practice' to ensure problems have a chance to develop if there was the potential for them to do so.

Figure 7.1: Flow diagram of one of the arabica parchment processing/inhibited drying experiments.



7.5 Experimental Results and Discussion

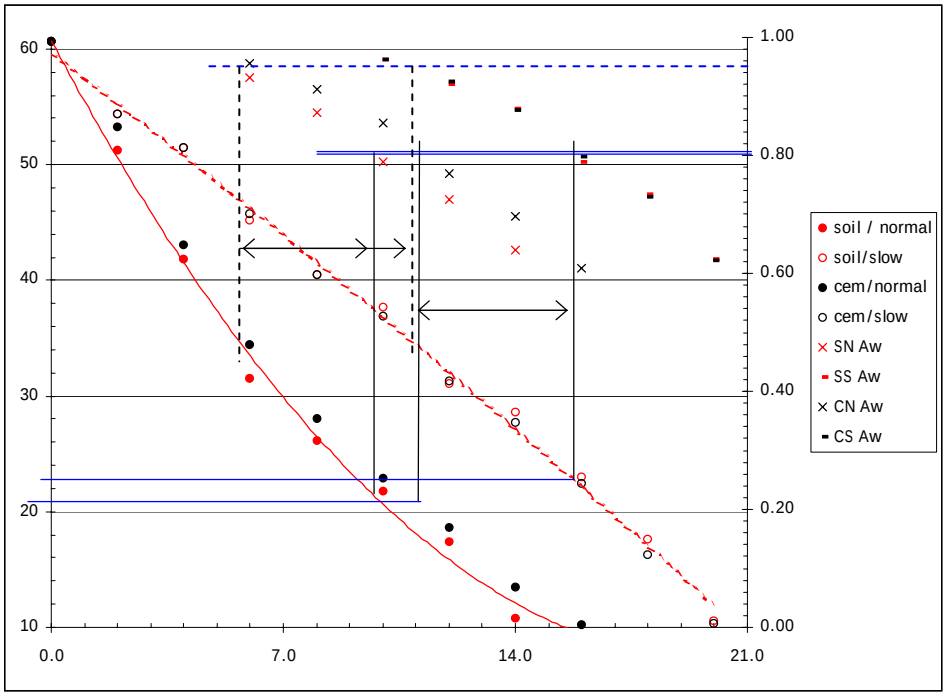
7.5.1 Robusta cherry

Figure 7.2 shows the drying time-course of normal drying and shade inhibited drying in an experiment run using robusta cherry in Uganda. This experimental design provided a side-by-side comparison between drying on cement compared to soil, both when drying is normal and when it is slow.

Under good drying conditions the use of bare soil is generally satisfactory, and this tests the possibility that the less sanitary soil surface may only be adverse to the outcome of drying when the drying period is extended beyond some critical point.

The difference between the two time courses is primarily while the coffee is above 55% m.c., corresponding to an A_w of above about 0.95. Although it can be said that the shaded treatment delayed drying to an A_w below 0.80 (the OTA production limit) by about 5 days, the period spent in the A_w range between 0.95 and of 0.80 is the same for both shaded and un-shaded on cement, and one day shorter on soil. The period spent reaching 0.95 in the shaded treatment is longer by 5 days. The time required for the shaded treatments to reach 13% m.c. is 21 days compared with 14 days unshaded drying.

Figure 7.2: Drying time-course (m.c. [wb] and A_w vs. days) on soil and cement with and without shade-cloth inhibition of drying during the entire period. First of 2 runs. The period in the OTA production window is indicated by double-headed arrows.



The mycological pattern of both runs is similar, and only the first one is reported here in detail. Drying of robusta cherry coffee resulted in a frequency of niger aspergilli infection approaching 100% in most cases, so it was not expected to be able to establish differences in the dynamics of this group. There is agreement with other drying trials showing that in robusta cherry drying there is low initial infection by

niger aspergilli and a rapid increase over the first several days. Here this group moved from about 5% infection at harvest to 80 to 100% infection after 7 days drying, irrespective of the drying rate and surface.

Ochre aspergilli were present at about the detection limit, and this group did not increase in any of the treatments. Ochre aspergilli were more frequently isolated from the 'hindered-drying on soil' treatment, but this outcome was highly irregular as can be seen by the very large standard errors, and again does not indicate any statistical differences (Table 7.1).

There is an indication that *Fusarium* survives better in the conditions of slow drying, but the differences are not statistically significant. Overall the differences in the treatments expressed in the frequency of occurrence of fungi are negligible.

OTA production can serve as an indication of the growth of OTA-producing species. These were manifestly present and active since there was OTA in the initial samples (Table 7.2). There is no significant OTA production during processing implying that the OTA-producers were growing little during the 10 to 16 days of drying spent in conditions with enough water to support OTA production.

Table 7.1: Fungal community dynamics in normal sun-drying on soil and cement, side-by-side with hindered drying produced by shade cloth. The medians are of the data expressed as % of bean infection, the standard error is of the transformed proportions (arcsin ($p^{0.5}$) a transformation to normally distribute percent. There are six replicates of each determination except 'dry' which was replicated 12 times. Medians of '0' are <0.5.

Treatment	Taxon	0d		7d		Dry	
		Median	Std error	Median	Std error	Median	Std error
Soil Normal	Total	15	15	90	15	100	4
	Black aspergilli	5	98	90	14	100	4
	Ochre aspergilli	Nil		Nil		<0.5	234
	Fusarium spp.	9	14	4	63	<0.5	131
Soil Hindered	Total	30	52	100	8	100	3
	Black aspergilli	4	68	100	8	100	3
	Ochre aspergilli	<0.5	245	<0.5	245	<0.5	234
	Fusarium spp.	11	37	2	99	5	81
Cement Normal	Total	34	23	90	16	100	47
	Black aspergilli	5	85	79	53	96	105
	Ochre aspergilli	Nil		Nil		Nil	
	Fusarium spp.	20	45	1	122	1	132
Cement Hindered	Total	17	39	100	9	100	6
	Black aspergilli	5	62	100	9	100	8
	Ochre aspergilli	Nil		<0.5	245	Nil	
	Fusarium spp.	4	68	<0.5	170	5	80

As Table 7.2 shows, OTA could readily be detected in the fresh coffee at an average level of nearly 3ppb (0-13 over 24 analyses) in the first run, and close to 4ppb (0-7.2 over 24 analyses) in the second run. These time=0 samples are replicates (for Run 1 and Run 2 separately, of course), since they were drawn from a mixed lot of coffee and had not yet been acted on by the treatments.

The coffee to be dried on cement in the first run has lower OTA levels than the other lots, though not at a level of statistical significance. However, the presence of OTA means the presence of OTA-producers (confirmed by viable counting), and the hindered drying treatment, designed to encourage their growth, did not cause an increase in OTA. In other words, OTA-producers were there but drying over three weeks on soil or cement did not cause an increase in OTA content.

Of further interest is the observation that the fresh cherry with the lower initial OTA levels produce dry product with lower final levels of OTA, though not at the level of statistical significance. Similarly, the four analysis points with means of 5.0 or above also have four of the five highest spreads, indicating that the higher levels correspond to higher variation.

If we analyse the results using numerical differences greater than 1ppb (see Table 7.3), we find that in the second run there are nominal increases of OTA of 2.9ppb during normal/soil drying and agreement with the 7 days sample, and of 2ppb during normal/cement drying, without agreement of the 7 days sample. In the first run there was a reduction in OTA of 2.2ppb with slow drying/soil with agreement of the 7 days sample.

A hypothesis that would explain these changes is impossible to frame, and with standard deviation of the final sample in all cases greater than the difference from the initial sample the conclusion must be that the treatments did not affect net OTA formation even though OTA producers were obviously present. Whereas there can be no doubt that adequate time at levels of A_w that permit mould growth is an essential condition for OTA contamination there seem to be other factors that determine whether the OTA is actually produced.

Table 7.2: OTA in ppb present in samples from both hindered drying time-courses. Values are means of six determinations and 'spread' is the difference between the highest and lowest values of each set of replicates. All time 0 samples are replicates, so a fuller idea of the inherent sampling + analysis variation can be judged by considering these. The treatments are described first by the drying surface and then by drying rate.

Treatment		Run 1			Run 2		
		0d	7d	Dry	0d	7d	Dry
Soil Normal	Mean	**4.6**	**3.5**	**3.6**	**3.9**	**5.4**	**6.8**
	s.d.	6.2	4.2	1.2	1.7	5.8	3.8
	Spread	13.0	8.7	1.2	4.7	13.0	12.8
Soil Hindered	Mean	**4.1**	**1.3**	**1.9**	**3.7**	**1.9**	**3.9**
	s.d.	0.7	2.0	1.6	2.5	1.5	0.8
	Spread	2.1	4.4	3.6	7.2	4.2	4.5
Cement Normal	Mean	**0.5**	**1.6**	**0.0**	**3.2**	**1.0**	**5.3**
	s.d.	1.2	2.5	0.0	1.9	1.8	3.9
	Spread	3.0	5.0	0.0	4.7	5.0	10.0
Cement Hindered	Mean	**1.8**	**1.9**	**0.9**	**4.2**	**1.6**	**5.0**
	s.d.	3.0	2.3	1.4	1.8	1.5	3.7
	Spread	7.2	5.1	3.1	4.7	3.6	11.0

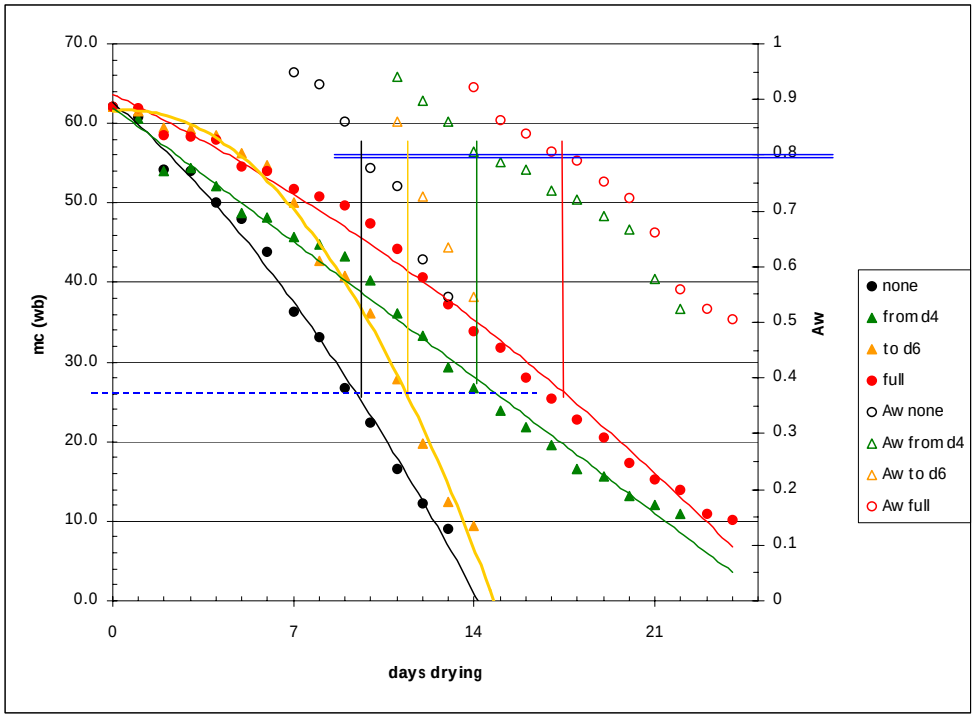
Table 7.3: Change in OTA (in ppb) from initial level in the final product of robusta cherry being dried at different rates on soil and cement surfaces.

Treatment	1st Run	2nd Run
Soil / Normal	- 1.0	+ 2.9
Soil / Hindered	- 2.2	+ 0.2
Cement / Normal	- 0.5	+ 2.1
Cement / Hindered	- 0.9	+ 0.8

A second hindered drying protocol attacks the proposition that coffee remaining very wet is safer than coffee remaining partially dry. Four treatments were run side-by-side: one treatment was hindered with shade-cloth for the first six days; a second treatment was allowed to dry normally for four days and then shaded until dryness; a first control was dried normally throughout; a second control was shaded throughout.

Figure 7.3 shows that the moisture conditions imparted by the treatments were what was intended. Note in particular that if we take a m.c. of about 50 to 55% as corresponding to an A_w of 0.95, the period of residence in the OTA production window is 5 days preceded by 4 days above 0.95 under normal drying; 7 days in the window preceded by 5 days under the late-slow treatment; 5 days in the window preceded by 7 days, and 7 days preceded by 9 days in the all shaded treatments. Here an A_w of 0.80 corresponds to about 26% m.c. (wb) in all the treatments.

Figure 7.3: Drying time-course of wet/dry hindered drying protocol in which similar periods of drying inhibition are applied at different parts of the drying cycle. The experiment was with robusta cherry drying on cement in Uganda.



The fungi produced the usual pattern of 80 to 100% niger aspergilli infection rate (*A. carbonarius* was not detected) by the end of drying in all treatments. Ochre aspergilli was only detected in the final sample of the fully shaded treatment (data not shown).

OTA analysis was completed in Uganda using the VICAM system, and shows little contamination throughout the study (data not shown). There is the odd higher value but these are not of any significance. In this experiment it is not clear whether there were any OTA producers in the system until possibly the end of one treatment, depending on what species of the ochre group was isolated. The VICAM system, at least with robusta coffee, throws up many false positives, and this probably accounts for the odd higher value reported.

7.5.2 Arabica parchment and cherry

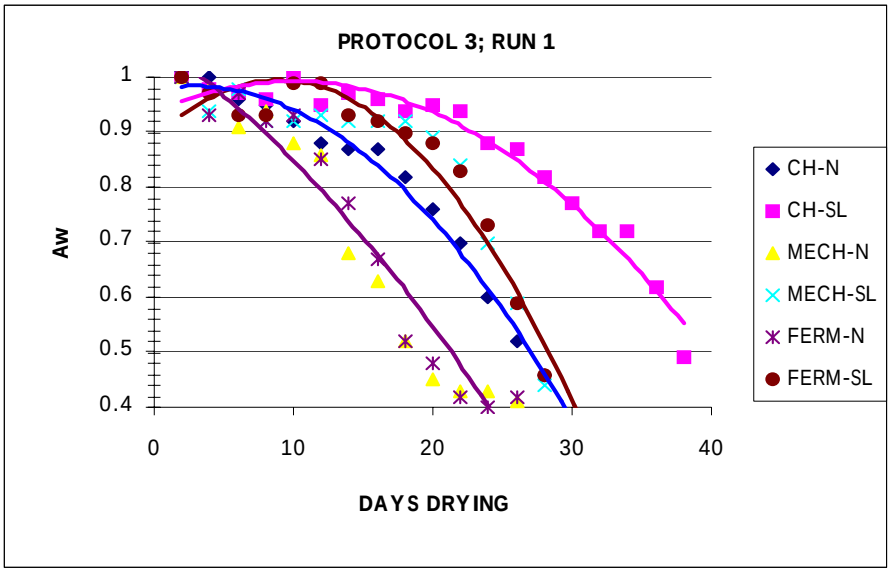
In the first protocol to be discussed, cherry drying was retained as a treatment for reference and as an alternative to wet processing. Intermediate samples were taken at specified points along the drying time-course according to expectations of what type of fungal community would be favoured. Hence, a sample at the end of the mesophilic interval (A_w at 0.80), a fresh fully dry sample and a final dry sample after a period of storage.

The normal drying time-courses all approximated linearity, characteristic of fairly rapid drying (Figure 7.4). Hindered drying treatments are disproportionately delayed in the high A_w part of the time-course, but once the delayed treatments dry to about A_w of 0.90, they dry at rates approaching normal drying. Parchment dried to 0.65 (corresponding to about 12% m.c.) required about 17 days in all the trials, with

cherries requiring an additional 6 to 10 days. The hindered parchment required 23 to 26 days by this criterion.

The residence time in the 0.95/0.80 window of these tests was, unlike the total drying time, remarkably uniform in comparison. Typically this period was 6 to 8 days for all parchment treatments, and 7 to 10 days for all cherry treatments. Note that the normal cherry drying resembles hindered parchment drying so that any differences between the two can be ascribed primarily to the difference in kind, rather than an indirect affect of different drying.

Figure 7.4: One of the three drying time-courses showing the typical relative and absolute rates for the treatments in the cherry/parchment inhibited drying protocol run in Kenya.



The results of the extensive mycological analyses are presented in Tables 7.4, 7.5 and 7.6, corresponding to three sequential runs of the protocol, as well as in Figures 7.5 through to 7.9, below.

The presence of niger aspergilli, though not rare, was of no numerical significance in these studies, the normal case with arabica coffee, especially parchment. Of the taxa tabulated in the tables below, 'pink yeasts' (probably *Rhodotorula* spp.) is primarily important in the parchment washate, rather than in the bean, although it does occasionally appear as a bean-infecting organism. This is interesting because it is favoured in wet processing by mechanical washing regardless of the drying regime, and is not important in cherry drying.

Continuing with this comparison, *Fusarium* is clearly inhibited by fermentation and occurs at much higher frequencies and survives drying much better in the mechanically washed wet processing regime. Though *Fusarium* does well in cherry-drying, infection rates are almost always higher in the mechanically washed treatments. The effect is enhanced in the hindered drying treatment.

Cladosporium is a consistent member of the coffee community through processing, but the processing variations do not seem to affect its frequency at all. *Penicillium* is also not consistently favoured by any particular treatment in these tests, but showed an increase in one of the slow drying treatments (in both fermented and

mechanically washed) and in one of the mechanically washed (as compared to fermented) treatments.

Ochre aspergilli were consistently more frequent in the mechanically washed treatments than fermented or cherry-dried ones. These differences are not large and are unlikely to reach a level of statistical significance, but the uniformity of the means from good replication is convincing. Of 18 replicates of each treatment over the three runs ochre aspergilli could be detected in 17 of those mechanically washed, 10 of those fermented and 8 cherry coffee samples. The grand means are 6%; 2.5%; 3%, respectively with the cherry grand mean being two-thirds attributable to one high sample.

Another interesting observation is that from the normally dried material, there is only one washate/fruit tissue analysis with detectable ochre aspergilli, compared to seven in the slowly dried coffee. This may indicate that these fungi require extra time to sporulate, or to produce a significant layer of superficial growth on the drying yard. They comprised a maximum of about 3% of the fungi in these niches where the total population varied between about $6x10^2$ to $1x10^6$/particle (either cherry or parchment).

The significance of this regular isolation of ochre aspergilli may not be as great as it first appears. As has been shown elsewhere, a large proportion of ochre aspergilli isolated from beans in East Africa have proven to be *A. melleus*, a non-producer of OTA.

The bar graphs of Figures 7.5 to 7.9 illustrate selections from the data tables. Two (Figures 7.5 and 7.6) make the comparison between normal and slow drying following dry processing (cherry), mechanical washing and fermentation with respect to total fungi, *Fusarium*, yeasts and ochre aspergilli. Perhaps the most salient feature of the first run is that most of the infection of the fermented/normal treatment is accounted for by yeasts, and in the mechanical washing/normal treatment by *Fusarium*, while the high infection of the mechanical washing/slow is provided by *Penicillium*.

Another method of illustrating this experiment is to chart the response of taxa one at a time, from day 0 to fully dry, and fully dry plus 3 weeks, graphed against the six alternative processing regimes (see Figures 7.7 to 7.9). Here it can be seen that ochre aspergilli are favoured by mechanical washing and hindered drying, but the match-up is not perfect, so this must be considered as more of a tendency.

The cupping results showed some impact on coffee quality relating to hindered drying, with a total of three cup defects recorded against nine hindered drying tests. Two of these were from the dry processing regime. Against this, two of the three best overall grades were awarded to hindered drying treatments, both following mechanical washing. The main difference in the mycology between these treatments is the lack of yeast and the augmented presence of *Fusarium* in mechanical washing. In Run 3 both treatments had a great deal of *Fusarium*.

Table 7.4: Cupping results from the dry processing vs. mechanical washing vs. fermentation followed by normal and slow drying regimes. 'Sour' is 'common sour' and is not a defect. The grades are from 1 to 10 (1 being the highest).

Processing	Drying	Overall quality			Cup defects		
		Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Fermentation	**Normal**	5	6	6	Sour	Sour	Sour
Fermentation	**Slow**	6	6	6	Sour	Sour	Sour
Mechanical	**Normal**	6	6	7	Sour	Sour	Sour
Mechanical	**Slow**	5	5	7	Sour	Sour	Onion sour
Dry	**Normal**	6	7	7	Sour	Sour	Sour
Dry	**Slow**	8	7	8	Sour tainted	Onion sour	Sour

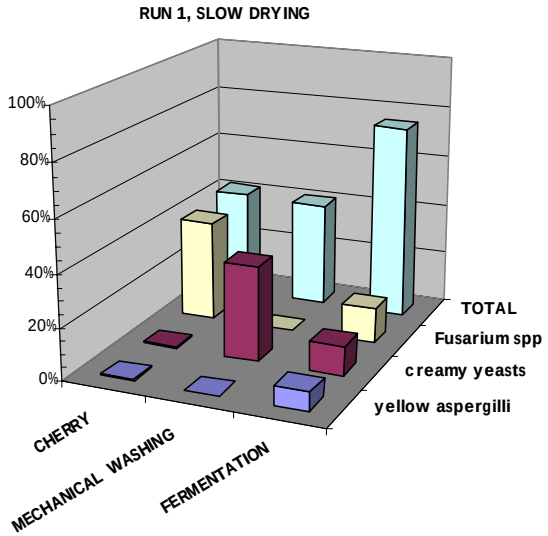
Table 7.5: Hindered drying data tables (1 of 3 tables) from wet/dry processing trials in Kenya. All values presented are means. NB - Replicate 2 was sampled approximately 3 weeks after the trial was completed.

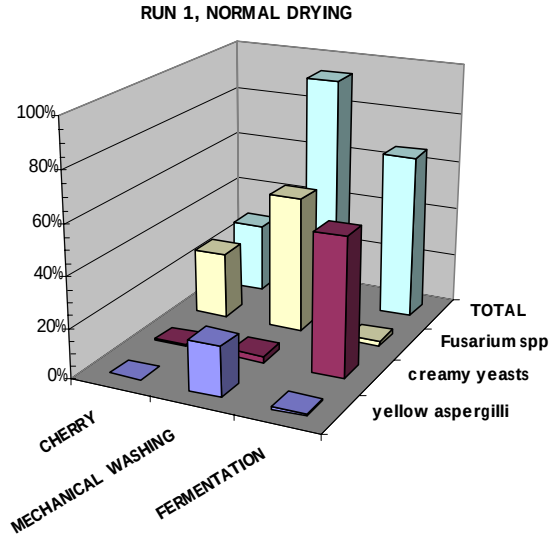
Run 1	**i Analysis** (% infection)						**x+m Analysis** (total cfu/part and proportion of community)			
	Normal drying			**Hindered drying**			**Normal drying**		**Hindered drying**	
	0.8	**Dry (Rep 1)**	**Dry (Rep 2)**	**0.8**	**Dry (Rep 1)**	**Dry (Rep 2)**	**Dry (Rep 1)**	**Dry (Rep 2)**	**Dry (Rep 1)**	**Dry (Rep 2)**
Cherry										
Total	41%	28%	31%	ND	42%	60%	2.5E+05	2.9E+06	3.1E+04	2.4E+04
Creamy yeasts	2%	1%	3%		1%	0%	0.88	0.68	0.41	0.40
Yellow aspergilli	1%	0%	0%		1%	0%	0.00	0.00	0.01	0.00
Penicillium spp.	2%	0%	0%		0%	1%	0.02	0.05	0.08	0.05
Fusarium spp.	36%	27%	21%		39%	46%	0.08	0.07	0.43	0.09
Cladosporium spp.	1%	0%	5%		1%	9%	0.01	0.13	0.01	0.21
Pink yeasts	0%	0%	0%		0%	0%	0.00	0.01	0.00	0.06
Mechanical washing										
Total	84%	94%	92%	92%	41%	98%	7.4E+02	1.6E+03	5.4E+03	3.3E+03
Creamy yeasts	27%	3%	6%	16%	37%	1%	0.32	0.37	0.40	0.44
Yellow aspergilli	10%	20%	16%	6%	0%	10%	0.00	0.00	0.01	0.03
Penicillium spp.	13%	27%	36%	26%	1%	35%	0.21	0.19	0.13	0.08
Fusarium spp.	39%	55%	46%	55%	0%	82%	0.14	0.03	0.11	0.12
Cladosporium spp.	2%	5%	2%	4%	1%	7%	0.14	0.04	0.06	0.12
Pink yeasts	3%	2%	3%	0%	0%	0%	0.19	0.35	0.27	0.21
Fermentation										
Total	28%	66%	24%	83%	76%	77%	1.2E+02	4.6E+03	6.4E+02	6.3E+02
Creamy yeasts	26%	56%	8%	18%	11%	10%	0.77	0.61	0.42	0.43
Yellow aspergilli	1%	1%	0%	5%	7%	5%	0.00	0.00	0.02	0.02
Penicillium spp.	3%	4%	1%	14%	49%	48%	0.09	0.00	0.11	0.50
Fusarium spp.	1%	2%	13%	54%	14%	18%	0.00	0.01	0.03	0.01
Cladosporium spp.	3%	1%	2%	5%	6%	3%	0.02	0.12	0.04	0.04
Pink yeasts	0%	1%	1%	2%	0%	0%	0.02	0.21	0.01	0.00

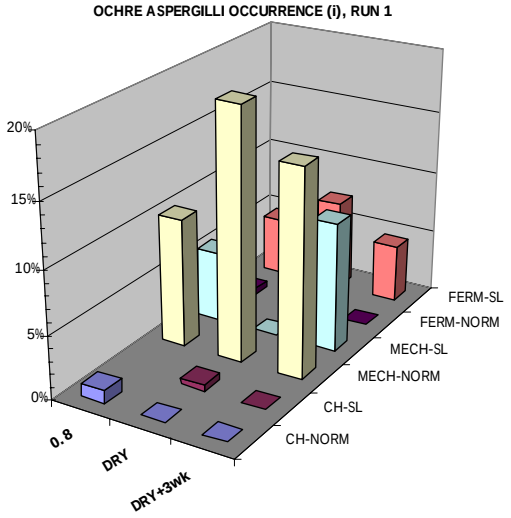
Table 7.6: Hindered drying data tables from wet/dry processing trials in Kenya. All values presented are means.

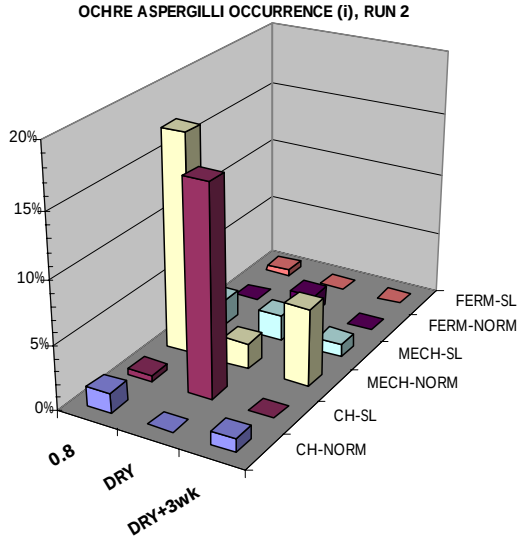
Run 2	**i Analysis** (% infection)						**x+m Analysis** (total cfu/part and proportion of community)			
	Normal drying			**Hindered drying**			**Normal drying**		**Hindered drying**	
	0.8	**Dry (Rep 1)**	**Dry (Rep 2)**	**0.8**	**Dry (Rep 1)**	**Dry (Rep 2)**	**Dry (Rep 1)**	**Dry (Rep 2)**	**Dry (Rep 1)**	**Dry (Rep 2)**
Cherry										
Total	48%	18%	29%	48%	59%	35%	1.8E+05	1.6E+05	1.3E+06	2.7E+04
Creamy yeasts	23%	1%	6%	3%	2%	0%	0.89	0.54	0.33	0.71
Yellow aspergilli	2%	0%	1%	1%	16%	0%	0.00	0.00	0.02	0.00
Penicillium spp.	2%	6%	0%	6%	11%	0%	0.05	0.06	0.22	0.00
Fusarium spp.	12%	12%	17%	38%	33%	10%	0.04	0.09	0.20	0.17
Cladosporium spp.	1%	1%	3%	0%	1%	10%	0.00	0.10	0.03	0.08
Pink yeasts	0%	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00
Mechanical washing										
Total	91%	95%	93%	98%	96%	92%	3.4E+03	2.9E+03	3.4E+03	3.5E+03
Creamy yeasts	3%	39%	28%	2%	5%	6%	0.68	0.45	0.53	0.53
Yellow aspergilli	17%	2%	6%	2%	2%	1%	0.00	0.00	0.00	0.00
Penicillium spp.	23%	4%	10%	24%	5%	8%	0.08	0.01	0.02	0.01
Fusarium spp.	41%	29%	52%	79%	87%	74%	0.00	0.02	0.09	0.12
Cladosporium spp.	5%	2%	5%	4%	3%	3%	0.00	0.00	0.03	0.02
Pink yeasts	1%	0%	10%	0%	1%	0%	0.23	0.49	0.32	0.32
Fermentation										
Total	79%	84%	61%	77%	87%	28%	2.0E+04	1.7E+02	6.6E+03	1.3E+03
Creamy yeasts	67%	37%	44%	39%	39%	11%	0.88	0.76	0.85	0.62
Yellow aspergilli	0%	2%	0%	1%	1%	0%	0.00	0.06	0.00	0.00
Penicillium spp.	6%	15%	11%	32%	18%	10%	0.02	0.00	0.09	0.06
Fusarium spp.	6%	6%	3%	12%	3%	5%	0.00	0.00	0.01	0.01
Cladosporium spp.	2%	29%	1%	3%	2%	1%	0.04	0.09	0.00	0.01
Pink yeasts	0%	2%	1%	0%	0%	1%	0.06	0.03	0.05	0.07

Figures 7.5 to 7.9: Two different graphical approaches to illustrate mycological results of above protocol - cherry, mechanical washing.









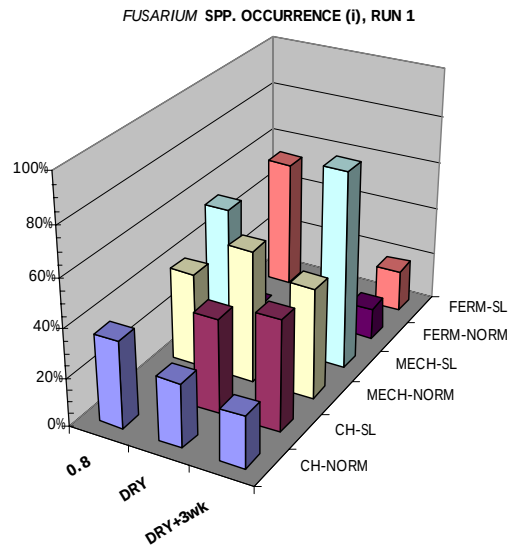


Table 7.7: Hindered drying data tables from wet/dry processing trials in Kenya. All values presented are means.

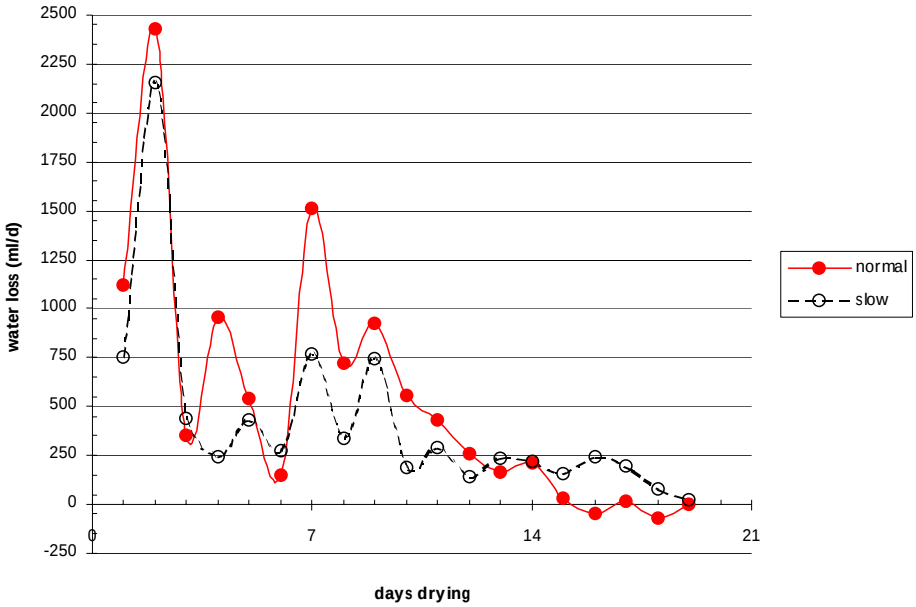
Run 3	**i Analysis** (% infection)						**x+m Analysis** (total cfu/part and proportion of community)			
	Normal drying			**Hindered drying**			**Normal drying**		**Hindered drying**	
	0.8	**Dry**	**Dry + 3wk**	**0.8**	**Dry**	**Dry + 3wk**	**Dry**	**Dry + 3wk**	**Dry**	**Dry + 3wk**
Cherry										
Total	74%	80%	70%	83%	96%	28%	8.2E+04	4.1E+05	2.9E+05	4.1E+04
Creamy yeasts	3%	2%	0%	1%	0%	1%	0.64	0.83	0.77	0.50
Yellow aspergilli	0%	1%	0%	1%	0%	0%	0.00	0.00	0.00	0.03
Penicillium spp.	1%	0%	1%	1%	29%	2%	0.01	0.00	0.02	0.01
Fusarium spp.	68%	67%	64%	71%	74%	20%	0.05	0.05	0.09	0.33
Cladosporium spp.	2%	8%	2%	3%	6%	6%	0.05	0.09	0.08	0.08
Pink yeasts	0%	0%	0%	0%	0%	0%	0.02	0.00	0.02	0.00
Mechanical washing										
Total	90%	99%	94%	98%	99%	96%	4.6E+04	8.0E+03	7.6E+03	3.6E+03
Creamy yeasts	14%	14%	5%	12%	2%	1%	0.47	0.53	0.59	0.47
Yellow aspergilli	1%	4%	4%	2%	1%	2%	0.00	0.00	0.00	0.00
Penicillium spp.	3%	2%	6%	1%	0%	5%	0.02	0.02	0.02	0.01
Fusarium spp.	84%	90%	74%	93%	100%	89%	0.02	0.01	0.12	0.05
Cladosporium spp.	4%	7%	8%	1%	2%	8%	0.11	0.02	0.03	0.04
Pink yeasts	0%	1%	3%	0%	0%	1%	0.33	0.38	0.24	0.41
Fermentation										
Total	97%	95%	85%	98%	99%	93%	2.1E+03	3.7E+03	1.1E+03	1.0E+04
Creamy yeasts	80%	41%	42%	12%	3%	7%	0.80	0.69	0.75	0.70
Yellow aspergilli	0%	1%	0%	1%	0%	0%	0.01	0.00	0.00	0.00
Penicillium spp.	0%	0%	1%	2%	1%	1%	0.02	0.02	0.00	0.00
Fusarium spp.	32%	45%	33%	82%	89%	78%	0.01	0.03	0.03	0.02
Cladosporium spp.	2%	3%	9%	3%	7%	21%	0.01	0.03	0.02	0.11
Pink yeasts	0%	3%	5%	1%	0%	1%	0.15	0.21	0.18	0.17

A similar protocol omitted the cherry drying comparator, and focused on the mechanical washing/fermentation comparison followed by normal sun-drying conditions or the inhibited drying provided by shade cloth. It was replicated in four runs. A notable feature of this protocol is that drying was monitored by weight loss from a set quantity of coffee. This was so that real-time moisture determinations could be calculated in order to guide sampling. Samples representing the initial conditions, after drying to $A_w \cong 0.95$, after drying further to $A_w \cong 0.80$, and lastly at full dryness, were taken. The mean data from one run is given below in Figure 7.10.

Water loss is generally higher under normal drying conditions when the coffee is wettest, and later water loss is greater under shaded conditions. However, the magnitude of daily differences in the first eight days or so can be as high as 750ml, whereas after two weeks the differences are less than 250ml. This shows the extent to which evaporation from the coffee is inhibited by the matrix potential/respiration of the coffee as full dryness approaches - solar input is no longer rate limiting.

The large fluctuation in the early part of the drying time-course indicates that wet coffee loses water more readily than semi-dry coffee, and is more affected by drying conditions. The energy required to evaporate a kilogramme of water is constant, but the energy required to remove water changes with the extent of drying.

Figure 7.10: Mean daily water loss rates of the shaded and normal drying treatments during the first run of hindered drying of parchment coffee following mechanical washing or fermentation.



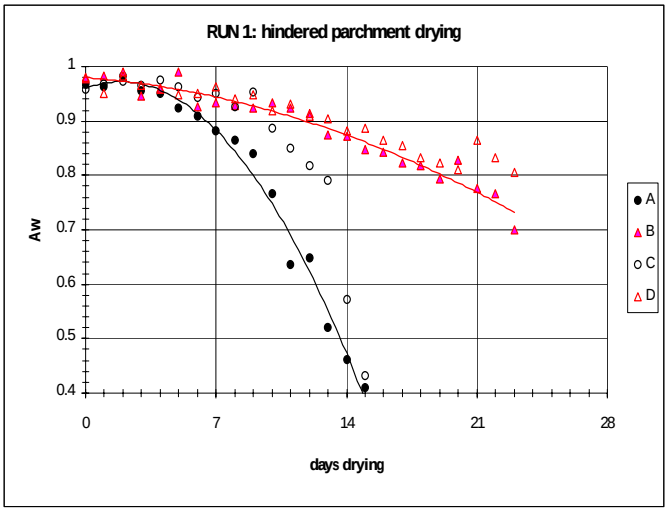
Looking at water loss rather than the usual measurement of moisture content (wb) one gets a different picture of what takes place in sun-drying. The effect of water loss from a unit of coffee of the size used in the hindered drying experiment on m.c. is provided in Table 7.8. The loss of 1kg (1 litre) of water is less than 3% from 17 to 16kg of total weight, but the same loss amounts to 8.4% as water is lost from 10kg to 9kg. In fact, it takes a water loss of 3kg from the initial 18kg total to achieve the reduction in m.c. of 8.4% that 1kg produces near the end of drying.

The drying conditions were poor for the three runs for which drying data is available. In fact the inhibited coffee didn't reach an A_w that would confer stability on the product in two of the three reported runs. The differences between replicates are substantial, though the drying times to and A_w of 0.65 are similar when this point is reached.

Table 7.8: Water loss/moisture content table showing the impact of progressive water loss on the moisture content of 18kg of coffee at an initial m.c. of 58% (wb) corresponding to initial water and dry matter contents of 10.44 and 7.56 kg, respectively, in the 18kg.

Total weight in Kg	Cumulative water loss in litres	m.c. (wb) %	m.c. (db) %
18	0	58.0	138.1
17	-1	55.5	124.9
16.75	-1.25	54.9	121.6
16.50	-1.5	54.2	118.3
16.25	-1.75	53.5	114.9
16	-2	52.8	111.6
15	-3	49.6	98.4
14	-4	46.0	85.2
13	-5	41.8	72.0
12	-6	37.0	58.7
11	-7	31.3	45.5
10	-8	24.4	32.3
9.5	-8.5	20.4	25.7
9	-9	16.0	19.0
8.75	-9.25	13.6	15.7
8.50	-9.5	11.1	12.4
8.25	-9.75	8.4	9.1

Figure 7.11: Drying time-course as measured by A_w of one of three runs. This represents the best drying conditions of the three. A/C are the normally dried mechanically washed replicates and B/D the corresponding slow-dried ones. Fermented treatments are similar. In the table, the final average A_w is given where 0.65 was not reached.



Period to A_w = 0.65		
	Normal	**Hindered**
Run 1	12 days	>24 days (0.72)
Run 2	15 days	>24 days (0.88)
Run 3	21 days	>24 days (0.85)

Mycological results are provided both graphically and in tables in the pages below. No OTA analysis was completed because ochre aspergilli were not reliably present in these studies, and could not be detected at all in the third run. There were 24 sets of samples (not counting the initial samples) in the four runs. Two positives for ochre aspergilli were from mechanically washed parchment, and four from fermented parchment. Only one of these six positives occurred in the normal drying treatments, but all were at the detection limit. There were also six positive washate samples (representing the surface of the parch and bean), half from normal drying treatments, but all at very low levels.

In Run 4 ochre aspergilli were detected infecting beans in the fresh cherry and was re-isolated from the first sampling (A_w 0.95) of the mechanically washed, hindered drying treatment, and two washate samples, but no others of the set. Ochre aspergilli were only detected in two of the fully dried samples, the A_w 0.80 samples providing 7 out of a total of 13 positives. This could be taken to indicate that some growth/infection of ochre aspergilli was occurring between A_w 0.95 and 0.80, but that the infection was susceptible to the desiccation experienced on the drying yard.

Many of the trends in population dynamics of the common fungal species identified in the other protocol can be seen in these studies. With more data points, plotting selected species' frequency of infection against the A_w of the samples produces a kind of time-course (drying correlates with the passage of time) and grouping the fermented and mechanically washed treatments on single charts makes a convenient assessment of the impact of fermentation. Runs 1 and 2 are presented below in this manner.

Fusarium is again clearly inhibited by fermentation and yeasts promoted, but the pink yeasts' response on the parchment surface is less marked than in other trials. Overall infection rates are lower in the fermented, dry parchment, compared to mechanically washed, but since this is accounted for by the increase in *Fusarium*, there is little direct significance of this observation on safety of the product.

However, it could represent a general case that the yeasts involved in fermentation generally suppress hyphomycetes and their absence renders this parchment inherently less stable. *Fusarium* could also stabilise the bean against the species of *Aspergillus* and *Penicillium* that are found in coffee. In only one case in these runs did *Penicillium* rise to levels as high as 20%.

The hindered drying was again demonstrated to be effective in providing different drying rates among treatments. Unlike in the previous hindered drying experiment, there is a large difference in residence time between A_w 0.95 and 0.80 in the two drying routines, and the normal drying (to A_w 0.65) was quicker at 12 and 15 days in Runs 1 and 2 as opposed to the 17 days required previously. Run 3 required more than 20 days, and corresponds to the one run where no OTA producers were detected. Drying data was not provided for Run 4.

The external fungi numbered between 10^3 and 10^5 with differences most marked between runs, ***not treatments***. This niche is dominated by white yeasts with the red yeasts important in some runs. The fermented process, strangely, was not consistently higher than the machine washed coffee. Some runs showed a fall in numbers through drying but this trend was not always expressed.

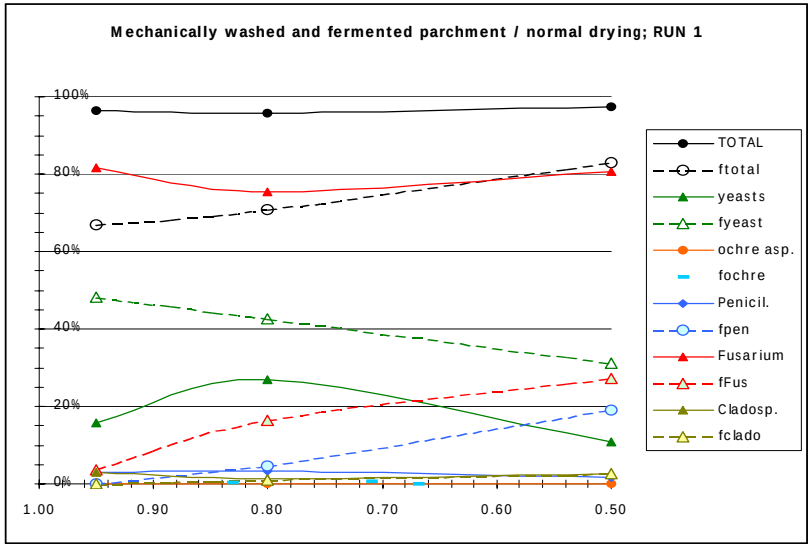
Tables 7.9 & 7.10: Selected means from hindered drying of parchment following machine washing or fermentation. The y-analysis is the surface fungi of the bean and parch and is given as a proportion of the total, the total being given at the bottom of each column. Ochre aspergilli are highlighted in yellow.

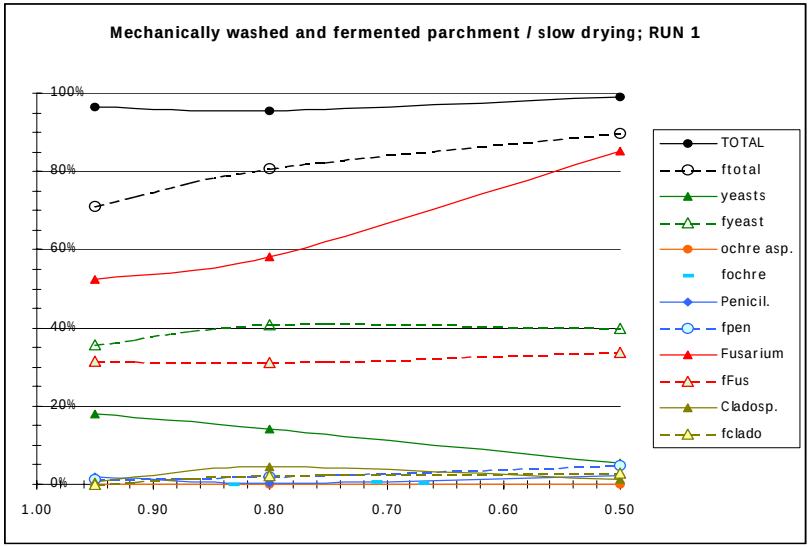
		Mechanically washed						Fermented					
Run 1		**Normal**			**Slow**			**Normal**			**Slow**		
i-analysis	**Initial**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**
Total		96%	96%	97%	96%	95%	99%	67%	71%	83%	71%	81%	90%
Creamy yeasts	**N/A**	16%	27%	11%	18%	14%	5%	48%	43%	31%	36%	41%	40%
Ochre aspergilli		0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	1%	0%
Penicillium spp.		3%	3%	2%	2%	0%	2%	0%	5%	19%	1%	2%	5%
Fusarium spp.		82%	76%	81%	53%	58%	85%	4%	16%	27%	32%	31%	34%
y-analysis													
Creamy yeasts	**N/A**	0.88	0.74	0.59	0.96	0.79	0.38	0.97	0.87	0.65	0.95	0.68	0.49
Ochre aspergilli		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.01	0.01
Penicillium spp.		0.02	0.00	0.01	0.01	0.01	0.00	0.00	0.02	0.08	0.01	0.02	0.01
Fusarium spp.		0.01	0.02	0.03	0.01	0.03	0.08	0.01	0.01	0.03	0.00	0.05	0.05
Pink yeasts		0.03	0.23	0.34	0.03	0.11	0.46	0.02	0.04	0.19	0.04	0.13	0.39
Total		4.1E+04	3.7E+04	2.8E+04	4.4E+04	6.1E+04	1.6E+04	6.7E+04	2.4E+04	2.2E+04	4.3E+04	3.5E+04	3.3E+03
Run 2	**Initial**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.08**	**0.50**
Total	93%	84%	96%	91%	87%	99%	97%	86%	73%	85%	81%	81%	88%
Creamy yeasts	57%	32%	2%	7%	22%	5%	16%	73%	38%	46%	40%	41%	28%
Ochre aspergilli	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1%	1%	0%
Penicillium spp.	0%	4%	1%	1%	3%	0%	4%	0%	6%	7%	0%	2%	4%
Fusarium spp.	47%	37%	71%	83%	38%	91%	90%	12%	36%	32%	35%	31%	45%
y-analysis	**x+m**												
Creamy yeasts	0.82	0.70	0.59	0.48	0.93	0.52	0.47	0.78	0.90	0.74	0.72	0.77	0.51
Ochre aspergilli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Penicillium spp.	0.00	0.15	0.01	0.00	0.00	0.00	0.00	0.15	0.01	0.01	0.01	0.02	0.01
Fusarium spp.	0.03	0.02	0.06	0.06	0.01	0.06	0.09	0.02	0.02	0.03	0.00	0.03	0.12
Pink yeasts	0.05	0.10	0.29	0.38	0.04	0.38	0.39	0.00	0.06	0.14	0.04	0.15	0.28
Total	3.9E+04	4.1E+04	2.2E+03	4.0E+03	5.3E+03	7.5E+03	2.4E+03	3.2E+03	6.1E+04	7.2E+02	8.9E+03	3.6E+03	1.7E+03

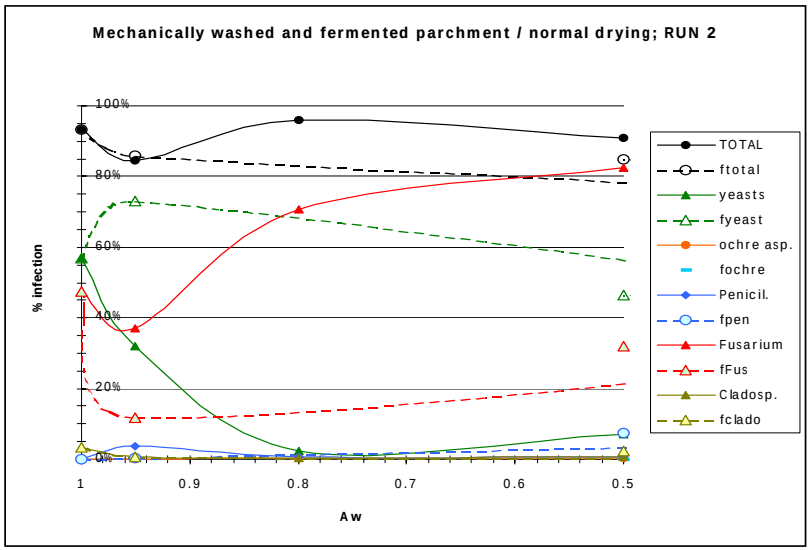
Tables 7.11 & 7.12: Selected means from hindered drying of parchment following machine washing or fermentation. The y-analysis is the surface fungi of the bean and parch and is given as a proportion of the total, the total being given at the bottom of each column. Ochre aspergilli are highlighted in yellow.

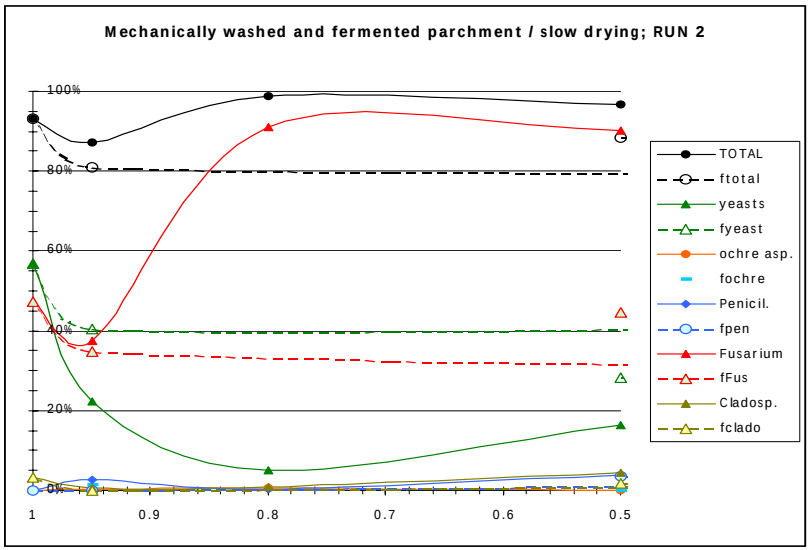
		Mechanically washed						Fermented					
Run 1		**Normal**			**Slow**			**Normal**			**Slow**		
i-analysis	**Initial**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**
Total	68%	58%	79%	69%	70%	84%	86%	72%	77%	76%	63%	87%	83%
Creamy yeasts	54%	30%	13%	9%	5%	3%	4%	45%	18%	14%	20%	13%	12%
Ochre aspergilli	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Penicillium spp.	0%	1%	2%	0%	2%	3%	3%	0%	5%	1%	1%	1%	2%
Fusarium spp.	0%	11%	39%	33%	50%	66%	69%	7%	37%	35%	20%	60%	40%
y-analysis	**x+m**												
Creamy yeasts	0.65	0.72	0.63	0.47	0.52	0.55	0.50	0.84	0.69	0.51	0.63	0.66	0.40
Ochre aspergilli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Penicillium spp.	0.00	0.00	0.02	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01
Fusarium spp.	0.07	0.01	0.02	0.05	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00
Pink yeasts	0.01	0.19	0.21	0.27	0.42	0.24	0.30	0.13	0.24	0.44	0.34	0.27	0.43
Total	4.1E+04	1.3E+04	1.0E+04	1.0E+03	1.0E+05	2.7E+04	1.8E+04	2.6E+04	7.5E+04	4.1E+03	8.4E+04	4.9E+04	4.0E+03
Run 2	**Initial**	**0.95**	**0.80**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.08**	**0.50**	**0.95**	**0.08**	**0.50**
Total	89%	34%	46%	81%	91%	93%	95%	48%	44%	74%	86%	83%	86%
Creamy yeasts	7%	16%	13%	23%	10%	3%	4%	33%	13%	31%	35%	42%	35%
Ochre aspergilli	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
Penicillium spp.	1%	1%	11%	45%	6%	14%	19%	1%	26%	27%	19%	6%	19%
Fusarium spp.	17%	3%	13%	11%	26%	73%	65%	4%	3%	4%	6%	21%	6%
y-analysis	**x+m**												
Creamy yeasts	0.47	0.85	0.81	0.60	0.84	0.69	0.62	0.92	0.59	0.49	0.86	0.75	0.58
Ochre aspergilli	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Penicillium spp.	0.00	0.03	0.06	0.22	0.02	0.15	0.15	0.04	0.19	0.37	0.05	0.18	0.34
Fusarium spp.	0.06	0.01	0.02	0.01	0.01	0.03	0.04	0.00	0.00	0.00	0.00	0.01	0.01
Pink yeasts	0.27	0.03	0.07	0.04	0.09	0.06	0.14	0.01	0.01	0.00	0.02	0.02	0.04
Total	9.1E+03	2.7E+03	9.0E+02	2.6E+03	1.5E+04	7.4E+03	1.4E+04	1.9E+03	2.3E+03	1.8E+03	3.0E+03	1.1E+04	5.0E+03

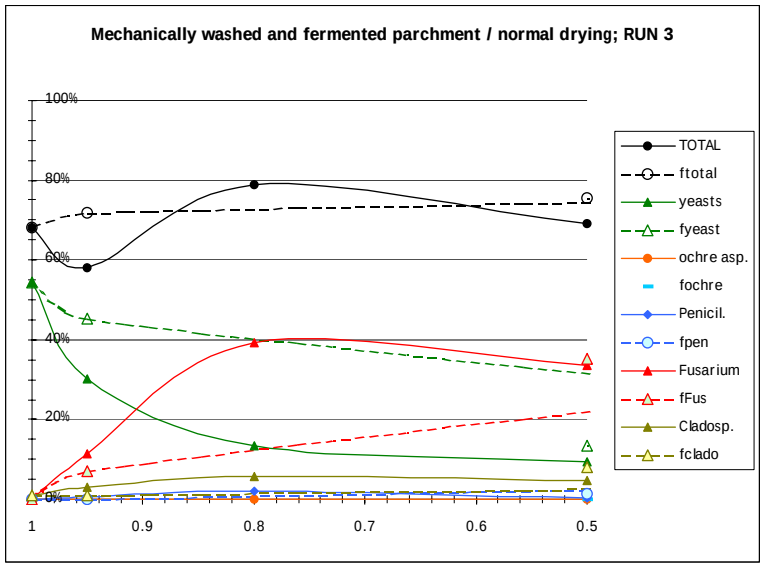
Figures 7.12 to 7.19: Plots of the infection rates (in percent of beans) of selected fungal taxa of all runs from the protocol comparing machine washed and fermented parchment dried normally or hindered by shading. Each plot has the machine washed and fermented outcomes for either normal or hindered drying. No initial analysis was completed for the first run.

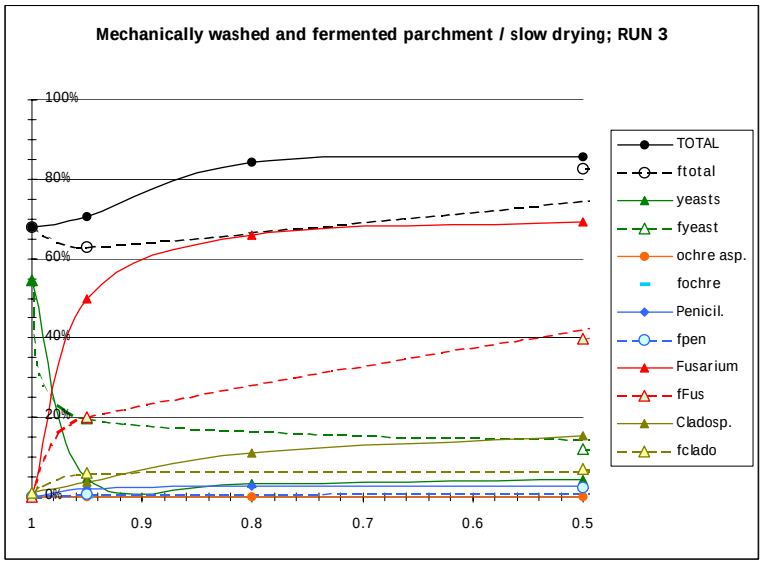


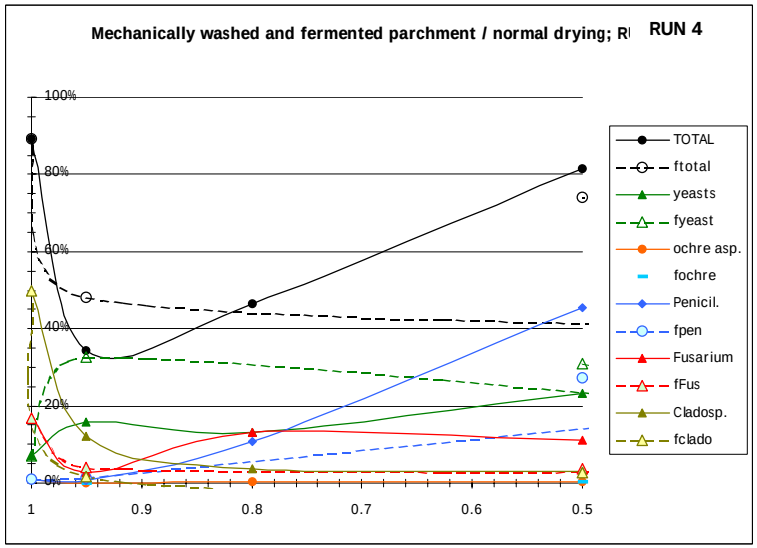


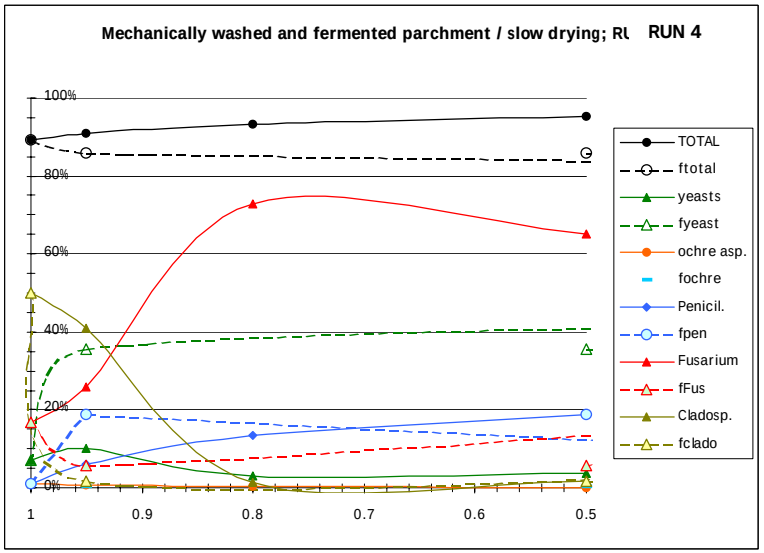





Mechanically washed and fermented parchment / normal drying; RUN 3
100%
80%
60%
40%
20%
0%
1
0.9
0.8
0.7
0.6
0.5
TOTAL
ftotal
yeasts
fyeast
ochre asp.
fochre
Penicil.
fpen
Fusarium
fFus
Cladosp.
fclado


Mechanically washed and fermented parchment / slow drying; RUN 3
100%
80%
60%
40%
20%
0%
1
0.9
0.8
0.7
0.6
0.5
TOTAL
ftotal
yeasts
fyeast
ochre asp.
fochre
Penicil.
fpen
Fusarium
fFus
Cladosp.
fclado


Mechanically washed and fermented parchment / normal drying; RUN 4
100%
80%
60%
40%
20%
0%
1
0.9
0.8
0.7
0.6
0.5
TOTAL
ftotal
yeasts
fyeast
ochre asp.
fochre
Penicil.
fpen
Fusarium
fFus
Cladosp.
fclado


Mechanically washed and fermented parchment / slow drying; RUN 4
100%
80%
60%
40%
20%
0%
1
0.9
0.8
0.7
0.6
0.5
TOTAL
ftotal
yeasts
fyeast
ochre asp.
fochre
Penicil.
fpen
Fusarium
fFus
Cladosp.
fclado

In Colombia a complicated protocol was run. T4 – T6 are the treatments of interest to us here (see 'Experimental Design', Section 7.4, above, for details). The full set of treatments are discussed fully elsewhere in Part C, Section 5 'Delays between Harvest and Processing'.

T4 was to impose a 36 hour post-harvest delay followed by fermentation and slow drying. This was controlled by T5 which was dried normally. Likewise, T7 had a 60 hour post-harvest delay followed by mechanical washing and is controlled by T6, dried normally. For reference T1 and T2 are included here which are mechanical washing and fermentation, respectively, followed by normal sun-drying.

Referring to Table 7.13, the weight required to get 70kg of green coffee (i.e. out-turn of husking) is higher in the two slow drying treatments indicating a loss of mass in addition to that suffered drying the post-harvest delay. There is no increase in black beans attributable to slow drying in the 36 hour delay treatments, but there is after a 60 hour delay. The black colouration is produced by the seed itself in response to injury. Fungal activity can undoubtedly produce this effect, but it may simply be exposure to the high temperatures experienced in the sacks during the post-harvest delay.

As with black beans, the vinegar defect is increased by slow drying only after the 60 hour delay. Total processing defects and total defects are both increased in the slow drying treatments over their normal drying comparators. It is clear that slow drying after post-harvest delay reduces the physical quality of the product.

Table 7.13: Affect of delay of processing on physical quality of parchment coffee. T1 and T2 are the reference process; '*Becolsub*' is mechanical mucilage removal; all fermentation treatments were washed in channels; sun drying in ambient conditions; plus shade for T 5 and T7.
T1 = 0 delay, *Becolsub*, sun; T2 = fermentation, sun; T3 = 40h delay, *Becolsub*, sun; T4 = 40h delay, fermentation, sun; T5 = 40h delay, fermentation, slow drying; T6 = 64h delay, *Becolsub*, sun; T7 = 64h delay, *Becolsub*, slow drying.

May Run

Treatment	Naked	p wt/70	Black beans	Vinegar beans	Discoloured beans	Tot proc D	Tot D
T1-2	**2.0-1.7**	**104.0**	**0.2**	**1.2**	**6.3**	**7.7**	**16.8**
Difference from reference mean							
T4	2.0	8.3	0.1	0.8	1.9	2.8	5.1
T5	2.0	1.7	0.6	1.8	-3.8	-1.4	-1.8
T6	5.9	4.8	0.7	2.3	-2.6	0.4	3.0
T7	6.4	8.7	1.1	7.1	-2.3	5.9	5.8

Naked=naked beans or '*pelado*'; p wt/70 = the wt. of parchment required to get 70kg of green coffee; Tot proc D=percent of defects in the bulk that could have been affected by processing; Tot D = percent of total defects in bulk. T5 and T7 are not reported in the September run.

Cup quality all but disappears after a 60 hour delay, so destroying any possible differences between normal and slow drying (Table 7.14). T5 compares well with

even the good processing practices of T1 and T2. It is almost uniformly slightly better in all categories, except bitterness, than the slow drying comparator.

Here is some evidence of deterioration on the drying yard influenced by slow drying which may or may not be attributable solely to fungal activity. The seed itself and its respiratory and enzymatic systems also have the capacity to alter taste. Another approach is to examine the tasters' qualitative evaluation (Table 7.15).

Taking good and very good together, T4 and T5 both received about 28 comments, but T5, normally dried, led the very good category 19 to 9. Neither showed much in the way of microbial defects but they were even again in terms of chemical defects with about ten. Again, slow drying has not intensified a quality loss suffered in the post-harvest delay.

With the longer post-harvest delay the slow drying may have added a marginal deterioration but not in the microbial defects aside from stinker bean which is attributed to bacterial activity, rather than fungal metabolism.

Table 7.14: Cupping evaluation of coffee samples from the coffee physically described above in Table 7.13. Twenty cups were tested for each of two replicates for each treatment.

T1 = 0 delay, *Becolsub*, sun; T2 = fermentation, sun; T3 = 40h delay, *Becolsub*, sun; T4 = 40h delay, fermentation, sun; T5 = 40h delay, fermentation, slow drying; T6 = 64h delay, *Becolsub*, sun; T7 = 64h delay, *Becolsub*, slow drying.

May Run

	Fragrance	Aroma	Acidity	Bitterness	Body	Overall	Overall s.d.
T1	6.7	6.7	6.6	6.4	6.4	6.4	0.8
T1	6.6	6.2	5.8	5.8	5.9	5.7	1.5
T2	6.3	6.3	4.4	4.2	4.1	4.2	2.1
T2	6.5	6.3	6.5	6.6	6.3	6.5	0.5
T4	6.3	6.3	5.5	4.5	5.0	5.3	1.3
T4	6.4	6.2	6.2	6.7	6.3	6.3	0.6
T5	6.5	6.4	6.2	6.1	6.1	6.1	0.8
T5	6.7	6.2	6.3	6.5	6.3	6.2	1.3
T6	2.6	2.0	2.0	2.0	2.0	2.0	0.0
T6	5.2	3.9	3.9	3.9	3.2	3.0	1.8
T7	4.8	3.5	2.9	2.9	2.9	2.7	1.8
T7	4.2	1.9	2.8	2.3	2.2	2.2	1.2

Table 7.15: Tabulation of the tasters' qualitative comments from the cupping evaluation described quantitatively in Table 7.14. Microbial=defects including mouldy, sour, earthy, fermented, dirty; Chemical=defects including phenolic, metallic, woody, astringent.

T1 = 0 delay, *Becolsub*, sun; T2 = fermentation, sun; T3 = 40h delay, *Becolsub*, sun; T4 = 40h delay, fermentation, sun; T5 = 40h delay, fermentation, slow drying; T6 = 64h delay, *Becolsub*, sun; T7 = 64h delay, *Becolsub*, slow drying.

May Run

	Very good	Good	Microbial	Chemical	Stinker
T1	11	6	0	3	0
T1	7	7	2	3	0
T2	3	3	8	5	0
T2	9	11	0	0	0
T4	3	5	2	10	0
T4	6	13	0	1	0
T5	7	7	0	6	0
T5	12	3	1	4	0
T6	0	0	11	0	0
T6	0	4	14	2	0
T7	0	2	7	4	5
T7	0	1	13	1	0

Traditional fermentation conditions (T2) are more conducive to bean infection by yeasts than the sacking fermentation (T3), but the yeast infection increases again with further delay (T4/5 and T6/7). In this case the increased yeast infection could be an effect of pH since bacteria rapidly reduce the pH to about 4.5 (close to the *Lactobacillus* minimum) and then yeasts are capable of further reductions. At the beginning of drying T4-7 had high yeast infection rates compared to normal processing routines. This was sustained in both normal and slow drying after the 60 hour delay, but the yeast infection rate fell away in the slow drying after 36 hour delay and fermentation.

Ochre aspergilli were isolated regularly and at levels where OTA has been found in other regions. Consistent with other studies, ochre aspergilli were detected during the sacking period and often persisted through drying. The highest level (12%) was reached in traditional fermentation where there was a remarkably low level of yeast infection. The ochre aspergilli were distributed more or less evenly, with no discernable reduction, in all of the products. Apparently, there are no treatment effects on infection rate of ochre aspergilli. Of course, it is possible that this fungus could grow and produce OTA in some of the treatment conditions without any impact on infection rate.

The only probable difference between the slow and normal drying is in the infection rate of *Penicillium* in treatments T6 and T7 where slow drying appears to favour its development. It increases from the initial conditions at the time of introduction to the drying yard, and has about twice the rate of the normal drying comparator.

Table 7.16: Internal fungal communities during processing and in the final product with delayed processing, Colombia, May run. T 4-5 are called 60h because this is the sum of 36h in sacks and 24h in traditional fermentation.

Time	Treatment	Yeast	Ochre	Niger	Flavi	*Penicillium*	*Fusarium*	*Cladosporium*
0	**T1**	22.4	0.0	0.0	0.0	25.5	0.0	0.0
36	**T2**	14.3	12.2	23.5	2.0	64.3	0.0	2.0
36	**T3**	3.1	2.0	13.3	2.0	90.8	0.0	0.0
60	**T4/T5**	57.1	3.1	3.1	0.0	26.5	0.0	0.0
60	**T6/T7**	61.2	0.0	3.1	0.0	39.8	0.0	0.0
Dry	**T1**	17.5	2.1	2.1	0.4	56.1	32.1	3.9
Dry	**T2**	9.6	1.1	1.4	0.4	38.6	24.3	2.5
Dry	**T3**	3.6	1.4	0.0	0.7	40.7	8.9	1.1
Dry	**T4**	3.6	1.1	0.0	0.7	76.8	6.4	0.4
Dry	**T5**	27.1	2.9	0.0	0.0	93.2	5.7	0.0
Dry	**T6**	60.4	0.4	0.7	2.1	53.9	3.6	0.0
Dry	**T7**	72.1	0.7	0.0	0.4	99.6	0.7	0.7

OTA was only detected in a few samples at < 0.3ppb.

Unexpectedly, *Fusarium* was only detected in the final product, which flies against much other data on the dynamics of this fungus through processing. The apparent success in both runs of *Penicillium* during the sacking period, and its persistence during drying, has been sporadically seen in other studies. The persistence of the yeasts during drying is also notable.

Section 8
Cherry Quality

8.1 Introduction

The wet weight of the coffee cherry is about equally divided between fruit and bean tissue, and the weight of the dried beans is approximately 20% of the fresh weight of the harvested cherry. The maturity and perhaps, to some extent, the quality of the coffee beans is linked to that of the fruit.

The flowering pattern and long development period (between seven and eleven months) lead to uneven fruit maturation which means that harvesting is potentially heterogeneous.

Traditionally, selective picking, buoyancy separation and garbling have all been used to control this potential heterogeneity. Selective picking and hand sorting are expensive and time-consuming, so it should be no surprise that during the project (between 2000 and 2005), when prices were low, stripping (or selective stripping) was often observed to have replaced selective picking.

Control of harvesting is an important parameter in coffee production. It is determined by the rate of fruit development to maturity, and by the rate at which processing proceeds. The rate of processing is constrained in all production chains by a combination of drying rates and capacity of drying facilities available. Additionally, in wet processing, the pulping step can sometimes limit throughput of the harvest.

The impact of a heterogeneous harvest depends on the processing method. Ripe cherries are a requirement for wet processing because it is only at this stage that the fruit can be pulped. In dry processing, the inclusion of immature coffee causes a reduction in cup quality, while ripe and immature coffee can re-wet tree-dried coffee if dried in the same layer.

Tree-drying has become an issue that needs evaluation, since some important regions have gradually begun to rely on tree-drying for the bulk of their production. This is due to high labour costs motivating, initially, strip-picking, which has more recently followed by widespread machine harvesting of coffee.

Late harvesting minimises the proportion of immature cherries picked, but means that the bulk of coffee harvested is dried to below 18% m.c. on the tree.

Once ripening begins, the peak is reached within a month so. Ideally the farmer should accelerate his harvesting and processing to conform to this pattern. In reality, however, it is often not economic to maintain a peak production work plan for the whole period. Therefore, on large farms the early harvest often contains a lot of immature fruit, and the late harvest a lot of dried fruit. This is an especially clear trend where the strip picking method is employed.

For wet processing, harvesting must be carefully managed and completed using numerous picking passes, so as to minimise the proportion of secondary product produced (i.e. cherry coffee).

8.2 Findings and Application

8.2.1 Coffee maturity

As coffee matures, it changes in appearance, in physical properties and in the mycological community both in and on it.

In general, 50% of ripe cherries will progress to the over-ripe stage after 3 weeks. This figure is likely to be influenced by climatic conditions, but gives an idea of the rate of ripening once ripening has begun. Of course, it takes 7 to 9 months for this process to begin.

There are increases in both the seed and fruit inhabiting fungi as cherries mature, but the diversity of fungi is highest in the immature fruit (Section 8.5.2, below).

The drying rate of tree dried cherries is lower than is seen for other maturity classes. It is, however, more important to note that since the tree dried cherries contain less water at the start of sun drying, in mixed populations the drying of these cherries will be inhibited. Tree dried cherries should therefore be separated from ripe and immature cherries before drying.

8.2.2 Tree-drying

These results also suggest that there is a tendency for *bóia* to have a higher frequency of ochre aspergilli contamination than ripe coffee at harvest, but that the difference in ochre group infection disappears or is reduced after normal terrace drying (Section 8.5.1, below)

The data also shows that OTA can increase much faster on the terrace than on the tree.

In direct comparison (but with limited data), ripe and over-ripe coffee appear to be similar regarding OTA contamination, though it should be noted that contamination rates of OTA-producing fungi are higher in over-ripe than ripe beans. This suggests the attached fruit and bean play a role in controlling the growth of the accompanying fungi.

In direct comparison, the development of niger aspergilli is inhibited in tree drying as compared with terrace drying. The general principle here is that a different adaptation is required of the fungi for the terrace and the tree, as is evidenced by the different relative performances of the most common species.

The evidence from direct comparison indicates that tree-drying is not overly affected by humidity, in that the measured parameters of coffee from a lakeside position were indistinguishable from those from a hill-top position on the same farm.

Evidence regarding any hazard relating to tree-drying in dry climates is contradictory. Some surveys[1] suggest OTA is more frequently found in *bóia*, but direct comparison has failed to demonstrate that *bóia* after drying is worse than *cereja/verde* after drying. It may be that other phenomena are involved here, and that the *bóia* associated problems are due to coffee that has been re-wetted or insect-damaged.

8.3 Additional Notes

Although the coffee harvest season usually lasts for about three months, at the smallholder farm level it is usually much shorter. With a smallholder production, it is not practical to process and sell several small lots of coffee throughout the harvest period, so compromises are made. Often only one or two collection passes are used to bring in the harvest. With low prices, and in areas not sought after by the quality market, practices often become lax.

As fruit matures there is little question but that the accompanying fungal and bacterial communities will also change. However, the course of maturation will be influenced by whether or not the fruit is harvested. Many kinds of fruit maintain their integrity after harvest, but coffee is not one of them. In coffee, the fungal succession upon detachment from the tree is demonstrably different from fruit left *in situ*.

The decision on when to begin harvesting is affected by many non-horticultural considerations. These may include a farmer's short-term requirement for cash, security fears, labour availability, and commitments later on in the harvesting period.

The processing method, wet or dry processing, will also affect this judgement since both immature and dry cherries are lost to the wet method.

In controlling the continuing harvest, drying rate must be balanced against harvesting rate and with a typical 18 and 14 day residence on the drying yard for cherry and parchment respectively, this requires careful planning. A spell of poor weather can easily add a week onto expected drying times.

8.4 Experimental Design

To study changes produced by the progress of the season is difficult. It did not prove possible to conduct the kind of design really called for: where fruit at the same stage of development from the same trees was sampled throughout the season.

The default design was merely to compare samples that were harvested early in the season with other samples that were collected late in the season. The weakness of this design is, of course, that you compare samples that differ in many particulars and attribute any observed differences to one particular difference. Bearing in mind that with two 'treatments', one must be numerically higher than the other, this cannot be very persuasive.

[1] Vargas *et al*. (2004). Influence of coffee processing and defects on the incidence and occurrence of ochratoxin A: A short report (unpublished).

An attempt to look at tree-drying using repeated harvest from an identified cohort of trees only partially succeeded as too few samples were taken. The concept was to compare drying conducted on the terrace with that conducted on the tree. The same set of trees were sampled at three times (0 day, 30 days, and 60 days) with a sub-sample analysed and a second sample dried on the terrace. By 60 days all the coffee was dry at the time of harvest. In effect, the initial sample, after drying, represents the outcome with terrace drying and the 60 day sample represents the outcome with tree drying. OTA analyses were also conducted for this experiment.

Comparing maturity stages is easily done by stripping a cohort of trees mid-season when there is an availability of cherries at all stages and dividing them first into tree dried (*bóia*) and then hand sort the remainder into maturity classes.

8.5 Experimental Results and Discussion

8.5.1 Tree-drying studies

In some Brazilian areas tree-dried coffee is the largest proportion of green coffee produced. Most of this crop is separated into CV (i.e. *cereja/verde* = ripe/green) and *bóia* (tree-dried) by water floatation. Typically, *bóia* has an A_w of between 0.70 and 0.80 or between about 15 and 22% m.c.. It may spend as little as three days on a drying yard then is often finished by a period in a mechanical dryer.

It would seem likely that prevailing humidity could influence the outcome of such a production system, so an experiment was set up to compare a humid locale near a lake with a relatively dry one on a hill 1km distant, but on the same farm. Humidity of the atmosphere was not monitored.

Unfortunately, sampling was not conducted with sufficient frequency to fully establish conditions and rates of change in the orchard, neither were the samples for A_w and m.c. well done. Apparently the first sampling was of *cereja* and the last two of *bóia*. Note that an A_w determination of a mixed sample of *bóia* and *cereja* would not have been meaningful since the system could not have been in equilibrium, a pre-condition for making an A_w measurement.

As normal, the *cereja* was at between 60 and 62% m.c. at the initial sampling, characterised as 'ideal harvest conditions' which was 51% ripeness at the lake site and 69% on the hill (Table 8.1). 30 days later the *bóia* had reached about 22% m.c. (A_w for the lake reported at 0.82, and for the hill at 0.80). 60 days later the *bóia* had reached about 12% (A_w = 0.67 for the lake and 0.58 for the hill).

From the limitations of the data set, the best estimate of ripening rates is calculated in the table below. Note that 84 and 92% of the *verde* had ripened at the first sampling at the two sites, but that 39 and 25% of this had dried to the extent of *bóia*.

29 and 42% of cherries considered to be ripe (*cereja*) at the first sampling were still *cereja* 30 days later (Table 8.2), meaning that about 70 and 60% of ripe cherries dried to *bóia* during this period. This implies a *cereja* category half-life of about 25 days on the hill and about 20 days by the lake. The change in % *cereja* that dries suggests that

such values depend on the progress of the season since a progressively larger proportion dries in the interval as the season progresses.

Table 8.1: Proportion of maturity classes harvested 0, 30, 60 days after the ideal point of harvest at a humid, lake-side site and relatively dry site on a farm in Sul do Minas, Brazil.

Days after start of trial	% *Verde*	% *Cereja*	% *Bóia*
0	16.4	50.9	32.7
30	2.8	27.6	69.6
60	0.0	2.4	97.6
0	7.3	69.4	23.3
30	1.1	51.0	47.9
60	0.0	2.5	97.5

Table 8.2: Cherry ripening trends at a humid, lake-side site and relatively dry site on a farm in Sul do Minas, Brazil.

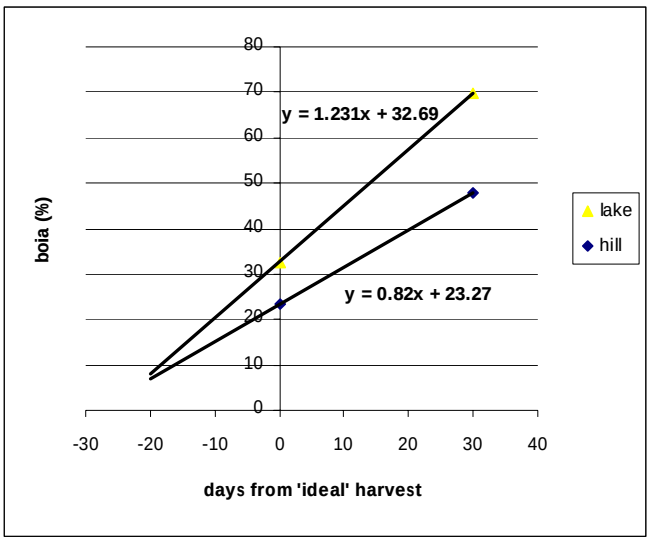
	% *Verde* **ripens**	% *Cereja* **remains ripe**	% *Cereja* **dries**
Pre	84	61	39
1st 30 days	81	29	73
2nd 30 days	100	0	100
Pre	92	75	25
1st 30 days	86	65	36
2nd 30 days	100	2	98

Figure 8.1 below shows the bóia generation rates, another way of considering ripening. Perhaps the most interesting features of this interpretation are that the two sites apparently differ in respect of this rate, and that the lines converge at about minus three weeks.

This means that cherry development and maturation was similar at both sites up until drying to *bóia*. One implication of this pattern is that the first *bóia* has dried some three weeks before the 'ideal' harvest point.

Data that is missing in this study is what degree of dryness is implied by *bóia* cherry that is positively buoyant? Other work suggests that *bóia*, just after the peak season, has an A_w around 0.70, so it would not be susceptible to most fungal growth and OTA production at the *bóia* stage.

Figure 8.1: Ripening as measured by generation of *bóia*. The last data point (+60 days) was omitted because more or less all of the coffee was *bóia* at this time which strongly implies *bóia* generation had run to completion at some (unspecified) time previously.



Bóia and *cereja* should have been analysed separately, but this was not completed. As a result, the interpretation of the mycological data is complicated by the fact that the proportion of *cereja* to *bóia* changes throughout the study.

The first sampling is 75%/25% and 61%/39% from the hill and lake sites, respectively, and the last is essentially fully *bóia*. However, the experimental design tests the process of drying whereby product that has been dried from day –21 to day +60, as described in Figure 8.1, with various proportions of the process having taken place on the tree and the terrace. All the coffee began as *verde* and finished as dried cherry, the question is, what is the impact of the different sites and tree-drying durations on the outcome?

As the coffee spends more time on the tree it becomes dryer and the dominant seed-inhabiting flora (*Cladosporium, Penicillium*) increases aside from *Fusarium,* which in this study was steady at about 50% infection rate throughout residence on the tree.

The seed infecting communities at the two sites after 60 days are indistinguishable, except possibly in respect of ochre aspergilli. The 6% difference between the sites, though, is difficult to accept as statistically significant or functionally meaningful. The appearance of this group as the season progresses may indicate that this group is better adapted to *bóia* since an increase in *bóia* is one of the phenomena to which this increase correlates.

The humidity of the location had little impact on the usual x+m inhabiting fungi, except that *Fusarium* was consistently more numerous at the drier site. The ochre group was detected only during the last sampling, comprising almost pure *bóia*, again an indication that these mesophilic fungi are relatively more successful in *bóia* coffee.

Table 8.3: Mycological results from tree drying experiment discussed above. The cherry ripeness categories were not analysed separately so presumably reflect the differing proportions of *cereja* and *bóia* reported in Table 8.1. Drying was conducted as normal terrace drying.

At time of harvest							At time of harvest						
	Period after harvest (d)	x + m analysis (cfu/fruit)						Period after harvest (d)	i-analysis, % infection of grain				
		Yeasts	*A.ochr*	*Fusarium*	*Cladosp.*	*Penicillium*			*A. ochr*	*A. niger*	*Fusarium*	*Cladosp.*	*Penicillium*
Hill	0	4.2E+03	nd	1.9E+02	2.4E+02	1.8E+02	**Hill**	0.0	0.0	8.3	49.0	44.1	16.5
	30	1.0E+04	nd	2.2E+04	9.6E+03	3.0E+03		30.0	3.3	1.7	32.5	35.8	99.6
	60	8.1E+03	1.5E+02	8.4E+03	9.8E+03	2.3E+03		60.0	2.5	0.0	50.0	15.4	42.5
Lake	0	4.9E+03	nd	8.2E+01	2.8E+02	5.2E+01	**Lake**	0.0	0.0	0.8	55.8	25.8	6.3
	30	6.2E+04	nd	8.3E+03	1.1E+04	3.2E+03		30.0	1.3	1.3	62.1	86.7	100.0
	60	1.4E+04	7.5E+01	2.0E+03	6.9E+03	4.4E+03		60.0	8.8	1.3	44.2	19.6	44.6

After drying							After drying						
	Period after harvest (d)	x + m analysis (cfu/fruit)						Period after harvest (d)	i-analysis, % infection of grain				
		Yeasts	*A.ochr*	*Fusarium*	*Cladosp.*	*Penicillium*			*A. ochr*	*A. niger*	*Fusarium*	*Cladosp.*	*Penicillium*
Hill	0	4.1E+01	2.5E+01	2.2E+02	2.8E+01	3.4E+02	**Hill**	0.0	7.9	5.9	6.4	10.1	20.7
	30	2.7E+03	nd	1.3E+03	1.0E+03	4.7E+02		30.0	12.0	0.8	20.3	4.1	33.6
	60	1.5E+03	nd	2.1E+03	7.1E+03	2.5E+01		60.0	2.5	0.0	28.3	4.2	14.6
Lake	0	4.6E+01	1.5E+01	7.5E+02	1.1E+02	9.0E+01	**Lake**	0.0	0.8	2.9	14.3	5.5	24.0
	30	4.6E+03	7.5E+01	4.7E+03	2.1E+03	1.0E+03		30.0	16.7	0.4	27.2	13.6	18.7
	60	5.0E+02	nd	1.9E+03	3.4E+03	nd		60.0	0.8	1.3	30.0	2.1	6.7

Considering the i-community through to complete drying, here again the coffee from the two sites is broadly indistinguishable.

The usual pattern of a reduction of infection rate of the seed-inhabiting fungi during residence on the drying terrace is clear, but the samples with more *bóia* perhaps change proportionately less.

The ochre aspergilli, which were detected in all six of the dried samples, show numerical increases during terrace drying in all but the 60 days treatments. Some of these increases develop from harvested coffee with no detectable ochre infection. Some of these numerical increases may be significant, particularly those arising from the 30 days treatment where 3 and 1% on the hill and lake, respectively, became 12 and 17% after drying. All six treatments produced coffee with detectable rates of ochre infection, but only half of these also contained detectable levels of ochre aspergilli in the x+m community. The two fresh samples that contained these fungi (+60 days from both sites) no longer produced them by the end of terrace drying. Conversely, three of the four fresh samples that did not show detectable levels of ochre aspergilli, did so after terrace drying.

These results show that there is a tendency for *bóia* to contain more ochre aspergilli than ripe coffee. However, the situation is changed somewhat after sun drying in the drying yard when ochre group infection consistently increased in optimally ripened cherry. Niger aspergilli were present in small numbers but these were not *A. carbonarius*. The worst scenario seemed to be for harvest to be delayed until there is between 40 and 70% *bóia* on the trees.

Table 8.4: OTA content of beans and husk from cherries at harvest and after terrace drying.

Days from Optimum	Hill site				Lake site			
	At harvest		After terrace		At harvest		After terrace	
	Bean	Husk	Bean	Husk	Bean	Husk	Bean	Husk
0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	2.0	0	0
	0	0	0	0	0	0	0	0
30	0	0	0	0.5	0	0	0	1.1
	0	0	0.5	6.5	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	1.2	0	0
60	4.3	0	0	0	0	0	0	0
	0.2	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0.9	0	0	0	0	0	0	0

Interpretation of the OTA data presented in Table 8.4 is difficult. OTA-is detected in 3 out of 4 '60-day' samples from the Hill site immediately after harvest but not after terrace drying.

One might postulate that this could suggest destruction of OTA, but it could also simply be due to sampling error. With the exception of one of the '30-day Hill site' samples, bean and husk contamination occur independently which is in keeping with much of the other data obtained during this project indicating that contamination of the bean by outgrowth of external contamination is not the only contamination route for OTA producers.

8.5.2 Microbiological infection of maturity classes

Cherries change in both physically and biologically through the maturation process. Immature or green cherries are hard and can be crushed by a pulping machine. Yellow cherries are said to bear mature seeds and can be pulped, so we group these with the red cherries as being ripe. Left on the tree beyond ripeness, the fruit becomes purple and shrivels slightly due to water loss. Some of this coffee can still be pulped, but we characterised this as over-ripe (= *paso*). Tree-dried cherry (= *bóia*) cannot be pulped, and floats in water.

A small survey of farms in Brazil, where stripping is almost universal, sought to document the effect of maturation on fungal communities (Tables 8.5 and 8.5a).

Generally the microbiological load of the fruit and of the beans increases with ripeness. The speciation itself is more stable than the enumeration except in the unripe fruit. It appears that the introduction of *Fusarium* to the bean can occur early and persist, whereas other fungi disappear with the onset of ripeness. However, the samples from *fazenda* 'B' show that the pattern of dominance by *F. stilboides* is not invariant.

The loss of diversity as the fruit matures could be indicative of more stringent conditions. The basis for the increasingly stringent conditions could be competitive pressure from the two apparently best-adapted fungal species, *F. stilboides* and *Penicillium brevicompactum*. In addition, *Cryptococcus albidus* is prominent in the x niche and *Candida edax* is prominent in the m niche as well as occasionally in the i niche. Species of *Cladosporium* occur relatively frequently, but in a patchy and unpredictable fashion, a pattern which could be indicative of some special circumstances, which are unclear.

Table 8.5: Changes in fungal communities related to the stage of ripeness on farms from two regions of Brazil (Pinhal = A & B; Araguari = PS). x = external fungi; m = mesocarp fungi; i = internal fungi; C = *Cryptococcus*; Can = *Candida*.

Sample	x	m	i			
	cfu/ch	cfu/ch	% infection	Speciation 1st dominant	Speciation 2nd dominant	ochre aspergilli
A - green	nd	nd	63	7 species/9 isolates incl. *F. stilboides* and *Colletotrichum*		
A - ripe	$3.6x10^4$	$1.2x10^3$	19	0.11 *F.stilboides*	0.06 *Can. edax*	<1/70
A - *bóia*	$2.6x10^6$	$3.8x10^5$	63	0.50 *F.stilboides*	0.11 *Can. edax*	<1/70
B - ripe	$> 10^6$	nd	69	0.29 *F.stilboides*	0.21 *P.brevicom*	<1/70
B - *bóia*	$> 10^6$	nd	84	0.71 *P.brevicom*	0.16 *F.stilboides*	<1/70
PS - ripe	$2.2x10^5$	$1.0x10^3$	48	0.44 *F.stilboides*	0.03 *Cladosporium*	<1/70
PS - *bóia*	$4.1x10^7$	nd	84	0.83 *F.stilboides*	0.01 *P.brevcom*	<1/70

Table 8.5a: Speciation of x and m fruit tissues related to the stage of ripeness. nd = not determined.

Sample	x		m	
	1st dominant	2nd dominant	1st dominant	2nd dominant
A - ripe	0.66 *F.stil* + *P.brevi*	0.44 *C.albidus*	1.0 *F.stilboides*	Nil
A - *bóia*	0.61 *C.albidus*;	0.39 *F.stil* + *P.brevi*	0.88 *Can. edax*	0.12 *F.stilboides*
PS - ripe	0.53 *F.stilboides*	0.44 *C.albidus*	0.80 *F.stilboides*	0.20 *Can. edax*
PS - *bóia*	0.83 *F.stilboides*	0.01 *P.brevicom*	nd.	nd.

8.5.3 Drying of different maturity classes

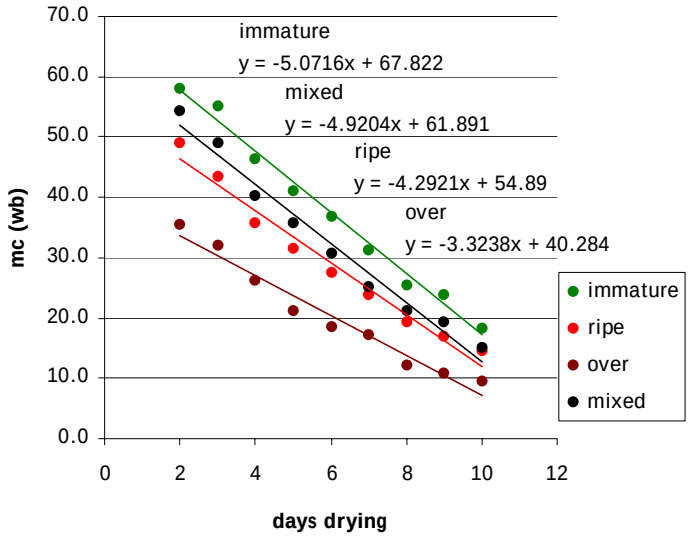
In this experiment, strip picked cherries were divided into immature, ripe and over-ripe classes and dried separately, as well as in the original mixture for comparison.

The linear parts of the drying curves (Figure 8.2, below) show a divergence in drying rates, measured as Δ m.c. (wb)/day, a difference that amounts to nearly 2%/d or almost 40% of the maximum rate.

Drying rate differences between maturity groups was not corroborated by other work such as that shown in Figure 8.3 below, where the slopes are approximately equal The expected differences in initial moisture content between classes can be inferred by the differences in y-intercept.

The more important consideration in relation to the behaviour of the different maturity classes in the drying yard, is that the tree dried cherries enter the drying the yard with a much lower moisture content than the other cherries. If they are mixed on the drying yard, then the tree-dried cherry can be re-wet allowing more time for growth of OTA producers.

Figure 8.2: Drying of maturity classes between day 2 and 10, the linear portion of the time-course. R^2 values range between 0.96 and 0.99.



There were no ochre aspergilli detected in this experiment. The niger infection is less in the over-ripe cherry, consistent with the idea that drying on the tree shows a different fungal population dynamic than on the terrace.

Table 8.6: Fungal community development during sun-drying of robusta cherry in maturity classes. Units are % infection; '<' = below detection.

		0d	8d	Dry
Immature	Niger	<	86	100
	Fusarium	34	<	6
Ripe	Niger	6	72	97
	Fusarium	11	<	<
Over-ripe	Niger	<	<	72
	Fusarium	<	23	29

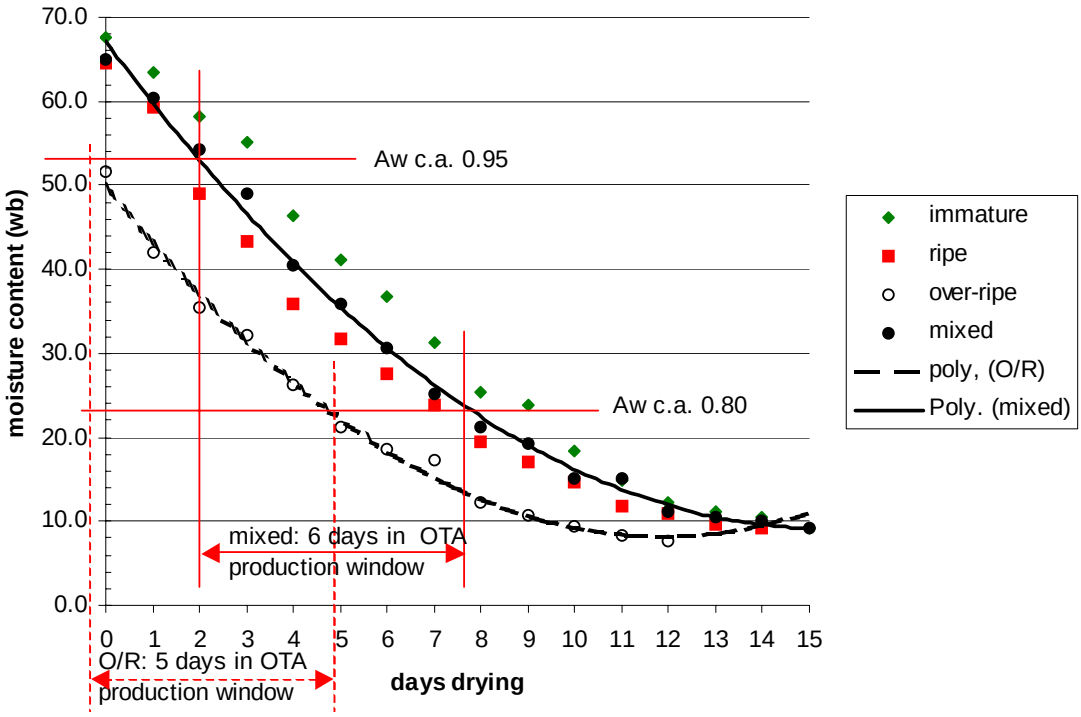
Over-ripe cherry is at an average m.c. of 35% which corresponds to about 1 week of drying under good conditions. Note that there is no niger infection reported in this class. Since, after a week on the yard there is usually more than 50% (and often more than 80%) infection in robusta coffee, this strongly suggests that drying ripe cherries on the tree is not mycologically equivalent to drying ripe cherries on the yard. Niger

aspergilli eventually appear at a substantial frequency, but the inference must be that with less time to grow, these fungi are represented by a smaller biomass than in the terrace-dried ripe cherry.

8.5.4 Cherry maturity and harvest period differences

In this comparison, strip picked cherries were harvested, separated into maturity classes and processed, as described above, using the mixed cherry for comparison. This is done twice, the second time, of course, being later in the season.

Figure 8.3: Comparison of drying of different maturity classes and polynomials fitted to over ripe and mixed classes. The surface on which this was conducted is not reported.



Differences between these two runs may have something to do with progression of the season. However, the coffee came from different locations at different times so it could, with equal justification, be attributed to other factors.

An experiment to test this would have to exploit the same trees which would be harvested using a minimum of three passes to produce the necessary maturity classes for a true comparison. Obviously, since the over-ripe fruit is dryer than the other classes, it requires less time on the drying yard than the other classes. Figure 8.3 shows over-ripe cherries also spend less time in the OTA production window , in this case by some 15-20%. The drying rate (slope) is identical for all the classes, as is the overall shape of the curves. The make-up of the harvested coffee with respect to maturity classes, early and late season, was remarkably similar (data not shown). This was not expected since there should be a shift to a greater proportion of over-mature fruit as the season progresses.

Analysis showed small amounts of OTA in the final product of this trial, though the mycological analysis did not reveal any OTA-producers except *A. niger* complex, and these were at 50–100% (i.e. normal levels) for all maturity classes.

Ripe and over-ripe contained about 3ppb OTA (Table 8.7), the mixed lot about 1ppb OTA and the immature sample contained a trace. Given the usual degree of variation in OTA results, it is very unlikely that differences of this magnitude would be significant.. Certainly it does not make numerical sense that if the vast majority of the coffee (ripe plus over-ripe) is above 3ppb that the mixed coffee could be at 1ppb. Indeed, accurate weighting cannot be calculated in the absence of the relative amounts represented by the three classes.

Table 8.7: Two cases of unreplicated OTA analysis of cherries during and after drying in maturity classes. nd = not determined.

Maturity	OTA µg/kg dry	Days drying		
		0	6 (or 5)	9-10 (dry)
Mixed	1,0	nd	nd	nd
Green	0,2	0.8	5.9	4.5
Ripe	3,2		81.5	126.2
Over-ripe	3,4	3.5	82.2	81.3
Tree dried	nd	52.3	32.5	73.9

In the second set of data presented in Table 8.7, a great deal of OTA was produced. The tree-dried coffee here was badly contaminated but apparently the potential of this coffee to support OTA production is realised to a greater extent on the drying yard than when dried on the tree. Note that the over-ripe cherry has ceased to produce OTA after 6 days, presumably because it has already reached an A_w below 0.80 by that point.

If the 6 day measure of the tree-dried sample suggests the 0 day measurement was exaggerated, then the final day figure suggests the 5 day determination is an underestimate. We wouldn't expect there to be enough water available to support OTA production toward the end of terrace drying in the tree-dried coffee. The reasons why this coffee should become so contaminated is far from clear since these levels are exceptional, and there was no treatment conducted beyond some hand-sorting.

All the drying studies used consecutive runs as a form of replication and could therefore be used to compare later with earlier harvests. However, one of the major findings of this design is that the greatest difference in drying performance is attributable to differences in conditions experienced during the respective drying periods. One example is presented in Table 8.8 below.

Table 8.8: Period of drying and final OTA content of samples dried in Lugogo, Kampala early and late season using various drying technologies. The fact that drying is much faster in the second trial should not be attributed to changes in the nature of the coffee.

Drying technology	Early season		Late season	
	Period (d)	OTA (ppb)	Period (d)	OTA (ppb)
Soil	15	1.0	10	5.0
Cement	17	2.1	8	3.3
Tarpaulin	15	0.5	8	39.6
Mat table	14	1.3	9	7.0
Mesh table	17	0.9	10	1.5
Cabinet	12	0.3	8	0.5
Box	15	0.3	11	0.3
Green house	11	1.2	7	0.4

In this example, the drying is much more efficient in the late season than the early season so would be expected to permit less OTA development. The background occurrence of OTA is about 1.5ppb ±100% and three values could be entertained as being higher than this level, all in the late season: soil, tarpaulin, and mat table.

However, seasonal influence is also exerted in the orchard, so the most likely scenario, if these possible differences were real, would be for a raising of the background level in the later run. It is possible that there is an undetected difference that is only subsequently expresses under certain processing conditions but given the speed of drying, the support in the data for this is very thin.

Section 9
Defects and OTA in Coffee

9.1. Introduction

'Defect' is the term applied to any one of a number of visually observable properties of coffee beans that are taken as contra-indications of quality. Defects include specified visible blemishes, as well as cherry hull/parchment fragments or non-coffee foreign matter. Although there are international definitions and standards[1], different national sectors commonly operate their own standards which are not necessarily easily compared. Harmonisation of the 'language of defects' would facilitate communication between trading partners in particular and the international coffee community in general.

Bean defects indicate an upstream problem and some inherently produce a problem in cup quality of themselves. Some defects are considered more important than others and the ISO 10470:2004 defect chart includes coefficients that give more weight to defects that are thought to have a greater impact on quality.

The European Coffee Federation (ECF) '*OTA risk management: Guidelines for green coffee buying*' state that standard quality characteristics, like an 'earthy off-flavour', 'visually damaged' or presence of 'beans in cherry' are indicators of OTA risk and should be used in developing effective and targeted OTA monitoring programmes. According to the ECF, the correlation between the defects and off-flavours noted above and OTA content is based on the results of analyses of large numbers of coffee samples by the industry.

This project set out to further investigate possible correlations between OTA contamination levels in coffee and certain defects. If such correlations are indeed shown to exist, then this knowledge would have ready application in reducing OTA contamination of coffee reaching the market.

Clearly some, if not most, defects are produced by the physiological activity of the seed. Some of these are likely to be causable by several different circumstances or organisms. As far as is known, OTA-producing fungi do not produce any defect. However, it is possible that conditions that encourage these fungi to grow might overlap conditions that cause other fungi that do generate defects to grow, or that certain types of damage may pre-dispose the affected beans to recruit OTA-producers. Thus a correlation could exist between certain defects and OTA contamination without OTA-producers being directly involved in the generation of the defect. Equally, defects that can be produced by a variety of circumstances are unlikely to serve as effective indicators of risk.

[1] ISO International Standard 3509 contains an official vocabulary of coffee including terms related to defects. ISO 10470:2004 provides a defect chart which lists the main types of defect and rates them according to their impact on quality parameters. The ICO minimum coffee quality standard includes a maximum defect count.

It is important to note that whilst there are a great number of opinions as to the causes of defects most of these are not evidence-based. Furthermore, it is critical to recognise the fact that a particular defect does not necessarily correspond to a unique 'condition' or 'state'. Different regions have distinct profiles of defects arising from production systems operating within different environmental conditions so there is no reason to assume that similar defects from different regions are necessarily comparable. This complicates the investigation of correlations between OTA content and defect class. To complicate the picture even further we can consider the simple fact that many defects are difficult to discern and this could introduce more difficulty in picking up on associations between defects and OTA contamination.

Bulk coffee is stored for considerable periods before further curing which is usually done after the sale of the coffee is agreed - thus defects are stored intermixed with sound coffee. It may be that these defect particles have a shorter shelf life or that they provide spoilage foci in stored coffee. A study to investigate associations between defects and OTA contamination should also consider this possibility.

Of course, even if some correlation between certain defects and OTA risk exists, it is clear that full control of OTA contamination cannot be exerted through control of defects. The best approach to minimising OTA in coffee remains the application of good hygiene practices throughout the coffee chain. However, knowledge about the possible associations between certain defects and OTA should be directly applied to the development of risk-based safety and quality management programmes as appropriate to the situation. This might include:

- Improved production practice to reduce occurrence of defects;
- Outsorting to remove 'high-risk' coffee from the food supply;
- More targeted OTA screening by using visual indicators of higher-risk coffee to trigger closer scrutiny of suspect lots, etc.

During the project, the project team found no evidence that outsorted defects were actually destroyed or in some other way removed from the food chain. There was, on the other hand, plenty of evidence that the outsorted defects were added back into lower quality coffees which found its way into the marketing chain. In some producing countries with a reasonable level of coffee consumption, these high-defect coffees were actually used by local roasters. If there is a suggestion that OTA is disproportionately present in defects, there is an implication for public health in the domestic markets of coffee producers, and national authorities will have to pay special attention to the handling of any defects shown to be associated with OTA contamination.

Coffee seeds are viable entities capable of responding to external factors through a significant part of their transit through the production chain. Defects in green coffee are symptoms of some prior exposure to influences that have elicited a response from the seed or directly imposed a visible change. General rules regarding these interactions are almost sure to have numerous exceptions but only a much more thorough and systematic grasp of them will reveal what role they may have to play in an integrated system of quality and safety management.

9.2. Findings and Application

9.2.1 Association between OTA contamination and certain defects

Preliminary evidence suggests a strong association between certain defects and OTA contamination in some cases. This association, however, seems to be strictly related to the existence of certain conditions during production and primary processing of the coffee.

The design of the first study in Kenya provided reasonable assurance that the defects and sound beans being compared actually shared a common production/processing history. The findings showed that in samples of P3 and *m'buni* coming from cooperative factories, over 96% of the total OTA content of the sample was found to be concentrated in two defect classes. In *m'buni* the two defect categories implicated were 'foxy bean' and 'diseased bean', while in the P3, they were 'diseased' and 'insect damaged' (refer to Section 9.5.1 below for further discussion of this trial).

A wider study in Kenya covering estate and cooperative sectors revealed a much less marked association of defects with OTA, but still demonstrated that diseased beans had a significantly higher OTA content than sound beans. The cases where large OTA differentials between defect classes occurred tended to be those where contamination levels were high (refer to Section 9.5.2 below for discussion of this study).

Limited surveys carried out in some countries did not reveal any association between defect classes and OTA. Other work provided some evidence that relatively high (ca. 15%) Coffee Berry Borer (CBB) infestation can enhance ochre aspergilli infection rates where there is a substantial infection in the area (refer to Section 9.5.4 for further discussion on this).

As production/processing conditions change, the association of defects with OTA contamination also changes. This means that findings of OTA accumulation in defects in any particular situation cannot be extrapolated to the 'universe' of coffee. This is clearly demonstrated by the defect studies carried out under the project

The existence of strong associations between certain defect classes and OTA contamination in local situations would have important and direct implications for management of OTA risk in those situations. For this reason further work to confirm the findings of the first study in Kenya, and to investigate other coffee production systems using the approach employed in that study, is urgently required.

These findings would also have implications for risk management at national or international levels. At these higher levels, defects arising from a range of production/processing systems are mixed, and associations between defects and OTA are very weak if demonstrable at all. A much clearer picture of OTA development in specific defects under defined situations is required to inform rational decisions about managing food safety risks associated with coffee defects in international trade.

9.2.2 Storage of unsorted coffee: impact of defects on OTA risk during storage

Only the first study carried out in Kenya was designed to evaluate the impact of storage on OTA content and distribution in unsorted coffee. The findings showed no effect of storage.

Storage conditions (humidity and temperature) were not monitored but water activity levels in the coffee at the end of the 1-year storage period were well below the limits for mould growth. No conclusions can be drawn about the impact of storage under poor conditions (results are presented in Section 9.5.3).

Another storage trial showed that contamination of defects with ochre group fungi declined substantially over a five month storage period, with A_w levels below 0.80.

9.2.3 Further work required

Further work is required to ascertain whether the results from low quality arabica classes (P3 and *m'buni* from the cooperative processing sector in Kenya) are valid for well-controlled processing.

The results of this work should provide a focus for future studies of processing since the conditions that encourage these defects to arise apparently also encourage the growth of OTA-producers as evidenced by the production of their characteristic metabolite. One element of these studies should compare diseased beans oven-dried at harvest with the same material prepared badly as *m'buni*. This would clarify the relative contribution of production factors (pest and disease control during primary production) as compared with processing factors (such as poor management of drying) in the accumulation of OTA.

More work also needs to be done on the nature of the variation experienced in the defect samples. Understanding the distribution of OTA between beans could yield valuable information relating to the mechanism of the accumulation of high OTA levels.

Careful consideration of the coffee bean microflora in these defect studies could provide useful clues about the interactions between different groups of micro-organisms and implications of these for risk of OTA contamination.

More well-founded information on the origin and consequences of the common defects is needed to underpin a rational quality/safety evaluation system that would benefit the whole of the coffee sector.

9.3. Additional Notes

Kenya uses a system of parchment grading where, of the main green coffee classes, A and B are from the densest parchment, C is also referred to as P3 and/or lights and contains beans from small cherries. *M'buni* is dry processed cherry. It is cherry coffee that has been removed before pulping through hand sorting and comprises fruit affected by Coffee Berry Disease (CBD), tree-dried fruit and fruit too small to be

pulped, and which is hand sorted from P3. Floatation separation is all but nonexistent so there is no 'floats' coffee.

In Kenya it was decided to work with low grades of coffee (P3 and *m'buni*) to increase the chance of finding OTA and OTA-producers in the research material. It is particularly important to note that *m'buni* and to some extent P3 or lights coffee, is of very low value and is handled with very little care. It is invariably dried in very thick layers which may be on the grass or on mesh tables depending on demand for table space but it is essentially a long and uncontrolled process.

Many defects have been attributed causes but these are rarely based on sound or verifiable evidence. Some defects are said to be pre-harvest problems, others caused by processing problems. Coffee Berry Borer damage is one that begins in the field but can become much worse on the drying yard under conditions of slow drying. Some defects represent some kind of general symptom, like 'black beans', are of more than one type and undoubtedly have a number of causes. Black beans are of at least three types with immature beans (especially when exposed to mechanical drying) becoming superficially black. A second form is when the entire bean becomes black and the third form is when a part of the bean becomes black extending at least partly through the tissue layers.

Several defects are only significant in certain origins which suggests there are special conditions of processing or horticulture that lead to specific changes in the bean. For example, in Lampung, Indonesia, brown beans are the most common defect but this is not seen in Uganda which also produces robusta cherry, much of which is dried on soil surfaces. Of course, where origins differ in respect of harvesting method and/or processing method, the defects are bound to differ but even when there is uniformity of processing relative proportions of defects and their forms can differ.

9.4. Experimental Design

The main focus in designing experiments relating to defects is to assure that there is an appropriate comparator or control. This means there must be a treatment or component which has a shared processing history and could have been, but is not, a defect. Therefore, single lots were divided so to compare the constituent defect categories. Due to practical constraints facing the collaborators involved in the project, there was a substantial difference in the degree of homogeneity within the initial lots of bulk coffee from which defects were sorted. These differences are outlined below and have important implications on the interpretation of data from the different trials.

9.4.1 Study of defects from coffee beans with a common history

In the first study in Kenya, samples (100kg each) of unsorted *m'buni* and unsorted P3 coffee were each taken from a single processing batch from two cooperative factories and from one estate factory. The defects from each coffee sample were hand sorted in such a way that the OTA and fungal contamination of each defect could be compared with contamination of the sound bean from the same coffee sample. The design of the experiment was intended to provide reasonable assurance that defects

and sound beans being compared actually shared a common production/processing history.

Duplicate samples of P3 and *m'buni* from each of the three sources described above were placed in storage for 1 year prior to sorting and analysis of defect classes.

In Côte d'Ivoire duplicate samples of unsorted coffee bean (100 kg each) were taken from each of three farms. The handling of the coffee was as outlined in the Kenyan study above.

9.4.2 Other defect studies

The use of commercial systems to provide the coffee fractions was advantageous in that it provided fractions that represented a broader commercial reality. However an important disadvantage is that defects and sound beans being compared do not have a common history, and the validity of the comparison is therefore compromised.

Taking samples from low grade fractions at milling/sorting facilities also reduced the work associated with hand sorting the bulk sample at the start of the experiment.

In the second larger survey in Kenya, a total of 96 samples of 1-2kg were taken from coffee milling and sorting factories, P3 samples were taken from the SB low-grade fraction and *m'buni* samples were taken from the '*m'buni* light' fraction. The samples were then hand sorted into sound bean and different defect classes for OTA analysis.

Given the system of coffee marketing in Kenya, the samples taken from the coffee milling/processing operations can be traced to a particular estate or to a cooperative factory or group of factories. Of course, the coffee taken in this way is comes from many different processing batches.

In Uganda, coffee samples for the defect study were collected from milling/processing factories from the low-grade fractions resulting from the sorting process. The marketing system in Uganda is much less structured than in Kenya, and therefore the 'history' of the coffee within any sample is likely to be more heterogeneous.

In Colombia, a small coffee curing company was employed to sort coffee for storage and defect experiments so that the sorting and handling operations were realistic and relevant to actual commercial practice.

9.5. Experimental Results and Discussion

9.5.1 First study in Kenya

Each sample (100kg) of P3 and *m'buni* used in this trial came from a single processing batch from either the estate sector or the cooperative sector in Kenya. The results of the mycological and OTA analyses carried out in this study are summarised below.

Table 9.1: Percentage overall infection and percentage infection by ochre group fungi of sound bean and individual defect classes of P3 and *m'buni* coffees taken from two cooperative factories and one estate factory.

	% infection								% ochre group							
	P3				*M'buni*				P3				*M'buni*			
	Coop		Est.	**Mean**	Coop		Est.	**Mean**	Coop		Est.	**Mean**	Coop		Est.	**Mean**
Insect damage (CBB)	85	92	22	**66.3**	92	97	94	**94.3**	17	23	8	**16.00**	35	22	8	**21.67**
Diseased[2] beans	69	93	30	**64.0**	92	98	78	**89.3**	7	23	6	**12.00**	21	24	0	**15.00**
Hulled ears	42	40	32	**38.0**	81	85	37	**67.7**	8	7	5	**6.67**	14	12	5	**10.33**
Black beans	91	85	27	**67.7**	88	100	92	**93.3**	5	29	4	**12.67**	43	57	2	**34.00**
Stinkers	73	N/A	N/A	**73.0**	89	100	87	**92.0**	8	N/A	N/A	**8.00**	20	29	2	**17.00**
Foxy beans	69	81	N/A	**50.0**	88	100	97	**95.0**	11	16	N/A	**9.00**	18	6	2	**8.67**
Sound beans	63	66	14	**47.7**	91	100	78	**89.7**	4	17	7	**9.33**	10	27	0	**12.33**

The mycological data shows there is a consistent occurrence of ochre aspergilli at a level that has shown in the past to be adequate to produce significant quantities of OTA but no 'dose response' form of correlation arises: the highly infected samples do not reliably predict the highly OTA-contaminated ones (see Table 9.2). The sound beans are reported to have comparable rates of ochre aspergilli infection to many of the defects. Kenyan coffee, in other studies, has yielded a high proportion of *A. melleus*, the OTA non-producer, to *A. ochraceus*. The overall infection rates of coffee from the cooperative sector were over 90% for *m'buni* and over 60% for P3, somewhat high for washed arabica. Overall infection rates were lower in the washed arabica from the estate sector.

The overall proportion of defects found in the samples (Parchment P3 with 25-30% defects, and *m'buni* with 35-40% defects) is quite representative of the national average, as can be seen in Table 9.2, below.

Levels of OTA contamination in the defects in P3 and *m'buni* coming from the cooperative sector are much higher than the levels of contamination observed in the defects from samples taken from the estate sector. OTA samples from the estate sector were low and approximately uniform, and these are not discussed further here. All samples were at an A_w of less than 0.56 at the time of collection.

[2] In the Kenyan context, 'diseased bean' is primarily beans from fruit affected by CBD, a big problem, but one restricted to East Africa. The mucilage collapses in this disease and the skin adheres to the parch and the parch often adheres to the bean. It cannot be pulped efficiently and so is largely found in the *m'buni* and P3/lights.

Table 9.2: Proportion of each defect in bulk sample and OTA content.

Defect Class	P3						*M'buni*					
	Weight (kg)		OTA (ppb)				Weight (kg)		OTA (ppb)			
	Kirura	Gathiruini	Kirura		Gathiruini		Barikongo	Kiambu	Barikongo		Kiambu	
Insect damaged (CBB)	0.54	0.46	10.0	0.5	499.3	75.1	1.0	1.2	2.7	10.9	1.2	0.9
Diseased bean	15.38	6.76	152.6	0.3	2.3	130.0	21.9	17.7	2.3	0.4	21.4	146.0
Hulled ears	8.98	11.04	0.2	0.3	0.5	0.5	0.4	7.1	0.5	0.6	1.7	0.6
Black bean	2.30	1.26	0.2	0.9	3.9	4.5	7.5	7.2	2.2	0.3	0.6	3.5
Stinker	0.22	0.1	5.1	2.5	1.5	3.5	0.3	0.3	3.7	1.3	1.8	2.4
Foxy	1.04	0.20	0.3	3.1	5.0	11.6	1.2	1.5	28.1	169.5	727.0	241.9
Sound	68.00	79.00	0.1	0.2	0.2	0.1	61.6	63.3	0.2	0.4	0.9	0.4

Each lot was sorted into defect sub-samples and each sub-sample was represented by two analyses. These samples are represented by two analyses. Statistics are not possible with a population of just two, but it is clear that variation between the two samples can be extreme and this aspect will be discussed first.

The P3 Gathiruni CBB sample is only 460g in its entirety and the analytical sample will represent over 5% of the entire fraction so it would be expected to represent the 'true' OTA content very well. On the contrary, one replicate has 500ppb and the other 75ppb. These replicates vary by about a factor of 6 representing a difference of 425ppb. In a 25g analytical sample this equates to about 2µg in the low replicate and 10µg in the high replicate. For reference, in axenic culture, many mycotoxins can be produced in mg quantities in a week or ten days.

We could imagine that this high variation is due to a low background OTA content with a small but significant number of highly contaminated beans. Without several more analytical determinations it is impossible to be sure of what value the mean of the two replicates has, though it is the only tool available for interpreting the data.

Sound beans show a low OTA contamination rate in all of the 12 determinations of the 6 samples. In the P3 defects from the Gathiruini sample the mean OTA content of CBB and diseased beans is high and foxy beans somewhat elevated, with all of the other defects possibly higher than the sound beans. The sample from Kirura have higher OTA in diseased beans and CBB beans, and possibly in stinker beans.

Both *m'buni* samples show a strong elevation in OTA content of defects, especially that from Kiambu. Here foxy beans and diseased beans are very high with stinker beans as the only other defect to possibly register a higher value than the sound beans. In the Barikongo sample it is only foxy beans that are strikingly elevated with CBB possibly slightly higher than the sound beans.

With the sample weights available, a picture of the distribution of OTA in the bulk coffee can be generated while recalling that the reliability of the mean OTA content values is poor (Table 9.3). The first four columns represent the weighted means of the defect categories and represent the amount of OTA with its origin in that defect if the lot could be homogenized and perfectly mixed. The second four columns show the percent contribution of each category.

In terms of total contribution, foxy beans and diseased beans account for most of the OTA in the *m'buni* samples from the Cooperative sector. In the case of P3, diseased beans and insect damaged (CBB) beans account for almost all of the OTA in the bulk sample. These data imply that if, for example, all black beans were removed from a lot of coffee, representing 2 to 8% of its weight, no more than 5% and probably less, of the OTA would be removed. If diseased beans and foxy beans were removed, representing perhaps 10 -15% of weight, probably more than 80% of the OTA would be removed.

Table 9.3: Calculations of relative contributions of defects to total OTA load. The weighted means are used to calculate the distribution of the OTA in coffee class categories of each lot. CBB = coffee berry borer

	Defect contribution (µg OTA per kg of bulk coffee)				% of total OTA in bulk sample			
	P3		*M'buni*		P3		*M'buni*	
	Kirura	Gathiruini	Barikongo	Kiambu	Kirura	Gathiruini	Barikongo	Kiambu
CBB	0.03	1.32	0.07	0.01	0%	22% [3]	4%	0%
Diseased bean	11.8	4.47	0.30	14.8	98%	74%	15%	64%
Hulled ears	0.02	0.06	0.0	0.08	0%	1%	0%	0%
Black beans	0.01	0.05	0.09	0.15	0%	1%	5%	1%
Stinkers	0.01	0.0	0.01	0.01	0%	0%	0%	0%
Foxy	0.02	0.02	1.21	7.56	0%	0%	63%	33%
Sound beans	**0.10**	**0.12**	**0.25**	**0.41**	**1%**	**2%**	**13%**	**2%**
Total	11.95	6.04	1.92	23.05				

[3] It was noted that several of the beans that were classified as 'insect damaged' also showed signs of disease. The lack of precision in the reporting of defect categories should be taken into account in interpreting the data.

9.5.2 OTA associations with defects in downstream coffee marketing

Two collections from the trading chain were made, one in Kenya where arabica parchment is the only significant product and one in Uganda where robusta cherry is the main product.

Because of the central auction and payment method applied in the Kenyan coffee sector, lots are maintained as delivered through the milling process. This allowed samples taken at the mills to be traced to the coffee factory of origin in the case of the Estate sector and either to a single factory or small group of factories in the case of the cooperative sector. Each lot represents a mixture of processing batches over the season using coffee from a fairly large number of growers around the Cooperative factories.

Table 9.4 gives the overall analysis of the samples broken into defect types and coffee class of origin, some 188 samples in all. A very few sample from A and B and no defects from these were analysed. There is no difference between the sound beans from A/B and C and little difference between A/B and *m'buni* or SB. The slightly higher average is due to seven samples of *m'buni* between 2 and 7ppb and in the case of SB, four samples between 2 and 8ppb.

Table 9.4: Mean OTA content (ppb) of samples collected at mills (curing works) in Kenya. The samples could be identified as to the coffee factory (or group of factories) of origin but represent amalgamations of many farmers' crops and many processing batches. The defects do not always have corresponding sound beans, and vice versa, in the collection.

	All samples		AB		C		*M'buni*		SB[4]	
	n.	OTA	n.	OTA	n.	OTA	n.	OTA	n.	OTA
Sound	79	1.2	6	0.2	18	0.3	27	2.0	33	1.4
Diseased	63	12.3			14	10.2	18	31.3	30	2.3
Black beans	22	17.0			3	104.7	14	3.7	5	0.94
Insect damaged	10	1.3			7	0.9	3	2.2		
Ears	4	0.5			3	0.6	1	0.1		
Faded	1	1.2			1	1.2				
Foxy	5	11.1			4	13.9	1	nil		
Green water	2	7.2			2	7.2				
Pulp damaged	1	nil			1	nil				
Stinker	1	4.4					1	4.4		

'AB' = from parchment 1 and 2;'C' = from parchment 3/lights; 'SB' = green bean removed from ABC during milling; '*m'buni*' = reject fruit prepared as cherry coffee.

Before looking at the pattern of OTA contamination in the defects a few points should be noted. Firstly, the samples taken in this survey are quite heterogeneous in

[4] The SB fraction is a collection of low grade coffee from parchment coffee processing.

origin. Defects and sound beans from a single sample taken at the mill are likely to have experienced a number of differences in their production handling and processing. The greater the heterogeneity of the sample, the greater the difficulty in identifying associations between 'the defect factor' and OTA content. However this 'heterogeneity' is representative of the reality in downstream coffee marketing and therefore gives an idea of the overall significance of defects to OTA risk.

Secondly, one of the defects that was of major interest in the first study – foxy beans from *m'buni* – could not be examined in the second survey. This defect was absent from the samples taken from the mills. This might be related to the way in which the samples were taken, or may simply indicate that the defect is rare is some seasons. This question should be investigated. There is also a relatively small number of insect damaged samples available in the second survey. This defect category was another of the apparently important risk categories.

Thirdly, the first study showed that there was big difference between the estate and cooperative sectors in terms of the level of contamination of defects. Table 9.4 provides averages across these two sectors. This tends to reduce apparent differences between diseased and sound beans when all sources are pooled. In fact, when the data are segregated according to the source of the coffee we see that diseased beans are more highly contaminated than sound beans from the cooperative sector, but there is no difference in contamination between diseased and sound bean produced by the estate sector (see Annex C.8 on the enclosed CD-Rom).

Of the more common defects, three look to have a higher risk of OTA contamination by this data set: diseased beans, foxy beans and black beans. The defect that has the most consistent high OTA level with a fair number of analyses is diseased beans from both *m'buni* and P3/lights (class C). A statistical analysis of the ***overall*** data set shows that the diseased beans are significantly more highly contaminated than the sound beans ($p>0.05$). The highest average contamination is in black beans from class C coffee, but there are only three such examples. Foxy beans are only represented by 5 samples.

The elevated average OTA content of diseased beans from this study is consistent with the findings of the earlier trial discussed in Section 9.5.1 above. In many cases, samples obtained from the mill did not contain adequate amounts of defects for an analysis of defect classes to be possible – in these cases, only OTA data on the sound bean is available.

In other cases, OTA analysis of a defect class was presented without an analysis of sound bean from the same sample. Table 9.5 collates the cases where OTA data for diseased and sound beans from the same sample are available. This table provides some insight into the question of variability of OTA content in this defect class and also of the relationship between OTA levels in the defect and in a comparator sound bean sample.

Table 9.5: Individual comparisons of OTA content of diseased and black beans to sound beans for samples where a sound bean comparator sample could be identified.

M'buni			**Parchment**			**Parchment**		
Sound	**Diseased**	**Black**	**Sound**	**Diseased**	**Black**	**Sound**	**Diseased**	**Black**
Traces	Traces	0.1	Traces	Traces	Traces	0.2	0.9	1.6
0.2	2.8		Traces	0.5		*4.6*	*0.1*	
3.2	57.1	1.7	0.2	23.4		0.2	0.3	
7.8	87.1	16.9	Traces	1.0		0.1	0.3	
0.7	3.1	1.2	0.5	5.0		0.1	Trace	
1.0	79.6	1.8	Traces	Traces		0.3	1.3	
4.3	47.7	0.9	Traces	10.9		0.6	2.5	
4.1	1.8	5	0.1	2.5		0.2	Trace	
3.6	3.8	3	0.2	2.7		1.3	0.4	
0.3	34.9	16.8	0.3	0.1		0.3	0.3	
1.9	6.7	1.5	Traces	0.2		0.2	0.4	
1.0	89.3	1.4	2.2	0.1		Trace	0.9	
3.2		1.7	0.1	4.2		2.5	3.5	
0.1	2.3		0.6	0.1		0.4	5	0.2
2.1	4.4	2.3	0.5	26.8		4.0	5.5	0.3
0.7	0.7		0.1	0.7		0.4	7.9	0.3

The highlighted cells indicate cases where the OTA content of the defect is more than five times that of the sound bean comparator. Using this criterion for identifying substantial differences in contamination, we see that 8/15 diseased *m'buni* samples are substantially more contaminated than the comparator sound bean. In the case of parchment, the figure is 13/32. Conversely, in 7/15 cases for *m'buni* and in 18/32 cases for parchment, there is either no difference or a minor difference in contamination level. In one case out of 47 is the sound bean substantially more contaminated than the diseased bean.

Notably, in all cases of high contamination, the OTA is highly concentrated in the diseased bean defect.

Annex C.8 on the enclosed CD-Rom contains the results of statistical analyses carried out on all samples analysed in Kenya in the two defect trials. It shows that diseased beans coming from the cooperative sector were significantly more contaminated than diseased beans from the estate sector. One factor that might contribute to this observed difference is the system of CBD control practiced on the estates as compared with the small-scale farmers. This hypothesis requires investigation.

Data from a market chain survey carried out in Uganda can also be considered here. In this relatively small survey, samples were taken at exporters' warehouses and a few farms in Uganda. Two points are immediately obvious: that there is no indication of any association between OTA contamination and any of the defect

classes; and that the farm gate samples are less contaminated than the exporter samples.

Table 9.6: Analysis of bean defects sorted from samples removed from the trading chain and farm gate in Uganda.

Source of coffee	Sound beans		Defect categories				
	SC 18	BHP 1899	Immature	Blacks	Broken	CBB	Floats
UGACOF	2.6	3.1	--	3.2	4.1	8.6	2.8
Great Lakes	3.8	11	--	1.8	2.8	11.0	7.1
OLAM	2.2	2	--	2.8	4	5.6	3
PANAFRIC	3.9	8.6	--	2.9	3.4	7.2	5.6
Kyagalanyi	2.6	7.8	--	2.5	3.7	4.5	2.9
Farm 1	tr		tr	1.9	tr	0.2	--
Farm 2	1.2		0.2	0.2	0.3	0.2	--
Farm 3	0.2		0.3	0.3	--	--	--

There is some direct evidence of reduction of frequencies of, infection by OTA producers, as coffee is cured or milled. Several lots were sampled before and after curing (grading, sorting, polishing etc.) and analysed for fungal infection. OTA was not measured.

All samples had detectable amounts of niger aspergilli and all the cherry samples showed ochre aspergilli. All samples had comparable total infection rates between 82 and 100%. In the case of the cherry coffee it is possible that the post-curing samples had a slightly reduced frequency of niger and ochre aspergilli (Table 9.7). If the change is real, it could be understood as reflecting the removal of certain defects that have an increased frequency of infection. Black beans and the Indian category 'cuts and bits' would be the primary classes of bean to have been removed from the bulk of the cured coffee.

Table 9.7: Changes in niger and ochre aspergilli bean infection rate pre- and post-curing.

Arabica	Cherry		Parchment
	Ochre	Niger	Niger
Pre curing	14	55	17
Mean post-curing	7 ± 1*	41 ± 2*	12 ± 3**
Robusta			
Pre curing	11	58	24
Mean post-curing	4 ± 1*	39 ± 3*	20 ± 1***

* n=3; ** n=5; *** n=4

9.5.3 Changes in OTA content and moulds in defect beans on storage

In Kenya, 100kg samples of P3 and *m'buni* (sub-samples of the coffee from trials discussed in Section 9.5.1, above), were stored for one year. There was little or no increase in A_w over the storage period with final values typically below A_w 0.60. There was no overall increase in OTA in any of the six consignments and no indication of an increase in the weight of defects during the storage period. As was seen in the samples before storage, the defects account for most of the OTA in the bulk sample. There are, however, notable differences in the levels of OTA contamination of individual defects as compared with levels of contamination of the same defect class before storage (see Annex C.9 on the enclosed CD-Rom). The low replication and the high variability in OTA analytical data limit the interpretation of this observation. It is highly possible that the inconsistent classification of 'multiple defect' beans (for example beans that are both 'diseased' and 'black') might contribute to this confusing picture. Future trials should pay close attention to this.

An experiment was carried out in Colombia to characterize defects in bulk coffee before and after a period of storage (and Table 9.8). *A. carbonarius* was detected in only one sample at the detection limit. Only clean and bulk beans were analysed for OTA and none was detected before or after storage.

Table 9.8: Infection rates of beans, sorted into defect categories, of representing replicate lots of arabica parchment coffee. Elapsed time between initial and final is about 5 months and there were only very minor increases in m.c. with no A_w exceeding 0.68.

	Means					Maxima				
	Clean	**Black beans**	**Broken**	**CBB**	**Vinegar**	**Clean**	**Black beans**	**Broken**	**CBB**	**Vinegar**
Initial										
Total	22.4	80.6	76.5	77.8	70.4	55	100	95	100	99
Aspergilli										
Ochre	**0.4**	**15.3**	**15.5**	**13.3**	**13.1**	**2**	**43**	**33**	**39**	**27**
Niger	0.6	7.8	7.3	6.3	3.7	1	11	12	14	8
Flavi	3.5	17.6	13.3	16.3	8.8	8	35	26	33	18
Penicillium	16.1	61.2	56.3	62.4	55.3	46	91	73	92	92
Eurotium	0.0	2.2	3.9	1.4	4.7	0	5	8	4	11
5 month										
Total	8.0	33.0	19.0	39.6	18.0	10	57	27	61	36
Aspergilli										
Ochre	**0.0**	**2.2**	**1.2**	**1.6**	**0.6**	**0**	**7**	**5**	**4**	**2**
Niger	0.8	9.6	5.9	13.9	6.3	2	13	10	21	10
Flavi	0.8	7.1	3.5	9.6	4.3	2	14	7	23	10
Penicillium	2.9	1.9	4.3	10.2	3.3	7	6	9	22	10
Eurotium	1.6	12.5	5.1	8.4	6.3	7	43	10	22	16

There was no marked difference between the initial samples to indicate any had experienced particular problems in processing. The means give the rather

improbable picture that all of the defects are more contaminated, and to the same extent, compared to the sound beans. In particular there is the near absence of ochre aspergilli in sound beans and its uniform significant presence in all defect categories at about 15%. The maximum values recorded fall between 27 and 43%. The two major issues with this picture is that it is not consistent with other iterations of this kind of analysis and it implies that all of the defects are produced by conditions that equally favour the development of these fungi, i.e. the various defects are produced in a single set of conditions.

There is a decrease in viable fungi through storage though of the aspergilli, only ochre section shows a consistent decrease. As is known, *Penicillium* is susceptible to storage conditions in coffee and *Eurotium* probably increases during the five months despite the average A_w indicating that there is too little moisture to permit even this xerophile to grow.

Under the storage conditions, which were in a storage facility in Caldas, Colombia, not far from a river, there was little uptake of moisture despite some very humid periods. At least under these good conditions there was no outgrowth of the fungi in the defect categories and, by extension, no suggestion that defects curtail shelf life in well-stored coffee.

9.5.4 Investigations of some specific defects

Coffee berry borer (CBB) damage: Robust insects can breach physical barriers to fungal invasion and passively or actively introduce fungi to the inner tissues of plants. Their passive activity is by carrying fungal propagules that happen to have become temporarily attached to the insect's exoskeleton so can be almost anything. Active introduction describes fungi that is in association with the insect such as certain yeasts and bacteria that inhabit an insect gut. There is some circumstantial evidence that *Candida edax* falls in this category with the CBB.

Beetle feeding galleries in coffee generally develop a greenish colouration that could be mistaken for fungal growth. Direct observation shows that this region is not colonized by fungus and that the coloration is probably a chlorogenic acid produced by the seed itself. in response to mechanical damage.

A. ochraceus would not be expected to be commonly carried by the beetles except where it had become common. In the plantation it is most commonly found associated with coffee either in the rhizosphere soil or the seed. Of course CBB is only found in the coffee seed, or on its way between seeds so its activities might be expected to amplify a field infection of this fungus. The samples analysed in Table 9.9 show this.

Table 9.9: Mycological analysis of two samples from a farm, taken at different points in the season, with high ochre aspergilli occurrence and a CBB rate of about 15%.

Ob34/35	Sound beans	CBB beans
Overall infection rate	50%	60%
Ochre grp rate	9%	23%
Rate neglecting ochre grp	41%	37%
Ob25/26		
Overall infection rate	63%	86%
Ochre grp rate	17%	33%
Rate neglecting ochre grp	46%	53%

Note that the underlying infection rate (neglecting ochre aspergilli) is the same in the CBB-attacked and CBB-free beans and the additional infection seen in the overall rate is accounted for by ochre aspergilli. This suggests that despite the beetles potentially introducing a host of common environmental fungi, in particular those on the surface of the coffee plant, it was the ochre aspergilli alone that were augmented. Apparently only the fungi adapted to the niche are successful colonizers.

Stinker beans: A collection of stinker beans from arabica cherry showed an infection rate of 35%. The fungi appear to be uninvolved with this defect. Not only is the fungal infection rate only low to moderate, there is no association of the dark green discoloration with the point of emergence of the fungal contaminant. Furthermore, no species emerged as characteristic from the sample.

Black beans: A collection of black and black-spotted beans from arabica parchment were collected from the washing step. They showed a 95% infection rate, about half of which was yeast. Yeast infection immediately after wet processing is common and tends to disappear quickly on the drying yard. In this particular season there was a very high rate, about 20%, of this defect for no apparent reason. Table 9.10 shows that several different fungi are of significant occurrence in this defect. That 5% of these beans carried no fungus could be taken to mean that the coloration is a disease response that is sometimes successful in eliminating the invader, or it could be taken that fungi are not the only cause of black beans. Probably both of these are true statements and various injuries can produce this defect, which is mediated by the seed itself, in any case. Whether yeast could be involved in generating black and black-spotted defect beans is moot.

Table 9.10: Percent infection in black beans and black-spotted beans of arabica parchment collected before drying.

Taxon	Total	*Fusarium*	*Penicillium*	*Aspergillus*	*Cladosporium*	Yeast spp.
i-infection	95	12	33	2	7	53

NB - % of total infection comprised by taxonomic grouping; there can be more than one fungus/bean.

Section 10
Storage and Conditioning

10.1 Introduction

Storage of coffee is a universal practice found throughout the production chain with each stakeholder of the chain potentially retaining the coffee for days, weeks or many months. Conditioning, commonly associated with the Kenyan coffee sector, is conducted only by the stakeholders who conduct processing and lasts for a few weeks or less.

Generally speaking coffee is produced in upland regions and since most is exported it is ultimately traded from lowland coastal areas. This represents a large change in ambient conditions especially since, by the nature of the crop, coffee is grown in the wet tropics. To some extent, coffee trading is the mechanism of collecting small lots of coffee and moving it from the producing regions to the ports, so mixing of disparate lots is inherent in coffee trading.

On-farm storage may be brief, only long enough to negotiate the sale of the coffee, or it may last longer than a year as the farmer gambles on a better price. Smallholders store their coffee as cherry coffee (*en casca*) as parchment (*en parch*) or as unsorted bulk green coffee. This very much depends on the way that the market is set up to function, though how and why a particular tradition becomes established is not clear.

Storage facilities on the farm vary from purpose built 'go downs' to the living quarters of the home or lean-to shelters built against the house. Often the farmers' options on how long he might like to hold coffee is limited by security fears and this factor can be a serious issue leading to changes in practice.

Local traders are often not specialist coffee traders and have the capacity to concentrate their activities in other commodities if they see higher risk or less advantage in coffee. This tends to put pressure on the coffee producers' price since he has already converted his investment into the coffee he now holds and has no such alternative. It also means that some traders are not coffee specialists and may function in a different way than a specialist might.

One primary role of the local trader in regions with a predominance of smallholders is to accumulate lots of coffee of sufficient size to be efficiently transportable and of interest to larger traders or exporters. In doing this he mixes coffee from many sources and qualities. This may mean different moisture contents with the potential this implies of re-wetting well processed coffee with poorly dried wet coffee. It also implies the possibility of diluting mouldy coffee into sound coffee so the blended result is still of an acceptable overall quality. The blending of different coffees has clear implications for the storability of the batch.

Aside from collecting and mixing the traders role may also include re-drying and cleaning of coffee depending on the structure of the local sector and certainly

includes transportation. These activities also have implications for the basic storability of coffee in the chain.

The market has a natural relative price cycle related to the stage of harvest which international changes, fuelled by factors independent of the local market (aside from within the Brazilian domestic market), modify. The middlemen can buy in the glut market during peak of harvest and hold coffee for several months to try to take advantage of the rise in prices expected in the off-season. So this is one reason why storage in coffee production areas, both by farmers and traders, can sometimes be for extended periods.

10.2 Findings and Application

10.2.1 Moisture uptake during storage

Since storage may occur over long periods, and even slow changes could have significant impact, a main objective of storage must be to maintain the moisture at levels that cannot support mould growth. Monitoring of moisture in well designed and managed storage facilities over a 6-month period showed that there were only very slight increases in moisture content with A_w values remaining below 0.70 – well below the level that would allow any risk of OTA contamination.

In coffee-producing countries, coffee storage often takes place in poor, improvised, facilities. In these cases, coffee rehydration behaviour is variable. In some cases, even after lengthy storage periods, the moisture content remained at safe levels while in other cases rehydration to levels that could allow mould activity were noted.

When storage of coffee cherry in heaps within a trader's storage facility was compared with storage in sacks, there was a consistent trend whereby the coffee at the centre of the heaps had a moisture content 2-3% higher than at the surface or than the coffee in sacks (see Section 10.5.2).

10.2.2 Fungal contamination during storage below 12% m.c.

During storage of well dried coffee there is a general reduction in fungal infection rates as mesophilic fungi tend to die off. In cases where there is a relatively high frequency of ochre aspergilli infection, this group has also been shown to decline during storage. In most cases, the initial level of ochre infections is low – around the detection limit of the methods used – and remains at this low level after storage.

Under dry conditions of storage the moulds exist in a dormant state. Notably, when doing the viable counting of fungi after storage, a much longer time is required for the fungi to grow out – up to 2 weeks as compared with 3-4 days with other coffee samples. This would have implications on the conditions necessary to have re-activation of OTA-producers and accumulation of OTA after a period of storage.

The fungal contamination of the bean was found to depend to some extent on the prior handling of the coffee (see Section 10.5.3).

Sampling of air in storage facilities demonstrates predominantly xerophillic organisms not implicated in OTA risk, but ochre group aspergilli are also present though at low frequencies (Section 10.5.1).

10.2.3 Re-wetting of coffee during storage

Two common reasons for re-wetting of coffee during storage are the passive uptake of moisture from the environment and the mixing of wet coffee with dry batches during pooling of coffee prior to storage.

In trials where passive rehydration of coffee occurred, water activity levels after 6 months of storage reached 0.80 – 0.85. In these cases there was a nominal increase in frequency of ochre group fungi, but no increase in OTA was observed (see Sections 10.5.2, 10.5.3 and 10.5.5).

In one trial where there was moisture uptake during storage, but where measured A_w values remained in a range that would not support mould-mediated deterioration, there was a reported increase in defects (Section 10.5.6). The implication is that the seeds themselves mediate these defects. This cannot be true of all defects, and it would be useful to know which defects are involved and the mechanism of their generation (refer to Part C, Section 9 on 'Defects and OTA in Coffee').

In another trial small amounts of wet coffee were mixed in with dry coffee prior to storage to simulate rewetting that might be expected to arise due to the pooling of coffee dried to different levels. In this trial the treatment batches remained with elevated moisture contents (12-15%) as compared with the control batches over the 6-month duration of the trial. Mycological analysis of the samples revealed some differences. The main differences in the re-wetted coffee were that *Penicillium* survived better and that *Cladosporium* failed to increase. The quality implications of these differences were not investigated. There was also a small but consistent increase in ochre aspergilli but no OTA formation (Section 10.5.3).

Where greater re-wetting was experienced as in monsooning (>0.90 m.c.) there was greatly increased occurrence of ochre group fungi over approximately a one month period. Measured OTA levels, however, remained low (Section 10.5.7).

10.2.4 Temporary storage of wet parchment in conditioning bins

Observation of common practice at some cooperative factories in Kenya revealed that incompletely dry parchment (20-25% moisture) was being placed in conditioning bins when drying table space was required for freshly processed parchment.

The moisture and A_w profile of such parchment in the conditioning bins was monitored so as to ascertain the magnitude of risk that this practice posed for OTA contamination. It was confirmed that the usual methods of mixing coffee in the conditioning bins are not adequate to bring about drying of the central bulk of the coffee. Very little drying takes place even in the outer layers during the entire period of confinement in the conditioning bin (Section 10.5.8).

10.2.5 Overall comments on storage

These studies, like other published work (P. Bucheli, et al., 1998[1]), failed to generate anything that looked like a storage problem, in terms of OTA accumulation, despite purposely applying questionable practices to the storage.

We know problems can arise during storage and we know the approximate physical conditions under which fungi can grow. The trials have demonstrated that ochre aspergilli remain viable even after periods of storage with the water activity is maintained below 0.70 and that nominal increases in the frequency of infection by these OTA-producers are seen under poor conditions of storage with rehydration to moisture content around 15% (wb). High infection frequencies with niger group aspergilli are also maintained during storage.

We have also seen, however, that in well managed stores, the moisture content of stored coffee can be maintained below 12% m.c. and under these conditions risk in minimised. Measures should be applied in producing countries to see that good storage practices are applied.

10.3 Additional Notes

Studying processes that take place at the edge of physiological capability, and that are therefore slow and slight, are most difficult. If, by their nature, they increase the heterogeneity of an already heterogeneous system, experimental problems become greater. We know that sometimes problems arise in the storage of coffee but it has proven difficult to 'generate' the problems even when imposing storage conditions that should allow mould-mediated deterioration.

The reality of storage in producer countries covers an expanse of conditions that makes devising realistic experiments difficult. By their nature, problems in storage do not occur uniformly throughout the lot but only in certain parts of it. The classical spoilage pattern referred to as 'hot spots' where rampant microbial and insect activity generates water from stored carbohydrates and spreads out from a point is the clearest example of how heterogeneous storage spoilage can be.

Furthermore, since the length of time a lot can be stored can be long, very slight activity can be important and the physiological data from relatively short-term laboratory experiments are of questionable value. Of course it is very difficult first to detect small, slow changes and second, unambiguously attribute differences to treatments. For example, a study that shows there is no OTA production at an A_w of 0.80 over one month does not necessarily mean there will be none over twelve months. Likewise, an A_w of 0.78 or 0.82 may be quite different over a long period as assuredly as will be the response of two different isolates to these marginal conditions.

In close-up, the activity of fungi in a commodity takes place in millions of (more or less) isolated systems. The particles are surrounded, though not suspended, in a relative desert. The hyphal growth habit allows fungi to move across these inhospitable areas but only if there is a relative oasis to grow from, hence by its

[1] Bucheli, P., Meyer, I., Pittet, A., Vuataz, G., and Viani, R. 1998. J. Agric. Fd. Chem. 46: 4507-4511.

nature, a storage problem is fragmentary and maximizes heterogeneity as it develops.

So storage trials to be useful in representing real world conditions and predicting outcomes have to represent a scenario that is understood in light of known 'real' conditions. The sampling issue cannot be overcome if realistic quantities are to be used, as they must represent how a bulk commodity changes in response to ambient conditions, so this must be allowed for in the interpretation of results.

In effect, the ingredients for the spoilage of stored coffee are within the lot. There are plenty of viable fungi, nutrients and water. What keeps the fungi from utilizing the nutrients is a low ***concentration*** of water. In a tonne of well-dried coffee there is between 100 and 120 litres of water. If this is evenly distributed there is no problem. However if a part of this volume accumulates, and the easiest way for this to happen is through condensation, then growth can begin. Given enough growth, there will be a significant generation of water from microbial (or insect) respiration. The whole key, then, is to begin with evenly dried coffee and store in such a way as to prevent a redistribution of water.

10.4 Experimental Design

Several experimental designs were used, some to simulate particular field conditions and others to characterise changes in coffee that was a part of the market. The storability of products is affected by their initial quality or condition as well as the physical conditions of storage. This rationale provides the guiding principle for the experimental designs.

A second issue is that amounts of coffee have to be large enough to make the storage physically realistic and sometime other coffee was used to accomplish this. Coffee is a valuable commodity and larger amounts have serious cost implications but also there is also a trade-off to be made between realistic volumes for storage and taking representative samples from a large bulk. A tonne or two may be a better mimic of a trader's store but your ability to accurately and meaningfully measure the outcome is diminished.

The storage facility was problematic in many cases. Often the only alternative was an out-building on a research station that more or less resembles what is found in use by local traders or farmers. Where possible using a real coffee store is preferable because this introduces the possibility of cross contamination and standing populations of specialist fungi and insects found in coffee stores.

Some trials utilized coffee produced in other research under the project, in particular processing trials. The initial material is very well characterized, its history known in detail and moving from processing to storage realistically follows the natural pattern in the sector. In one form different processes were amalgamated into three categories as best, intermediate and worst. These lots were divided and sub-samples removed and sterile water added to these sub-samples before they were blended back into the bulk. This gave three quality levels and three quality levels with added water in the form of re-wetted low quality coffee or re-wetted high quality coffee.

A simpler version of this approach was simply to compare lots of coffee prepared in different ways, either dried on different surfaces or processed as cherry, split cherry or parchment.

Storing methods were touched on in comparing storage in sacks with storage in heaps. This was in response to field observations indicating that heaping coffee is quite common in some regions.

Since one would expect damaged beans to be more susceptible to spoilage and providing a source of contamination, some trials were aimed at defects in storage of bulk coffee. The procedure here was to purchase two sacks of a lot of coffee and divide it into two parts. One part would be sorted immediately into its various defects and clean coffee and all categories characterised. The second sack is stored for a designated period and then it is sorted and characterised in the sane way. This ascertains whether any defects may be particularly unstable and to judge whether they have a wider effect on the rest of the coffee bulk.

In Indonesia it proved possible to secure coffee in local traders' stores and to return to sample this periodically. This was repeated with three local traders in each of three regions and provides a realistic, if small, sampling of real coffee stored in actual facilities. Since coffee is traded at a moisture content that would be regarded as too wet by most norms, these lots were divided and half re-dried to within the recommended level (12% wb) so a further comparison could be made between well dried and normal practice, that is bulk green coffee traded between 13 and 18% m.c..

10.5 Experimental Results and Discussion

10.5.1 Mycological observations on storage and storage facilities

The air spora from storage and processing facilities differs from that of the drying yard although the two locations can be compared on the basis that coffee provides the major source of the airborne spores. Where the storage facility is also a cleaning and grading facility, the normal case in smaller-scale operations, *Cladosporium* spp. and *P. brevicompactum* can predominate. If the facility serves purely a storage function as is normal on farms, these two fungi are less significant in the air and *Wallemia sebi* and *Eurotium* spp. can predominate. Ochraceus and niger group aspergilli are usually present but not usually numerous. Here yeasts and *Fusarium* are virtually absent.

Table 10.1 shows fungal infection and OTA contamination data from coffee taken from an on-farm storage facility. The storage facility took the form of a lean-to shelter and could be considered very poor. The coffee was stored as dry cherry after stripping and drying without floatation separation. A_w measures of several sacks varied between 0.70 and 0.76. The dry cherry comprised a mixture of coffee accumulated over three years. Although the coffee is not directly comparable, coming as it does from different seasons, it is clear that the 'field' species have been replaced by a combination of xerophilic species (*A. penicilliodes* is an obligate xerophile) and generalist saprophytes such as *Cladosporium*. Although ochre aspergilli persist in the coffee as suggested by the air sampling, there has been little or no OTA production over the three years of storage under these poor conditions.

Table 10.1: OTA and the internal fungi of coffee beans from on-farm storage, sampled in the 1996 season.

Sample	OTA (ppb)	i speciation					Ochre group
	Bean / hull	% infection	1st dominant (proportion of fungi)		2nd dominant (proportion of fungi)		
F, 1996	0.7 / 1.0	96	0.86	*P. brevicompactum*	0.51	*F. stilboides*	1/84
F, 1995	(<0.5)	Not available					
F, 1994	0.7 / 0.8	43	0.35	*A. penicilloides*	0.07	*Cladosporium*	1/84

10.5.2 Comparison of heaped cherry with bagged cherry storage

This study seeks to emulate relatively short-term storage of poorly dried coffee in conditions as might be experienced on a small farm of a robust cherry producing region. However, the moisture data (both m.c. and more importantly A_w) show the two intended treatments are both too dry and that they overlap so the study becomes a 12 week storage in sacks and heaps of coffee dried to between 11 and 15% m.c. (wb).

Over this 6-month period the heaped storage facilitated moisture uptake to a greater extent than bagged storage (Table 10.2). The final 12 to 18 weeks of these treatments were spent at an A_w at which OTA could be produced and certainly where many mesophilic, not to mention Xerophilic, fungi can grow. The bagged coffee remained below this (0.80) level throughout the storage period.

Table 10.2: A_w of four lots of robusta cherry stored either in bags or in heaps for 24 weeks.

Storage period	Bagging 1	Bagging 2	Heaping 1	Heaping 2
0 wks	0.68	0.78	0.72	0.79
6	0.71	0.78	0.78	0.81
12 wks	0.73	0.77	0.82	0.83
18 wks	0.74	0.77	0.85	0.84
24 wks	0.71	0.74	0.81	0.82

The mycological picture (Table 10.3) does not, apparently, conform to this in that ochre aspergilli, which are present in this coffee, do not make consistent increases in the stored coffee. Both the heaped and the bagged coffees show modest temporary increases and decreases, consistent with sampling error around the detection limit. Also surprising is the slight consistent reduction of niger aspergilli in the heaped coffee and its disappearance in one of the dryer bagged samples, apparently replaced by *Fusarium* which is surprising at the levels of A_w reported in this treatment.

Work reported above shows that fungi do die in prolonged storage. The appearance and disappearance of some fungi during the 24 week storage suggests that

persistently marginal water availability is problematic for mould growth. Dormancy may be broken but if conditions do not allow the mould to grow it quickly dies off again. *Fusarium* consistently showing an increase under conditions where niger aspergilli showed even a modest decrease is unexpected and does not follow the textbook.

Table 10.3: Mycological analysis of four lots of robusta cherry stored either in bags or in heaps for 24 weeks in Ugandan conditions. '<'=below detection of 1.4%.

	Weeks of storage in bags					Weeks of storage in heaps				
	0	**6**	**12**	**18**	**24**	**0**	**6**	**12**	**18**	**24**
	Replicate 1									
Niger	100	100	100	98	98	100	100	93	100	86
Ochre	<	<	<	16	<	<	<	<	2	<
Penicillium	<	1	2	<	<	5	24	5	<	<
Rhizopus	51	11	6	<	<	<	<	12	<	<
Flavi	<	6	8	2	<	<	<	<	<	8
Fusarium	<	<	<	48	6	<	<	<	<	4
	Replicate 2									
Niger	99	97	98	29	<	99	93	82	65	84
Ochre	<	2	8	7	<	<	1	7	<	<
Penicillium	23	6	2	<	<	23	14	29	<	8
Rhizopus	<	<	<	5	<	<	<	<	<	2
Flavi	<	<	<	<	<	<	<	<	8	4
Fusarium	1	3	2	62	100	1		14	<	12

A second procedure that utilized the same type of robusta cherry and storage conditions sought to test the relative storability of coffee with different processing histories. Coffee that had been dried less well or had been produced using methods not recommended could contain a greater fungal biomass that could affect storability.

The initial m.c. was 10-11.5% (wb), corresponding to an A_w in the range of 0.6 to 0.68. With three exceptions all m.c. levels of the sample from the centre are higher than the surface sample. This difference can be substantial at up to 3.5% and about 1.5% on average. But this masks the fact that the average difference between the centre and surface of heaps is 2.4% and of sacks 0.5%.

Table 10.4: Moisture parameters of robusta cherry, after storage in sacks or heaps, which had been processed with and without a post harvest delay in processing. Initial moisture content was 10-11.5%.

Storage Mode		Processing		Centre m.c.	Surface m.c.
		Surface	Delay		
Run 1	Heap	Cement	0	14.0	12.6
			4	15.4	11.8
		Tarpaulin	0	15.5	13.5
			4	15.1	11.7
	Bag	Cement	0	12.6	11.4
			4	11.4	11.6
		Tarpaulin	0	11.8	12.0
			4	11.3	12.2
Run 2	Heap	Cement	0	15.8	13.8
			4	15.7	12.9
		Tarpaulin	0	17.0	14.4
			4	13.5	12.0
	Bag	Cement	0	12.8	12.4
			4	12.6	11.3
		Tarpaulin	0	11.9	10.9
			4	12.6	11.6

After six months storage niger aspergilli was consistently higher in the coffee that had been exposed to 4-day delay between harvest and drying (Table 10.5). Conversely, *Penicillium* was more numerous in the no delay processing. Ochre and flavi aspergilli showed no clear pattern, however, there may be a more consistent presence of the ochre group fungi in the 'heaped' coffee. Seven out of eight heaped samples showed viable ochre group fungi, with at least one showing an elevated infection rate, as compared with three out of eight of the bagged samples all of which were around the detection limit.

One sample that had been dried without delay on tarpaulin had a heavy infection by the mycotoxigenic aspergilli groups, ochre and flavi, which seems to have displaced the niger group to some extent. The glaucus group of aspergilli are generally xerophilic and are characteristic of stored grains of all kinds. Perhaps the storage period is too short but there is little indication that these fungi are actively growing in these conditions.

Once again it appears that heaped coffee takes up water faster than coffee in sacks. Whether this is due to moisture coming up through the floor, consistent with the higher central m.c. compared to the peripheral layer's lower moisture level, or some aspect of the geometry of stacks is not clear. In practical terms it appears that medium-term storage should not be conducted in heaps.

Table 10.5: Seed infecting fungal community makeup after six months of storage in sacks and heaps of robusta cherry that had been processed with and without a 4day post-harvest delay. *Cladosporium*, *Mucor* and *Fusarium* were occasionally present in small numbers. ('<'=below detection of 1.4%)

	Storage mode	Processing		Total	Niger grp	Flavi grp	Glaucus grp	Ochre grp	*Penicillium*
		Surface	Delay						
Run 1	**Heap**	Cement	0	94	66	<	2	3	38
			4	100	58	<	<	<	20
		Tarpaulin	0	84	40	<	6	2	44
			4	100	99	1	<	6	34
	Bag	Cement	0	94	66	<	2	3	38
			4	89	88	<	<	<	1
		Tarpaulin	0	53	53	<	<	<	<
			4	99	99	1	<	<	<
Run 2	**Heap**	Cement	0	100	81	<	<	2	40
			4	100	100	5	1	9	27
		Tarpaulin	0	77	2	26	<	30	28
			4	99	61	<	1	1	43
	Bag	Cement	0	100	81	<	<	2	40
			4	93	92	1	<	1	<
		Tarpaulin	0	54	37	<	6	<	13
			4	100	100	<	<	<	<

10.5.3 Blending poorly dried and well dried coffee before storage

This procedure was designed to evaluate the impact of arabica parchment quality and blending of wet with well dried coffee on its storability. The products from fermentation studies because they were very well documented and of various qualities, were ideal for this purpose.

The processing variations of the fermentation study were grouped into putative quality classes. 'Best' (B) = immediate processing with normal drying; 'Intermediate' (I) = the various 36 and 60h delays with normal drying; 'Worst' (W) = the various 36 and 60h delays with shaded drying. Sub-samples of the best and worst material was re-hydrated to about 20% m.c. with sterile, distilled water and mixed (at a 5% level) with all three quality classes yielding six treatments as can be seen in Table 10.6, below.

The mycological pattern of the control treatments shows the known pattern of mortality during storage, particularly of yeasts, *Penicillium* and *Fusarium*. *Cladosporium* increased to compensate, ochre aspergilli did not change and the xerophilic genera *Wallemia* and *Eurotium*, commonly isolated in the coffee storage context, failed to become significant. The poor coffee behaved in the same fashion as the best coffee except it was exclusively here that an increase in m.c. was recorded.

Moisture content of the bulk treatment samples was still higher, even after the four month storage period during which time there was probably moisture loss. The highest value was over 15% and, all bar one, were over the recommended level of 12%.

There was no difference between the addition of the 'worst' coffee compared to addition of the 'best'. In other words, the differences in the treatments compared to the control can be attributed purely to the addition of water. These differences are that *Penicillium* survived better and that *Cladosporium* failed to increase. Infection with ochre group aspergilli shows what might be a slight increase since, though small, it is consistent.

There was no OTA detected in either the initial samples (from the previous experiment) or at the end of the storage experiment.

Table 10.6: Fungi infecting the beans of different qualities of washed arabica coffee before and after a four month storage period. B = best; I = worst; W = worst; +b = a proportion of re-hydrated 'best' coffee mixed in; +w = a proportion of re-hydrated 'worst' coffee mixed in. Analyses were of 140 or 196 beans.

	% Infection					
Sample code	**B initial**	**B final**	**I initial**	**I final**	**W initial**	**W final**
Total infection	79.1	55	74.2	39	98.8	41
Ochre aspergilli	1.6	1	1	2	1.8	1
A. niger	1.8	1	0.2	2	0.0	0
A. flavus	0.4	0	1.2	0	0.2	0
Penicillium spp.	47.3	0	57.1	0	96.4	1
Fusarium spp.	28.2	7	6.3	3	3.2	3
Cladosporium spp.	3.2	45	0.5	29	0.4	32
Yeast	13.6	3	22.5	1	49.6	3
Syncephalastrum	0.0	3	0.0	5	0.0	2
Mucoraceous	2.1	2	1.9	2	1.1	2
Aureobasidium	0.0	0	0.0	0	0.2	2
Eurotium	0.0	1	0.0	0	0.0	1
Alternaria spp.	0.2	0	0.4	0	0.7	0
m.c.	11.7	11.7	11.5	11.5	11.8	12.6
Sample code	**B+b**	**B+w**	**I+b**	**I+w**	**W+b**	**W+w**
Total infection	51	45	39	34	57	50
Ochre aspergilli	3	10	5	5	4	1
A. niger	0	2	0	0	1	0
A. flavus	6	3	3	1	4	5
Penicillium spp.	36	26	26	22	44	36
Fusarium spp.	2	5	1	3	2	1
Cladosporium spp.	4	4	7	12	3	2
Yeast	2	0	2	0	2	3
Syncephalastrum	0	0	0	0	0	0
Mucoraceous	1	0	1	0	0	0
Aureobasidium	0	0	0	0	1	1
Eurotium	0	0	0	0	2	1
Alternaria spp.	0	0	0	0	0	0
m.c.	13.4	12.7	12.2	11.6	15.5	13.3

10.5.4 Storage of unsorted green bean

A second foreseeable issue of storage, especially in the local trading chain is the behaviour of defects in bulk coffee during storage. A commercial lot of bulk coffee was purchased and a commercial miller hired to prepare half of the coffee. The other half was stored and the same firm contracted to prepare the other half after storage.

The mycological analysis is characterised with the means, medians and maximum values of the significant taxa in Table 10.7. *A. carbonarius* was detected in only one sample at the detection limit. Analysis for OTA was conducted on samples of the bulk and clean coffee, before and after storage and none was detected.

Table 10.7: Mycological evaluation of sound and defect categories of green coffee produced as arabica parchment. Elapsed time between initial and final is about 6 months and there were only very minor increases in m.c., no A_w exceeding 0.68.

	Means					Medians				
	Clean	Black beans	Broken	CBB	Vinegar	Clean	Black beans	Broken	CBB	Vinegar
	Initial									
Total infection	22.4	80.6	76.5	77.8	70.4	15	94	81	94	83
Aspergilli Ochre	0.4	15.3	15.5	13.3	13.1	0	10	17	11	13
Niger	0.6	7.8	7.3	6.3	3.7	1	10	8	5	3
Flavi	3.5	17.6	13.3	16.3	8.8	4	15	12	11	6
Penicillium	16.1	61.2	56.3	62.4	55.3	11	69	54	66	59
Eurotium	0.0	2.2	3.9	1.4	4.7	0	1	5	1	1
	Final									
Total infection	8.0	33.0	19.0	39.6	18.0	9	32	22	44	20
Aspergilli Ochre	0.0	2.2	1.2	1.6	0.6	0	2	0	1	0
Niger	0.8	9.6	5.9	13.9	6.3	1	10	6	14	8
Flavi	0.8	7.1	3.5	9.6	4.3	0	6	3	8	3
Penicillium	2.9	1.9	4.3	10.2	3.3	2	0	4	7	2
Eurotium	1.6	12.5	5.1	8.4	6.3	0	5	4	7	5

Table 10.7 contd.: Mycological evaluation of sound and defect categories of green coffee produced as arabica parchment. Elapsed time between initial and final is about 6 months and there were only very minor increases in m.c., no A_w exceeding 0.68.

	Maximum				
	Clean	**Black beans**	**Broken**	**CBB**	**Vinegar**
	Initial				
Total infection	55	100	95	100	99
Aspergilli Ochre	2	43	33	39	27
Niger	1	11	12	14	8
Flavi	8	35	26	33	18
Penicillium	46	91	73	92	92
Eurotium	0	5	8	4	11
	Final				
Total infection	10	57	27	61	36
Aspergilli Ochre	0	7	5	4	2
Niger	2	13	10	21	10
Flavi	2	14	7	23	10
Penicillium	7	6	9	22	10
Eurotium	7	43	10	22	16

The storage facility was a very good building but sited near to a small river at the foot of a forested hill. There had been floods in the past, though in exceptional circumstances. The position in respect of the forested hill was such that one could anticipate cold, moist air running off the hill at night and accumulating around the warehouse. Despite this, there was little re-hydration of the coffee.

The means give the rather improbable picture that all of the defects are more contaminated, and to the same extent, compared to the sound beans. In particular there is the near absence of ochre aspergilli in sound beans and its uniform significant presence in all defect categories approaching or exceeding a maximum of 35% in all categories.

The two major issues with this picture is that it is not consistent with other such analyses and it implies that all of the defects are produced by conditions that equally favour the development of these fungi, i.e. the same conditions, within fairly narrow bounds.

We see the usual decrease in viable fungi through storage though of the aspergilli, only ochre section shows a consistent decrease. *Penicillium* is susceptible to storage conditions in coffee and *Eurotium* appears to increases during the five months despite the average A_w indicating that there is too little moisture to permit even this xerophile to grow.

The median values suggest that the data has a broadly normal distribution though allowances have to be made for the relatively small number of determinations used for this measure which is more sensitive than the mean to small sample numbers. The maximum values show that the means are not overly distorted by the odd massive result balancing zeros. Taken together, these two measures give weight to the validity and usefulness of the means in this study for its interpretation.

Image 10.1: Top: hand-sorting of coffee at a commercial miller's. Bottom: the storage facility where the storage of parchment trials were conducted, also a commercial warehouse, Colombia.

10.5.5 Comparing the storage performance of coffees processed in different ways

Modifications of storage trials already discussed were run in Indonesia using both robusta cherry and parchment coffee. The coffee products generated in the processing and drying trials, which included cherry, split cherry and parchment, .was stored and re-analysed after 6 months.

The different products from the processing trials showed no differences over 6 months of storage with respect to m.c. and A_w at the three locations. The coffee had been over-dried at 7-10.4% m.c. initially. After 6 months the coffee in Silo had taken up the most water and was then over 14% m.c. with that at Kaliwining the driest at about 12.6% and Liwa at about 13.5%. This shows a much greater extent of water

uptake than in other regions but this is attributable to the low starting point (see Figure 10.1 below). The final m.c. levels are in line with other trials. The final A_w measurements were seen to have increased to between 0.65 and 0.70 for all treatments after the six months. This is still well below the minimum A_w required for mesophilic growth.

There was a general increase in defect counts over the storage period with a few exceptions. There is no consistent difference between the forms of coffee stored. Liwa had a much higher defect rate to begin with but it is difficult to generalize about the changes here because there is one very large change, one large negative change, and two negligible changes. Taking all the coffee forms and locations into account It appears that one could expect an increase of about 20-30 defects per 300g during six months storage of very dry coffee. Given the low water availability, it would seem that fungal growth could not be the cause of this increase.

Table 10.8: Moisture measures and defect counts over the six month storage period of robusta coffee stored in different forms.

		m.c. (% wb)		A_w		Defects (# per 300g)		Change
		0	6	0	6	0	6	
Liwa	Parchment	8.1	13.5	0.43	0.67	116	143	27
	Split	8.2	13.4	0.36	0.67	191	273	82
	Cherry	9.0	13.6	0.38	0.67	112	136	24
Kaliwining	Parchment	6.7	12.7	0.31	0.65	53	62	9
	Split	7.2	12.6	0.31	0.64	43	72	29
	Cherry	7.9	12.6	0.34	0.65	50	81	31
Silo	Parchment	7.4	14.1	0.31	0.69	43	59	16
	Split	7.3	14.5	0.33	0.70	56	74	17
	Cherry	8.1	13.9	0.52	0.68	48	60	12
Liwa	Solar	9.4	13.5	0.38	0.67	184	114	-69
(all cherry)	Tarpaulin	8.4	13.6	0.36	0.67	140	145	5
	Cement	9.3	13.5	0.39	0.67	130	157	27
	Soil	10.4	13.6	0.57	0.68	144	142	-1

Table 10.9: Mycological analysis corresponding to the treatments described in Table 10.8 above.

		Niger		Ochre		Flavi	
		0	6	0	6	0	6
Liwa	Parchment	76	99	0	0	1	2
	Split	97	100	0	0	2	0
	Cherry	96	100	0	0	1	0
	Parchment						
Kaliwining	Split	84	72	1	2	16	2
	Cherry	99	93	0	0	57	2
	Parchment	54	13	0	0	20	2
	Split						
Silo	Cherry	81	72	0	1	10	2
	Solar	99	85	0	1	60	4
	Tarpaulin	32	13	4	1	11	1
	Cement						
Liwa	Soil	98	100	0	0	1	0
(all cherry)	Parchment	100	99	0	0	5	0
	Split	100	96	0	0	5	5
	Cherry	99	100	0	0	0	0

There is an indication that niger aspergilli infecting cherry coffee sometimes dies within six months of storage. The fact that both instances of this were in coffee that had low initial infection may indicate that high infection rates correspond to more vegetative growth, hence better survival. It appears that flavi aspergilli are more susceptible or, following on from the logic of the last point, had infected but had not become strongly established. Ochre aspergilli were found at about the detection limit both before and after the storage period.

10.5.6 Storage of re-dried coffee

Coffee lots were taken from three traders in three regions, re-dried half to within recommended m.c. levels and re-analysed both the wetter and dryer lots after 3 and 6 months. Replicate lots were created within each treatment and the different sources and regions, in the case of the trader experiment, serve as additional layers of replication.

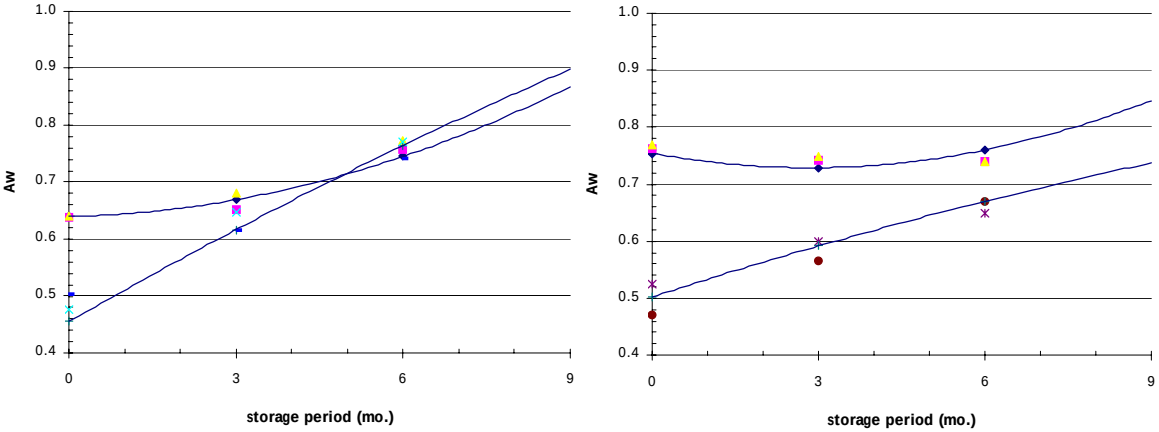
This coffee used was *'asalan'*, unsorted bulk coffee as traded in the Indonesian internal market supplied by middlemen in Malang (East Java), Pupuan (Bali) as well as in Liwa and West Lampung.

The re-dried coffee took up moisture faster than the wetter coffee and the two lots converged or got close to convergence in the six months. In Pupuan the re-dried coffee was .03 A_w lower on average (about 3% m.c. (wb)) and at Liwa it was almost 0.1 A_w lower (about 4.5% m.c. (wb)). Several of the lots stored at their original

moisture content dried further over the first three months. This could only be in response to a season of low humidity. All or most of the 0 to 6 month change actually took place in the final three months.

The re-dried lots had an initial A_w of about 0.50 and the 'as is' samples varied from 0.64 to 0.76, initially. None of the eventual water activity levels, as measured, are high enough to suggest that anything but the hardest xerophiles could have produced any spoilage toward the end of the storage period, thus any differences are unlikely to be attributable to mould growth. Again, although the m.c. tended toward A_w levels where growth would be possible after 7 to 8 months, it was not shown that the coffee would actually reach those levels.

Figure 10.1: Moisture uptake patterns of coffee during storage in Pupuan (left) and Liwa (right) extrapolated to 9 months. One lot was bought from each of three local traders, each divided into half and one of each half lot was re-dried before the original and re-dried lots were stored.



Defect count increased significantly and consistently in Pupuan and Liwa while it was stable and may even have fallen in the re-dried samples of Malang (Table 10.10). Exactly how defects disappear is not clear so this result may give an indication of the confidence limits of the counting procedure. All samples had been done in duplicate and though standard error with two replicates is not very meaningful, it indicates that a std error of 15% is typical though with some pairs showing very large differences between them.

In general the re-dried lots performed, at least numerically speaking, better than the 'as is' lots and some samples showed serious deterioration. The Liwa material was remarkably poor and the defects at this level are likely to be the result of poor hulling, i.e. high levels of husk and broken beans. Details about the make-up of the defect counts does not appear to have been submitted so it is not known what proportion of defects are of a non-bean origin.

OTA was low throughout with a numerical fall in the Liwa samples and a numerical increase in one of the Malang samples but no significant changes. Considering the high frequency of positive samples in this collection compared to previous results from Indonesia, it is difficult not to assume there are false positives here. There was a dramatic increase in the defect counts in two of the three locations, Malang providing the exception. There is no correlation between the relative size of the defect increases with that of moisture.

Table 10.10: Six month storage of coffee (*asalan*) in three production regions of Indonesia. The 'as is' (a.i.) treatment was to store the coffee at the m.c. at which it was being traded and the '<12%' treatment was to re-dry a sub-sample of this lot to specifications for comparison. 'Defects' are the counts of defects according to standard Indonesian practice, 150-225 is Grade 6, above 225 is un-graded.

	Initial			After 3 months			After 6 months		
	A_w	Defects	OTA (ppb)	A_w	Defects	OTA (ppb)	A_w	Defects	OTA (ppb)
Malang									
a.i.	0.64	77	1.7	0.67	72	0.6	0.76	83	1.3
<12%	0.48	99	0.6	0.63	75	0.5	0.76	80	1.0
Pupuan									
a.i.	0.74	179	0.0	0.67	152	0.9	0.74	337	0.1
<12%	0.53	201	0.2	0.64	154	0.3	0.71	311	0.3
Liwa									
a.i.	0.76	425	1.0	0.74	413	0.6	0.75	758	0.3
<12%	0.50	525	1.6	0.59	481	0.6	0.66	751	0.4

OTA analysis was conducted on the defect categories before and after storage of the bulk coffee. The three replicate sources were analysed individually initially but combined as two treatments at the end of the storage period. The data is organised in table 11. Comparison of the treatments after 6 months shows there to be no difference in outcome between the two treatments. Comparing the initial with the final OTA determinations indicates that if there is any change, it is a loss of OTA. Most of the values are low enough to sustain doubt about whether they are reliably positive for OTA but the two broken bean categories and black beans from Malang and CBB 1 from Malang are reported at easily detectable levels and here there is certainly no increase. We can only conclude from this that asalan is stable over six months at A_w up to 0.75, allowing that 3 to 4 months was required to reach this level.

Table 10.11: OTA analysis of defect classes sorted from *asalan* stored in different regions for six months. '1, 2, 3' refer to three different traders in each region and the samples were initially analysed separately but combined for the final determination. 'a.i.' = 'as is', coffee stored at the m.c. at which it was purchased; '<12%' = sub samples of the corresponding 'a.i.' coffee that was re-dried prior to storage. 'A' means no sample was analysed; '0'= below detection.

Storage (months)	Defects	Malang			Pupuan			Liwa		
		1	2	3	1	2	3	1	2	3
0	CH	2.6	1.2	0.7	1.6	0	4.0	0	A	A
6	a.i. / <12%	A	A		A	A		0.1	A	
0	imm	0	1.2	A	0	0	0	A	0	1.6
6	a.i. / <12%	A	A		A	A		A	A	
0	pBB	1.5	1.1	0	0	0	0	A	0	1.6
6	a.i. / <12%	0.2	0.3		0.1	0		1.6	A	
0	CBB >1	0.8	0.7	0.3	1.1	0.1	0.1	A	0	1.4
6	a.i. / <12%	0.1	0.2		0.1	0.2		0.1	A	
0	B/BrB	2.3	5.4	0.8	0.3	0	0	0	0.1	6.0
6	a.i. / <12%	A	A		A	A		0.6	A	
0	Husk	2.1	2.9	A	0.5	0.2	1.6	0	0	9.4
6	a.i. / <12%	A	A		0.1	0.3		A	A	
0	BB	1.4	3.3	0.3	0.2	0	0	0	0.8	0.9
6	a.i. / <12%	A	0.3		0.1	0.2		0.8	0.2	
0	CBB 1	1.3	4.9	0.5	0.9	0	1.3	A	0	A
6	a.i. / <12%	0.2	0.4		0.3	0.1		0.5	0.1	
0	BrB	0.5	1.2	0.4	1.9	0	0.3	0	0	4.6
6	a.i. / <12%	0.1	0.5		0.2	0.3		0.6	A	

Codes for the defect categories: CH = unhulled cherry; imm = immature bean; pBB = part black bean; CBB>1 = beans bearing more than 1 hole; B/BrB = black and broken bean; husk = husk piece; BB = black bean; CBB 1 = bean bearing single hole; BrB = broken bean.

10.5.7 Monsooned coffee

Although not a form of storage as such, monsooning lasts 2 to 3 months and manages the gradual re-hydration of green coffee and its subsequent re-drying. As such it can serve as a model of passive and unmanaged re-hydration as could take place during storage. Changes in the nature of coffee and the measurement of moisture in coffee that has imbibed water from the air are more conveniently studied since the exposure to moist air is well managed.

Monsooned coffee is a unique product of India's south western or Malabar coast. It is a small but significant product now comprising about 6,000 tonnes per annum, a figure that has been rapidly increasing in recent years. The product arose as a consequence of the long exposure coffee experienced on its route by sailing ship around the Cape of Good Hope and to Europe from India. The taste became

established and when sailing ships were replaced by container transport and the Suez canal cut sailing times, a processing method was developed to satisfy demand for this speciality coffee. The factories are all in the Mangalore area where coffee is 'monsooned' during the monsoon period (June to August) and the following couple of months, then sorted and prepared for export.

The process uses cherry coffee, both robusta and arabica, bought from growers who have been producing good quality cherry especially for the company concerned, some for many years. The process consists of three rounds of spreading the coffee in layers with frequent turning over about three weeks. The coffee is then re-bagged and stacked in 'windrows' for a similar period.

Moisture from the humid monsoon air is absorbed by the coffee, swelling and bleaching it, and producing a distinct change in taste. As the monsoon abates, moisture falls with the humidity and the coffee is shipped at 12 to 14% m.c.. Before shipping, the coffee is polished, graded and sorting by hand. Hand sorting is a skilled occupation and can be done at a rate of about 150kg/man-day, depending on the quality of the input coffee. Processing costs are put at about 4 Indian Rupees/kg, excluding weight loss of defects etc. Colour sorters are available but, interestingly, it is reported to be less effective than for normal green coffee, though in the case observed it had been specially tuned by the manufacturer's engineers.

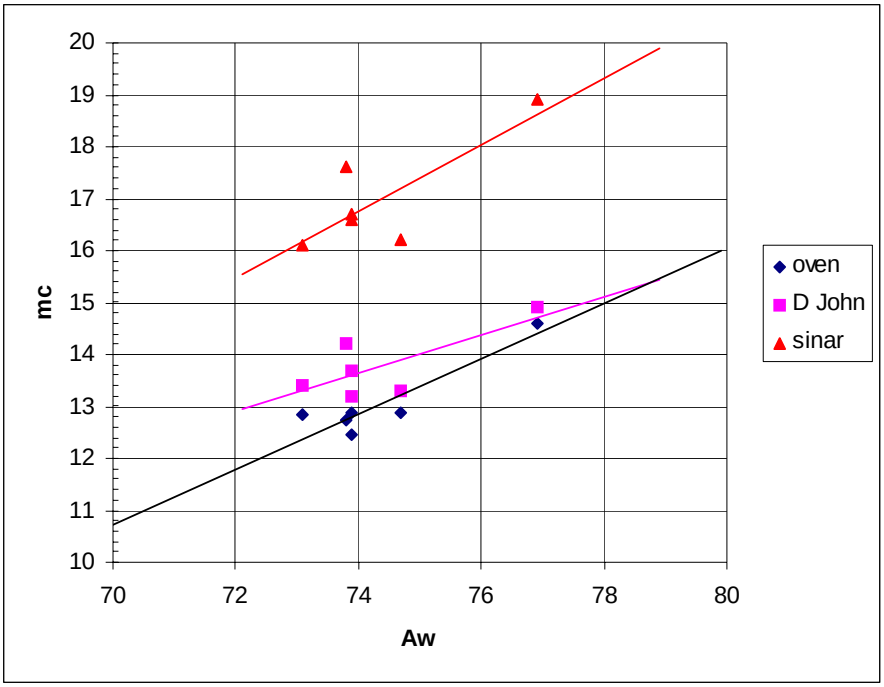
Image 10.2: A Mangalore monsooning factory: a) outside view of the monsooning halls; b) hand-sorting of monsooned coffee being done on the floor where the coffee is spread during the monsoon rains; c) sacks loosely packed and stacked in windrows for air exchange; d) notice over the entrance to the processing plant which lies between the arabica and the smaller robusta monsooning halls.

A lot of effort has been expended by the project in assessing the reliability of different methods of estimating moisture content and relating this to the more meaningful parameter of water activity, A_w.

Monsooned coffee is nominally green coffee but it has been modified by slow re-humidification in a process that resembles conditioning, albeit conditioning with humid air. The physical aspect of the coffee is altered so that it is off-white in colour and bolder, and a much less dense bean. In density it falls from around 1.2 to .85 specific gravity. It is very soft to bite and easily bit through, never mind dented as in the traditional criteria for adequate dryness.

Several samples from different origins and steps in the final milling were taken during a visit in late October 2004. These were described as per A_w, Sinar AP 6060 moisture meter (channel 3), Dickey John Multigrain moisture meter (channel 2 - both calibrated for green coffee), and oven moisture content which can be taken as actual moisture content.

Figure 10.2: Moisture content measured against A_w of monsooned coffee. The samples were from different lots. The sample with the highest moisture content is before polishing, the others are post polishing.



Of particular interest is the relation between A_w and oven moisture. In the monsooning process, coffee is allowed to hydrate to moisture contents of between 16 and 20% as measured with a specially calibrated moisture meter. The high moisture period lasts about three months of the four to five months required for the full process – plenty of time for mould development. The project's overall data suggests that a moisture content of 13% corresponds to 0.66 on average but at a 95% confidence limit this extends to between about 0.58 and 0.75. The monsooned coffee showed an A_w of between 0.72 and 0.75 at this m.c.. Monsooned coffee binds water less tightly than normal green coffee, the process makes it less hygroscopic meaning that there is greater water availability at a given m.c..

Wallemia was most commonly observed in the air spora of all the processing rooms from polishing onward though not isolated from air before this step. Ochre aspergilli occurred at a few cfu/min/plate except in the sorting area and store, with niger aspergilli more common at destoning and progressively falling off to nil at the store.

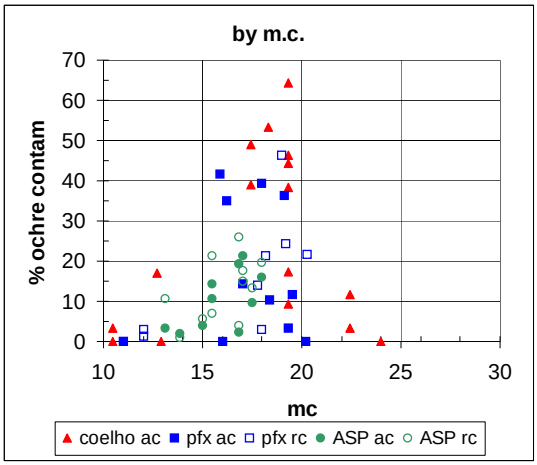
Table 10.12: OTA analysis of samples collected in October 2004 at a Mangalore monsooning works.

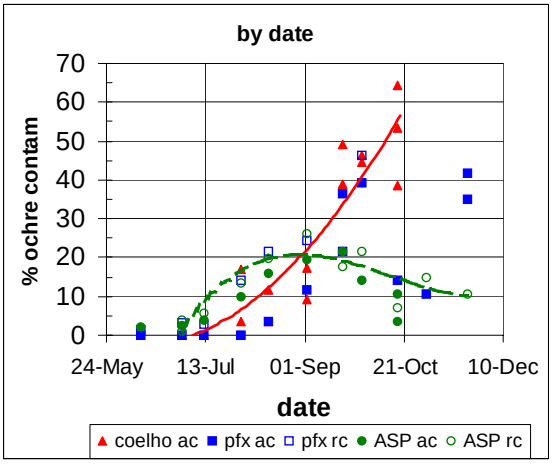
Sample description	Oven m.c. (wb)	OTA[!] Sub-sample 1	OTA Sub-sample 2
From Coorg	12.88	<0.1	4.3
From Chickmagalur	12.72	0.4	0.2
After grading1	12.84	<0.1	0.7
After grading2	12.89	<0.1*	1.0
Before grading	12.44	0.3	<0.1
Before polishing	14.61	0.3	0.3
Hand-sorted defects	12.41	0.9	0.7

[!] Evaluation of the chromatograms has indicated that several of the low level samples are either 'trace' or not OTA at all.
* The chromatogram for this sample seems to show a significant level of OTA. The reason for the discrepancy is not clear.

The Coffee Board of India had been conducting routine sampling at three monsooning curing works. Samples were taken periodically through the process during three seasons and both moisture (by meter) and mycological analyses were completed. The results for 2002 are interpreted in Figures 10.3 and 10.4 below.

Figures 10.3 and 10.4: Frequency of occurrence in beans of ochre group aspergilli related to the moisture content of the sample and the time of sampling. m.c. increases through 16% in mid-July and falls back below this level in mid September or early October. Coelho, PFX and ASP are three factory sites; ac = arabica; rc = robusta; both are prepared from cherry coffee.





Caution must be exercised in interpreting these results since it was found that the surface sterilization was being conducted inadequately so the results are likely to partly measure superficial contamination and infection. If the data are taken at face value, the lack of a relationship between m.c. and ochre contamination rate can be attributed to the fact that the material starts dry at a low contamination rate and returns to dryness often with a high rate. Contamination rates by other fungi were also high, especially niger aspergilli. If this material is low or free of OTA (see Table 10.12, above) with this mycological profile it must be because the high contamination rates are reflecting the presence of spores rather than growth, consistent with the significant ochre group spore load was recorded where polishing was taking place. Unlike the situation on the drying yard where time is rather limited, here one would expect growth of OTA producers to correlate with OTA production at least in the latter stages of the process where the A_w exceeds an A_w of about 0.82 or about 17% m.c. in this material.

10.5.8 Conditioning

The main purpose of conditioning is to ensure uniform moisture content throughout the coffee mass. However, during field visits to several cooperative factories, it was noted that it had commonly become primarily a drying yard management tool used in Kenya to relieve pressure on drying table space at peak of harvest by committing partly dried parchment into deep wire bins.

The coffee in the conditioning bins is periodically stirred by hand with paddles in the traditional wooden structure (Image 10.3) but newer technology uses air forced through the coffee bed from below to either dry or re-hydrate coffee if it has been inadvertently over-dried.

This trial monitored the moisture content of the parchment in the conditioning bins when the parchment was placed in the bins at a moisture content of between 20-25% as was being commonly practiced at the cooperative factories.

The salient features of the physical data of the time courses is the high moisture levels at both beginning and end of the entrainment in the bins and the difference in behaviour at the edge compared to the centre. Unfortunately, the distance between the two probes was too great to make any conclusions about lag-times or gradients through the coffee bulk. The A_w of the bulk of the coffee scarcely changes (superimposed black line) while that in the outer regions falls 0.03 to 0.05 (superimposed green line). Since the outer and inner regions diverge, one can infer that mixing is not adequate.

Image 10.3: Top: drying tables typical of Kenyan practice. Bottom: a traditional conditioning bin sited in a roofed and open-sided structure.

With diurnal temperature fluctuations of less than 5°C, it is not surprising that the A_w fluctuations are also modest – generally less than 0.05. These conditions would seem to be almost optimal for several species of *Eurotium* which can produce the aromatic metabolite geosmin that is distinctly musty or earthy.

One advantage of conditioning is said to allow the residual moisture to become uniformly distributed in the coffee bulk. As Table 10.13 shows, this is usually, but not always, accomplished. It appears that A_w is distributed less closely around the mean than m.c., which implies disequilibrium. One might note that A_w is generally more variable but this may be largely due to the measurement of A_w being inherently less precise than that of m.c.. The coffee in these bins was far too wet for storage and must be exposed to further drying.

Table 10.13: Evenness of moisture at the beginning and at the conclusion as determined by s.d.% of the transformed values [arcsin (ρ^0.5)] of % m.c. (wb). The oven moisture content and A_w of six sub-samples from each bin/run combination were determined.

	Std error of m.c. (wb)							
	Ga1-1	**Ga1-2**	**Ga2-1**	**Ga2-2**	**Gk1-1**	**Gk1-2**	**Gk2-1**	**Gk2-2**
Initial	2.48	0.77	0.46	2.10	0.49	0.59	5.97	3.39
Final	0.35	0.54	0.24	0.43	1.48	1.02	1.01	0.26
	A_w							
	Ga1-1	**Ga1-2**	**Ga2-1**	**Ga2-2**	**Ga1**	**Ga2**	**Gk1**	**Gk2**
Initial	2.72	1.95	1.30	1.52	1.83	2.32	1.91	2.29
Final	1.90	2.61	2.09	1.92	*No data*	*No data*	3.41	3.41

Humidity/temperature charts for two of the four runs are given below in Figures 10.5 and 10.6.

Figure 10.5: Gathiruini coffee factory conditioning time-course, first run. Average evaporation over the period was about 1100ml/m^2/d. Red is Bin 1 and blue is Bin 2, with 'x' the outside probe and points the central probe.

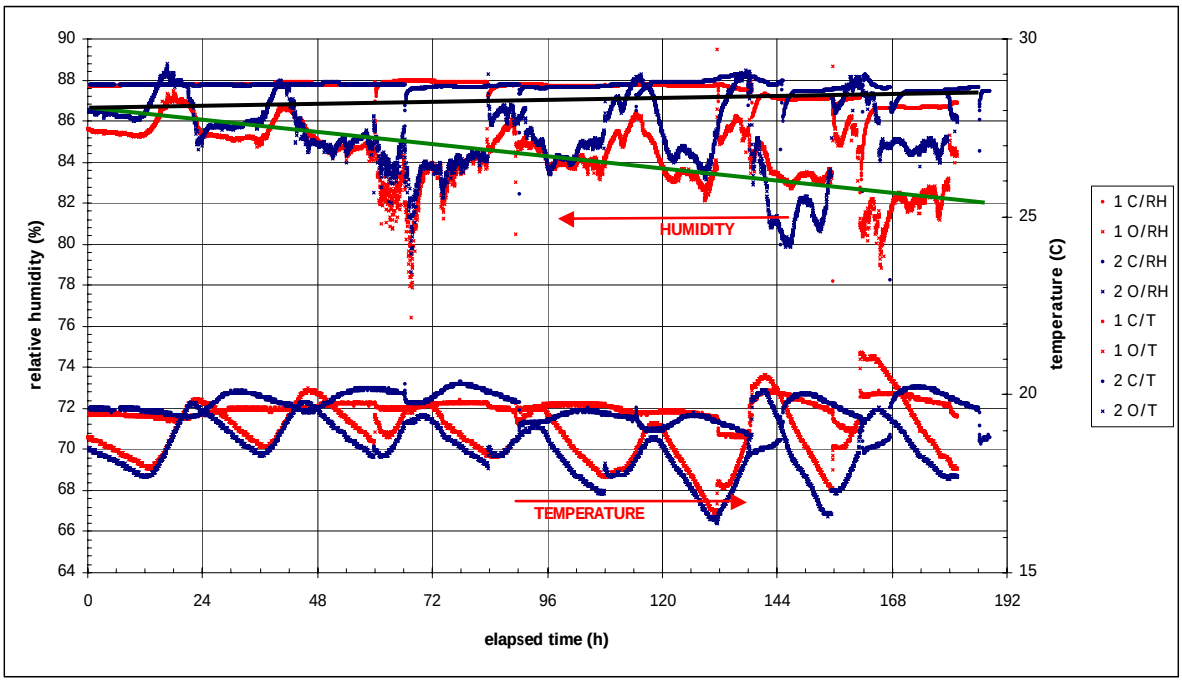
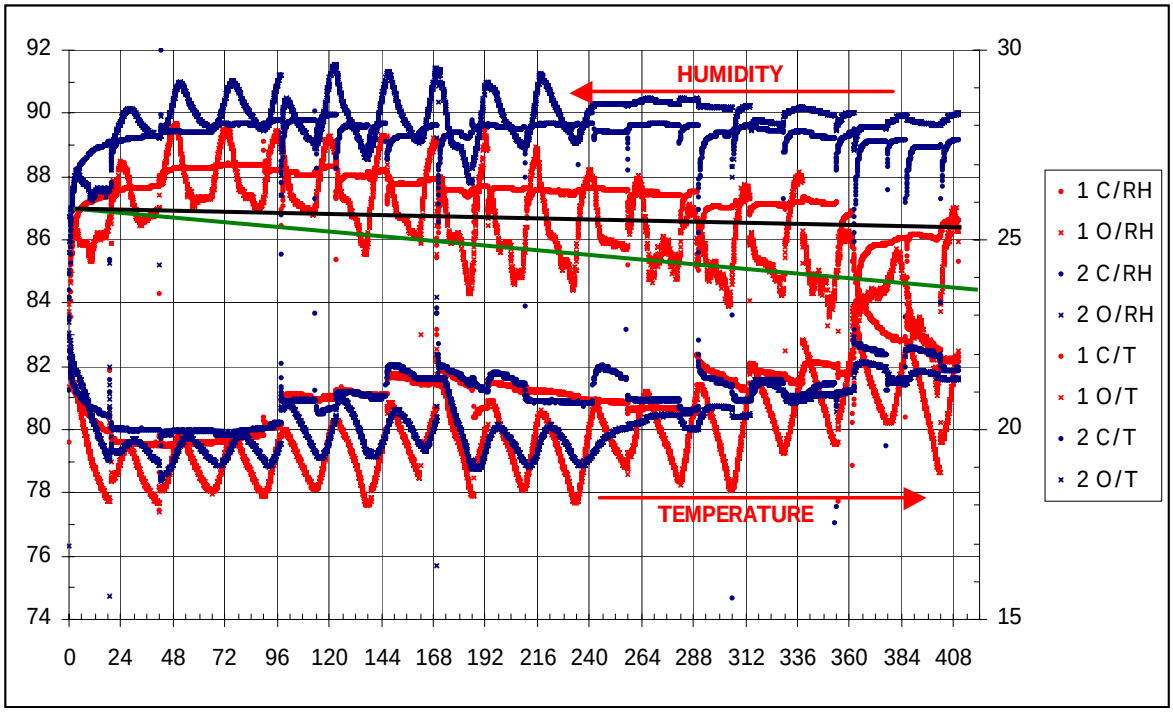


Figure 10.6: Gachika coffee factory conditioning time-course, second run. Average evaporation over the period was about 2400ml/m^2/d. Red is Bin 1 and blue is Bin 2, with 'x' the outside probe and points the central probe.



Section 11
International Transportation Trials

11.1 Introduction

A commonly reported problem in the shipment of food commodities is deterioration due to moisture migration within shipping containers creating 'wet spots' in the container that can support microbial growth and other forms of biochemically-mediated deterioration. In the case of coffee a main concern is avoiding conditions that would allow development of OTA-producers and accumulation of OTA.

The role of the global project in the area of international transportation was primarily one of facilitation and coordination in putting collaborators together with the 'OTA Taskforce' - an association of European coffee roasters who were beginning to study international transportation. The form these studies took was to locate temperature/humidity loggers in containers at the time of stuffing and then to recover them when opened in the European port. Generally samples were also taken for OTA and moisture analysis.

Given the importance of understanding the risk of OTA contamination associated with conditions of international transport, a recommendation of the independent mid-term project evaluation was that the project should arrange for additional trials to further investigate problems that might arise as a result of international transportation.

The project therefore organized the implementation of two additional trials monitoring changes during international transportation of coffee in collaboration with the OTA Taskforce. A separate simulated transportation trial was carried out in collaboration with the Instituto de Technologia de Alimentos (ITAL) in Campinas, Brazil.

11.2 Findings and Application

11.2.1 In relation to moisture migration in containers under realistic conditions of shipping

When a container is exposed to external ambiental conditions, the air in close contact with the exposed surfaces shows large fluctuations in temperature and relative humidity (RH) during transport and sometimes condensation conditions are reached. The maximum variation seems to be during overland transport or residence at the quayside. However, this may be partly due to coincidence of the position of the container in the ship.

The recognition that condensation within the container causing re-wetting of coffee could be a common problem led to the recommendation in the European Coffee Federation Code of Practice to protect the surface of the coffee during internaitonal transport.

None of the studies documented an adverse outcome in terms of OTA contamination but there were signs in one that a migration of water had occurred and a moisture content differential of about 2.5% was noted.

Although direct comparisons were not possible between transport in bags and in bulk both were monitored, and it appears that a more stable situation exists in bulk transport.

What has not yet been established is the cause of transport failures. The existence of 'hotspots' (that is, a point out from which spoilage spreads) is an indication that liquid water generated from condensation is a common mechanism. More effort is required to understand how these conditions develop and can be avoided.

11.2.2 In relation to the impact of container fill volume on moisture mediated quality changes

In a transportation simulation, carried out according to a real route from Santos in Brazil to Livorno in Italy, slight increases in moisture content of coffee from the initial value of about 10.2% were observed during the course of the 15-day trial. Water activity levels did not exceed 0.6, therefore fungal growth would not have been supported under the conditions of the trial.

Statistically significant but numerically small increases in the '*ardido*' defect and in bitterness were noted in some cases over the 15-day trial. Slightly less deterioration in these quality parameters was observed in the full containers as compared with the ¾ full or ½ full treatments.

The results of this trial suggest that the fill-volume is not an important consideration determining quality loss and OTA risk in international transport. It is considered, however, that the trials should be repeated over a longer timeframe and perhaps using coffee batches of coffee with different initial levels of moisture.

This simulated trial is discussed briefly in Section 11.5.2, below, and the full report of the trial is available as Annex C.10 on the enclosed CD-Rom.

11.3 Additional Notes

There is plenty enough water even in a well-dried consignment of coffee to produce copious microbial growth if some of it became concentrated. This is basically the issue in transportation: how is a commodity best moved to avoid the inevitable ambient variation that will produce a degree of disequilibrium in the container causing a redistribution of water into wet spots?

The main shortcoming of passive transportation trials is that conditions are left to develop according to circumstances.

Condensation and the effect of a standing gradient on water distribution, as might be expected to form if one side or end of a container is exposed to direct sun while the rest is shaded, are specific situations that can be predicted to cause a problem. Future work should concentrate on understanding what conditions are required to produce

these events and what their consequences might be regarding the re-distribution of water.

11.4 Experimental Design

11.4.1 Passive monitoring of transport conditions

The transportation trials monitored changes in temperature and humidity during actual exportation using data loggers. The variables in this sort of passive experiment, aside from the inherent conditions during shipping, were the location of the monitors and the way in which the container was stuffed.

Loggers were located so that comparisons of the air above the coffee with that within the coffee mass and/or between positions relative the door. Shipment in sacks as opposed to bulk was compared by preparing containers according to both methods and aspects such as the use of cardboard cladding or plastic liners were used. Often no direct comparisons set up, so no 'control' treatment existed in the classic sense.

Image 11.1: Data logger (°C and RH) mounted on the outside of plastic container sack containing coffee in bulk.

11.4.2 Transport simulation trial

The simulation was done in mini-containers with a capacity of about 600kg. Here conditions of a simulated voyage were imposed on the containers for 15 days (Santos, Brazil to Livorno, Italy), although what these conditions were or how they were imposed is not reported. The containers were filled, ¾ filled and ½ filled, thus providing the three treatments for comparison.

The coffee was placed in large perforated nylon bags four of which could be accommodated in a mini-container. There were, of course, 3 and 2 bags in the ¾ and ½ filled containers, respectively. Cup tasting, defect assessment and moisture measured in different ways were conducted on samples from the top, middle and bottom of each container after the 'voyage'.

11.5 Experimental Results and Discussion

11.5.1 Transportation monitoring trials

The six trials that were completed are summarized below:

Trial #1: Five data loggers were introduced in different places in a container of Ugandan robusta (from cherry) in bags that had arrived at Rotterdam harbour. It was monitored for 30 days (February/March 1999) in the open air (i.e. not warehoused) on the dockside.

Monitors 'one bag deep' into the coffee stack for the rear, middle and door-end of the container showed a gradient over time, temperature going slowly down and RH going up, but with no dramatic increases in RH. The registered temperatures and RHs within the stack of coffee bags corresponded to coffee moisture contents being up to 2.5 % apart at the same time.

The monitors in the middle, directly on top of the coffee, and the one taped to the roof, showed very marked day/night fluctuations, with temperatures between ~5 and ~35 °C and RH exceeding 95% several times. In total, 10 hours of condensation on nine different occasions were registered, all early in the morning.

Samples were taken from the front row of bags directly behind the door at the beginning and end of this trial. Mean moisture content at the start was 13.6% and 14.4% at the end. OTA levels did not differ beyond analytical accuracy.

Within the coffee stack, temperature and RH changed slowly and moisture also only moved slowly. Nevertheless, there were very clear differences in spot moisture contents. However, in the headspace over the coffee, the fluctuations show a day/night pattern and are much more pronounced.

Trial #2: Two monitors were placed in a container in Kampala, Uganda whilst it was being stuffed with coffee in bags to be transported to Bremen, Gemany (December 1999/January 2000). Walls of the container were lined, but there was no cover over the stack of bags in the container. One monitor was taped to the roof and the other one was placed one layer of bags into the stack of coffee.

Within the stack of coffee both temperature and RH changed slowly. The monitor taped to the roof registered strong day/night fluctuations both during transport from Kampala to the port in Mombasa and after arrival in Bremen. A maximum temperature of 50ºC was reached during terrestrial transport from Kampala to Mombasa and condensation conditions (RH of 100%) were experienced for two hours after 7 hours of temperatures above 40ºC. Condensation conditions were also experienced after arrival in Bremen. The temperature and RH fluctuations during maritime transport were relatively small. However, no information is available about the position of the container on the vessel while sailing.

Trial #3: Three monitors were placed in a container with Uganda robusta coffee in bulk in a plastic liner. The transport was from Kampala to an European harbour and left on the quayside for 9 more days (May/July 2000). One monitor was on top of the liner, one inside the liner on top of the coffee and the third one was place 30cm into

the coffee. The conditions of the overland transport seemed to be less extreme than the previous Kampala to Mombasa trial, and the highest RH in the headspace was 85%. There were no condensation episodes observed while the container was on the quayside in Europe.

A direct comparison with sacks is difficult to make since the ambient conditions were apparently les extreme as can be judged by conditions recorded on the ceiling of the container. The absence of condensation during stay at the quay in Europe could have been due to temperate summer weather.

Trial #4: Two containers of Santos coffee were monitored during transport from Santos, Brazil to Rotterdam, The Netherlands (November 2000/January 2001). One container contained bulk and the other bagged coffee. Two monitors were positioned in each of the containers, one within the stack of coffee and another on top of the coffee. A third monitor was placed on top of the liner (outer surface) in the bulk coffee.

The fluctuations observed in this trial were minor with one exception where the RH on top of the bags briefly reaching 90%.

Trial #5: Four containers stuffed with Vietnamese coffee in bulk were equipped with different arrangements of drying bags. Inside walls and roof were covered with cardboard and the coffee was contained in a plastic liner. Monitoring devices were positioned inside the coffee stack, on top of the coffee, on top of the liner and behind cardboard taped to the roof.

Trial #6: Indian robusta AA (screen 18) from cherry was stuffed in Bangalore, in bulk with cardboard cladding and a full plastic liner. The container moved by road over 3 days to Madras where it stayed for 6 days until shipping by sea to Salalah, Oman which required 7 days. After a further 4 days the cargo sailed to Spain over 12 days and was moved through Spanish waters for an additional 10 days before reaching its destination in Barcelona.

Three data loggers were positioned between the coffee and the container wall at various points, and a fourth one placed on top of the liner at about the middle of the container.

Periods of fluctuation were observed that coincided with overland transportation and residence on the quayside. Condensation conditions were approached only once - on the day before unloading in Barcelona. The periods corresponding to sea transportation showed little fluctuation, but it is not clear whether this was due to the fortuitous positioning of the container or something about the conditions during transport.

There were large temporary distinctions between monitors according to their position, but there was no indication that condensation conditions were reached. Six samples evaluated for m.c. varied between 12.5 and 13.1 before transport and between 12.6 and 12.9 afterwards. There was also no detectable OTA either before or after transportation.

Two of the monitors sandwiched between the container wall and the liner containing the mass of coffee showed that the heat was fairly efficiently absorbed by the coffee

with much larger fluctuations of both temperature and RH recorded by the other monitors that were not surrounded by coffee.

11.5.2 Simulation study

In this study, temperature conditions were simulated to correspond to the changes observed during transportation from Santos in Brazil to Livorno in Italy. The trial was conducted over a 15-day period with temperature and RH recorded constantly over the entire period. The 'coffee quality' parameters measured before and after shipping included cupping, defect analysis and moisture as measured by oven drying (ISO 6673), meter, A_w and weight change.

Most, but not all, of the post confinement samples were judged to contain a higher frequency of total defects and *ardido* beans, but this did not amount to a statistical difference except in the case of the half-filled container.

Most samples also showed increased bitterness at the end of the trial as compared with the initial condition. The observed changes, even though statistically significant ($p < 0.05$), were small.

The simulated transport conditions were taken from a time of year where maximum differences would be expected so as to impose a 'worst-case' scenario. With a duration of only 15 days, and with the coffee initially well-dried (m.c. approx. 10.3% wb), it would have been remarkable if there had been any fungal-mediated changes. Furthermore, RH of the headspace air rarely reached 90% during the trial.

It should be noted that during shipping, there are frequent delays and periods where the container is held at the port. The simulation should be repeated taking these likely extensions at either end of the journey into consideration.

Notably the coffee used in this trial was very dry at the start of the trial. This is undoubtedly a factor that contributed to the observed stability. The full report of the study is provided in Annex C.10 on the enclosed CD-Rom.

Section 12
Conclusions and Areas for Future Work

12.1 Overall Conclusions

12.1.1 General

The basic requirements for OTA contamination of coffee are known and were known before the start of this project: there must be an active population of OTA-producers and adequate time, at a water activity that permits growth of these moulds, to allow accumulation of OTA. The goal of the field trials described in the preceding Sections was to better characterise the conditions that lead to OTA contamination so that acceptable process controls could be more clearly defined and that efforts could be focused at the points where there is greatest risk of the hazards occurring in coffee.

A general observation from the trials is the overall difficulty in devising processing regimes that yield coffee highly contaminated with OTA. In some cases this could be attributed to the absence of OTA-producers in the experiments. However, in other cases, OTA-producers were demonstrably present, residence time at high water activity levels (> 0.80) was long enough for growth and OTA production to occur, yet no significant OTA accumulation could be detected. From these experiences it seems that there are other requirement(s) for OTA production that are poorly understood and commonly not met in a 'real world' situation.

In effect, and at our current level of understanding, there is a degree of unpredictability regarding OTA contamination and the relative importance of process control parameters in its prevention. Without a reliable relationship between control parameters and outcomes it was not possible to arrive at experimentally supported critical limits for identified critical control points, so this objective was largely not met.

The trials however, all enrich the general understanding of the mould and OTA contamination of coffee and make an essential contribution to the development of science-based recommendations on measures for improving coffee hygiene that are commensurate with the food safety risks. The findings of the trials have also lead to the formulation of 'further areas for investigation' that could further advance our understanding of the problem of OTA contamination of coffee, and help us to refine food safety management systems for its effective control.

12.1.2 Occurrence & activity of OTA-producing mould in coffee

A. ochraceus, the most important OTA-producer in coffee, is commonly found in coffee-growing regions although the infection rate of coffee beans is usually very low. On average the field infection rate by this mould is about 0.1 or 0.2% of beans.

Infection by niger group fungi, primarily by *A. niger*, *sensu stricto*, is almost universal in samples of robusta coffee, whether wet- or dry-processed. It is found in the fresh cherry and commonly reaches 100% infection of beans during the drying course of

the coffee. It is much less common in green arabica coffee and seldom reaches high frequencies of bean infection. Unlike the ochre group fungi, niger aspergilli are commonly isolated from stem tissue of coffee plants.

The main OTA-producing species in the Niger section is *A. carbonarius* which, though not common, is ***occasionally*** found in significant numbers. The percentage of OTA-producing strains of *A. niger*, the most common representative of the section in coffee, is reportedly low and only 2 of 70 isolates tested in this work produced traces of OTA in culture. Several new OTA-producing species were isolated in the course of this project[1]. Those of the Niger section are apparently rare, however, those of the Circumdati (ochre) section are common in coffee. This new information could alter our understanding if the physiological capacity or ecological performance if the new species was materially different from that of *A. ochraceus*, which they very closely resemble. According to the project's convention these new species would have been reported as 'ochre aspergilli' in the routine mycological work carried out under the project as most of the collaborators did not have the required skill to confidently identify the species within the group. The high frequency of the non-OTA-producing *A. melleus*, normally considered to be a soil organism, infecting beans in East Africa is another interesting and potentially important observation.

Aspergillus ochraceus is not uniformly distributed throughout coffee production areas, though the fungus seems to have some limited association or accommodation with coffee, and there is evidence of greater activity in certain regions. Efforts to correlate horticultural or processing practices with occurrence failed and although regional distribution patterns sometimes seemed to arise from our surveys, there must be some uncertainty around this difficult area not least because of the question of stability of such patterns from season to season. Nevertheless, systematic programmes of monitoring would be an important component of overall national programmes and the information derived from them would contribute to an understanding of these patterns.

OTA accumulation is undoubtedly subject to the influence of the activities of other organisms comprising the biological communities arising in the coffee production system. Clearly the coffee plant and its complement of commensal and parasitic organisms provide a context but not one that is constant. During processing microbial growth can strongly influence physical conditions around the OTA-producing fungi such as temperature, pH, nutritional factors and gas composition. The seed, of course, has a homeostatic property and a capacity to prevent saprophytic degeneration, something, if it occurred, that would be clearly evident in the coffee's general quality characteristics. This complex system may hold the key to the apparent unpredictability of OTA described above.

Mycological analysis during the project revealed a general picture of coffee mycology in which there are relatively few fungal species found in almost any sample of fresh (and the related dry) coffee: *Candida edax*, *Fusarium stilboides*, and, specifically in robusta, *Aspergillus niger*. Several additional species are also commonly found in all

[1] For recent discussion on OTA-producing species in the *Aspergillus* sections *Circumdati* and *Nigri*, see:

- Samson, R.A., Houbraken, J.A.M.P., Kuijpers, A.F.A, Frank, J.M., Frisvad, J.C. 2004. New ochratoxin A or slerotium producing species in *Aspergillus* section *Nigri*. *Studies in Mycology* 50:45-61.
- Frisvad, J.C., Frank, J.M., Houbraken, J.A.M.P., Kuijpers, A.F.A, Samson, R.A. 2004. New ochratoxin A producing species of *Aspergillus* section Circumdati. *Studies in Mycology* 50:23-43.

regions in both coffee species: *Cryptococcus albidus*, *Aureobasidium pullulans*, *Penicillium brevicompactum*, and *Cladosporium* spp. The species list of fungi found throughout the study is quite long, however, and sometimes samples from a particular region would have an unusual taxon not recorded in other locales or in other seasons. *A. carbonarius*, *A. japonicus* or *A. aculeatus* are Niger section aspergilli that are occasionally common. Likewise, *A. flavus* and *A. oryzae* can be numerous in a given sample. Seed-associated fungi such as *Alternaria*, *Scopulariopsis*, or *Nigrospora* were sometimes seen as well as plant pathogenic fungi such as *Colletotrichum* and *Phoma*.

12.1.3 Primary production factors influencing OTA contamination

No correlation of OTA-producing fungi or general mycological load to any horticultural or processing practice could be discerned from surveys carried out at the start of this project. It should be emphasised that this does not mean that correlations do not exist, but that they could not be identified given the design of the survey.

Data collected during the project showed that the beans of freshly harvested cherries commonly contain trace levels of OTA, and contamination levels of up to 20ppb have been recorded. Given the finding that OTA contamination in the field is much more common than previously thought it could be important to design a survey targeting this aspect and apply it in a carefully selected region to reveal if there are horticultural practices that favour the development of OTA-producing fungi.

There is good data deriving from the project's experiments that exposure of coffee flowers to spores of *A. ochraceus*, but not some other species common in coffee, leads to bean infection. However, this does not amount to proof that the observed field infection of coffee seeds is established via this route. Clearly it would be valuable to establish the significance of this mechanism and, if significant, any means of reducing its efficacy whether by coffee variety selection or horticultural practice.

It is logical that contamination of the bean by growth of superficial mould through the fruit can occur. The relative importance of this mechanism in bean contamination in the orchard was not systematically evaluated, but mycological analysis did not demonstrate a correlation of fruit with bean contamination.

12.1.4 Harvesting practices

The balance of evidence is that there is an enrichment of *Aspergillus ochraceus* in the rhizosphere soil of the coffee plant as compared with local non-coffee soils and that infection of the bean over time can be affected by contact with this soil. Contact of cherries with orchard soil for prolonged periods (exceeding 1-2 days) is a risk factor for OTA contamination and inclusion of such coffee in the marketing chain should not be permitted.

Evidence from the trials also suggest that there is a tendency for tree-dried cherry to have a higher frequency of ochre aspergilli contamination than ripe coffee at harvest, but that the difference in ochre group infection disappears or is reduced after normal terrace drying.

Tree dried cherries showed some other interesting mycological features. In direct comparison, the development of niger aspergilli is inhibited in tree drying as compared with terrace drying. The general principle here is that a different adaptation is required of the fungi for the terrace and the tree, as is evidenced by the different relative performances of the most common micro-organisms found in coffee.

Delays between harvesting and dry-processing of cherries was found to be a common practice in several of the collaborating countries and was initially suspected as capable of causing significant OTA accumulation. However, trials designed to document the impact of delays before wet- and dry-processing did not demonstrate it to be a determining factor in OTA accumulation. What was observed was a period of vigourous fermentation, primarily by bacteria but with a contribution of yeasts, that more often reduced general quality than produced OTA above control levels.

Post-harvest delay of processing represents a loss of control of the process so advice to producers must remain as reducing any delay to a minimum. Present evidence does not support a specific minimum acceptable delay but in any case the urgency of setting such a limit does not appears to be great.

12.1.5 Residence time between 0.95-0.80 A_w

The best theoretical measure to predict OTA formation in drying coffee is the length of time during which the coffee is wet to the extent of A_w = 0.95 to 0.80. The OTA-producing species are mesophiles that compete well in the presence of hydrophiles, yeasts and *Fusarium* in coffee, only when there is partial drying. As drying proceeds, the condition become too stringent and the fungus recedes into dormancy.

Water activity limits for growth and toxin production of *A. ochraceus* are 0.78 and 0.80, respectively, the lowest of the OTA-producing species known to occur in coffee. Since it is moisture content rather than A_w, a direct measure of water availability, that is routinely monitored in the coffee sector, the project investigated the relationship between these two parameters based on measurements of over 3,500 field samples. It was found that the average m.c. at which coffee can be said to be secure from growth and OTA production of *A. ochraceus* is higher than previously recognised at between 17-20% m.c. (wb), depending on coffee type. Taking into account the degree of variation in the A_w-m.c. relationship between samples and the variation between beans comprising a sample, the target drying end-point for preventing growth of OTA producers must be much lower than this. A moisture level of around 13% (wb) corresponds – at a confidence level of 0.98 - to an A_w that does not support growth of *A. ochraceus*. During storage of coffee the project recommends operating limits of 12.5% m.c. for cherry coffee and 11.5% m.c. (wb) for parchment and green coffee to ensure that there is neglibable risk of an increase in OTA content.

Trials were executed where the residence period of drying coffee in the 0.95 – 0.80 A_w zone was controlled in order to determine the longest safe period before drying coffee began to accumulate excessive OTA. In concept, this period corresponds to the critical limit of a critical control point for drying coffee. However, the results failed to produce any consistent or, given what is known from the result of other project work, plausible outcome so this parameter could not be calculated.

This, we think, is due to the balanced, multi-factorial system of coffee bean/fruit with active microbial and plant metabolism in which suitable moisture conditions is a pre-condition but where other factors – that are not fully understood – determine the ability of OTA-producers to grow and produce OTA. The trials did not therefore allow an experimentally determined value of the maximum safe time limit in the critical water activity window.

The maximum rates of net OTA production we have measured *in situ* are approximately 2µg/kg/d and 4µg/kg/d in two trials carried out under the project that showed high levels of OTA (see Annex C.7). These measurements imply that once established and in the production phase, OTA can accumulate very fast; 7 days at that rate would see OTA increase by as much as 28ppb. For context, in good conditions, cherry coffee spends 4 to 5 days in the 0.95-0.80 A_w zone and parchment may only spend 3 days.

Based on the fact of the presence of OTA in freshly harvested coffee and other observations, OTA is probably produced from beans bearing an infection at harvest in most cases. The accumulation rates we have measured relate to a given infection rate: only the beans containing the OTA-producer are accumulating OTA so the accumulation rate is proscribed by the proportion of infected beans in the lot. Assuming a 5% infection rate in the samples alluded to above (based on several studies from the same origin where similar levels of OTA were reported) a more typical infection rate of 0.5% implies an accumulation rate of 0.2-0.4µg/kg/d. At this rate of OTA accumulation seven days in the critical window would contain less than 3ppb OTA.

Conditions during drying are far from uniform and the fungi may be exposed to super-optimal temperatures, pH and nutritional variation and periods of diurnally fluctuating water availability. Shifting conditions may inhibit biosynthesis or alternatively, 'down regulation' of growth has been claimed to stimulate production of secondary metabolites such as OTA. In either case the response to such shifting conditions is unlikely to resemble the average conditions making prediction less certain.

If *de novo* contamination external to cherry coffee were responsible for the production of OTA, one might expect germination, growth through the fruit tissue and colonisation of bean tissue to require considerable time: estimates from atleast 5 days to around three times that (2 weeks) would be reasonable. For parchment probably something similar would be expected with the shorter route to the bean being compensated for by the lack of nutritional material on the surface of the parchment compared to the cherry. It should be noted that there was very little evidence produced during the project that supported the notion that *de novo* contamination made an important contribution to bean infection. In case of an active external population there could very well be OTA produced in the fruit tissue and this could diffuse into the bean without colonisatioin ever occurring, at least before the drying bean shrinks away from the fruit tissue.

12.1.6 Optimising drying

It remains a truism that Good Hygiene Practice requires rapid passage through the critical A_w window. Our extensive comparative trials, conducted in all the

collaborating countries, have shown that the single most influential factor in achieving good drying is the weather. This cannot be controlled but optimising drying procedures and drying yard management remain key functions in mould prevention, though this is not always sufficient to produce a good processing outcome.

No consistent differences in drying behaviour were observed when different surfaces used in sun drying were compared. The surfaces that were investigated included those most commonly utlilised in producing countries: raised tables (wire mesh or bamboo); compacted soil; concrete; and plastic sheets or tarpaulins. This means that the selection of the surface should be based on considerations of practicality, given the nature of the local climate. Of major practical concern would be the ease with which the coffee can be protected from re-wetting, particularly in case of rainfall. Optimising the location and surroundings can affect performance in so far as it selects the best available micro-climate for drying. The drying yard should be arranged so to expose it to the maximum amount of unimpeded sun and exposed to the prevailing wind.

Use of compacted soil drying yards could not be shown to lead to increased contamination by OTA-producers or OTA. However, the consideration noted above regarding ease of management would militate against drying of cherry in direct contact with the soil. This practice should therefore be discouraged.

Loading rates exceeding 30kg/m^2 of cherry or 40kg/m^2 of parchment led to reduced drying rates, so it is recommended that these loading rates should not be exceeded. The maximum expected daily harvest and the average expected drying time should be used to judge whether existing drying space is available to meet this guideline. Coordination of harvesting with drying is an important function not to be neglected. Raking of coffee between 1 to 4 times per day did not increase overall drying rates, but increased rate of defects with the lower stirring rate suggests that drying was less uniform so at least 4 rakings per day are recommended.

Tarpaulins have been strongly recommended in some countries as an affordable means of keeping cherries from prolonged contact with soil drying yards. Direct observation has indicated that condensation takes place on the tarpaulin surface, especially with thick layers and re-wetting can occur. Some outcomes using tarpaulins have been very poor, so the use of this material should be kept under review.

Splitting of cherries is a strategy that has been widely employed in some countries to reduce drying times. However, there are indications that split cherries may be more prone to fungal contamination which is probably attributable to the destruction of the physical barriers to fungal dispersal and growth posed by the fruit skin. It is supposed that re-wetting could be especially serious. Under conditions of slow drying, split cherry sometimes appeared to have a higher defect count as compared with parchment or cherry drying.

12.1.7 Defects and OTA contamination

Surveys carried out under the project documented instances where most of the OTA in the lot could be attributed to certain defect categories. This association, however,

seems to be strictly related to the existence of certain conditions during processing of the coffee.

As production/processing conditions change, the association of defects with OTA contamination also changes. This means that findings of OTA accumulation in defects in any particular situation cannot be extrapolated to the 'universe' of coffee. This is clearly demonstrated by the defect studies carried out under the project. In two generic processing systems a strong association was observed but in other studies weak or no association was observed.

More work on this is required as there are important implications for risk management measures at national or international levels. Clearly, guidelines on blending and sorting would have to account for any causal or statistical association between distinguishable bean categories and OTA occurrence. The fate of out-sorted and low grade coffee relating to public health in the domestic producer markets is a real concern if defects are generally more contaminated. With the pooling that occurs as coffee moves downstream in the market chain, defects arising from a range of production/processing systems are mixed, and associations between defects and OTA become very weak, if demonstrable at all.

A combination of upstream surveys and investigation of OTA development in specific defects under defined situations is required to inform rational decisions about managing food safety risks associated with coffee defects.

12.1.8 Storage

We know problems can arise during storage and we know the approximate physical conditions under which fungi can grow. However, trials undertaken in this project failed to generate a storage problem, in terms of OTA accumulation, despite purposely applying questionable practices to the storage including the introduction of additional water in the form of re-wetted coffee.

The trials have demonstrated that ochre aspergilli remain viable even after periods of storage with the water activity maintained below 0.70 and that nominal increases in the frequency of infection by these OTA producers are seen under poor conditions of storage with rehydration to moisture content around 15% wb. High infection frequencies with niger group aspergilli are also maintained during storage though instances where the infection frequency of these fungi fell were also noted.

Our storage trials demonstrated that A_w levels remained well below 0.80 in reasonably good storage conditions over 6 months in most of the collaborating countries and where re-hydration above this level eventually took place there was no evidence of OTA accumulation over the period studied.

12.1.9 International transportation

Moisture migration due to temperature gradients and diurnal fluctuations is a well known phenomenon in commodity transport. Using RH/Temperature data loggers in shipping containers demonstrated these changes can give rise to condensation conditions and therefore the risk of re-wetting through the redistribution of water in well dried coffee. Measures to avoid this risk, as proposed in the *European Coffee*

Federation Code of Practice, include protecting the surface of the coffee during international transport and ensuring that initial moisture levels are below 12.5%. There is no further guidance to be added in relation to this issue.

A simulated trans-Atlantic shipment over 15 days was run to test the impact of fill volume on moisture-mediated quality deterioration. Results suggested that the fill-volume is not an important consideration determining quality loss and OTA risk in international transport so there is no indication that existing advice for filling containers to 18 metric tonnes (bagged) or 21 metric tonnes (bulk) needs be changed.

It should be noted that this trial was very short and retention in containers during transport in more convoluted routes and after arrival can extend residence to much longer periods. Future simulated transport trials should take this into consideration.

12.2 Areas for Future Work

12.2.1 General

Below we suggest specific, mostly technical issues that require further attention to improve risk-based approaches to preventing OTA contamination of coffee. National bodies or, where possible, collaborative action by groups of producer countries need to prioritise and act to tackle the outstanding issues in continuing the process that the project initiated. Some of the project collaborators have formulated lists of work priorities and these are included as Annex C.11. However, pursuit of better answers to specific questions should be seen as a part of an overall strategy for supporting the capacity of the national coffee sector to meet evolving quality and safety requirements of strategic markets.

One particular area that requires attention is the mechanism of exchange of information and perspectives in the coffee sector, especially in the vertical direction. What marketing system provides optimal arrangements for producing and regulating a commodity can be argued but the exchange of information between stakeholders is essential for the function of any form of market. This is especially important to ensure fairness, to inform policy and to be able to adapt well to new challenges that can be expected as foreign and domestic markets continue to evolve.

Some aspects of coffee sector activity such as food safety, food security and environmental impact need to cut across crop delineation to be dealt with effectively and efficiently at the national level. Some of the capacity built by the project could be of value in food safety and quality of other commodities, both in the sense of satisfying export controls and of domestic public health.

Different producers have their own characteristic production chains and processing traditions. Of course, these are not fixed, but it does mean that the existing gaps in knowledge are not equally significant to all producers and each sector must prioritise future work. The topics within each heading below are arranged in a suggested priority order combining a judgement of the likelihood of success and impact of a successful project. However, certain general considerations might be helpfully discussed here.

Pre-harvest infection now seems to be commonly associated with OTA contamination of the bean. Unfortunately, it is also the area most difficult to study whether by survey or by ecological investigation. The potential benefits of pursuing some of the lines set out below are great but this work should be done through further international effort or where the resources and technical ability exist.

Processing requires two strands of work: better understanding of the impact of control parameters in the different climatic conditions, and further effort to understand the biological interactions in the resulting manmade environment. Parameters of general quality need to be re-evaluated objectively and used in concert with those of safety to evaluate experimental outcomes.

12.2.2 Pre-harvest

The project demonstrated that seed infection and OTA-production can take place before harvest and demonstrated a method capable of producing field infection by *Aspergillus ochraceus*. There was no correlation between field levels of OTA and specific horticultural practices in surveys.

- It would be useful to improve the method of artificial infection at flowering for research purposes and to reveal the mechanism of this infection route;
- Production of OTA in the field could then be studied as affected by plant disease and horticultural parameters such as drought and fertilization, for example;
- Possible use of *A. melleus* (an OTA non-producer) as a biological agent to prevent *A. ochraceus* infection could be evaluated utilising, in part, artificial flower infection;
- The importance of niger aspergilli species in OTA contamination of robusta coffee, in which it is ubiquitous, is not clear. Most black aspergilli in robusta coffee are *A. niger* and very few isolates of this species produce OTA but it is not clear whether its ubiquity overcomes the rareness of its ability to produce OTA. A systematic and rigorous survey for the distribution of *A. carbonarius,* a strong OTA-producing species, could be useful here;
- More work is required to understand the impact of fruit/seed maturation, through tree-drying (an aspect to be emphasised in Brazil) in different climates on OTA and general quality;
- There is a need to better understand spontaneous abscission of fruit and fungal infection of this fallen fruit on the ground. This is required to supplement further work on the effects of residence of normal fruit of various stages of maturity on the ground between harvest and final collection, after being dislodged during harvest;
- OTA-producing fungi are not uniformly distributed, so a monitoring programme could help optimise resources by identifying regions that require more attention and others that perhaps require less based purely on occurrence of OTA-producers.

12.2.3 Processing

Processing includes activities from harvest to dryness and can be divided into those activities where moisture remains high and those where the point is to remove moisture. Trials comparing processing methods transferred from other producing countries showed that ambient conditions prevailing in different regions or in the same region at different times generally have a greater influence on the suitability and performance of alternative methods than the differences between the methods.

With such a degree of variation in processing practices between regions as exists, it is difficult to be specific in recommending future research priorities. Each producer sector should gather and evaluate up-to-date information on prevalent processing practices and likely future developments and prioritise a programme of work as seems appropriate.

- More information is required relating to the source or origin of defects. Data shows that in some lots, defects contain a higher concentration of OTA than the sound beans of the lot. An investigation into the origin of these defects could also lead to an understanding of what conditions lead to OTA production *in situ* if any of the defects prove to correlate with the presence of OTA;

- Even with evidence of the presence of OTA-producers, a poor drying time-course does not ensure an increase in OTA accumulation. This suggests there are other key factors controlling OTA formation here. This needs to be understood. It could be investigated using coffee artificially contaminated with *A. ochraceus* at flowering, to reveal what the other important variable are;

- Cherry splitting produces an unstable intermediate product that is prone to developing quality problems despite slightly reducing drying time. However, descascado (unwashed parchment) is more promising and tests for its suitability in climates wetter and more humid than those in which the method is already known should be trailed in the field. This may be particularly suitable for robusta. Availability and accessability of appropriate equipment for use by small scale farmers and farmers' groups could be a key issue to be addressed;

- In wet processing, how pulper damage to beans and the inclusion of skins/un-pulped cherries in the fermentation mass contributes to quality problems has not been systematically studied. Since the monitoring of the operational parameters of the pulper form an important element of the control of operation of wet processing, this weakness in our knowledge needs to be addressed;

- Infection of the seed from air, soil or other fruit during processing has not been confirmed in our studies, including by artificial introduction of spores at the beginning of cherry drying. Consequently this infection route appears to be of little significance. This contention needs to be experimentally challenged in order to finally confirm it.

12.2.4 Handling

Handling includes post-drying operations such as hulling, blending, grading, sorting, polishing and domestic transport. It also includes marketing, storage in

farms and aling the marketing chain, and the routines used in selling and buying that control quality and set price. Surveys have suggested that OTA is higher in samples taken from the trading chain than from the farm gate. This implies OTA is formed during handling.

Moisture content must be controlled in order to assure stability of the commodity during its residence in the domestic market. To demonstrate control of moisture content it is necessary to be able to measure it reliably through the chain. Results from the project has demonstrated that the means for doing this are rarely in place.

- Gathering good information on the physical condition of the coffee and facilities in the domestic market and its residence time between the farm gate and exporter is an important goal. To do so meaningfully, the structure of the chain and the function of the stakeholders must be known. Bear in mind that the market and how it operates is not a fixed quantity but is both varied and flexible. Such knowledge allows the identification of potential problems so that attention could be focused on them;

- Sorting is an important function of marketing. There is a need to understand the significance of defects with regard to OTA content and general quality. Studies should be coordinated with processing studies so the origin of them can be documented. Here again, different origins see different arrays of defects so these studies must be appropriately tailored;

- Most available moisture meters do not function satisfactorily in the field. Though it may be debated whether this is because either the measurement technology or the training/calibration is inadequate, resolving this significant short-coming should be a priority;

- Several storage trials have shown coffee to be quite a stable and robust commodity. However, documented problems attributed to poor storage are not rare so it is important to ascertain what conditions lead to deterioration of coffee in storage, including the conditions that give rise to 'hot spots' by applying combinations of known faulty conditions;

- An important step in the sale of coffee is its evaluation by the prospective buyer. This could be considered to be a control step so, in tandem with work on defects, the various triage systems could be usefully evaluated for their true effectiveness in controlling the safety and quality of coffee lots.

12.2.5 International transportation

There is potential for conflict inherent in quality problems first diagnosed in the importer's locale. This has always existed, but is brought to the fore if legislated limits or criteria of any kind are in force. How is it to be established whether the problem existed at the time of loading or arose during transit? With large lots, a container holds between 18 and 21 tonnes depending on how it is loaded, even establishing the OTA content of a container is fraught with difficulty.

- Passive observation of normal consignments is unlikely to provide the opportunity to discover what happens when problems arise during

transportation since 'incidents' are rare. Smaller volumes of the scale of several hundred kilos of coffee should be used to study movement of water in different conditions and the development of fungi in response to these conditions;

- Fundamental measurements should be made for coffee mass such as its heat content, migration rate of water through a coffee mass in response to a given temperature gradient and evaporation rate from the mass into the headspace in response to a temperature change of a given size;

- Innovative instrumentation to make direct measurement of condensation events, CO_2 generation, and gas exchange should be developed.

Part D

Guidelines for the Prevention of Mould Formation in Coffee

Drying yard, Brazil

Part D
Guidelines for the Prevention of Mould Formation in Coffee

1.1 Preface

In a commodity like coffee where it is important to retain diversity in flavour and where sensory quality can create value, any code of practice must respect the differing traditional production methods. Of course, practices that can be shown to compromise public health must not be permitted, but beyond this benchmark there are few if any practices without safe alternatives. However, there are restraints on acceptance of potential solutions to identified problems such as the limited capital of small farmers, remoteness from support and marketing institutions, lack of financial incentive for change and the inertia of habit borne of long tradition.

These guidelines interpret and incorporate scientific findings into practical guidance. The studies themselves can be found in the supporting documentation. There is no intention or desire to strictly codify practice into narrow limits, a futile pursuit, in any case, given the diversity of practice and the essential variety and good quality of the product of these practices.

These guidelines are not intended for the direct use of every stakeholder, rather they aim to provide concerned authorities with the basis for developing national guidelines or codes of practice specifically tuned to their respective sector.

The first objective of these guidelines is to characterise factors associated with each production step throughout the 'coffee chain' that could contribute to the problem of OTA contamination, explain their relevance in different situations and propose means for their control. Recommendations, and the contra-indications of poor practice, need to be specific enough so that the concerned authority or stakeholder can develop his own solution appropriate to his circumstances.

It is not enough for the advice to be correct and practical, and the intention to apply it solid. As much thought must be given to how it will be implemented and assured in the day-to day rush that envelops any production system at the height of the harvest period. Successful implementation is tied to understanding how to structure and manage an operation. Providing advice on a safety/quality management system to aid the implementation forms the second objective of these guidelines.

These guidelines, and national guidelines or codes of practice that should be derived from them, will form the basis of national programmes for the reduction of OTA contamination in coffee. Concerned institutions must develop effective programmes of training to support the implementation of national guidelines. Policy-makers must ensure that regulatory and other relevant policies are consistent with achieving widespread stakeholder compliance with the recommended practices.

1.2 Introduction

OTA is a chemical product of the growth of a few specific fungi. It occurs where certain micro-fungi capable of producing it occur in concert with the conditions they require for growth and biosynthesis of this chemical for enough time for the product to accumulate. Fungal contamination along the coffee chain can impart a smell or taste to the product. However, the specific kinds of fungi involved in these taints are not the same as those involved in OTA production, so the causes of OTA remain essentially invisible.

Compared to staple crops, coffee has some advantages. Perhaps the most significant is the limited extent to which it is subject to pest attack in storage. Birds and rodents do not eat the seeds and only one significant insect, the coffee weevil *Araecerus fasciculatus*, attacks the dry product (Hill and Waller, 1988). In fact, the bulk of the carbon in coffee seeds is in fairly refractile forms such as the poly-mannan carbohydrate storage material, cellulose and pectin and allied to the high phenolic content of coffee probably restricts the diversity and rate of fungal spoilage. Importantly, there are no significant current alternative uses for coffee aside from human consumption as a beverage or flavour in other processed products.

However, fungal and bacterial spoilage does occur and though the almost universal use of high-temperature roasting before consumption means that food poisoning bacteria present a negligible risk to public health nor are the poly-peptide enterotoxins some produce likely to be sufficiently heat-stable to persist in the roasted product. Toxins produced by fungi, however, are known to survive roasting and present a potential hazard. Ochratoxin A (OTA) and to a lesser extent aflatoxin, both produced by species of the fungus *Aspergillus* in coffee (M. Nakajima, *et. al.*, 1997; C. P. Levi, 1980; I. Studer-Rohr, *et. al.*, 1995; H. Tsubouchi *et. al.*, 1984), can occur in raw and roasted coffee beans.

Practices that restrict the development of certain fungi tend also to preserve quality in both sensory and safety terms. The two specific tools available are, 1) managing water availability from the beginning of drying onward, and 2) facilitating the development of competitive micro-organisms and restrictive growth conditions that are not prejudicial to quality, before this point.

There are predominantly two commercial species and some inter-specific crosses used in coffee production. *Coffea arabica* (arabica coffee) requires a wet tropical highlands climate at altitudes between 600 and 1600m. *Coffea canephora* (robusta coffee) can be grown at sea level but it too is often grown in wet tropical highlands. The vigour and disease resistance of robusta is superior to arabica.

Although the chromosome number of these two species is different, crosses can be forced, and at least one spontaneous cross is known. Such crosses are primarily used to back-cross with arabica to improve disease resistance in arabica and most commercial arabica, outside of Ethiopia, are of this type. However, two inter-specific hybrids, 'arabusta' and 'congusta' the latter a robusta and *Coffea congensis* cross, are grown and marketed to a limited extent.

Robusta's vigour means that production costs are less than arabica, but its value is also considerably less. The bulk of robusta coffee is used in soluble coffee production

but there is a small outlet in the speciality coffee market, especially for the wet-processed product.

The commercial product is the seed and these are formed, usually, two per each small cherry-like fruit which are referred to as 'cherries'. The fruit is borne in either tight (robusta) or loose (arabica) bunches at the nodes of side branches. Both commercial species are large bushes and many commercial varieties have been selected for dwarfing character to simplify harvest which is done primarily by hand.

Processing is conducted on the farm with the overall objective of stabilising the product (the seeds) by drying to a level where microbial deterioration is prevented. This may involve prior separation of the product from the fruit tissues. Once dried, the coffee can be stored and transported and will also be 'cured', a series of steps that may include sizing (grading), sorting, polishing, cleaning and sacking. The commercial value of coffee is vested in its taste characteristics so the preservation of these qualities is central to processing methods.

There are two generic systems of coffee processing: wet processing and dry or natural processing. The dried product of the first is 'parchment coffee' which is the seed enclosed in the inner integument or endocarp and the dried product of the second is the seed enclosed in the complete dried fruit tissue. Parchment coffee has a higher market value, but is more expensive to produce, and has different sensory qualities than cherry coffee. Most robusta coffee is produced as cherry coffee; most arabica coffee is produced as parchment coffee, but with important regional exceptions. There is a limited market for washed robusta in the speciality market and arabica cherry is an essential component of espresso-style blends.

In wet processing, equipment is used to split the seeds out of the fruit, generating a significant by-product, 'pulp', the skin and part of the 'mucilage' (mesocarp) of the fruit, The main product is 'parchment' coated thickly with mucilage. The parchment is traditionally fermented in order to degrade the mucilage so that it can be easily washed but it can also be removed immediately by machine. After removal of the mucilage, the parchment is dried, usually by sun drying on cement, brick terrace or tables. There are many variations and technological innovations to this generic scheme but it is beyond the scope of this treatment to describe these.

In dry or natural processing, the fruit is laid directly out to dry in the sun with or without a step to separate floating cherries from sinking ones. Bare soil, cement, brick, bamboo mat and tarpaulin are all surfaces that are commonly used for sun drying. By this method, the separation of the fruit tissues from the seeds is accomplished later, in the dry state, generating a significant by-product: dried fruit tissue or hull. The hulling step is usually done on-farm or the hull is returned to the farm.

Though sun-drying is the most common drying method for coffee, mechanical drying is important in some regions, particularly in more capitalised sectors. Even here, sun drying is normally used for a significant part of the drying period since most mechanical dryers are designed to handle coffee with an initial water content of 35-40% m.c. (wb) from initial values of 60% or more.

Although the occurrence of OTA in coffee was reported in the 1970's, it did not become a public health concern until a revision of its mode of action was mooted in the 1990's. Although not proven, there was evidence published that OTA was a genotoxic carcinogen, like aflatoxin. The practical significance of this, if true, is that any exposure to OTA, increases risk of, in the case of OTA, kidney cancer. The accepted guidance for genotoxic agents is to reduce their occurrence to a level that is 'as low as reasonably achievable (ALARA)'.

OTA is a heat-stable mould metabolite produced by a proportion of isolates of a few species in the genera of Aspergillus and *Penicillium*. In coffee, only *Aspergillus* species in the ochre and niger sections are involved. The toxin is produced by a growing mycelium within certain physical restrictions of water activity, nutrition and temperature and these provide the potential areas of control. Most commercial samples do not contain detectable OTA with a current detection limit of 0.1–0.5µg/kg (= ppb) depending on the method in use. Of positive samples, most fall below 5ppb and anything above 20ppb is considered exceptionally high.

While these guidelines focus on reduction of OTA contamination, which is the primary food safety issue in the production of green coffee, industry food safety programmes must also effectively manage other potential hazards in the production, processing and handling of coffee.

1.3 Definitions

***Bóia*:** Cherry coffee separated by virtue of it being positively buoyant in water applied to a one-pass stripping harvest system where there is abundant tree-dried cherry.

Cherry (or Coffee cherry): The complete fruit of the coffee tree, can be either fresh or dry

Conditioning: The storage of dried beans in ventilated bins to achieve an even moisture content within the bulk of the coffee.

Conditioning bin: Large wire-mesh holding bins usually of 1 x 1 x 3m (or larger) that are used for conditioning coffee. Modern designs incorporate fan ventilation.

Curing: The final stage of preparing coffee, known as 'curing', usually takes place just before the coffee is sold for export. Coffee passes through a number of operations that may include cleaning, polishing, screening, sorting and grading.

Defects: The collective name for common but undesirable particles found in bulk green coffee. Defects can include various types of beans, or parts of beans, fruit tissue and foreign matter. Numerous terms are used to describe the various defects that can be present in both green/raw and roasted coffee beans, and sometimes these are used in some producing countries and not others. In general, bean defects are caused by faulty processing, pest damage, or inclement climatic conditions leading to poor fruit development. Defects are given a weighted value to assist in the classification and grading of coffee lots under various national and international systems.

Dry processing: Treatment consisting of drying coffee cherries to give husk coffee, followed by mechanical removal of the dried pericarp to produce green coffee. The product is called 'cherry coffee', 'unwashed coffee' or 'natural coffee'.

Floats coffee: Cherry coffee separated by virtue of it being positively buoyant in water applied to selectively picked coffee the vast majority of which is ripe or immature.

Gleaning (or Sweeping): Applies to the collection of coffee fruit found lying on the ground beneath coffee bushes, having either become detached during harvest or abscised during development. 'Gleanings' is the collective term for coffee collected in this manner.

Green coffee bean: The dried seed of the coffee plant, separated from non-food tissues of the fruit. Coffee is exported in this form.

Hull: The dried endocarp of the coffee fruit.

Husk: Waste material resulting from the hulling of parchment or dry cherry coffee, made up of the dried pulp and outer covering of the parchment.

***M'buni* (or *Buni*):** Cherry coffee that has been separated from selectively harvested fruit based on visual criteria such as evidence of CBD or CBB attack or being at a non-ripe stage of maturity (**Note:** '*Bun*' or '*Buni*' is also the generic name for coffee in Ethiopia, and is not to be confused with '*m'buni*').

Mechanical drying: Any of several drying technologies where heat is provided from combustion of a fuel.

Mechanical washing: Any of the mechanical methods for removing the mucilaginous mesocarp from the surface of the parchment, taking place after pulping without a fermentation step.

Mucilage: Common word to describe the fruit mesocarp, an intermediate layer of tissues between the epicarp and the endocarp (parchment). It consists mainly of pectinaceous mucilage and pulp.

Naked beans: Parchment coffee that has been partly or entirely peeled of its parch during pulping and/or washing.

Natural processing: See 'Dry processing'.

Parchment (or Parch): Common word to describe the endocarp of the coffee fruit. It lies between the fleshy part (or pulp) of the cherry and the silver skin. This is the thin, crumbly paper-like covering that is left on wet-processed coffee beans after pulping and fermentation. Subsequently removed during hulling.

Parchment coffee (or *Pergamino*): Wet-processed beans after pulping, dried to about 12% moisture content, but before hulling has removed their hard outer covering (the endocarp/parchment).

Processing: Steps involving the transformation of harvested coffee fruits to a dry and stable condition.

Pulp: The fleshy outer layer of the mesocarp, directly beneath and including the skin, removed with a pulping machine

Pulping: Mechanical treatment used in wet processing to remove the exocarp and as much of the mesocarp as possible

Wet process (or Wet processing): A method of processing coffee cherries into dried pergamino/parchment coffee. Treatment consists of mechanical removal of exocarp in the presence of water, removal of all the mesocarp by fermentation or other methods, and washing followed by drying to produce parchment coffee which is subsequently stripped of its parchment to produce green coffee.

1.4 Recommendations

1.4.1 Pre-harvest

There are serious fungal pathogens of coffee but fungi in general, and OTA-producing fungi in particular, are not responsible for plant disease. Many are or can be involved in fruit spoilage and several of these can grow and survive in viable, healthy seeds. Micro-organisms form a natural part of the plant, inside and out, and in the healthy plant there is a balance between these commensal organisms and the plant itself. There is good evidence now that infection of the seed by OTA-producing fungi can take place in the orchard and grow enough to produce OTA by the time of harvest. Further work is required to better understand the factors that lead to this contamination.

There are two documented infection routes: introduction through the flower producing no sign of this infection; introduction by casual carriage of spores into the bean on coffee berry borers (CBB) (*Hypothenemus hampei*) producing obvious signs, a hole in the cherry and one or more tunnels in the bean. More mature and dryer fruit and production by-products (husk and pulp) can contain increased levels of the spores and mycelium of OTA-producing species.

It is logical that contamination of the bean by growth of superficial mould through the fruit can occur. The relative importance of this mechanism in bean contamination in the orchard was not systematically evaluated, but mycological analysis did not demonstrate a correlation of fruit with bean contamination. If cherries become detached and reside on the ground, contamination and growth through the fruit is more likely. This process requires time but in the field, generally, the history of fallen fruit cannot be known. Fruit becomes detached through inclement weather, higher animals feeding on the fruit, disease or stress-induced abscission or accidentally through other farm activities such as weeding or spraying.

These considerations lead us to recommend practices designed to minimise spore load from OTA-producing fungi in the orchard, to minimise CBB occurrence and to ensure the vigour of the coffee trees so to minimise development of fungi residing within the tree and its fruit.

1. Use plant material from manual weeding to improve soil texture and fertility. Coffee production by-products can also be used but should first be composted until the material has reached a friable condition, requiring 3-6 months depending on temperature and moisture conditions. Avoid applying organic material during or just prior to flowering.

2. Do not use overhead irrigation around the flowering period. This could augment normal spore dispersal rates and increase the chance of infection of beans by OTA producers.

3. Clean the orchard of fallen cherries, especially in the off-season, and deploy alcohol traps for CBB control especially in the run-up to and throughout harvesting and processing. Programmes of integrated pest management (IPM) should be promoted.

4. Employ horticultural practices that contribute to vigorous trees: weeding; pruning; fertilization, pest and disease control; etc. In selecting a pruning system, do not neglect its impact on leaf area. This should be high since self-shading and high photosynthetic potential improves vigour in coffee.

5. Do not dispose of uncomposted coffee waste, household waste, waste from staple crops that may also be produced on the farm or animal feed in or around the orchard. Deposition of seed and seed-associated material could encourage proliferation of OTA producers since many are seed-borne fungi.

1.4.2 Harvest

The harvesting method is dictated by a combination of the requirements of the processing method, economic considerations and availability of labour. In general, four harvesting systems can be identified: 1) multi-pass selective picking (finger picking) where the picker takes only ripe cherries; 2) multi-pass stripping where whole bearing shoots are stripped off only if bearing predominantly ripe cherry; 3) single-pass stripping where everything is stripped off as the workers get through the orchard; 4) mechanical harvesting where machines, sometimes hand-operated, use vibration to knock the fruit from the trees.

In addition to these methods of bringing in the main harvest, there are other activities before and after main harvest, some of which bring in fruit. Often a 'fly harvest' collects prematurely ripened fruit. Weeding and cleaning the orchard floor to expedite the spreading of mats or the collection of fruit that goes to ground during harvest is conducted.

Subsequent to the main harvest there is usually a collection ('gleaning' or 'sweeping') of fruit missed during main harvest, some of which is still on the trees but mostly on the ground. This is an important element of Integrated Pest Management for CBB but, traditionally, this coffee joins the human food chain.

Brief contact with the ground is not problematic but becomes so if the contact period lengthens. According to some experimental findings, *in dry conditions* fungal development is not rapid and up to two weeks residence on the ground may not increase contamination with OTA producers. In wet or humid climates only collection from the ground on the same day should be considered acceptable. Measures to assure these limits are not violated must be in place if this coffee is to be used.

Irregular maturation of fruit is a problem for all farmers and processing methods because the physical properties and potential sensory quality of different maturity classes differ. If selective harvesting is used, the heterogeneity can be minimised but at the expense of higher harvesting costs. The timing of the harvest is therefore an issue of importance particularly with non-selective methods.

There is some indication that OTA can increase in the standing crop as the season passes and certainly CBB increases through the harvesting season. In early season there is a disproportionate frequency of immature cherries which have low cup quality, cannot be pulped and are not readily separated from ripe cherries by automatic means.

The proportion of over-ripe cherries increases as the season progresses and past a certain stage, they can no longer be pulped. The situation with over-mature and tree-dried cherries is complex but it appears that tree drying in regions with arid harvesting seasons is a safe practice. Tree-drying in other climates is probably less so and, in any case, has been implicated in certain cup defects such as fermented and *rioy* taste.

Coffee cherry should be processed without delay. Buffering methods such as retention of harvested cherry in sacks, holding cherries under water, removal of partly dried coffee from the drying yard to 'conditioning bins' or drying in excessively thick layers, are sometimes used to replace good planning but these are all problematic. Careful planning and anticipation is required because readiness to process depends on the completion of drying, itself usually dependent on weather conditions. The harvesting rate, along with processing performance and labour availability must be made to match drying rate.

Evidence thus far indicates that keeping fresh cherries temporarily under clean water is safe but this material rapidly becomes more difficult to pulp and wash. Indications are that retention in sacks may erratically produce high OTA levels and quality loss. Likewise, thick coffee layers during drying slow drying rates and consequently permits fungal growth and development. Tests have shown that little additional drying takes place in conditioning bins so the period in bins provides additional time in which spoilage can occur.

The coffee presented to the processing method should be uniform so that mixing different categories is avoided: wet with dry coffee in dry processing; pulpable with not pulpable in wet processing; sound fruit with other categories in all processing. The harvesting result must serve the processing intention and be evaluated by how well it does so. It is known that coffee seeds can contain OTA at harvest but the detection of these seeds is not feasible.

1. Removal of brush, fallen cherries and high weeds from the proximity of the trees is an important prelude to harvest. It improves picking efficiency, protects the pickers and is necessary to protect the main-crop from contamination by old fallen cherries that may be included when dropped cherries are retrieved from the ground.

2. Harvest should commence as soon as there are sufficient ripe cherries for the harvest to be economically viable.

3. Use picking mats beneath the trees where possible. They protect the main crop from contamination by old fallen cherries and improve harvesting efficiency. They are only practicable in flat or gently sloping terrain since the fruit rolls off the mat on steep slopes.

4. Exercise appropriate selection at the picking stage or before further processing or both to remove inferior fruit from the main production chain as is suited to the processing method.

    - Where CBD or *Phoma* commonly attack the fruit, only sorting by hand is possible. Here sorting is used to remove diseased and immature or over-mature fruit from the main harvest. Also remove immature fruit.

- Sorting based on buoyancy in water conveniently separates fruits with one or more diseased seeds, some multi-hole CBB attacked fruits and tree-dried fruit, all of which float, from a combination of ripe and immature fruit, which sink. There are indications that the superficial microbial load is reduced by brief agitation of cherries in water though it is questionable whether this reduces the risk of OTA contamination of the coffee bean.

5. Establish clear routines for processing and handling secondary products that arise from sorting or separation procedures in your production system.

6. Coffee that had been in contact with the orchard soil for longer than specified should be collected and destroyed.

7. Assure harvested coffee can be promptly moved through the processing steps without delay. An important management function is the co-ordination of harvesting activities with processing activities. In general, coffee is better left on the tree for a few days, rather than harvested and retained awaiting processing.

1.4.3 Post-harvest processing

Maturation and drying of cherries on the tree is quite distinct from drying after harvest. Coffee fruit, unlike many other fruits, has no capacity for dormancy – rapid change and senesce follow once the fruit is detached. Based on the available means of controlling processing, the post harvest period is characterised by two distinct phases joined by a transitional phase.

In the first or high moisture phase, which begins with harvest, the product is in an unstable state and spoilage can only be controlled by encouraging competitor micro-organisms, restricting oxygen and limiting the time in this state.

In the last or low moisture phase, which begins in the later part of drying and extends through to roasting, the commodity is in a stable condition and control is exerted by preventing the re-introduction or redistribution of water in the coffee bulk.

During the transition between these two phases, spoilage can only be controlled by time limitation because there is enough water for the growth of mesophilic and xerophilic spoilage organisms but not their hydrophilic competitors and aeration is an indispensable part of drying.

In wet processing the high moisture phase may be extended while being controlled with a fermentation step, but generally a process should seek to minimise the length of the high moisture phase.

The transitional phase is the least stable and most difficult to predict. During this period, certain hydrophilic microbes, known to be harmless, are replaced by mesophilic ones, some of which are known to be capable of OTA-production. It should be noted, however, that many of the harmless organisms still have the capacity to produce quality deterioration. Rapid drying is often not possible where harvest coincides with a rainy season or high prevailing humidity so measures to

optimise drying under these poor conditions must be taken (see Section 1.4.4 on 'Drying', below).

At some point during drying further growth becomes impossible as the commodity passes to the low moisture phase heralding the end of processing.

There are many claims relating good general quality to one or another aspect of processing and that partly determines market value. Usually these are not supported with evidence of objective comparison between alternatives and since they strongly influence practice, this area is in need of systematic review. A rational market needs such information to reward practices that are demonstrably beneficial either in safety terms or general quality. Resources or attention expended on non-beneficial activities at best divert effort from more important issues.

In the past, both fermentation and sun-drying were considered essential for good quality but this is now disputed as the use of mechanical washers and drying has become more widespread. Some workers recommend that parchment coffee should be protected from rapid drying in the mid-day sun at the early stages, but many origins have no such tradition.

1.4.3.1 Wet-processing

Wet processing has required uniformly ripe cherries, though new pulping technology has arisen that tolerates inclusion of immature cherries in the ripe cherry. Wet processing produces parchment coffee as the main product and cherry coffee as a secondary product.

This dry processed cherry coffee is derived from out-sorted cherries (floats coffee and *m'buni*) removed from the main production chain prior to pulping according to characteristic defects or incompatibility with the parchment processing technology. Typically, the low value secondary processing chain is very much neglected and this should not be the case – it too is destined for the human food chain. Analysis has shown this product, when neglected, can become highly contaminated with OTA. The out-sorted cherries are likely to contain a relatively high proportion of defects some of which, according to data from some surveys of defects, are associated with greater risk of OTA contamination than sound beans produced in the same batch.

Control of spoilage of parchment is exerted either by using a fermentation to limit oxygen availability and encourage harmless competitive micro-organisms while degrading the mucilage to permit washing followed by drying or applying mechanical removal of the mucilage to permit immediate drying. A recent innovation where the pulped parchment is immediately dried without mucilage removal (*descascado* or *cereja descascado*) provides a third alternative.

Extensive sampling has failed to show that pulping remnants strongly support the development of OTA-producers although they do support rapid growth of bacteria and yeasts the acidic by-products of which could damage the equipment. Adequate cleaning programmes are necessary to control unnecessary additional sources of contamination and also to safeguard the equipment. Likewise, recycled pulping water is safe for use for pulping. The largest reservoir of OTA-producers in wet processing is the coffee fruit, including the bean, itself.

The inclusion of skins, crushed immature cherries and un-pulped, under-sized cherries in fermentation and drying of parchment has long been considered to have serious general quality consequences. At high levels they could pose an OTA risk but the evidence for them having a significant impact on OTA accumulation, at frequencies of occurrence that are acceptable in terms of general quality, is weak.

Based on the rapidity with which naked or nipped beans become mouldy, the parch provides some tangible protection against mould contamination when wet. Although it does not automatically follow that this contamination would generally lead to OTA contamination, it is a clear cautionary point. Nipped and naked beans are much more common from low water use mechanical washers and unrefined pulpers, so special attention is required when operating these.

1. Any equipment, no matter how technologically basic, benefits from regular maintenance. Equipment failure could delay processing and compromise coffee quality and safety. In addition to regular cleaning and maintenance during harvest season:

    - In decommissioning, all processing equipment should be thoroughly cleaned and lubricated as appropriate and protected from water, dust and debris during the off-season. This is also the time to order replacement parts and conduct repairs. Check pulping surfaces for wear.

    - In re-commissioning, clean, reassemble, lubricate where appropriate and all processing equipment and inspect, installation, fittings and power and water supplies. Test for operational integrity well before use is required to provide time to retrench if faults arise.

2. Adopt acceptability criteria for each significant element of the process and unambiguously assign roles to staff to ensure that they are met. Pulping is a crucial and central activity in wet processing and you should assure it is being done as well as possible. There may be training implications for workers. A guide to these considerations follows:

    - Quality of input cherries: What is the maximum acceptable proportion of immature and over-mature/tree-dried cherries (if a siphon is not used) for your operation? How is the rate of immature and over-mature cherries estimated? Who should monitor this and how often? Prescribe remedial actions to be taken if norms are exceeded.

    - Quality of pulping I: What proportion of un-pulped cherries and, on the other side, nipped beans do you accept in your operation? How and how often do you monitor the amount of these categories? What corrective action is justified by the consequences of processing these unintended classes? Might measures to increase size uniformity of the input be cost effective? Prescribe remedial actions to be taken if norms are exceeded.

    - Quality of pulping II: Are skins being effectively separated? How and how often is monitoring required? How do you investigate the cause of poor skin separation - inadequate water supply, outflow blockage, worn pulping surfaces? Prescribe remedial actions to be taken if norms are exceeded.

- With such a scheme established, some of the measures may prove to be ineffective, too stringent or too lax. Recording the various estimates of the monitoring, as well as the quality and safety of the product could be used to improve the efficiency of the operation.

3. Although there is no evidence that poor water quality can lead to OTA contamination, coffee is a food and clean water should be used in processing it. If available, bore-hole or spring water should be used. Turbid water has been reported to ruin coffee sensory quality in wet processing.

4. The shortest fermentation required to loosen the mucilage sufficiently for washing is the optimal one. Establish how and when the fermentation should be sampled and assessed. Fermentation may contribute to coffee quality but its primary purpose is to enable the mucilage to be removed. The fermentation rate can vary due to variation in inoculum speciation and level (in the in-coming cherry) and ambient temperature.

5. Monitor the build-up of fruit flies and take measures to control them if their populations become extreme. In general they carry whatever micro-organisms that are present in their food but heavy infestations can unbalance fermentations.

6. Have a parallel programme for the processing of the dry-processed secondary cherry coffee and do not allow it to be controlled `by default'. Maintain separate facilities for cherry coffee drying and apply good drying practices (see below) to this product.

7. Establish criteria to judge washing efficiency and a routine to implement this control measure and whether water usage is well controlled and minimised.

   - Amount of non-coffee by-products after washing;

   - Amount of broken, nipped and naked beans after washing.

Drying and drying yard management elements are discussed below.

1.4.3.2 Dry-processing

In dry processing the whole cherry is dried with or without some preceding selection/separation step. Regional variations include retaining harvested coffee in sacks, heaps or thick unstirred layers before spreading to dry and the mechanical splitting of the cherry like pulping but where the parchment and skin is dried as an un-separated mass.

It should be emphasised that to get good results, cherry drying, although simple, requires the application of good practices and management as much as the more complicated wet processing method.

On a per kilogramme of green coffee basis, almost twice as much water must be removed on the drying yard in dry processing than wet processing. At the same time, whole cherries provide a greater degree of protection for the beans. Splitting cherries is a 'low-tech' compromise to reduce drying time without increasing

processing costs too much as with wet processing. If it is poorly executed, physical damage to the bean can increase opportunity for internal bean mould contamination and associated risk of OTA contamination and quality loss.

One very important variation on the usual method of presenting ripe cherries to the processing unit is to allow most of the fruit to dry on the tree. Results indicate that this method can produce safe and good quality coffee in regions where the harvest season is reliably arid. Its efficacy is to reduce the cost of harvest allowing one-pass stripping while minimising the amount of immature beans in the product.

Field surveys have revealed that it is common practice to hold harvested cherry in sacks or heaps for 3 to 7 days, especially amongst smallholders. Under these conditions, high temperatures are experienced and rapid fermentation takes place, different in kind to the fermentation employed in wet processing.

Direct studies have not produced consistent incontrovertible evidence to condemn this practice. It is clear, however, that the process is not controlled and alarming outcomes have sometimes been recorded. On this basis, it is recommended that fresh cherry should not be held beyond the day of harvest before spreading the cherry for drying. Furthermore, delays before processing often lead to substantial quality deterioration.

Wet processing operations also produce a certain amount of cherry coffee (see above) but this must not be compared to main-crop cherry coffee. Generically, this comes from, 1) 'floats coffee': where a siphon removes the ripe cherry that floats in water from the main crop and is combined with hand sortings (immature, over-mature), or 2) *'m'buni*': in endemic coffee berry disease (CBD) regions a siphon is not usually employed so floating cherries form a part of the main crop with visibly diseased, immature, and over-mature cherries dried as cherry.

In some regions, ripe cherry is selectively picked and dried. In most regions, especially after several years with very low prices, stripping is used in cherry coffee production, often with floatation separation. If harvesting of tree-dried coffee is common, a floatation step should be used to ensure that the tree-dried cherries are handled separately. This avoids their re-wetting on the drying yard due to mixing with fresh cherries. Even with uniformly ripe cherries, the frequency of defect beans can be reduced in the main crop by removing floats coffee. Analysis of defects have shown some of these to be associated with high levels of OTA contamination, therefore reduction of defect levels may *in some cases* be an important OTA control measure.

1. The primary equipment for the dry process is the drying equipment: the surfaces on which the coffee will be dried, mechanical dryers if used, covers and rakes as well as floatation separation facilities in some cases.

    - In decommissioning, all processing equipment should be thoroughly cleaned and lubricated as appropriate and protected from water, dust and debris during the off-season. This is also the time to order replacement parts and conduct repairs.

    - In re-commissioning, clean, reassemble, lubricate where appropriate, all processing equipment and inspect installation, fittings and power

supplies if used. Test for operational integrity well before use is required to provide time to retrench if faults arise.

2. Triage or floatation should be used to remove diseased or damaged cherry from the main processing stream.

3. If unselective picking is used, use floatation to separate ripe and immature cherries from tree-dried cherries

4. Establish measures so that the harvest activities are co-ordinated with drying facility availability and that it does not become necessary to delay further processing after arrival of the cherry at the processing unit.

Drying and drying yard management elements are discussed below.

1.4.4 Drying coffee

Strictly speaking, drying coffee is a part of processing but is dealt with separately here because cherry and parchment drying are more conveniently discussed together. Water relations in biological systems is a very complicated and important area in controlling the quality and safety of commodities. A great deal of effort was put into understanding all aspects of drying, control of drying and measurement of water content and a great deal of information and data is available in the supporting documentation.

World-wide, most coffee is sun-dried on some type of prepared surface such as tables covered in wire mesh, bamboo mat or sisal mat, cement or brick terraces, compacted earth, plastic sheets/tarpaulin or fish farm netting. Mechanical drying is also used after pre-drying in the sun to a moisture content of about 40%. Solar dryers are rare in the field, but the *parabolicos* and 'Maquesina' can be found fairly commonly in some regions. The value of the former design has been found to be highly dependant on prevailing weather conditions.

Three regions of a drying time-course (m.c. vs. days) can be identified: an initial lag period, a period of maximum change and a deceleration phase. Cherry coffee has a lag period of 1 to 3 days where m.c. changes little compared to a lag period of 1 day or less in parchment drying. OTA-producing fungi are at a competitive disadvantage in these hydrated conditions.

The next phase is linear and its steepness depends primarily on drying conditions and secondarily on drying yard technology. Cherry and parchment, under identical conditions, dry at the same maximum rate. OTA-producers are best suited to succeed during this period.

As the coffee approaches dryness the remaining water is tightly held by the seed and water loss rates fall producing a period of slow drying. Some fungi can grow well at these moisture levels but the OTA-producing fungi are not amongst them.

For OTA to be produced, one or more of the fungi capable of producing this toxin must be able to grow. For this to happen these fungi must experience favourable conditions for a sufficient period. An essential part of these conditions is water availability: too wet (above A_w of about 0.95) and fast-growing hydrophilic fungi,

including yeasts, will thrive and repress OTA-producing fungi; too dry (an A_w of less than about 0.80) and the OTA-producers are incapable of producing the toxin; dryer still (A_w below 0.78-0.76) and they are incapable of growth. The objective of control on the drying yard is to minimise the period the coffee spends in the range of water availability where OTA-producer growth is possible. Experimental results indicate that 5 days or less in this range is both generally attainable and effective in preventing OTA accumulation.

Rewetting may be more serious than slow drying. If the beans contain a level of contamination, it may have increased in biomass through the drying period so with more biomass the mycelium would be poised for rapid growth and OTA production if growth conditions become suitable.

Recent evidence confirms that the recommended maximum acceptable moisture content (12 and 13% (wb) for dry parchment and cherry coffee) protects coffee from growth of OTA-producers and includes a substantial safety margin. This assertion is based on the study of the relationship between A_w and moisture content, including over 2,000 samples from many sources, which shows that moisture contents for robusta cherry and arabica parchment of about 18 and 16%, respectively, correspond to an average A_w of 0.76 which is the minimum requirement for growth of OTA-producers. The data indicate that, at a confidence level of 99%, this figure becomes approximately 13% for both. It should be noted that the relationship A_w vs. m.c. was determined only by desorption a sorption isotherm might be expected to vary slightly.

This A_w vs. m.c. relationship is supported by evidence from storage experiments where moderate re-hydration has taken place without serious consequences. However, a lot of coffee with more water in it is inherently less stable than one with less and the recommended moisture content is not difficult to attain in most producer regions.

Different climates pose different problems for drying and suitability of equipment can only be assessed in light of prevailing harvest-season climate. This fact also makes generally applicable recommendations difficult to devise. Many well-replicated studies show that differences in sun-drying equipment produce very small differences in drying rates but that drying rates vary enormously depending on how the equipment is used and the prevailing meteorological conditions at the time of the drying run. Any operation can benefit from keeping track of what has been done and the consequence. This information can be used to improve practice or to identify batches that might have experienced particularly bad drying conditions and might be considered to be at risk.

Mechanical drying is most commonly used as an adjunct to sun-drying, employed at the end of drying to rapidly generate more space in the yard. In some regions, however, it is widely used as the primary means of drying. Most of the available types of driers are controlled with two parameters: duration and inlet temperature. The main concerns with mechanical drying is excessive inlet temperature generating black beans from immature beans and over drying causing a loss of value, through weight loss, for the producer.

The objective of drying is to remove water from the seed in the most efficient way in order to stabilise the commodity and preserve its quality.

1. Site the drying yard to maximise sun and wind. In sun-drying, energy for the evaporation of water from coffee beans is provided by the sun and is expedited by air circulation. The drying yard should be located where both are maximally available, avoid shade and low areas for drying.

2. Use a surface appropriate to the climate and product you are producing.

    - In extensive side-by-side tests, different surfaces sometimes showed differences in drying rate but these are generally small and not consistent.

    - Parchment coffee taints more easily so only cleanable and easily drained surfaces can be used.

    - These tests failed to condemn the use of any particular surface but all have advantages and disadvantages. Soil would not be recommended in rainy zones and impervious surfaces such as plastic have been observed to 'sweat' below the coffee layer and promote the superficial growth of moulds. In regions with wet or showery weather, consider the practical imperative that the coffee will have to be frequently covered and re-spread, once the surface has dried.

3. Plan the harvest based on the processing/drying yard capacity and the average required residence time for drying. Plan in a contingency since poor weather can occur and increase drying yard residence time.

4. Coffee committed to the drying yard must be carefully managed to make the most of prevailing conditions, on the one hand, and to avoid adverse possibilities that could occur in any outdoor process. The principle parameters available to control this process follow:

    - Keep different categories and different day's harvests separate and use a system of labelling to prevent confusion.

    - Do not dry coffee in thick layers. As a guide, the optimal load for sun drying is about the same for parchment and cherry drying at 25 to 35kg/m^2 when fresh. This corresponds to 3 or 5cm layer depth, respectively.

    - Better drying conditions (low humidity, good air circulation and sun intensity), allow thicker layers: in cloudy damp, still weather the optimum layer is thinner and coffee should be spread more thinly. Different regions could apply different norms based on climatic differences

    - Once the coffee is somewhat dry, on average one full day for parchment and three for cherry, heap and cover it at night. When fully wet, there can be water loss during the night and covering would produce condensation. This protects the coffee from re-wetting from dew or showers.

- During the day, turn the coffee layer four times per day if possible. Although it is difficult to demonstrate that raking more than once per day reduces the drying period, coffee in a static bed has been observed to become covered in mould.

- Take measures to prevent access of farm animals to the coffee. Coffee is a foodstuff and should not be exposed to agents commonly found on and in livestock and even local introduction of water to drying coffee must be avoided.

- Be aware and regularly monitor CBB populations on the drying yard, during cherry drying. The concentration of cherries can attract females from the surrounding area and extra damage to the crop can take place during drying. Use alcohol traps around the yard to help control them.

- In showery weather, be prepared to protect dry or part dried coffee from rain. Persistent re-wetting can produce unfit coffee. Cherry coffee that has been on the yard for less than about three days will be little affected by some re-wetting but parchment coffee should always be protected.

- Establish a routine, a standard approach for assessment of the dryness of coffee as it approaches full dryness (<13% or <12% (wb) for cherry and parchment, respectively). For guidance, and bearing in mind that over-drying is also undesirable from the producer's perspective, a lot should first be assessed two or three days before it is expected to be dry. Reassess at least daily, depending on subsequent drying conditions. Assemble a sample from several positions in the lot accounting for any shading across a part of the lot.

- Traditional methods, such as biting or shaking, can be effective in this assessment but stronger measures to 'verify' these measures against a reliable instrument should be undertaken than are usually in place in the field. It is imperative that if a meter is used, the person using it has been well trained and that the instrument is calibrated at least annually, preferably just prior to harvest season.

5. Organise the operations on the drying yard. Make sure the workers are trained in what they are expected to do. Have a ready reference available of what is supposed to be done. Clearly delegate responsibilities and make sure that essential tasks are recorded as completed so in the case of the absence of the designated person, the task will get covered. Most farms cannot afford to delegate a team or even a man to solely to oversee drying operations so communication between the workers should be facilitated to assure the best application that is possible.

6. Once dry, store the dry product in clean sisal sacks in appropriate storage conditions. Storage of the dried cherries or the dried parchment coffee ('*en casca*' or '*en parch*') is appropriate, especially if it is intended to retain the product for some time on the farm.

7. After the harvest season, clean and protect the drying surface and equipment as appropriate. Before drying commences, inspect, repair, clean and

commission the equipment, and the on-farm store or go-down. This includes easily over-looked items such as baskets, tarpaulins, rakes, barrows, sacks, stitching cord etc. – develop a checklist.

1.4.5 Cherry and parchment handling and local trading

Handling coffee in local trading varies a great deal in different producer countries both with respect to the chain structure and how the functions are executed. These functions include various value-added operations such as removal of remaining fruit tissues, cleaning, sorting, grading (into size classes) re-bagging, sometimes re-drying. It also includes storage and transport. In general it is in the form of green bean that coffee is traded.

Throughout this period the coffee must be protected from degradation, re-wetting, cross-contamination and, indeed should be improved through sorting and cleaning. Sale and shipment to a roaster ends this stage of the 'coffee chain'.

In storage, coffee will continue to dry if the air is drier than the coffee (a relative humidity less than about 60%) but if the air is more humid than the coffee (a relative humidity of more than about 80%) the coffee will begin to absorb water. Since storage periods can extend for a considerable length of time, even very slow changes can become problematic. Routes of re-wetting include moisture migration from damp floors and walls, leaks or wind-driven rain, dead air, and blending of dry with wet coffee. These are all controllable by following good practices in adequate facilities complemented with routine monitoring so to diagnose and act on a problem before the consequences emerge.

Moisture content is the principle parameter for predicting storability and an important part of assessing the current status of a coffee lot. Few farmers have moisture meters, used to make rapid determinations of moisture content, but they are more common amongst traders. Meters make an indirect estimate based on the electronic properties of coffee and are calibrated to one or a few samples of known (typically by the oven drying method) moisture content.

Their veracity is subject to several limitations aside from sampling, which affects all methods. Coffee can differ significantly between lots according to physiological and processing historical differences and these variations cause unexpected errors in the moisture determination by meters. In addition, the equipment drifts off calibration, could be maliciously adjusted off calibration and is subject to errors of use due to inadequate training. The use of these instruments is deceptively simple but it is not a foolproof or trivial measurement.

Aside from storage certain value-added functions will be conducted but it is impossible to generalise as to who and when the several functions are executed as coffee moves from the farm to the exporter because the various sectors differ widely. The coffee must be de-hulled or de-husked which may be done by the farmer or not, it may change hands several times, get blended with other coffees, get re-dried, get sorted in any one of several ways and graded (sorted into size classes) get cleaned polished and weighed into sacks.

Results, based on lower grades of coffee, have indicated that certain defect classes can contain highly elevated OTA content. This is by no means a universal observation and further investigation is urgently required to clarify the relationship between types of defects and OTA contamination. In the meanwhile there is a case for special handling of defects implicated in OTA risk. Tolerance for such defects in sorted green bean should be low and the out-sorted defect beans should not be re-blended into clean coffee or sold to directly to roasters unless direct OTA analysis, with a suitable sampling plan has shown them to be acceptable on public health grounds.

Between stakeholders there is, of course, a transportation step. Depending on the conditions of roads and remoteness, coffee may be transported locally from place to place moving around the highlands on motorbikes, jeeps, lorries or trains or taken directly to the harbour-based exporters. This last transfer implies a significant climatic change, which could require additional measures to avoid rewetting of coffee.

All parts of the production chain are, of course, sensitive to market forces. The local market is the part of the production chain, perhaps most sensitive to changes in demand. If there is a demand for coffee that has been handled according to hygiene recommendations, practices will be undertaken to supply it. This means the potential for influencing practices through regulatory and non-regulatory mechanisms is at its highest, a fact that should be taken under consideration by concerned authorities. Ensuring that producers reliably operate in a way that assures the safety of their product should be the overriding consideration of any intervention.

Each of the stakeholders can contribute to protection of the coffee as it passes along the chain by establishing procedures to avoid accepting suspect coffee and avoiding practices that could contribute to a downstream problem. Once dried, the coffee must be protected from re-absorbing water, whether through contact with liquid water, blending with wet lots of coffee, absorption from damp surfaces or air or through redistribution of water within the lot. Defects associated with high levels of OTA should be reduced to acceptable levels. Protection from contamination by other materials forms another imperative.

1. Each operator should establish minimum requirements related to the hygienic condition of coffee presented for sale as well as a method of rapid assessment, before purchase, to assure that coffee conforms to established minimum acceptable criteria.

    - To the extent possible, develop a list of approved suppliers who adhere to recommended hygiene practice.

    - Establish a routine for rapid assessment of in-coming coffee to include, a method of sampling that presents a representative sub-sample of the in-coming lot for moisture content determination, defect levels, general physical quality assessment and signs of mouldiness (visual or smell).

    - Use a spear to remove coffee from each sack and combine into one sub-sample. The sampling method must account for the fact that a lot may be an agglomeration or a blending of different sources so each bag must be

sampled. A spear is the most convenient tool for assembling a representative sample and the uniformity of the lot should be assessed by visual impression as this is made.

- If coffee is delivered in bulk, make up the sample by removing small aliquots regularly during unloading or with a specially adapted long spear if sampling is to precede unloading.

- Use a well maintained and calibrated moisture meter to estimate moisture content. Moisture content is a good predictor of storability but not of past handling.

- Aside from the basic records of purchase and sale where weights and prices will be recorded, maintain a complete record of the evaluations, moisture content, location of origin, and any feed-back from downstream (e.g. reports of cup quality, curing reports, complaints) that you may become aware of.

- Improve the criteria according to which in-coming coffee is assessed based on an annual review of the records. Match as much as possible the receiving assessments with the outcome of more detailed or specific assessments. Of course, the extent to which this is possible is limited by the need to pool batches of coffee.

2. The design and structure of the storage facilities play an important role in maintaining dryness and uniformity of the stored coffee. Storage facilities do not have to be expensive structures but they must be sound:

   - The best facility has a high ceiling and ample air circulation, cement floor with a damp-course and is not subject to local flooding, even during heavy rains. Assure the roof and any windows are sound and prevent water ingress. If possible, route any water supply around the storage area so any plumbing problems that might arise do not wet the coffee.

   - Stored coffee should not be exposed to direct sunlight or located where there could be local heating that could lead to temperature differentials and water migration.

   - If coffee is stored in bulk, the best arrangement is purpose-built silos with elevators. Less expensive but also effective for bulk storage is the slatted wooden bin (‘*tuhla*’), which are not part of the outside walls and chocked up above the floor. A door formed of removable short slats held in rails allows convenient filling and removal of the coffee.

3. The objective of operating a storage facility is to optimise the organisation of the facility so that cross contamination and the reintroduction of moisture is prevented and execution of receiving, sale and value-added operations are facilitated. The quality of the product has to be preserved until it is sold to the next stakeholder in the marketing chain.

   - Maintain records of receiving so that the initial condition and age of all stocks is known.

- If sacks are used for storage, do not stack them directly against walls and arrange them so that air can circulate freely. Use pallets to prevent direct contact with the floor.

- Cleaning and maintenance programmes should be implemented to ensure that storage facilities are periodically inspected, cleaned and renewed.

- Facility inspection should include checks for evidence of the coffee weevil. These insects can only survive in coffee too wet for suitable storage so an infestation signals the presence of wet coffee. Remedial measures should include eradication of the insect and correction of high-moisture problem.

- Many operations, including farms, will need to maintain a separation of coffee types so they should plan the storage area and labelling system to accommodate this requirement. Coffee stores, including on-farm storage areas, should not be used for the storage of non-food materials that could lead to contamination or taints.

- If appropriate to the period of storage, institute a monthly check of moisture content measurement of the stocks and take action accordingly. Possible remedial action in case of unacceptable moisture uptake from surrounding air could include activation of extractor fans or re-drying.

4. Cleaning and sorting of coffee should not result in physical damage to coffee that could make it more susceptible to contamination/deterioration, should not introduce new contamination and should assure reduction of undesirable materials to acceptable levels, in accordance with pre-determined criteria.

    - Cleaning and maintenance programmes should be implemented to ensure that the facility and equipment are inspected, maintained and thoroughly cleaned at regular intervals.

    - When cleaning and sorting coffee is combined with storage, consideration should be given to measures, such as partition walls or extractor fans, to avoid contamination of post-cured coffee with the curing by-products of dust and foreign matter.

    - Remove defects from main-crop production stream. Such off-grades should either be discarded or subject to screening before inclusion into to the human food chain. OTA can occur in coffee of any class, grade or origin and no certain pattern has emerged relating its occurrence to any region, practice or circumstance. However, its distribution within the classes of beans separated from bulk coffee is not uniform and there is evidence that defect beans and husk (also considered as a defect) sometimes contain substantially more OTA than the corresponding sound beans. Likewise, silverskin can contain a disproportionate amount of OTA compared to the sound bean. National authorities should provide clear guidance on the basis of further investigations of OTA contamination of defects.

5. Transport of coffee can be considered as an extension of storage of coffee but introduces distinct practical challenges in meeting the storage strictures of avoidance of re-wetting, from whatever source, maintaining uniformity of temperature and preventing contamination by non-food foreign materials.

    - Where appropriate operators should develop a list of approved transport service-providers who operate in a way that is consistent with Good Hygiene Practices for transport of coffee.

    - Inspect the vehicle for residues from previous loads and holes that could allow penetration of water or exhaust fumes to the cargo. Pay particular attention to the floor and around wheel wells since water from the road surface, even after light rains, could be channelled into the cargo bay by the wheels.

    - Regular maintenance of the vehicle is particularly important since a breakdown could lead to unexpected exposure in the open.

    - The longer the period in transport, the more important is the condition of the vehicle or container and the requirements of their use.

1.4.6 International transportation

Coffee is only transported from producer to consumer nations in bulk, mostly in containers holding from 18 to 22 tonnes depending on whether the coffee is loaded in sacks or as bulk. Even well-dried coffee in these volumes contains a great deal of water that, as long as it remains evenly distributed, poses no problem. However, temperature fluctuations can cause condensation and local re-wetting and standing temperature gradients can cause redistribution of water and lead to fungal outgrowth.

1. Loading and off-loading areas should be covered to protect coffee from rain.

2. Assure that coffee intended for export is dried uniformly and below 12% m.c. (wb). Check that the coffee is free of foreign matter and excessive defect beans, according to classification.

3. Inspect the empty containers for residues from previous loads or moisture. Check for obvious structural damage that could lead to a further failure during loading onto ship. Check for evidence of minor structural damage that, never the less, could allow the entry of water.

4. Load coffee preferentially in bulk in a sealable plastic liner – taking care that the liner is well away from the roof of the container.

5. If sacks are to be used, stack them so that the stacks cross over for mutual support and empty vertical columns (chimneys) are not formed. Cover the top layer of sacks with heavy cardboard to absorb any condensation that might form despite precautions. Silica gel packages are also sometimes used to absorb moisture in the air. Their efficacy is unknown and protection against contamination of the coffee by the silica gel must be assured.

6. Coffee is best shipped in a protected location aboard ship out of direct sun because direct sun will cause local heating of the container.

Part E

Surveys of the Market Chain and Socio-economic Studies of Selected Issues

Covering coffee sacks ready for overland transportation, Uganda

Part E
Surveys of the Market Chain and Socio-economic Studies of Selected Issues

1.1 Introduction

The project emphasised a systematic and evidence-based approach to understanding and addressing potential and actual food safety problems along the coffee chain. This approach has required the conduct of field trials and a number of surveys during the course in order to guide and inform activities.

Part C of this report outlines findings of the field trials carried out under the project, and examines their implications for food safety management at all stages of coffee production, processing and marketing. These findings, as well as a general understanding of the underlying issues affecting mould growth in commodities, led to the development of the '*Guidelines for the Prevention of Mould Formation in Coffee*' which are presented in Part D of this report.

Information from field surveys was very important in directing the design of experimental trials both in terms of relevance and focus. Furthermore, the data coming from market surveys, covering both producers and traders, is a valuable addition to the experimental results obtaind. Surveys carried out under the project sought to define the conditions under which coffee is handled and to record data on selected quality criteria as the coffee passes through existing market channels.

Several examples came to light during the course of the project of responsible national institutions being unaware of behaviours and trends affecting coffee handling in the local chain. This indicates that existing mechanisms for information collection should be rendered more effective. There is, in some cases, a tendency for unidirectional information flow when extension services meet with farmers. This is unfortunate as it is a lost opportunity to remain up-to-date with practices and problems confronting producers themselves - information that should be regularly harnessed by the authorities that plan sector development and advise on policies affecting the sector.

Other surveys were carried out during the project in response to specific country-level issues that were identified by the project team or determined by the counterparts themselves to fill information gaps that they deemed essential to support upcoming decisions to be made about the sector.

In the next Section the surveys that were completed under the project are outlined. The full reports of these surveys are available as Annexes to this report and can be found on the enclosed CD-Rom.

1.2 Market Chain Surveys

The planning of market chain surveys was based on a clear description of how coffee flows from producer to exporter, or roaster, in the domestic marketing chain. A sampling/interview plan was devised so that all the significant 'agents' were included and appropriately represented. Usually this was followed up by a second questionnaire and in some cases samples would be taken as part of the interview of these stakeholders. There were the inevitable financial constraints on the intensity of any of these studies, since the size of the sector invariably outstrips the capacity to sample it.

Any mycological analysis of the samples was conducted according to the standard methods of the project, defect analysis was made using local norms, m.c. by a validated oven drying method, and A_w using water activity meters provided by the project.

1.2.1 Market chain survey of various coffee producing districts in Uganda

In this study the marketing chain was characterised as comprising between one and three levels from the farmer to the exporter. The range of practices of stakeholders at different levels in the marketing chain is documented in the report of the national consultant which is available as Annex E.1 on the enclosed CD-Rom.

The survey revealed several aspects of existing practice that were not previously recognised, such as the trade in fresh cherry by some farmers and common delays between harvest and drying of roughly 3-4 days, motivated by the need of farmers to rationalise the time spent looking after drying operations.

The reports also provide useful information on the perceptions and concerns of farmers, traders and exporters. This is all information that should be taken into consideration by the responsible authorities in planning interventions geared at improving hygiene practices at all stages of the chain.

The surveys also focussed on the assessment of selected quality criteria of coffee samples taken at different stages of the chain.

Table 1.1: Characterisation of coffee in the Ugandan robusta cherry trading chain (Musaka and Mukono).

Stakeholder	m.c.	Ochre aspergilli (bean infect)				OTA
	(%wb ± std error)	Mean	Median	Proportion +ve	Spread	(ppb ± std error)
Farmer (S4)	17.4 ±25	5.9	2	0.59	51	1.2 ±79
Collector (S3)	18.7 ±27	18.9	8.2	0.57	75.5	3.6 ±111
Small Trader (S2)	14.5 ±19	14.7	4.3	0.64	75.5	2.4 ±110
Large trader (S1)	14.2 ±22	10.4	4.1	0.53	93.8	2.4 ±126

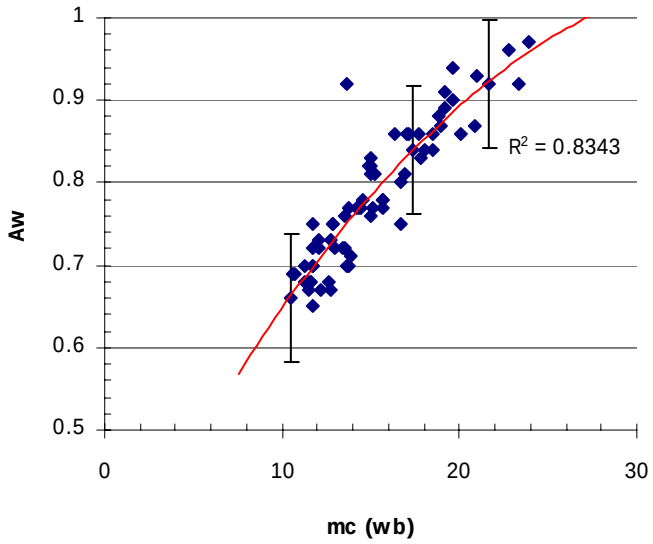
Because of high variability, there are no statistical differences in the calculations presented in Table 1.1, so the remarks that follow refer to nominal trends in the data.

This is as much as to say that, for example, ochre infection rates are lower at the farm gate than amongst collectors but a proportion of samples from farms have higher rates than a proportion of samples from collectors.

At the farm gate the Ugandan coffee apparently has a lower infection rate than later in the chain, and this corresponds to a lower OTA level. The frequency of samples positive for ochre occurrence above detection limit is fairly constant at somewhat over 50% so with a greater spread in the trading chain, there is the implication that a proportion of lots have become more infected while in the chain. Based on the m.c. data, it is at the collector level where this is most likely to happen though with a substantial standard error on 14.5% m.c., which corresponds to an A_w of about 0.78 in this material some 33% of lots from the S2 and S1 levels will have an A_w above 0.82 (see Figure 1.1 below). This is, of course, sufficient to allow slow growth and OTA production. A second survey covering additional coffee growing districts (Luwero, Jinja and Kamuli) showed an even wider range of moisture contents through the coffee chain 14% (S1) to 28% (S4).

With respect to OTA contamination reported in the first survey, there may be a slight increase in going from the farm gate to the trading chain.

Figure 1.1: Relationship between oven m.c. (wb) and A_w in samples from the Ugandan production chain. The regression is 2nd order polynomial and error bars are at one s.d.



The second market chain survey included OTA analysis of over 70 coffee samples. The levels of contamination range from 0-15ppb with an average of 2.3ppb. The contamination levels, however, showed no correlation with moisture levels or with stage of the marketing chain.

1.2.2 Market chain survey in India

In this survey 137 coffee holdings were visited in 5 main coffee producing districts to identify socio-economic factors that influence their practices relevant to the hygienic handling of coffee, and to characterise the coffee flows through the marketing chain. The second phase of the survey described practices of main stakeholders at different

stages of the marketing chain and assessed coffee samples for moisture, mould and OTA contamination.

The report of this survey presents many interesting statistics on handling practices that should inform local and national training and information plans (report available as Annex E.2 on the enclosed CD-Rom). As has been noted in many other cases, farmers point to the lack of incentive for improving practices. They note that the marketing system already readily absorbs their coffee and that no price premium is provided in the mainstream coffee market for better coffee.

An example of a widespread practice that could have significant implications for management of OTA risk is the inclusion of 'gleanings' in the coffee marketing stream. This coffee is generally left on the ground in the orchard for 2-3 weeks after harvesting prior to collection and drying. All farmers interviewed stated that they collected and sold this coffee – with some selling it separately and others mixing it in with coffee from the main harvest.

There is evidence from the project's field trials that this is a highly risky practice. Now that they have been alerted to this fact, the Coffee Board of India should work with other concerned food safety agencies to carry out targeted monitoring of these low quality coffees in the market chain. They must also take steps to correct farmers' practices.

Table 1.2: Prevalence of selected practices in dry processing of coffee in four districts in India.

Dry Processing Practices	Chickmagalur	Coorg	Hassan	Wynad
Sorting cherry (percent)	Nil	Nil	17	Nil
Mixing floats & gleaning with main cherry lot (percent)	75	50	83	14
Heaping harvested fruits before drying (percent)	75	100	83	100
Duration heaping (days)	2.75	1	2.59	3.57
Day for cherry drying (days)	14.38	13.00	12.00	10.14

Table 1.3 presents the average moisture contents and A_w values of coffee. There is a general decrease in moisture levels as coffee proceeds along the chain from farmer to exporter, as was seen in the case of Uganda. This is likely largely due to the fact that the larger traders and exporters re-dry coffee that they receive. It could also be in part related to the observation that the use of moisture meters was relatively rare among traders. The values of water activity measured during the survey were at all times below 0.80 which represents the lower limit for OTA production. It should be borne in mind that variability in the moisture content within a batch could render some areas of the coffee vulnerable to contamination even if the 'average' moisture level is safe.

OTA was detected in over half of the coffee samples but at low levels. The highest level of contamination recorded was 2ppb.

Table 1.3: Moisture content, A_w and OTA levels in coffee samples collected during market chain survey

Type of coffee	Source	Oven moisture (%)	A_w	OTA (ppb)
Arabica parchment	Grower	12.7-16.2	0.697-0.75	BDL-0.196
	Local agent	9.7-14.8	0.598-0.745	BDL-0.293
Arabica cherry	Grower	NA	0.755	0.20
	Local agent	12.9	0.704	BDL
	Exporter	10.4	0.687	BDL
Robusta cherry	Grower	10.9-13.1	0.718-0.781	BDL-0.819
	Local agent	11.50-13.8	0.698-0.76	0.408-1.31
	Exporter	10-11.9	0.421-0.774	BDL-1.98

BDL – Below detection limit (< 0.02 ppb).

1.3 Targeted Surveys and Socio-economic Studies

Targeted studies and surveys were conducted in some of the project countries in order to respond to the need for additional information to support decision-making on specific policy and programme issues. Several of these studies are outlined below, the full reports are available in Annexes E.3 to E.11 on the enclosed CD-Rom.

1.3.1 Targeted survey in Northern Rift Valley, Kenya

Background and objectives: There are two long-standing routes for the handling of coffee in the Kenya coffee sector: the estate sector and the cooperative sector. However, recently there has been a change in regulation allowing farms of two hectares or more, or groups of smaller farmers, to process and market their own coffee creating a 'new' sector.

There are 250 processing units registered with the Coffe Research Foundation (CRF) which are concentrated in the Northern Rift Valley. This is a new and relatively small coffee producing area but it is growing in importance accounting for over half of the new plantings over the last decade.

In light of this, and also in view of the national policy geared towards improvement of Kenyan coffee quality as part of the national strategy to regain a competitive edge in the international market, the CRF wanted to know the extent of on-farm processing in the Northern Rift valley and the implications on coffee quality and OTA contamination. The main objectives of the survey were the following:

- To determine the extent of on-farm primary coffee processing;
- To establish reasons that have promoted this practice;
- To assess the divergences from recommended processing practices;
- To propose interventions in order to limit the effects of the constraints and capture the opportunities towards quality coffee production.

Findings and application: The survey showed that the extent of on-farm processing was much higher than previously thought. Many farmers who are registered with cooperatives actually process their own coffee and deliver the parchment to the cooperatives for marketing. Only 30% of the on-farm processing units met the requirement established by the Coffee Act No. 9 of 2001, whereby registered farms should be at least two hectares in size.

The responsible authorities should either reconsider the appropriateness of the existing requirement or review the procedures in place for registration.

Nearly half of the farmers surveyed said that they would have opted for communal processing units if they existed within their localities. The others affirmed that they would continue processing their own coffee in order to achieve either prompt/higher payment, or to have greater decision-making power by working independently. Farmers' stated motivation for undertaking on-farm processing did not seem to have any bearing on their processing practices or the condition in which they maintained processing facilities. It is not clear how the expectation of 'higher prices' could be satisfied in the apparent absence of effort to improve coffee quality.

There was widespread use of poor processing practices among the on-farm processors with roughly ¼ of respondents encountering delays in excess of 17 hours between harvesting and onset of processing, non-observance of recommended fermentation practice, and only 15% having adequate drying table space for their production. There was a generally low level of cleanliness, poor maintenance of facilities and inadequate provisions for waste disposal. This was despite the fact that 75% of the operators reported frequent visits by extension staff.

The survey did not include any quality assessment of the coffee produced at the facilities surveyed.

If the national authorities want to support growing coffee production in the Northern Rift Valley and at the same time consider quality improvement to be a strategic necessity for the national coffee sector, then some action is clearly required. The CRF must identify what changes in farmer behaviour are most important in terms of OTA prevention and quality improvement and focus on facilitating these changes.

The findings of the survey (see Annex E.3) point to the fact that reversion to communal processing would not resolve the problems of poor practice observed in the region. Poor maintenance of facilities, inadequate drying table space and unreliable services (water and waste disposal) were among the problems observed at the cooperative factories included in the survey. Furthermore the high rate of staff turnover at the cooperative factories leads to the problem of poor knowledge and inexperience of factory managers.

1.3.2 Targeted survey in Lampung, Indonesia

Background and objectives: Approximately 70% (roughly 300,000 tonnes) of coffee produced in Indonesia comes from the 3 provinces of Lampung, South Sumatra and Bengkulu. This coffee typically has high defect levels with a number of different defect types, derived from coffee berry borer, poor harvest practices, poor drying and storage, and poor hulling. There are also defects such as 'brown' bean, the source of which is not clear.

Indigenous farmers account for 30% of the coffee produced in these provinces. These farmers use traditional practices such as heaping and 'composting' of coffee to facilitate its drying. The remainder of the coffee is produced by transmigrant farmers, originally from Java, who employ rudimentary systems for the processing of coffee.

One private sector initiative aimed at improving coffee quality has been operating in Lampung for a few years. Nestlé has established a buying programme in Ngarip Village and Tanggamus Village, where they work with farmers in order to source Grade 4 (the lowest exportable grade) robusta with low risk of OTA contamination. The Nestlé programme involves farm extension and price incentives and is said to produce about 2,000 tonnes of coffee per year.

Image 1.1: A team of socio-economists interview a farmer to understand 'the wider picture'.

The Indonesian Coffee and Cocoa Research institute (ICCRI) is concerned about the low quality of the coffee produced in these provinces and about the risk of OTA contamination. The survey was planned in order to provide information that could allow them to formulate an appropriate and sustainable approach to improving coffee quality in these areas. Due to practical constraints the survey was conducted in a single village where the Nestlé buying scheme operates side-by-side with the traditional marketing systems. The objectives of the survey were the following:

- To define the range of practices of each stakeholder group that is involved within each of the three main marketing systems currently operating in Lampung;
- Identify points of the chain associated with high risk of OTA contamination;
- Identify opportunities and constraints for sustainable approaches to improving coffee quality and safety in Lampung.

Findings and application: Coffee production is of great social and economic importance in the village surveyed, accounting for roughly 75% of farm income, however, low coffee prices has meant that the average income in the coffee producing area is below the poverty line. Family labour is generally used to accomplish tasks related to production and marketing of coffee and many other farm activities compete for the available labour.

The prevalent system of marketing does not distinguish between good and bad coffee so in this context there is no reason for farmers to adopt improved practices.

The experience of the Nestlé buying scheme suggests that strengthening/creation of small farmers groups could be an important element in developing opportunities for price incentives in coffee marketing, but also underlines that the opportunities for price incentive schemes is limited in the present marketing context.

Table 1.4: Analysis of asalan (bulk green coffee) as it moves between stakeholders in the Lampung trading chain.

Stakeholder	moisture content		defects		margin
	mean %wb	CV%	mean #/300g	CV%	Rp/kg
Farmer	19.4	14	211	92	- -
Collector	19.1	10.6	189	47	718
Trader	17.8	8.9	140	27	450
Exporter	12.7	5.9	58	20	400

Coffee quality is extremely low with ***average*** moisture contents of unsorted green bean in the marketing chain at around 19.5% and average defect counts of 210. The limited amounts of coffee traded according to the Nestlé system are of much higher quality. The period of time over which the vast majority of the coffee is maintained at unacceptably high moisture levels could support growth of OTA producing mould and OTA accumulation. Despite the presence of 'high risk' conditions, the actual levels of OTA found in the coffee during the survey were relatively low, with an average value of 0.74ppb and a maximum of 2.7ppb.

This lack of correspondence between poor handling practices (high moisture over long periods) and observed OTA contamination levels creates difficulties in generating the will – locally, nationally and internationally - to aggressively address the need for Good Hygiene Practices (GHPs) along the marketing chain. The very high defect levels found in this survey also raises some interesting questions. Specific surveys that were conducted elsewhere under the project noted associations in some cases between certain defect classes and OTA content (see to Part C, Section 9). Closer examination of the defects in this coffee could be of practical interest.

The report of the national consultant socio-economist (Annex E.4) contains a number of recommendations for consideration by the ICCRI in developing their programme for coffee improvement in Lampung.

1.3.3 Targeted survey in Northern Sumatra, Indonesia

Background and objectives: 'Mandheling coffee' is renowned in the world of speciality coffee. 'Mandheling' is the generic name for the range of coffees from Northern Sumatra, exported out of the port of Medan, which have a spicy flavour, complex, earthy body and low acidity. There is no clear understanding how this Mandheling character is derived. The character is likely to be complex relationship between the four key factors of: a) coffee variety, b) growing environment, c) farm

management and d) processing. The relative importance of each these factors and the interactions between them in the formation of Mandheling character is unclear.

The system of processing and marketing of this specialty coffee involves maintenance of the coffee at high moisture contents for long periods. Theoretically, this is an important risk factor in mould formation and OTA contamination.

Demand for this specialty coffee has grown steadily from 12,000 tonnes in 1992 to almost 30,000 tonnes in 2004. However, complaints about inconsistencies in cup quality threaten to reduce demand and premiums for this specialty coffee.

The best solution for this dilemma is to develop processing and trading systems that maintain the organoleptic characteristics of the coffee and at the same time minimize the risks associated with physical coffee quality and mould formation. In response to this problem, a survey was organized in three Mandheling-producing districts, Humbang Hasundutan, Tapanuli Utara and Toba Samosir in order to:

- Define, describe and verify the key steps in the processing and trading system of typical Mandheling coffee;
- Identify points in processing and trading system where there is risk of OTA contamination;
- Guide further studies to support the development of sound quality assurance programmes that will prevent OTA contamination and promote consistent quality.

Findings and application: The survey found that in all three districts surveyed, the basic steps in the production of Mandheling coffee were: picking, floating, pulping, fermentation, washing, drying, and storing. The schematic diagramme of coffee processing steps in shown in Figure 1.2 below.

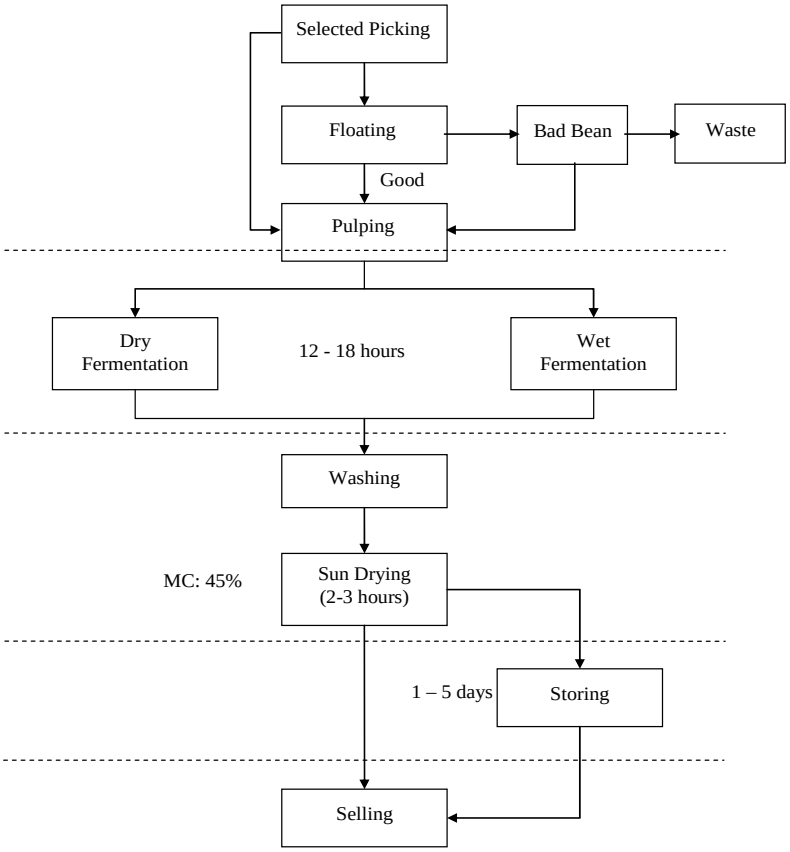
Some degree of variability was noted at each of the processing steps. Delays between harvesting and pulping sometimes occurred in one of the districts surveyed. The delay did not exceed 24 hours.

Fermentation times of between 18-24 hours were reported. Most districts practice dry fermentation while others carried out the fermentation step under water. In some cases farmers keep their wet parchment in plastic bags overnight before putting the parchment to dry.

Washing of parchment takes place at rivers or swamps and the parchment is put to sun dry for 2-3 hours before direct sale to trader or temporary storage until 'market day'.

Parchment is stored and marketed at very high moisture levels - between 40 -50 %wb. Farmers sell coffee in small quantities to collectors who sell to traders who in turn sell to exporters. The period of time that the parchment remains at elevated moisture contents (40-50 %) is typically between 3 days to 1 week, but could be longer. Hulling of wet parchment followed by drying of green bean may be carried out either at trader or exporter level. Storage of green bean was commonly carried out at moisture levels of 17%. The full report of the national consultant socio-economist is available as Annex E.5.

Figure 1.2: Generalised schematic diagramme of Mandheling coffee processing at farmer level in N. Sumatra.



Following this survey, a preliminary study was carried to consider the impact of 'residence time of parchment at high moisture content' on quality and OTA content of coffee. The report of this preliminary study is available in Annex E.6. The study investigated the impact of varying storage time of wet parchment (1, 7 and 14 days) on quality and safety of the coffee.

Mandheling flavour of coffee produced in this trial was not strong and was not correlated with any of the processing treatment considered. The processing factors considered do not, therefore, seem to be determinant in the development of the 'Mandheling' flavour.

Infection by ochre group fungi, which include the strong OTA producer *Aspergillus ochraceus*, was found to be present in 5 out of 6 wet parchment samples stored for 2 weeks. For all other storage periods, ochre group aspergilli were not detected in the wet parchments. The practice of holding wet parchment for periods exceeding one week could constitute an OTA risk.

A greater understanding of the factors that produce this Mandheling character are needed. Further work is thus required on aspects of food safety, as well as on coffee character, quality and consistency.

1.3.4 Targeted study in East Java, Indonesia

Background and objectives: East Java produces approximately 40,000 tonnes of robusta coffee, roughly half of which comes from large estates in the form of wet processed robust and the remainder produced by smallholder farmers as dry processed robusta commonly utilising the 'coffee splitting' technique to hasten drying.

Unfortunately, farm income of coffee farmers in East Java is relatively low due to problems both in the production and marketing of coffee. The ICCRI is considering a programme to promote conversion to wet processing of robusta among small holder farmers. This approach is inspired by the success story of coffee farmers in Pupuan, Bali where a similar programme was successfully undertaken with ICCRI support.

Wet processed robusta enjoys, on average, a 50% price premium as compared with the dry processed coffee. Moreover, the declining export of WIB from the large estates in East Java, from 10,306 tonnes in 2001 to only 6,848 tonnes in 2004, could provide a market opportunity for the small farmers in East Java. These plans are consistent with policy at local government level which has identified the coffee industry as one of main agribusinesses of strategic importance in the region.

The ICCRI requested support from the global project to assess the feasibility of various aspects of the planned conversion to wet processing of robusta by small holder farmers.

Garahan Village is one of main coffee-producing centres in Jember Sub-District of East Java, it is close to ICCRI, and a good working relationship already exists between the farmers and the ICCRI staff. Additionally, it is considered representative of smallholder robusta growers in East Java. This site was therefore chosen for the study.

The overall objective of the study is to assess the feasibility of implementing wet processing of robusta coffee in Garahan, East Java. The feasibility assessment included technical, management and organization, market opportunity, and financial considerations.

Findings and application: The study confirmed the interest of farmers in Garahan village to process their coffee using the wet method. It also concluded that the proposed plan was financially feasible based on current prices for wet processed robusta. It does, however, point out several issues that would have to be dealt with if such a programme is to be successfully implemented. These are briefly outlined below:

- Based on technical considerations and farmers aspirations, the wet processing equipment to be adopted by farmers' groups in Garahan should have the following characteristics: (i) medium size, (ii) mobile, (iii) be motorised, and (iv) have an integrated pulper and huller. The technical feasibility of developing wet processing in the region will be strongly dependent on the availability of suitable equipment. ICCRI should have a critical role in developing this equipment and increasing farmers' skills on technology improvement;

- In terms of management and organization, the farmers in Garahan need some training and supervision, especially in planning, reporting and effective leadership;
- Considering the potential supply and demand of wet processed robusta coffee in Garahan, it can be concluded that there is a significant market opportunity. However, the development of production has to be managed carefully to avoid over-supply. In other words, the production schedule has to follow the real market that has been clearly identified. Assistance from ICCRI or other governmental or non-governmental institutions in understanding and accessing markets and negotiating prices is important;
- Most farmers are in an interlocked market situation that blocks them from participating in new marketing initiatives to improve their coffee quality and increase their incomes. To break this vicious circle, credit availability is a determining factor. Under the new government, which has announced greater attention to agriculture in general, the provision of soft loan for farmers is expected to increase and would be an important factor for the feasibility of the wet processing initiative

The full report of the study is available in Annex E.7. An Executive Summary of the preceeding three targeted studies in Indonesia discussed above (Annexes E.5, E.6 and E.7) can be found in Annexes E.8 andf E.9a and E.9b.

1.3.5 Socio-economic study in Uganda

Background and objectives: Coffee is primarily produced by smallholders with an average farm size of 0.23 ha and is a source of income for more than 500,000 Ugandan smallholders. It is the country's leading export crop and accounts for 43% of total exports. Coffee is critical to the incomes of 20% of the country's population, with over 4 million people directly or indirectly depending on coffee growing and trading for part of their livelihood.

Uganda mainly exports robusta coffee which accounts for roughly 80% of total coffee exports, with the rest being arabica. Furthermore, Uganda's soils, climate and attitude make it ideal area to grow relatively high quality robusta coffee that normally commands a premium on the international market.

Despite Uganda's inherent advantage in producing high-quality coffee, it is generally recognised within the sector that liberalisation has brought with it a reduction of quality of Uganda coffee. This is explained by the high degree of competition in the local marketing chain which lead to low margins for coffee traders. Traders therefore make money on handling large volumes of coffee - buying policy in the local marketing chain is much more concerned with quantity than quality.

UCDA considers that it is of strategic importance for Uganda to recover its image as a supplier of high quality coffee, as well as being a source of coffee with a low risk of OTA contamination. In support of this objective, it recently embarked on a programme to promote wet processing of robusta coffee. However, the programme was initiated without the benefit of either economic or viability analysis. The project counterparts therefore agreed that an evaluation of the proposed system by the project would be useful.

As part of project activities, national consultants were hired to design and build solar driers, suitable for use by small-scale coffee farmers, which would then be tested under the project. The terms of reference for the consultants clearly stated that there should be a participatory approach to the design of the driers so as to be sure that their functional characteristics and cost met the requirements of the target users. Despite these instructions there was no evidence of participatory approach in the design of the driers, and the project therefore required an assessment of their feasibility after they had been built and tested.

The general objectives of the study were to:

- Analyse the structure of the commodity chain and the potential consequences of OTA standards on the coffee commodity chain as well as opportunities for creating price incentives for quality coffee;
- Study the feasibility of technological alternatives proposed for reducing contamination risks.

The full report of this socio-economic study, completed in collaboration with CIRAD, is available in Annex E.10.

Findings and application:

Coffee flows

A schematic diagramme illustrating coffee flows through the local marketing chain is provided in Figure 1.3. Entry barriers to coffee trading activities are low and a large number of collectors and traders are involved in this highly liberalised marketing system.

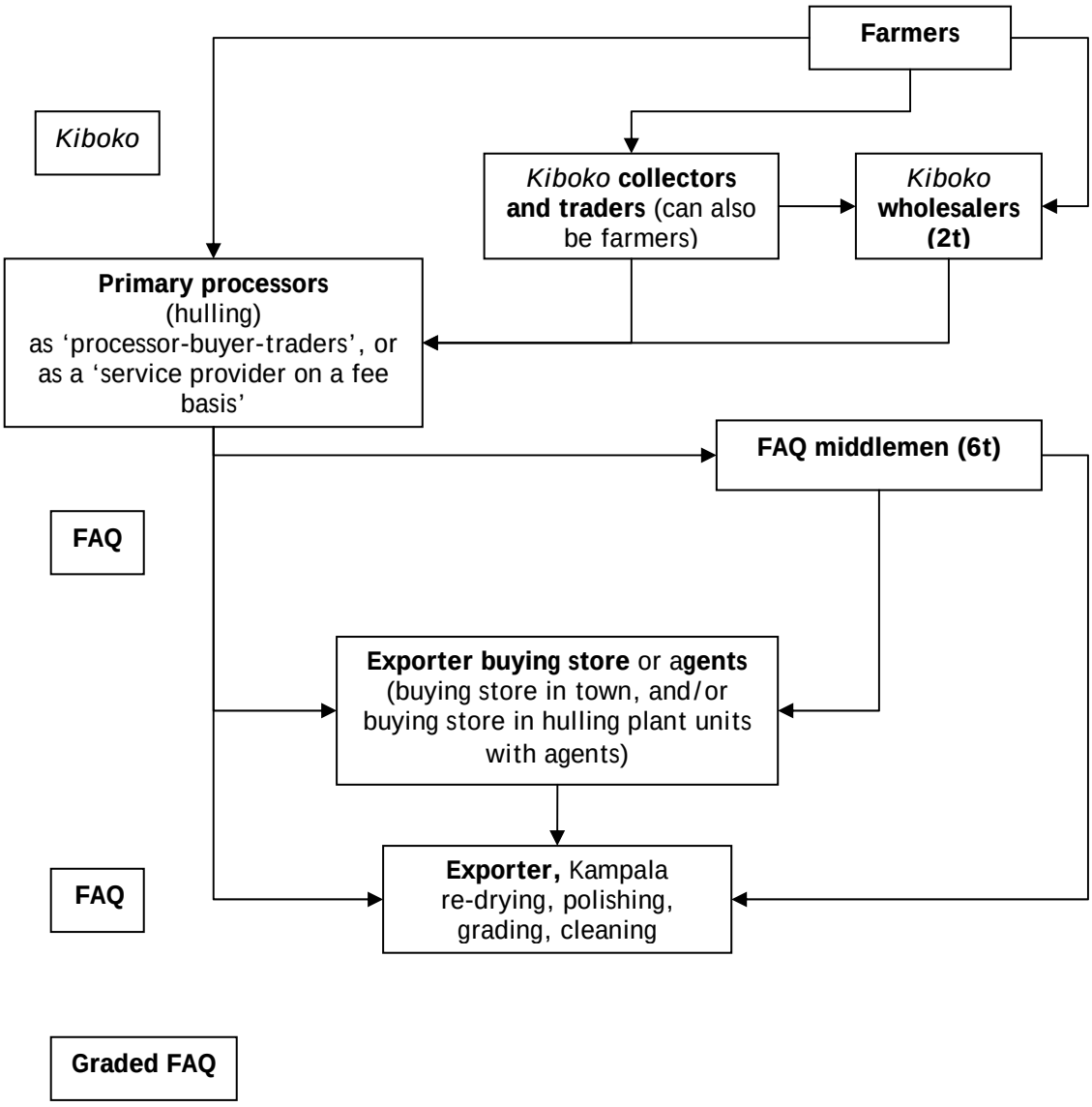
The survey revealed that fresh cherries were mostly sold by farmers. In general, only small traders (‘*Ddebeman*’), middlemen for other traders, and farmer-traders were involved in this form of trade. Trade of wet cherry is most likely linked to delays before drying. Even though the trials carried out under the project to evaluate the impact of this practice on OTA contamination failed to demonstrate that delays are a determining factors in coffee contamination, the practice is poorly understood in microbiological terms and must be avoided (see Part C, Section 5).

‘Semi-dried’, which is coffee in the process of being dried, was also found to be supplied by farmers, small traders and *kiboko* traders. Coffee in this semi-dry state is most likely to be at greatest risk of contamination by mesophilic fungi such as *Aspergillus ochraceus*, the most important OTA-producer in coffee. Trade in semi-dried and fresh cherry is not only risky, it is also less remunerative for the small farmers. Farmers considered that they made the biggest margins when trading in FAQ.

Competition and quality deterioration

All stakeholders agreed that an increase in adulteration and reduction in coffee quality could be seen (stone, husk, etc., and high m.c.) and attributed this to competition in the highly liberalised market. The low margins established for traders in the local chain mean that money can only be made through trading large volumes. The emphasis is therefore on getting as much coffee as possible, regardless of quality.

Figure 1.3: Commodity chain organization in Uganda.



Note: A *kiboko* trader is also called a *Ddebeman*.

The current functioning in the commodity chain is based on volume and rapid transactions, and this militates against effective control of moisture and other parameters that influence OTA risk.

Quality premiums in the local marketing chain are rare, and criteria other than moisture content or number of defects are rarely reflected in premiums for FAQ, never for *kiboko*.

Discounts, on the other hand, are commonly applied in the coffee trade. Traders use standard discount formulae for weight loss that are disadvantageous to sellers in reality: the formulae deduct slightly more weight than the true weight loss. This tends to increase the margin of the buyer to increase his margin and/or to protect himself from risk.

Feasibility of quality coffee initiatives

The study investigated one 'Quality coffee scheme' that was in operation in the Masaka coffee district with the assistance of USAID, and under the management of IBERO, a coffee exporting company. According to that scheme, farmers were provided with training and were required to apply agreed harvesting and drying practices. They received a 'premium' of between Ugandan Shillings (USH) 50 – 150 (approximately US$0.02 – 0.06) for their dried *kiboko* coffee (i.e. dried cherry).

The price differential may have partly corresponded to the profit margins of middlemen between farmers and exporters, in which case it would not really be a question of quality premiums. That said, it was difficult to assess the additional costs generated by direct buying from farmers, and to find out who funded them (e.g. subsidies or companies).

The high degree of competitivity and the lack of adequate controls in the marketing chain created difficulties for the implementation of this scheme. Traders reportedly purchased this coffee at the premium price and then added in foreign bodies and black beans to the acceptable level of 5%. There was also, reportedly, widespread fraud by unscrupulous traders in the weighing the coffee delivered to them so that they actually received considerably more than they paid for.

It is important to note that, according to IBERO, this quality coffee initiative would not be viable without subsidies as the coffee did not benefit from a premium price on the international market.

The study also specifically considered the issue of the UCDA's wet robusta processing initiative. UCDA is proceeding with this programme and has guaranteed bank loans for the purchase of 20 wet processing plants imported from Brazil, which were distributed to farmers who had agreed to use their farms as lead farms for coffee villages. The coffee villages will be the source of cherry for these plants. Out of the 20 plants imported eight are in the final stages of installation.

Interviews with farmers and management of two of the wet processing plants revealed several problems. The management indicated difficulties in getting fresh cherry of the required quality for pulping, and in getting adequate quantities given the stiff competition from traders. Key concerns of the farmers were the lack of incentive prices and inconvenient organization of cherry collection by the processing facilities.

Despite the challenges the study concludes that the wet processing plan offers important opportunities to improve coffee quality and increase incomes of farmers and other local stakeholders. The opportunities focus on the need for greater involvement of farmers and the sharing of risk and benefits among the stakeholder groups. This in turn requires better organization of farmers into 'Quality producer groups' and farmers' associations.

Regarding the feasibility of improved drying systems designed or tested during the project, the study concluded that adoption of these by small-scale farmers is not feasible under the current market conditions.

Emerging structures in a liberalised market

The study reports the emergence of new organizations that could play an important role in 'overseeing' marketing practices. These are outlined below.

A Masaka District Task Force has been established which is comprises a government security apparatus, coffee stakeholders such as farmers, millers and exporters who work together to ensure that only properly dried coffee goes through Masaka district to Kampala. Masaka is strategically located as a gateway to and from Kampala for coffee that comes from Tanzania, west and south-western parts of Uganda, which grow almost 30% of the total coffee produced in Uganda.

The National Union of Coffee Agribusiness and Farm Enterprise (NUCAFE) is an association of coffee farmers in Uganda representing the interests of about 80 coffee farmer associations. NUCAFE is still very young, about 5 years old, and due to lack of funds it is not very effective on the ground. However, they represent a potential to empower farmers to own their coffee and add as much value as possible within the coffee supply chain, and to negotiate prices for their coffee.

The Uganda Coffee Traders Federation was founded in 1996. UCTF, which comprises the major coffee traders in Uganda, has in place a code of conduct to ensure that coffee regulations are followed. UCTF works closely with UCDA to ensure that there is compliance with the coffee regulation among exporters. They also participate in discussions with NUCAFE relating largely to price incentives for farmers.

While the development of such organizations may be critical for the improvement of functioning in the market chain, the report of the national consultant Dr. Kulaba (see Annex E.1), underlines the sensitivity of the question of farmer organization in Uganda. He points out that many operators particularly those outside Masaka region, are very sceptical about the idea of forming associations, probably due to a bad historical experience with defunct cooperative societies and unions of the 1960s and 1970s where corruption and mis-management were rife. His survey revealed that approximately 69% of respondents were not in favour of farmers' groups with the remaining 31% recognizing that such associations could be beneficial but would have to be organized on completely different lines from those of the old cooperative societies and unions.

1.3.6 Socio-economic study in Côte d'Ivoire

Background and objectives: As in the other project countries, the problems of poor practices in the production, processing and handling of coffee is not just a technical matter but is strongly linked to a number of social and economic issues affecting the coffee chain. The study sought to:

- Identify different stakeholder groups within the coffee sector in Côte d'Ivoire, and clarify their roles;
- Define the range of current practice within the marketing chain, identifying points of the chain where there is greatest risk of introducing problems of mould and OTA contamination;

- Identify opportunities for creating sustainable incentives for farmers and other stakeholders to apply Good Hygiene Practices in the production and handling of coffee so as to reduce OTA contamination.

The study was intended to include careful consideration of current policy initiatives by the government in support of the establishment and operation of cooperatives and the implications of this policy for quality and safety of coffee and price incentives for coffee farmers.

Findings and application: The study provides an account of current practices by farmers, and shows that less than half of the producers follow basic hygiene practices in the harvesting and drying of their coffee. The main constraint to the improvement of practices by the farmer is reported to be the lack of price incentive in the marketing of coffee.

The study concludes that the solar driers that were evaluated by the project are not feasible for use by smallholder farmers.

The report highlights general policy issues that are negatively affecting the coffee chain such as: the need for better rural infrastructure, the need for upgrading the transport sector nationally and the need to resolve the problems caused by security road blocks.

Unfortunately the report does not sufficiently address the policy of strengthening the cooperatives and its possible implications for quality and safety of coffee. Such guidance would have had a much greater utility.

The full report of the study (in French) is available as Annex E.11 on the enclosed CD-Rom.

1.4 Concluding Comments

Poor post-harvest practices are certainly implicated in the problem of OTA contamination of coffee. Awareness of current practices is important to enable responsible authorities to know where main risks lie and to plan effective and efficient actions to address the problems.

The experience of the project clearly demonstrates that purely technical solutions will not solve the problems of poor practices along the chain. The problems and sustainable approaches to address them have important social and economic dimensions. The surveys and studies reported above will help guide decisions about the national programmes of OTA prevention.

The core problem is that the mainstream market doesn't distinguish between coffee that is handled according to Good Hygiene Practices and coffee that is not. This is largely driven by marketing forces in the international coffee sector.

In recognition of this, the project commissioned a socio-economic study on the feasibility of enforcement of Good Hygiene Practices along the coffee chain in the international context. The complete report of this study by LMC International Ltd. is available as Annex E.12 on the enclosed CD-Rom.

This study concludes that there is a marked difference between scenarios where there is limited enforcement of hygiene requirements compared with strict enforcement scenarios.

Under a limited enforcement scenario there is a negative impact on grower returns whereas, under a strict enforcement scenario, the returns to farmers are greater. In the latter case, it is not the improvement in quality that increases price but the reduction in volumes due to the elimination of poorly handled coffee. The strict enforcement scenario would have significant monitoring costs that were not incorporated into the analysis.

The analysis suggests that in a limited enforcement scenario, the benefit to farmers of upgrading coffee depends upon how much costs can be reduced along the supply chain, how much of this cost reduction is passed on to growers, and whether this increase in income makes it financially beneficial to upgrade coffee. Export premiums are unlikely in the mainstream coffee market.

The study emphasises that while the 'strict enforcement' scenario leads to higher prices, most industry participants remain sceptical about the political desirability of such programmes (among both producers and consumers) and the ability of the authorities in producing countries to enforce hygiene requirements.

Decisions on OTA prevention in coffee producing countries will, of course, be influenced by broader reality of the international coffee trade.

Part F

Training and Capacity-building

Field visit as part of a ToT course, Indonesia

Section 1
Training and Capacity-building to Improve Hygiene Practices along the Coffee Chain

1.1 Introduction

A main objective of this project was to promote practices throughout the coffee chain that would prevent or minimise contamination of coffee with OTA. Achieving that objective requires that there be a high level of awareness within the sector of food hygiene principles, and how they should be applied, by each of the actors along the chain. It also requires that there be commitment on the part of policy-makers to create a policy framework, and support programmes, that promote the use of good practice by all stakeholders.

In light of this, training activities under the project focussed on developing groups of trainers to continuously provide training and information on good practices in the production and handling of green coffee as required by the different target groups. It also emphasised awareness-raising of policy-makers of the need for adequate attention to the safety of coffee.

1.2 Training of Trainers' (ToT) Courses on the 'Application of Food Hygiene and HACCP Principles to the Coffee Chain'

The situation at the start of the project was a general lack of awareness of about food hygiene among professionals in the main technical institutes supporting the coffee sector. In most countries the coffee sector has evolved quite separately from the rest of the food sector, and coffee sector institutions were largely uninformed about the handling of food safety issues at national and international levels.

For the most part, these institutions have focussed on 'production' issues such as the development of new varieties, pest management techniques, productivity and other aspects of overall farm management and, to some extent, on selected quality issues during post-harvest handling. The emergence of hygiene considerations in the coffee chain means that coffee institutions must be prepared to provide guidance on these issues as well.

1.2.1 ToT objectives and content

The Training of Trainers' (ToT) courses aimed:

- To inform participants of the internationally recognised responsibilities of food producers to apply Good Hygiene Practices;
- To make the participants aware of the role of government to ensure that producers meet national food hygiene requirements;

- To familiarise the participants with the Codex General Principles of Food Hygiene and make them understand how they should be applied to the coffee sector;
- To explain the HACCP principles and enable participants to use a HACCP-based approach to design effective national OTA prevention programmes;
- To motivate the trainers to advise and guide coffee sector entrepreneurs to adopt recommended practices.

Image 1.1: Learning is improved when various techniques are used during training. A learning game has been included in this training session to keep participants' attention and energy levels high. A question and three possible multiple choice solutions are written on each box which contains three compartments labelled 'A', 'B' and 'C'. Each trainee has to put his name into the compartment that he thinks corresponds to the correct answer.

1.2.2 Selection of ToT participants

The participants of the ToT courses were carefully selected in recognition of the fact that a blend of expertise would be required to sustain strong national training and awareness-raising programmes.

Typically, the participants included senior technical staff from coffee extension services (private and public sector); technical/scientific staff from the coffee staff from national coffee institutes; and, in some cases, officials from government agencies involved in food safety control and representatives from academic and research institutions that could provide support to the coffee sector in finding innovative solutions to the problems facing entrepreneurs along the coffee chain.

1.2.3 The ToT courses

A total of eight national and sub-regional Training of Trainers' courses were held under the project. Six other ToT courses, utilising the central project team and the training materials developed under the project, have also been held (see Box 1.2 for details).

Image 1.2: ToT course participants observe cherry sorting operations and take notes during a field visit in Indonesia.

Box 1.1

Since 2001 approximately 93% of all coffee export origins[1] have had trainees participate in one of the ToT events organised under either the 'global' project or associated FAO-funded TCP projects.

[1] Calculation based on total average exports and global production figures between 1995 and 2004. Source: ICO.

Box 1.2

Summary of Training of Trainers' (ToT) courses 2001-2006 'Application of Food Hygiene and HACCP Principles to the Coffee Chain'

1. India (November 2001) – National ToT course for 10 extension officers and 10 coffee scientists working at the central and the regional offices of the Coffee Board of India. *Trainers: Renata Clarke (FAO), Rinantonio Viani (Consultant, Switzerland), Daniel Duris (CIRAD, France) and Coffee Board of India staff.*
2. Indonesia (August 2002) – National ToT course involving 22 participants from the Indonesian coffee sector. *Trainers: Renata Clarke (FAO), Daniel Duris (CIRAD, France), Rinantonio Viani (Consultant, Switzerland), Okky S. Dharmaputra (Consultant, Indonesia), Ir. Surono (Consultant, Indonesia) and ICCRI staff.*
3. Ecuador (April-May 2003) – Sub-regional ToT course involving 24 participants from Costa Rica, Ecuador, Panama and Peru. *Trainers: Mick Frank (Consultant, UK) and Gloria Puerta (Cenicafé, Colombia).*
4. Kenya (May 2003) – Sub-regional ToT course involving 28 participants from Côte d'Ivoire, Ethiopia, Kenya and Uganda. *Trainers: Renata Clarke (FAO), Mick Frank (Consultant, UK), Daniel Duris (CIRAD, France) and Gerrit van der Stegen (Consultant, The Netherlands).*
5. Indonesia (August-September 2003) – Sub-regional ToT course involving 20 participants from Lao PDR, Papua New Guinea, Thailand and Timor Leste. *Trainers: Ezzeddine Boutrif (FAO), Gerrit van der Stegen (Consultant, The Netherlands), Ir. Surono (Consultant, Indonesia), Abdul Cholil (Consultant, Indonesia) and ICCRI staff.*
6. Guatemala (October 2004) – Sub-regional ToT course involving 30 participants from Costa Rica, Cuba, Dominican Republic, El Salvador Guatemala, Honduras, Jamaica and Nicaragua. *Trainers: Gloria Puerta (Cenicafé, Colombia) and Mirna Quiroz (Consultant, Mexico).*
7. India (November 2004) – National 'refresher' ToT course for extension officers and coffee scientists working at the central and the regional offices of the Coffee Board of India. *Trainers: Renata Clarke (FAO), Mick Frank (Consultant, UK) and Coffee Board of India staff.*
8. Rwanda (May-June 2005) A sub-regional ToT course for participants from the eight main African Francophone coffee producing countries: Burundi, Cameroon, Côte d'Ivoire, Democratic Republic of the Congo, Guinea, Madagascar, and Rwanda. (Togo also invited by no participants nominated). *Trainers: Louis Ban Koffi (CNRA, Côte d'Ivoire), Jean Nemlin (CNRA, Côte d'Ivoire) and Daniel Duris (CIRAD, France).*
9. In addition to the above, similar Training of Trainers' courses have also been completed under the auspices of various FAO-funded Technical Cooperation Projects using material developed under the global project (year of course(s) in brackets): Ecuador (2005), Thailand (2005, 2006), Uganda (1999, 2002), Vietnam (2003)

1.2.4 ToT feedback and follow-up

Formal and informal feedback at the end of the ToT courses confirmed that the participant learned a lot from the courses and that they intended to apply their new knowledge to their regular work.

1.2.4.1 The first steps

The counterparts have all, to varying degrees, reported on follow-up training activities. The nature of the follow-up activities depended very much on the existing mechanism for training and information dissemination at each of the collaborating centres.

In many cases emphasis was placed on training of extension staff in coffee-growing regions that could then ensure the passage of guidance on improved hygiene practices along with other guidance required by coffee farmers. In several cases, funding provided through the project has also been used in the printing of brochures and posters, targeting mainly small-holder farmers, to convey simple messages about recommended improvements in the national coffee chain that have been identified as problematic.

Some highlights of these follow-on activities in core project countries, and elsewhere, are outlined below.

Brazil: During 2002, EMBRAPA finalised their collaborative GAP/HACCP training video – '*Segurança Alimentar e Qualidade na Produção de Café*'.

A number of training activities were held in Brazil from December 2003 under the auspices of the national Food Safety Programme (PAS) run by Embrapa-Café and Embrapa-CTAA in conjunction with SENAI (an industry funded body), covering quality issues, good practices and HACCP principles along the coffee chain.

In addition in 2004 Embrapa-Café, in conjunction with the Consorcio Brasileiro de Pesquisa e Desenvolvimento do Café, the Programa Nacional de Pesquisa e Desenvolvimento do Café and the Delegacia Federal da Agricultura de Minas Gerais arranged a one-day seminar on OTA regulations and the Brazilian coffee industry, with participation by USDA, and EC DG-SANCO.

Colombia: National training activities incorporating the OTA prevention message started in Colombia in 2003. Lectures and training courses concerning the risk of moulds and OTA in coffee production, good practices and HACCP principles in coffee processing, storage and transportation were delivered to around 678 people in 2003 alone.

The main audiences for these courses were coffee farmers, extensionists, students and officers of the Colombian Coffee Federation. The personnel of the extension services in five 'Comites' (Caldas, Antioquia, Risaralda, Quindío and Cundinamarca) all received training. Various exporters also received advice concerning OTA prevention in coffee from Cenicafé. In addition, special editions of Cenicafé's '*Avances Técnicos*' were published between 2003 and 2005 dealing with OTA prevention.

Ecuador: A US$ 60,000 'sub-project', funded entirely by the CFC (project CFC/ICO/25FT), was designed and implemented as a one-year training and dissemination package on OTA prevention, in conjunction with the Asociación Nacional de Exportadores de Café (Anecafé). Dr Duris (CIRAD) completed the initial training of extensionists in November 2001, and Anecafé subsequently produced a number of posters and other training materials (see image, right) to promote the OTA prevention message nationally, including the use of radio and TV slots.

Image 1.3: OTA related publicity material prepared by Anecafé, Ecuador.

The sensitisation of stakeholders was also a focus of activities in Ecuador, and Anecafé estimates that over 8,000 *caficultores*, exporters, technicians, extensionists, students and others involved in the coffee sector have benefited from OTA-awareness raising, with some 880 meetings and workshops held in 2002.

Indonesia: A national training manual in *Bahasa* was finalised in 2003, drawing on the material used in the initial project ToT course. Further Training of Trainers' courses were completed by ICCRI extension staff between May and July 2003 in Jember (covering Central Java, East Java, Bali and East Nusa Tenggara), Liwa (covering Lampung, South Sumatra and Bengkulu), and Enrekang (covering Central and South Sulawesi), benefiting some 76 extensionists. Additional local ToT courses were completed in Northern Sumatra in late 2004. Farmers' school activities were also initiated in 2003 – an important step in ensuring that a sustained impact on coffee quality enhancement in Indonesia is achieved.

ICCRI also established programmes linking farmers' groups that had received training on improved practices to coffee buyers that were willing to pay premiums for coffee meeting higher quality standards. Indeed, at the time of writing, the ICCRI run quality improvement programme in Bali (for arabica and robusta) is ongoing and attracting more farmer groups. ICCRI are also identifying the possibility of implementing a similar programme in East Java (for arabica and robusta) and on Flores island (for arabica).

India: Training of coffee extension officers in India started in 2002, and was organized in all three traditional coffee growing states viz., Karnataka, Kerala and Tamil Nadu. These two day training courses were conducted by the Indian project team, comprising trainees who had attended the 2001 ToT programme run by the central project team (see Table 1.1, below).

Table 1.1: Details of Level 2 ToT Programme, India, 2002.

Venue	Regions covered	No. of participants
Central Coffee Research Station, Balehonnur, Chikmagalur, Karnataka	Chikmagalur and Hassan	25 Extension officers
Coffee Research Sub-station, Chettalli, Coorg, Karnataka	Coorg	15 Extension officers
Regional Coffee Research Station, Chundale, Kerala	Wayanad and South Kerala	20 Extension officers
Regional Coffee Research Station, Thandigudi, Tamil Nadu	Pulneys, Shevaroys, Nilgiris and Bodinayakanur	15 Extension officers

During the following season 55 training courses for farmers were held between October and December 2003. The Coffee Board of India also completed an additional eight training courses for extensionists during 2003.

Kenya: A comprehensive series of national awareness raising and training events were implemented between September and December 2003:

- Farmer's field days in 21 of Kenya's 42 coffee growing districts, attended by 6,694 farmers;
- Two courses for 62 officers at technical support level 1;
- Four courses for 130 officers at technical support level 2.

The project, through these courses, has built up the capacity of technical staff to train farmers and other stakeholders on coffee quality and safety improvement from an initial 10 trainers (trained under the central project) to 195. In addition, 383 coffee factory managers and coffee society chairmen and secretaries attended related training events across the country between 2003 and 2005.

Peru: Five participants from across the Peruvian coffee sector attended a sub-regional ToT course held in Ecuador in May 2003. As a result of attending this course the national Comisión para la Promoción de Exportaciones (PROMPEX), the Junta Nacional del Café and the Cámara Peruana de Café secured USAID funding to implement a national training programme on the 'Application of Good Hygiene Practices and HACCP Principles to the Coffee Chain' during 2004.

Three courses were held, using project-based training material adapted in Peru, involving more than 150 trainees representing over 60 coffee organizations. This comprised the training element of a national action and information plan for the prevention of OTA in coffee, which has benefited from cross-Ministerial and joint public-private sector support.

Uganda: OTA prevention information was rolled-out in Uganda from 2003 onwards. Apart from training of extension staff and development of brochures to raise awareness of stakeholders, the Uganda dissemination programme also incorporated keys OTA prevention messages into national radio information slots.

Working closely with the Quality, Regulatory and Information staff at UCDA, the National Union of Coffee Agribusinesses and Farm Enterprises (NUCAFE) developed an interactive radio programme transmitted over the coffee year, with one of four technical officers on hand to field questions, and 'model' farmers to share their experiences.

Calendars for 2004 were designed and distributed by UCDA in late 2003, highlighting themes associated with coffee quality and relating them to activities during the coffee year.

Other: Finally, several thousand copies of '*COFFEA*', a copyright free 'wordless' graphic training booklet[2] targeting smallholder farmers, and detailing good practices to avoid mould formation, were disseminated in September 2003 to all seven project collaborators and UNDP/FAO-Representations in the 45 main coffee producing countries (according to annual production volume) for onward distribution to farmers, extension workers and national trainers.

1.2.4.2 The next steps

More elaborated guidance to several key stakeholders is still required. The '*Guidelines for the Prevention of Mould Formation in Coffee*'[3] that were developed under the project advocate the implementation of systems of quality and safety management – as appropriate to the situation – at coffee processing and handling facilities. Support in the design of suitable quality and safety assurance programmes and training to the small-scale operators to apply them should be provided by the technical institutions whose mandate it is to support the sector. This is not a simple job and it is the next step that should be taken within coffee producing countries by the main coffee institutes.

Any hygiene programmes developed for implementation by small-scale coffee entrepreneurs must focus on those points where there is a substantial risk and should take into account the capacity of the entrepreneurs themselves. The application of the programme should not impose unreasonable and/or overly onerous requirements.

A comprehensive CD-Rom based coffee hygiene resource tool ('*Good Hygiene Practices along the Coffee Chain*') has also been developed under the project to assist coffee institutes develop appropriate hygiene programmes. This training resource can be accessed from www.coffee-ota.org in English, French and Spanish.

The project has already provided some assistance to the CRF in Kenya to prepare them for these next steps. Factory managers at cooperative processing facilities were identified as key players in determining the quality of Kenyan coffee. The CRF has in the past provided training to this target group but without clear training objectives and without an established training programme. A course outline was proposed under the project as a starting point for elaborating a detailed course programme and training materials that would enable the factory managers to uniformly implement quality and safety management programmes throughout the Kenyan cooperative

[2] Developed under the project in association with the Fundação Educar Daterra, an education foundation based in Brazil.
[3] These can be found in full under Part D of this report.

coffee sector. The CRF management expressed full satisfaction with the proposed course outline, but now much work lies ahead to build this into an effective programme of training. The course outline is included in the above-mentioned *'Good Hygiene Practices along the Coffee Chain'* CD-Rom as an example to others on how to go about training course development.

1.2.4.3 Obstacle to hygiene improvement

Project counterpart institutions have noted that the issue of incentive payments to farmers who do apply good practices in the production and handling of coffee is an important factor in changing farmers' behaviour.

The absence of distinction between poorly handled coffee and well handled coffee in mainstream marketing is an obstacle to achieving good hygiene practices along the coffee chain. If the ultimate goals of training are not met, then the money and time spent on designing and implementing training programmes are wasted. The technical experts in the coffee sector have to liaise with decision-makers within the sector to ensure that such obstacles to hygiene improvement are addressed.

1.3 Awareness-raising Among Decision-makers

1.3.1 Why the need for awareness-raising?

Decision-makers have several levels of responsibility in addressing the question of OTA in coffee.

Impending regulation on maximum levels of OTA to be tolerated in coffee was the motivation for formulating and implementing this project. Within the EU, a decision is expected on the need for OTA maximum limits in green coffee. At the international level, the Codex Alimentarius Commission is discussing the need for a Code of Practice for the prevention of OTA contamination in cocoa and coffee.

Governments of coffee producing countries must react appropriately to this situation to ensure that unjustifiable requirements are not placed on coffee exporters creating obstacles to trade. Senior policy-makers must, first of all, be aware that they have a critical role to play in international food safety regulation. Then they must be aware of the international mechanisms that exist that facilitate their role.

The section above focussed on programmes of national training to promote Good Hygiene Practices by stakeholders along the coffee chain. This is an essential component to any national programme for OTA prevention in coffee. Policy-makers must understand the need to support such programmes through adequate provision of financial and staff resources - otherwise the efforts of this global project to build the capacities required for OTA prevention will have been wasted.

1.3.2 Awareness-raising seminars

Four seminars were organized during the project to raise the awareness of influential stakeholders within the coffee chain of the need to address the issue of OTA contamination of coffee: three of these at national level in project countries and one at a sub-regional level. These seminars are detailed in Box 1.3, below.

Box 1.3

Sensitization of stakeholder seminars and workshops

1. **Regional OTA Workshop, Indonesia (October 2002) –** Attended by representatives from FAO, CFC, Brazil, Dutch Govt., the European coffee industry and regional representatives from India, Indonesia, Lao PDR, Papua New Guinea, Philippines, Thailand, Timor Leste, and Vietnam.

2. **National Sensitization of Stakeholders Seminar, Kenya (April 2003) –** Over 70 participants from various responsible national organizations, institutes, and ministries (including CRF, KPCU, CBK, MoA and Co-operatives)

3. **National Sensitization Seminar for Policy- and Decision-makers, Uganda (October 2003) –** attended by senior policy makers within the coffee sector at national and local levels, as well as several parliamentarians and the head of the parliamentary commission on agricultural development.

4. **National Sensitization of Stakeholders Seminar, Guatemala (October 2004) –** Approximately 50 attendees from the Guatemalan coffee sector, selected and invited by Anacafé.

The project also participated in an awareness-raising seminar in India (November 2001) and in national seminars organized under other FAO projects dealing with the question of coffee safety and quality in Thailand (Chumpon, January 2005) and Vietnam (Hanoi, July 2003; Ho Chi Minh City, October 2003).

The main messages to the decision makers of the sector at these seminars focussed on:

- The World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures and a demonstration of the relevance of its provisions in dealing with the question of OTA limits in coffee;
- The responsibility of coffee farmers, traders and exporter for the safety of their products;
- The role of government in ensuring food safety by establishing appropriate national regulations and guidelines, providing training and other technical support to coffee entrepreneurs to make sure that they are able to meet food safety requirements, and by implementing programmes of monitoring and enforcement to verify that food safety policies are effective and are being met.

1.3.3 Indicators of impact

The initial plans expressed by the EU for the regulation of OTA in coffee have been modified over the years, and this is arguably partly due to the reaction of coffee producing countries with support from strategic allies within the 'coffee world'. The global project can reasonably be attributed some credit for the high level of awareness of policy-makers regarding this issue.

Discussions on maximum OTA limits in green coffee, and on the development of a Code of Practice for preventing OTA contamination in coffee, are ongoing. Continued involvement of coffee producing countries – particularly the core project countries – will be a further indicator of the impact of the sensitization seminars discussed above.

Continued support of national coffee institutions to national OTA programmes will be another important indicator of the impact of awareness-raising seminars. The project funded most of the training activities that have been held as follow-up to the ToT courses. The institutes themselves must now assume responsibility for this by providing the budgetary and staff resources necessary for national training programmes as well as for other components of an overall national OTA prevention programme discussed during the sensitization seminars.

The involvement of the ICO in the global project provides an important opportunity to ensure and verify that decision-makers continue to build on the project's activities after its closure.

1.4 CD-Rom Based Resource on Hygiene Practices Along the Coffee Chain

1.4.1 Background to the development of the support tool

The technical institutions that work in support of coffee sector development in producer countries have a fundamental role to play in ensuring that hygiene issues are properly addressed by all parties. During project implementation it became clear that the relevant institutions needed a comprehensive 'coffee hygiene' resource tool to help them fulfil this role.

An outline for a CD-Rom based support tool on '*Good Hygiene Practices along the Coffee Chain*' was developed and discussed at a planning meeting held in Brazil in February 2004. With broad approval of the meeting on the proposed outline, the tool was developed fully, coordinated by FAO. The CD-Rom utilises and builds on the training materials that were developed during the ToT courses held under the project. It is available in English, French and Spanish in hardcopy, and from the website www.coffee-ota.org.

1.4.2 Scope of the CD-Rom support tool

The CD-Rom is organized into six Sections. The information content for each Section of the CD-Rom is briefly outlined below:

Section 1: 'Putting the OTA problem in context' gives the background to the question of OTA contamination of coffee and the international attention it has aroused. It introduces the general and widespread problem of mould growth and mycotoxin contamination in foods and feeds. It also provides background information on market and economic aspects of the coffee sector to highlight the fact that these issues have an important impact on the ability and the interest of stakeholders to comply with hygiene recommendations.

Section 2: 'The international framework for handling food safety issues' outlines the international framework for decisions on safety of foods in international trade and how governments participate in this decision-making process.

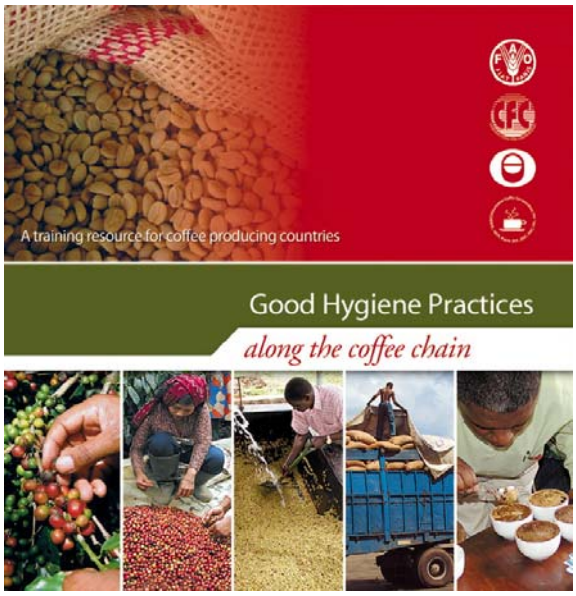
Section 3: 'Applying food hygiene principles to the coffee chain' illustrates how the general provisions for food hygiene in the 'Codex International Code of Practice - General principles of food hygiene' should be applied to the production, handling and processing of green coffee.

Section 4: 'HACCP and its application to the coffee chain' introduces the Hazard Analysis Critical Control Point (HACCP) system of hygiene control, explains its relevance in the international context and shows how the approach could be systematically applied in the production of green coffee.

Section 5: 'Training in good hygiene practices for the coffee chain' provides guidance for the development of effective programmes of training to improve hygiene measures along the coffee chain.

Section 6: 'Monitoring and analysis programmes' introduces the role of monitoring programmes as part of the overall strategy to prevent food contamination. Reliable food analysis capacity is an essential requirement for the implementation of OTA monitoring programmes. The Section provides information to support decision-making on programme development by senior managers as well as material that will be useful to laboratory specialists in building reliable OTA analysis capability.

Image 1.4: Front cover of '*Good Hygiene Practices along the Coffee Chain*' CD-Rom, available in English, French and Spanish.



1.5 Improving Information Access and Networking

The project website (www.coffee-ota.org) was originally conceived to present the activities of the global project to the widest possible audience, both specialist and non-specialist, and as an entry point to understanding the context of ochratoxin A (OTA) in coffee.

Essentially, for the duration of the project, it has been a project information site where implementation progress has been updated. However, as the project has now concluded, it will become a more issue-focused resource, both to assist in post-project dissemination of findings and recommendations and to encourage greater international cooperation amongst initiatives on OTA in coffee.

Indeed, the '*Guidelines for the Prevention of Mould Formation in Coffee*' can now be accessed from the site in three languages, and during the course of 2006 the following improvements will be completed:

- Full online access to the '*Good Hygiene Practices along the Coffee Chain*' training resource;
- Access to PDF versions of the project completion reports and annexes.

With this in mind, the PEA hopes that www.coffee-ota.org will help foster networking amongst the technical coffee community on food safety issues. Depending on how this cooperation develops, there is scope for the site to become more of a 'portal' with increased information exchange, as well as provision of guidance and updates on policy, regulatory and other developments. Interested parties can contact the PEA through the email coffee-ota@fao.org.

Section 2
Mycological Training and Capacity-building

2.1 Introduction

The ability of the collaborating centres to reliably conduct specific mycological analyses was a necessary precondition for the successful implementation of field trials and surveys required under the project.

At the start of the project collaborating countries had varying technical capacity in the area of mycological analysis. This ranged from no research experience and no laboratory facilities, to scattered university and governmental facilities, through to equipped and experienced, publicly funded coffee research institutes working in the area of coffee mycology.

The improvement of mycological training and facilities was an essential first step for the research programme under the project. Methodology had to be conducted uniformly, and well, and the facilities in which the work was to be completed had to reach an acceptable standard to minimise the chances of spurious results.

In brief, training staff at the national centres to an acceptable standard was necessary to ensure the results were of value in their own right, and, importantly, comparable between groups. The methodology also had to be well understood because the result of the application of these methods is contingent on the way the method is executed.

All mycological work requires sound aseptic technique, but even good technique can be undone by inadequate facilities. Above this minimum standard, the facilities and equipment had to be suited to handling large sample volumes. For example, simple, practical matters such as having sufficient quantities of appropriate glassware for mycological analysis had to be addressed, and often re-addressed, during the course of the project.

The approach to understanding problems of mould-related deterioration of coffee quality was another area of work under the project that was undoubtedly new for the project collaborators. It is hoped that the collaborating centres will continue to improve their capacity to carry out mycological work after the global project *per se* closes, to support programmes of coffee quality improvement that are ongoing in many producing countries.

The project also raised awareness of the potential usefulness of mycological farm audits and monitoring within OTA prevention programmes (see also Part C, Section 1.2 of this report).

The skills and experience developed through this project - a study of a consumer health, mycotoxin-based issue - will be available for transfer to other commodities where similar health risks exist.

2.2 Facilities for Mycological Analysis

A standard list of equipment, chemicals and supplies was created at the start of the project (see Annex F.1 on the enclosed CD-Rom). In those cases where materials essential for project mycological work were not available for purchase locally, they were supplied centrally by the project through the FAO procurement system.

The list reflected certain procedures that would be required of the collaborators, and was cognisant of the high sample throughput that would occasionally be required of the laboratories. Therefore, many of these items were selected to replace more cumbersome old-fashioned alternatives which were familiar to the collaborators. The development of these kinds of standardised lists will no doubt assist other centres in developing their own capacity in this regard.

Funds were made available for the essential upgrading of existing laboratory facilities, and the project mycology consultant helped to plan these developments during 2001 and 2002.

In Kenya there was no mycology laboratory *per se* at the Coffee Research Foundation (CRF), so an unused laboratory was fitted out for this purpose. In both India and Indonesia laboratories at the Coffee Board of India (CBI) and the Indonesian Coffee Cocoa Research Institution (ICCRI) were expanded and re-fitted. In Colombia, existing facilities at Cenicafé were repaired, and flooring and lighting improved.

Image 2.1: Improvement of flooring and lighting in Cenicafé laboratory, 2002.

In Uganda, an alternative solution was found, with laboratory facilities being hired from the Ugandan National Bureau of Standards (UNBS) as needed, due to lack of readily available space for the development of a laboratory at the Ugandan Coffee Development Authority. However, towards the end of the project, in 2004/2005, UCDA secured additional room to convert into a laboratory space, and this now houses equipment and materials required for mycological work as well as HPLC equipment purchased under the project.

Emphasis was placed on the need for attention to basic matters such as the state of walls, windows, insect control in the lab, temperature control, and, where necessary, water quality e.g. convenient sources of distilled, reverse osmosis or deionised water. These kinds of basic developments had to be ensured otherwise subsequent

expenditure on laboratory development would have been wasted. Training and facility development are summarized, country-by-country, below.

2.3 Training in Mycological Analysis

2.3.1 Handbook of mycological methods

The handbook of mycological methods is a collection of methods specifically designed for mycological studies in coffee aimed at the investigation of where, why and how OTA arises in this crop. Understanding these aspects provides the groundwork for developing effective prevention measures.

Having standard methods is essential for the comparison of results from different groups, and its promulgation provides a basis for future cooperation. It should be considered a living document to which new procedures can be added as the emphasis of studies evolves, or new ideas of looking at existing research issues arise.

Aside from practical instructions and media recipes, the document discusses recording and reporting data, standardised calculations relating to procedures and the meaning of the calculations. Along these lines, details of the weaknesses and strengths of the procedures are discussed to help the reader interpret results from experiments that are essentially ecological field studies, albeit 'the ecosystem' being in this case a coffee farm and processing unit.

Because it was expected that the groups working in the project would become autonomous as the project finished and their familiarity with the mycotoxin problem and methods increased, aspects of experimental design is also discussed. Over the years, the project continued to develop new protocols and methods and the document could be up-dated to reflect this.

The full version of the handbook of mycological methods is provided as a PDF file on the enclosed CD-Rom, under Annex F.2, 'Handbook of Mycological Methods'.

2.3.2 Informal in-country mycology training

Such was the deficit in mycological analysis and skills to be addressed, the project mycological consultant provided one-on-one training on all suitable occasions and, to some degree, during all of his missions.

General instruction on laboratory maintenance and planning of experiments was undertaken as part of the preparation for running experiments. Field methods, aseptic technique, labelling, recording observations and the specific methods described in the mycological handbook were taught by working side-by-side with national collaborators. In both cases, 'learning by doing' was employed. The reading and recording of mycological plates were likewise covered once the results of the experiment began to come in.

Fungal identification was treated as thoroughly as was reasonable. Hyphomycete identification is largely a specialist area, and it is essential to record only what is actually known reliably. For example, the OTA producer *Aspergillus ochraceus* can

only be reliably identified by a specialist, using specific techniques following isolation and purification. Therefore, the collaborators were taught to identify the group (i.e. ochre aspergilli) so as to avoid misidentification.

A benefit of the process of informal training is that there is considerable, and important, feedback. Often, useful information emerges slowly, sometimes because of cultural differences and sometimes merely due to things not being thought worthy of mention. Informal training of this sort is, by its nature, 'customized' and will fill in gaps left, even by the best thought-out formal training programmes. Often questions emerge that would only do so as experiments are actually put into practice. Even demonstrating problem-solving while finding a 'plan B' when problems arise, as they often do in field work, has a valuable training dimension.

2.3.3 Training workshops

A mycological training workshop was conducted for the African collaborators under the project in April 2002, with 13 participants drawn from Kenya, Uganda and Côte d'Ivoire, as well as from Tanzania. This was conducted at the Coffee Research Foundation and Coffee College, Ruiru, Kenya. Field work was conducted over three days in Kirinyaga. The workshop sought to acquaint the participants with:

- The laboratory and field methods of the mycological handbook;
- Address basic mycological skills;
- Introduce the identification of the more prominent fungal species and significant players in OTA in coffee;
- Introduce mycotoxin studies generally.

The workshop programme and contacts list are provided as Annex F.3 to this report, and can be found on the enclosed CD-Rom as a PDF file.

Image 2.2: A laboratory analyst gets hands-on practice at taking soil samples for mycological analysis.

There was also a strong element of fostering inter-African collaboration, particularly between the neighbouring countries of Kenya and Uganda. A return trip of staff from the Coffee Research Foundation in Kenyan workers to the Ugandan Coffee Development Agency was organised as a result of the relationships fostered during this workshop.

It has become clear during the course of the project that there is a requirement for additional training in fungal identification (which is notoriously difficult to master) and in improving basic laboratory skills and management. FAO will be investigating the possibility of mobilising post-project donor support for the implementation of a workshop to address this.

2.4 Country-by-country Summaries of Training and Facility Development

2.4.1 Brazil

After having been introduced to the detail of the methods adopted for the project by the project mycological consultant, Dr. Ludwig Pfenning (University of Lavras) held a two-day training and orientation meeting in Lavras, Minas Gerais in March 2002. Other meetings were organised under the project amongst the national Brazilian team.

Additionally, consultants from CIRAD and the project mycological consultant held a one afternoon colloquium with the Lavras team, Sara Chalfoun's group at EPIMIG, and other interested parties in July 2002. Several of the Brazilian team also attended the mid-term project meeting held in Uganda in March 2003, where key mycological training messages were further reinforced.

The laboratories at the counterpart institutions in Brazil are generally in a good, or at least satisfactory, condition so funds have generally been spent on individual pieces of equipment (e.g. an autoclave, incubator and laboratory oven), as well as general non-expendable laboratory supplies.

2.4.2 Colombia

The Colombian team began working from the handbook of mycological methods before the project mycological consultant's visit in December 2001. Field and laboratory methods were covered as the consultant participated in scheduled survey activities.

Mould identification was also introduced, and the rationale behind the study was discussed. The team subsequently attended the workshop held in Lavras, Brazil organised by Dr. Ludwig Pfenning, as noted above. Here the methodology described in the mycological handbook was revisited, and further instruction on fungal taxonomy was provided.

Several recommendations for improvements to the already good laboratory facilities at Cenicafé were made in the consultant's Back-To-Office-Report and these improvements were made in 2002. They related to improved lighting and bench space in the microbiology section, with a new floor and five windows installed. Various small items of laboratory equipment were also purchased with project funds including portable A_w and moisture meters, analytical balances and a forced air oven.

2.4.3 Côte d'Ivoire

Dr. Louis Ban Koffi (CNRA) attended the East African mycology workshop in April 2002 (see Section 2.3.3, above). Together with Dr. Nemlin of CNRA they also attended an 'Application of food hygiene and HACCP principles to the coffee chain' Training of Trainers' (ToT) course held at the Kenyan Coffee Research Foundation in May 2003. This ToT course was followed by a four day refresher course in mycological methods conducted by the project consultant mycologist, Dr. John M Frank.

Image 2.3: Informal in-lab training – the national project coordinator at CNRA, Côte d'Ivoire gives hands-on training in mycological analysis to laboratory staff.

The laboratories at CNRA were generally of a good standard, so funds have been concentrated on the provision of supplies and small non-expendable laboratory equipment such as portable A_w and moisture meters, a homogeniser, analytical balances, incubators, a shaking water bath, a forced air oven and incubators.

2.4.4 India

Mycological training was conducted on the ground whilst the project mycologist collaborated directly with the Indian team during two seasons of work.

The Coffee Board of India has numerous research facilities in the country's coffee-growing areas, and a large staff. Funding from the project has contributed to the improvement and development of lab facilities in several of these sites. In Bangalore, new microbiological and analytical laboratories have been built. At CCRI, near Balehannur, existing labs have been upgraded and equipped. At CRSS, near Chetthali, a microbiological laboratory was developed and equipped. The laboratories at RCRS in Wayanad, Kerela, have seen some improvement and the expansion of the laboratory at Tandigudi, Tamil Nadu, has also been supported by the project.

As with the other centres, the project has also supported the procurement of various items of laboratory equipment, including microscopes, portable A_w and moisture meters, analytical balances, laminar flow unit, a shaking water bath, and a forced air oven.

2.4.5 Indonesia

The Indonesian team has been the same since the first visit to ICCRI during the pilot study, so the training on mycological methods had previously taken place. Only advice and demonstration has been necessary on an *ad hoc* basis, usually during the participation of the mycological consultant in field activities.

The laboratories in Jember town have been renovated according to advice from the consultant and are now working efficiently with a good separation of functions and satisfactory facilities in place.

The laboratories were also furnished with the necessary equipment and supplies for the completion of mycological investigation, including an autoclave, analytical balances, forced air oven and portable A_w and moisture meters.

2.4.6 Kenya

During the pilot project both mycological methods and analytical methods were taught to staff members at CRF who, unfortunately for the later global project, subsequently left the institution. In April and May 2001 the mycologist assigned to the global project worked with Dr. Frank for ten days, being trained in mycological methods and the approaches and goals of the project.

In April 2002 an intensive two-week mycological methods workshop was held, based at the CRF and attended by Kenyan, Ugandan, Tanzanian and Côte d'Ivoirian scientists. Both laboratory and field methods described in the project's 'Handbook of Mycological Methods' were taught, with an emphasis on practical skills. This curriculum included practical aspects of record-keeping and reporting as well as theoretical considerations relating to the background and approach of the research effort.

In early June 2002, a few extra days of additional discussion, instruction and advice were provided primarily to the Côte d'Ivoirian team. This period also provided the opportunity for discussion, useful to all parties, and the addressing of problems that had arisen during execution of experimental protocols that the Kenyan team had in progress at that time.

The project has funded the development of a new suite of rooms for use by the project. The layout, developed in consultation with Dr. Frank, protects axenic functions from excessive traffic and provides a separation of functions related to office/computer facilities, reagent and supply storage, wash-up, sample preparation and moisture measurement, media preparation and chemical procedures.

Under the project CRF received a number of non-expendable laboratory equipment items including portable moisture and A_w meters, data loggers, analytical balances, an ultrasonic and water baths, magnetic stirrer, water deioniser and a laboratory refrigerator.

2.4.7 Uganda

The UCDA sent two participants to the East African mycology workshop held at the Coffee Research Foundation, Kenya in April 2002 (as described above). Prior to this the project mycological consultant had worked with UCDA staff in the field and taught the methodology of the mycological handbook during activities in the first field season. OTA analysis training and some supplementary mycological training was also provided by CIRAD in France.

Laboratory development at UCDA was not possible. Therefore, after an inspection of potential alternative labs, an arrangement to hire the food microbiology laboratory at the Ugandan National Bureau of Standards was completed. However, towards the end of the project, in 2004/2005, UCDA secured additional room to convert into a laboratory space, and this now houses the equipment and material provided for mycological analysis as well as HPLC equipment purchased under the project.

2.5 Lessons Learned and Future Needs

2.5.1 Laboratory craft

Training in mycological enumeration, identification and issues related to mycotoxins in food was envisaged from the outset of this project. However, general areas of 'laboratory craft', including experimental design, data handling and interpretation also needed to be addressed. Provision for a concerted mycological training course to deal with these issues would have been beneficial.

Experience of working with scientists and technicians from developing countries, indicates that 'laboratory craft' (i.e. the ability to maintain and calibrate routine equipment, diagnose problems and trouble-shoot them, and organise the laboratory so that it is adopted for the work at hand), is a consistent issue. In the course of discussing issues and problems with collaborators it became clear that a major reason for this is that many universities in the developing world do not teach *practical* laboratory skills. Indeed, the education they offer is somewhat more theoretical.

This causes an enormous problem for research-based projects such as this one. Practical skills are not so easily mastered and can only be mastered through a will to do so and consistent, repetitive use of them over a considerable time. It is an unglamorous area and, although it means that basic things like compounding media, measuring pH, A_w, and absorbance are often not done reliably, it is neglected. There is also often both a cultural and institutional bias against this sort of work. As it is essentially manual work, in many places it is not deemed suitable for a 'scientist grade' worker to undertake.

2.5.2 Laboratory infrastructure

Any plan to develop a laboratory facility should take full cognisance of the condition of the infrastructure, as well as the technical level, of the institution. Investment in laboratory equipment without initially securing a suitable location to house it will invariably result in the deterioration of the equipment, and with little or nothing to show for the investment after a relatively short period of time.

Furthermore, if the development plan includes the introduction of modern electronic or computer-based equipment, the laboratory must be proofed against power spikes, dust, temperature fluctuations and high humidity. This also applies to any optical equipment, e.g. microscopes, spectrometers or certain HPLC detectors. One observation is that local research institutions are often more interested in getting prestige equipment than in maintaining them or in providing for well-trained personnel to operate them. Also donors often more readily fund new equipment than

the surroundings and personnel to sustain them. This kind of mistake must be avoided by all means possible.

2.5.3 Continuity and focus

Though not strictly a training issue, motivation of national staff was often a serious problem faced by the Project Executing Agency. This was partly a consequence of a lack of regular personal contact with the central team, and partly due to the occasional heavy workload required by our ambitious procedures.

It is imperative for large-scale research-based projects such as these, which demand a large commitment of effort in terms of practical research, that international project experts work on the ground as much as possible. Morale and the dedication to not only do the work but do it well are fostered best by example, especially if it is provided by a respected outside expert.

In some countries there was a policy of staff turnover which meant that data from consecutive seasons often lacked continuity because of local in-house retraining of new students or trainees. Often pressure was brought to bear on staff (sometimes with 'political' motivation) to divert attention from the programme intended for the project. Invariably, this caused problems.

2.5.4 Experimental design

The understanding of how experiments work, their structure and how the measurement of different parameters is used in concert to characterise the outcome was often inadequate. This meant that written reports were poor and, just as importantly, the likelihood of being able to devise appropriate follow-up studies without outside input was minimal.

The distinction between data and calculation is not generally appreciated, and data was mixed with calculated values in completely inappropriate ways; often no actual data was presented. Some concepts (which, admittedly, are not particularly obvious ones) in how to use different types of measurements in a unified interpretation of the single phenomenon under study is another area where training is still required.

2.5.5 Computer software skills

Allied to the issues noted above is a lack of practical skill with computer spreadsheets. More often than not Microsoft Excel spreadsheets submitted by the national counterparts contained calculated values, but no underlying formulas. Obviously, calculations had been done by hand elsewhere and transferred into the spreadsheet. This method of working invites errors that can neither be diagnosed as errors, nor corrected.

Spreadsheets are seen by the neophyte as a means of drawing up tables, not as a powerful means to help one evaluate and interpret data. Simple but crucial rules in laying out spreadsheets also need to be emphasised since the power of electronic databases can easily be neutralised if the layout is wrong.

The project introduced Excel data forms with formulas pre-incorporated to address this situation, and, in some cases, provided training in the use of Excel.

In retrospect, however, the provision of data forms was probably a mistake. Although it alleviated some aspects of reporting problems, it thus avoided the root cause which was a basic lack of understanding. It divorced the people conducting the experiments from the output or meaning of their work, and the objective of the experiment became one of simply completing the data forms provided by the central project team.

2.5.6 Statistics

Spreadsheets make the application of statistical tests available to all but this does not help to guide the user to the *appropriate* application.

Knowledge of the basic rules of significant figures and application of concepts such as detection limits and sampling/analysis error would help avoid nonsensical assertions of numerical differences representing actual differences. A good understanding of 'standard error' and the importance of normal distribution and constancy of variation in standard statistical methods would represent a large improvement in this area.

Again, confidence is important here: investigations and treatments often produce no measurable or no consistent effect. This is a valid, and often useful, outcome but one that takes confidence to state.

2.5.7 Management and planning

Some problems with the conduct of mycological activities under the project might have been avoided if selected staff from some of the collaborating institutes had received general management training. Poor planning of procurement of basic laboratory supplies was a regular occurrence, and meant that laboratories were not stocked with enough material for the investigations scheduled.

Once this poor management issue was highlighted, much of the material required was ordered and delivered by the Project Executing Agency. However, the inability to manage stock and supplies and to plan sufficiently in advance for replenishment persisted, and adversely affected execution of some trials throughout the project.

Section 3
Building Capacity for OTA Analysis in Coffee-producing Countries

3.1 Introduction

The ability to reliably measure OTA content of coffee is an essential requirement for the implementation of effective national programmes for prevention of OTA contamination in coffee.

OTA monitoring – of a routine or targeted nature – will be an important component of national OTA prevention programmes. They will verify the effectiveness of recommended practices and they can signal problems in specific areas that require the attention of authorities. These monitoring programmes must be supported by reliable and accurate OTA analysis.

Furthermore, many questions about OTA accumulation in coffee at different stages of the chain remain to be answered. If coffee institutes are to play a role in developing science-based answers to questions that arise on 'best practice', particularly those of direct interest to the industry in their countries, then they will need to be able to carry out OTA analyses.

In light of the above, the project focussed considerable attention and resources on building capacity at the collaborating centres for OTA analysis in coffee. The capacity building activities included[1]:

- Provision of equipment and materials for OTA testing by both High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) methods and upgrading of facilities to accommodate this equipment;
- Training of collaborators to carry out OTA testing and to establish programmes of analytical quality assurance to ensure the reliability of determinations;
- Inter-laboratory proficiency testing to verify the reliability of OTA analyses at each of the centres or to point out areas for improvement.

3.2 Equipment Provision and Facility Upgrading

The project considered that was important for each of the collaborating centres to have the capacity to carry out the HPLC and TLC analysis of OTA using methods that have been internationally validated.

In most cases this required purchase of the HPLC instrumentation and other equipment required for sample preparation and analysis. In cases where the institution already had the required instrument, the project provided for its refurbishment, or accessories, as necessary.

[1] NB – all these activities relate to project Objective 4, Output 4.2, Activities 4.2.1 and 4.2.2 under Component II of the Project Appraisal Report.

In sourcing HPLC equipment for the collaborating centres, proximity of technical servicing for the equipment was an important consideration. Installation and operational training in the use of the equipment was also provided in all cases to ensure that equipment purchased was left with the counterparts in good working order.

Due to the security situation in Côte d'Ivoire, the equipment supplier could not provide an agent to install the equipment. The supplier therefore agreed to train a local technician (who was already familiar with Shimadzu systems) on installation and operation of the specific instrument purchased for the CNRA in Côte d'Ivoire. The local technician then carried out the installation and remains a readily accessible resource if problems arise with the equipment at a later date.

A real concern of the project is long-term impact. HPLC equipment must be held under conditions that will not lead to its deterioration or malfunctioning. In some cases, upgrading of the laboratory was required in advance of receiving this sophisticated instrumentation. The project provided technical guidance on the required upgrading and in some cases financial support for the work.

An account of equipment provided and the facility upgrading at each of the collaborating institutions is outlined below. A full list of capital equipment purchased under the project is provided in the separate Final Management Report, Annex 4, which is included on the CD-Rom.

3.2.1 Colombia

The existing analytical facilities were well established and suitable for OTA detection with the exception of a suitable detector. A Waters 2475 Multi-channel fluorescence detector was therefore purchased in 2003.

3.2.2 Côte d'Ivoire

In 2003, basic equipment for carrying out TLC analysis of OTA was procured for CNRA (including fume hood, spectrophotometer, vacuum manifold, Chromato-Vue UV and associated expendable supplies).

Under a scientific cooperation programme between Spain and Côte d'Ivoire, CNRA had already received a low-pressure gradient unit, on-line degasser, UV detector and solvent delivery module. Additional equipment was needed to allow them to carry out the HPLC OTA analysis method, including an injector, data processing system and fluorescence detector.

A decision was taken in 2004 to purchase various modular Shimadzu HPLC components to complete an HPLC set-up at CNRA, at a cost of US$45,000. A Romer RAS sample mill for grinding the green coffee samples was also provided at a cost of US$7,317.

Facilities at CNRA were upgraded to accommodate this equipment (mainly through the provision of air conditioning), and installation of the HPLC system at CNRA was completed in mid-2005.

3.2.3 India

New analytical laboratories were constructed during the project at the Coffee Board's headquarters in Bangalore, funded by the Coffee Board of India. The project did not procure HPLC analytical equipment for India as this was already in place, but expendable equipment (IAC columns and chemicals) was provided. A Romer RAS sample mill was purchased to ensure satisfactory grinding of green coffee samples in the sample preparation phase.

3.2.4 Indonesia

In late 2002, Indonesian firm PT Ditek Jaya successfully tendered for the provision of a Shimadzu HPLC system, for installation in ICCRI's Jember town laboratory facility. The HPLC system was successfully installed in early 2003. The laboratory housing the HPLC equipment was refurbished during 2002 in preparation for its arrival, and included the installation of air conditioning, and appropriate divisions between work areas.

A Romer RAS sample mill was also purchased to ensure efficient and satisfactory grinding of coffee samples.

3.2.5 Kenya

Refurbishment and rehabilitation of an existing Perkins Elmer HPLC system owned by CRF was undertaken during 2004 as a low-cost option to upgrade Kenya's national capacity in OTA analysis.

New software was purchased and integrated into the existing set-up, and a complete overhaul of the equipment was completed by a Perkins Elmer representative.

3.2.6 Uganda

Regarding the development of HPLC OTA analytical laboratory capacity at UCDA, civil works to covert existing UCDA office space into a laboratory were completed in mid-2004, funded by UCDA under the guidance of the central project team. The project procured and arranged for the installation of a Shimadzu modular HPLC system in late 2004, at a total cost of US$66,000. A Romer RAS sample mill was also purchased.

The project thus enabled a permanent solution for an OTA analysis facility controlled by UCDA both with good infrastructure, and support for maintenance beyond the life of the project.

3.3 Training in OTA Analysis and Analytical Quality Assurance

3.3.1 OTA analysis

Hands-on training on TLC and HPLC methods of OTA analysis was provided to laboratory staff of all collaborating institutions that were in need of such training.

The Brazilian project team, specifically Drs Eugenia Vargas and Eliene Santos from the LACQSA laboratory in Belo Horizonte, Brazil, provided the expertise for this training. The selection of the Brazilian team to carry out the training was not only motivated by the recognition of their competence to undertake the task but also because it was considered that their involvement could reinforce the informal international network of coffee professionals. Such a network should help coffee producing countries deal effectively with problems of a technical or scientific nature that arise in the future.

Image 3.1: Hands-on training with HPLC equipment, Kenya.

The coffee industry also provided support to the project by facilitating study tours to the Nestlé Quality Assurance laboratory in Singapore.

A list of training events on OTA analysis organized and funded by the project is provided below:

- Thin Layer Chromatography (TLC) OTA analytical training for Côte d'Ivoire, Kenya and Uganda completed in Uganda (December 2002). *Trainers: Eugenia Vargas (LACQSA, Brazil) and Eliene Santos (LACQSA, Brazil)*;
- Coffee Board of India study tour of Nestlé NQAC, Singapore, for 1 week. Received training in HPLC analysis and exposure to an industry standard laboratory (2002);
- A study tour for two ICCRI laboratory staff members to the Nestlé laboratory in Singapore for training in HPLC analysis of OTA in green coffee (July–August 2003);
- Coffee Board of India study tour to Brazil covering OTA analytical techniques and regulatory systems. Included visits to Embrapa Agroindústria de Alimentos, Embrapa Café and Cenargen, as well as the LACQSA/MAPA, Universidad Federal de Lavras (UFLA) and EPAMIG laboratories (September 2003).
- HPLC analysis training completed for Côte d'Ivoire, Kenya and Uganda in Kenya (April 2004). *Trainers: Eugenia Vargas (LACQSA, Brazil) and Eliene Santos (LACQSA, Brazil)*;
- Three Cenicafé staff trained in HPLC analysis techniques at Micotox, Bogotá, Colombia (2005).

3.3.2 Analytical quality assurance

If laboratory results are to be credible, the laboratories concerned must be able demonstrate that they have an adequate quality assurance system in place. The LACQSA training team not only focused on enabling the collaborators to be able to carry out OTA analysis, but also to be able to establish a system of quality assurance that allow reliable results.

The counterparts were provided with a manual on '*Mycotoxin analysis and laboratory management*' prepared by the LACQSA team. The manual is based on ISO 17025 - '*General requirements for the competence of calibration and testing laboratories*' - and on the already implemented Quality Assurance System of the Laboratory for Quality Control and Food Safety (LACQSA/LAV-MG), the reference laboratory for mycotoxin analysis of the Ministry of Agriculture, Livestock and Supply of Brazil. This manual is a model to be adapted by each of the collaborating centres according to the specificities of their own situation.

The manual is available for laboratories in any coffee producing country interested in improving the functioning of their analytical services. The manual can be found in Annex F.4 to this report, and it has also been included in the CD-Rom based resource tool on coffee hygiene which has been widely disseminated among coffee producing countries, and is also available from www.coffee-ota.org.

3.4 Proficiency Testing

3.4.1 Planning of proficiency testing

Proficiency testing schemes serve as a means of verifying the accuracy and reliability of the participating laboratories' analytical results. They are also a useful tool for identifying problems in laboratory procedures so that these can be addressed. There are a number of commercial proficiency testing schemes that are run on a regular basis.

In 2004 the project arranged for Dr. Vargas' team at LACQSA, Brazil to implement four rounds of inter-laboratory proficiency testing involving the laboratories at all collaborating centres based on the harmonized methodologies and procedures that were covered during the hands-on training courses implemented earlier in the project.

The decision of the project to have LACQSA run the proficiency rounds, rather than another commercial entity, aimed to reinforce the informal network of coffee professionals mentioned elsewhere. The project provided LACQSA with an ampoule sealer (US$5,000) and laboratory consumables to enable them to carry out the testing.

3.4.2 Implementation of proficiency testing

Implementation of the proficiency rounds involved the preparation of OTA test materials, testing them for homogeneity, and sending the samples to the participant laboratories for analysis. LACQSA subsequently analysed the results submitted by

the participating laboratories, reported on the accuracy of the analyses and pointed out problems to be addressed by each laboratory.

The 5 rounds of proficiency testing run during 2005 involved the analysis of over 80 samples by each of the participating labs. Nine labs from the seven countries participating directly in the project were involved in the proficiency testing.

The proficiency testing showed that the results of some labs did not meet the desired degree of accuracy. The overall proportion of satisfactory results (those falling within 2 standards deviations as determined by LACQSA in the homogeniety testing) obtained by each of the laboratories ranged from 46% to 97%. The laboratories, however, all showed a high degree of repeatability in the analyses which suggests good standardisation of analytical procedures.

LACQSA also noted, over the course of the five proficiency rounds, improved adherence to good laboratory practices concerning the raw data (analytical) recording and reporting.

LACQSA identified problems that were likely to be responsible for the inaccuracy of the analytical determinations that should be addressed by the project collaborators. These included:

- Inefficiency of the extraction procedure and/or losses during clean-up step, probably due to immunoaffinity column flow rate. Some laboratories have reported problems during the filtration step;
- Problems related to standard manipulation during preparation of the OTA calibration curve;
- Problems related to instrument use such as setting the chromatographic conditions, injection volume and column pressure;
- Problems with peak integration in the HPLC quantification step.

These difficulties must be addressed in order to allow the collaborators to improve their skills in OTA analysis and reliably provide accurate OTA results.

3.4.3 Feedback on proficiency testing

At the closing project meeting held in Salvador, Brazil in September 2005, there was a unanimous request by the representatives of the collaborating insitutions for support for their participation in another series of proficiency testing rounds. They all considered that they had benefited from the earlier rounds and welcomed the opportunity for external verification of their analytical skills. The experience of the first rounds had boosted their confidence and they all expressed the expectation that their responsibilitites for OTA analysis would increase.

3.5 Sustainable Impact of OTA Analysis Capacity-building

FAO has provided funding for the implementation of a second series of post-project proficiency testing rounds supervised by the LACQSA team. This will provide crucial feedback to the collaborating laboratories on their ongoing performance and stimulus for improving their performances. The LACQSA team has also carried out a further on-site assessment of the laboratory facilities and operating procedures in Uganda and Kenya in order to provide specific guidance on laboratory upgrading and direct instruction/training as required.

Sustainable impact of the OTA analysis capacity-building requires that:

- The management of the collaborating institutes make the necessary budgetary provisions to implement the facility improvements recommended by the project and by the subsequent laboratory assessment funded by FAO;
- The laboratory staff regularly engage in the OTA analysis so as to maintain or improve their skill levels;
- The management ensures that adequate funds are available for replacement of laboratory consumables needed for OTA analyses.

There should be follow up with the collaborating agencies to ensure that these requirements for sustained impact are met.

Part G

Systems for National Control and Management of Coffee Quality and Safety

Laboratory glassware being used for mycological analysis

Part G
Systems for National Control and Management of Coffee Quality and Safety

1.1 Introduction

For a variety of reasons the coffee sector in most coffee-producing countries has evolved distinctly from the rest of the food sector. Furthermore, food hygiene considerations have received very little attention in the production, handling and marketing of green coffee: criteria in the assessment of green bean quality have been traditionally limited to moisture content, bean size, bean appearance and sensory evaluation of the cup. For these reasons, professionals within the coffee sector have very little experience in the management and control of coffee hygiene.

Effective management of coffee hygiene along the coffee chain faces another challenge as well – that of the 'mind set' of liberalization. Market liberalization, which took place in many coffee-producing in the early 1990's, represented a profound change in the running of the coffee sector. Many in the coffee sector view with great suspicion any form of control which is considered as being antithetical to principles of market liberalization.

This project has played an important role in ensuring decision-makers within the coffee sector are aware of internationally accepted principles of food safety regulation and food safety management and their relevance to the coffee sector.

Food safety regulation is ***not*** a departure from free market principles - it is a necessary complement to free trade if public health is to be protected.

Food safety is the direct responsibility of the enterprises producing the food – this is a critical fact that cannot be overstated. In the context of this particular issue this means that coffee farmers, processors, traders and exporters must actively ensure that ochratoxin A (OTA) levels in the coffee that they trade are maintained as low as reasonably achievable and certainly below any maximum limits that may be set for OTA in green coffee. This does not mean, however, that there is not a critical role for governments to play. Governments must safeguard public interests and taking measures to assure citizens that the national food supply will not cause harm certainly constitutes an important aspect of public protection. As coffee is an important export commodity that provides livelihoods to millions of farming families in producing countries, the interest of governments in safeguarding the reputation of the country's coffee has major additional economic and social dimensions.

Generally speaking, a range of measures are at the disposal of governments to ensure that food is produced hygienically, and these can be either regulatory or non-regulatory. The characteristics of the coffee sector in most producing countries – very large numbers of small holders, and numerous small traders and other market intermediaries – makes it untenable to rely heavily of regulatory checks. Therefore, emphasis must be placed on helping the stakeholders to apply effective hygiene

programmes. Development of training programmes, provision of technical support units to advise on food safety management programmes and facilitating access to various 'inputs' necessary for the upgrading of facilities or processes are among a range of non-regulatory interventions that might be considered by governments according to their diagnosis of the problems and possible solutions for reducing OTA in coffee.

Regulatory measures to addressing the problem could include the establishment of codes of practice defining acceptable practice in the handling of coffee, maximum limits of contamination, definition of official methods of analysis of OTA in green coffee and associated sampling plans, maximum limits for moisture in coffee along the marketing chain, registration or licensing procedures for coffee enterprises, programmes and procedures for inspection and certification of coffee or coffee enterprises.

The following Sections discuss the contribution of the project to strengthening the capacity of coffee institutions in producing countries to manage and control the quality and safety of green coffee.

1.2 Guidelines for Mould Prevention in Coffee

The best way of reducing levels of contamination of OTA contamination in coffee is to prevent its formation in the first place. This can only be achieved if all operators along the coffee chain observe Good Hygiene Practices and minimise opportunities for the growth of mould and accumulation of OTA.

An important step in achieving this is the elaboration by the relevant authorities in coffee-producing countries of an explicit statement of what practices are required of each operator. This statement, or code of practice, can serve both an advisory function as well as being the basis for regulation of the sector.

'*Guidelines for the Prevention of Mould Formation in Coffee*' were formulated during the project, and are presented in Part D of this report. Essential features of the guidelines are that:

- They are based on a systematic consideration of potential food safety risks supported by experimental trials and field assessments;
- They cover all aspects of the coffee chain, from pre-production throughout all the marketing stages of green coffee;
- They promote the application of food safety management systems for effective control of potential hazards rather than emphasis on isolated and unverifiable actions.

The development of the guidelines represents the beginning of the process of providing clear risk-based guidance to the actual operators involved in the production and marketing of green coffee.

The next step should be the formulation of national guidelines, or codes of practice, at the national level based on the project's guidelines. The national codes of practice must be practicable within the national context. The process of considering the project guidelines as the basis for a national code must involve active participation

by farmers, traders, processors and exporters. Several governmental agencies must also be involved, according to the institutional framework for food safety in each country. This is discussed in more detail in Section 4, below.

At the closing project meeting held in Salvador, Brazil in September 2005, a working group session was held with the representatives from each of the project collaborating countries to initiate planning for the elaboration of national codes of practice. The conclusions of that working group session were the following:

- The participants planned to develop more specific draft national codes of practice on the basis of the *'Guidelines for the Prevention of Mould Formation in Coffee'* developed by the project ***over a 1 year period***. They all agreed that this work would start immediately;
- According to the institutional framework for food safety, they recognised the need to liaise very early in the process with the bodies responsible for preparation and adoption of national food standards;
- They estimated that the draft code of practice produced in Year 1 would be subjected to rigorous discussion with all concerned stakeholder groups and then the final version would be ready ***by the end of Year 2***;
- The participants recognised that the elaboration of national codes of practice was just one step in the process of ensuring the good practices are actually applied and that carefully planned and sustained programmes – comprising regulatory and non-regulatory measures – are required to ensure application of good practices.

There should be follow up with each of the participating countries to ensure that this work is moving ahead as planned.

It is essential that national level deliberations are attuned to the discussions within the Codex Alimentarius Commission on OTA contamination in coffee. The significance of Codex Alimentarius in the regulation of food safety in international trade has been emphasised in all of the awareness-raising seminars held for senior policy-makers during the project (see Part F, Section 1 of this report). It is crucial that coffee-producing countries participate actively, and effectively represent their interests at the Codex deliberations. In turn, national codes must be harmonised with any eventual Codex texts.

At the 37th Session of the Codex Committee on Food Additives and Contaminants (CCFAC), the Delegation of the European Community proposed to start new work on a *'Code of Practice for the Prevention and Reduction of Ochratoxin A (OTA) Contamination in Coffee and Cocoa'*. However, it was agreed that there should be a discussion paper on the issue before deciding on the need for a Codex Code of Practice.

The Committee agreed to establish an electronic Working Group, led by Ghana, to prepare a *'Discussion Paper on Ochratoxin A Contamination in Coffee and Cocoa'*, taking into account the JECFA evaluation, the outputs of the FAO-executed project 'Enhancement of Coffee Quality through the Prevention of Mould Formation', as well as other relevant information.

At its 38th Session, the CCFAC agreed to establish two electronic working groups, led by Brazil and Ghana, to prepare separate discussion papers on OTA in coffee and OTA in cocoa, respectively, for circulation, comments and consideration at its next Session, that would allow the Committee to decide if the development of Codes of Practice was appropriate.

It is essential for the project counterparts in all of the project countries to actively contribute to this process. The importance of such participation was agreed at the closing project meeting. The conclusions of a working group session that was organized to discuss this issue included the following:

- The group recognised the need to closely follow all developments, using the Codex website and national Codex structures, in order to provide the input needed into the deliberations;
- The group recognised the need to thoroughly brief country delegates to CCFAC sessions on the issue of OTA in coffee if the coffee institutions themselves cannot attend;
- It is important to encourage collaboration among coffee producing countries in developing common positions on Codex/SPS issues, not only in relation to OTA, but including, for example, addressing problems with pesticide residue regulations (e.g. priorities for establishment of international MRLs).

The International Coffee Organization (ICO) is an ideal forum for developing common positions on issues affecting the coffee sector. The fact that the ICO has observer status in Codex enhances its potential role in following up on hygiene issues that are relevant to the sector at its regular sessions.

ICO obtained observer status in Codex on 20th January 2006 as a direct result of the awareness-raising activities carried out under this project.

1.3 Training and Technical Support to Stakeholders in the Coffee Sector

Coffee production is largely carried out by smallholders, and small traders feature prominently in the local marketing of coffee. Ensuring good practices along the coffee chain cannot be based primarily on regulatory inspections – particularly given the highly fragmented nature of the 'upstream' activities in the chain. Promoting good practices must therefore be largely based on training the various stakeholders to handle the operations under their control in a way that does not introduce hazards or allow them to increase to unacceptable levels. This must be supported by the creation of an environment that facilitates the application of good practices by all stakeholders.

Optimising support to the coffee sector to support quality and hygiene objectives will be an ongoing process. The starting point of this process has been upgrading the capacity of technical staff in main coffee institutions in the area of food hygiene and, in particular, control of mould contamination. Capacity building activities carried out under the project are outlined in Part F of this report. They included training of groups of trainers that are responsible for the continued provision of technical

support for all stakeholders, as well as support to initial activities within national programmes of training and information dissemination.

An important question to be addressed by concerned authorities in the project countries is how sustainability of national training programmes can be assured. One side-effect of the recent 'coffee crisis' has been the dramatic down-sizing of the specialised institutions created to provide required technical support. This was necessitated partly by the decline in export earnings from the coffee trade which are used, through the application of levies, to fund these institutions.

In Kenya, a retrenchment exercise at the Coffee Research Foundation (CRF) reduced staff by from 700 down to just over 200 without any new recruitment. The CRF is mandated to conduct research, including research on quality factors, provide advisory services, training and to produce publications as well as make recommendations regarding the quality of inputs like chemicals and seeds. There is clearly a need to understand how effective training can be designed and implemented in such situations of greatly decreased staffing. This will call for increased inter-agency collaboration including cooperation between public and private sectors, and a high level of planning to make sure that the training activities respond to real needs and actually achieve the desired impact.

1.3.1 Collaborative training programmes

The review of systems of management and control of coffee quality in Kenya and Uganda highlighted the need for improved collaboration and coordination among agencies concerned with the provision of training, extension and other technical support to the coffee sector (the full report of this review is available in Annex G.1).

In Kenya, the CRF, the Coffee Board of Kenya and Ministry of Agriculture all deliver extension services to coffee farmers and all face severe resource limitations. It was reported that sometimes the messages conflict because the three bodies act independently of each other with no prior coordination to synchronise the advice provided. Clarity of roles and responsibilities by the parent Ministry for the institutions involved would streamline training and enhance effectiveness and efficiency.

With adequate collaboration and communication among CBK, CRF, extension services and cooperatives there is also a unique opportunity for identifying training needs and monitoring the impact of training in Kenya. The organization of the coffee marketing system in Kenya allows traceability from cooperative societies and estates right up to the auction. Quality trends can be monitored and this information can facilitate the targeting of training where it is most needed, and also provide an opportunity for objective indicators of impact.

In Uganda, up to now, UCDA has been the agency that carries out training on quality and safety management along the coffee chain from the farm to the export stage. However, recent restructuring of UCDA leaves it with regulatory and supervisory roles and divests the implementation of extension services to the local governments and National Agricultural Advisory Services (NAADS) and other technical support functions to the National Agricultural Research Organization

(NARO). So far, these latter organizations have not significantly addressed this responsibility.

The 'global project' anticipated the need for coordinated involvement of these various parties, and representatives from these institutions were included in the first Training of Trainers' (ToT) course on hygiene along the coffee chain. UCDA should build on this to ensure effective development and delivery of training and information dissemination programmes.

The first ToT course held in Uganda also included a representative from the Uganda Coffee Trade Federation (UCTF). The UCTF membership comprises the major coffee exporters in Uganda, and this private sector plays an important role in ensuring that members respect existing coffee regulations. Given the limited government capacity to provide training on quality and safety management, coordination with private sector bodies is imperative.

Another private sector body that could play a significant role in training and information dissemination is the National Union of Coffee Agribusiness and Farm Enterprises (NUCAFE). This organization was sub-contracted under the global project to develop information dissemination materials on OTA prevention for farmers. Their continued involvement in such activities will be critical.

In all of the ToT courses on food hygiene practices along the coffee chain, care was taken to include representatives of all key agencies from both public and private sectors. In Côte d'Ivoire, representatives from the national extension services were included along with the project counterparts from CNRA. In Indonesia, the representatives from the influential Coffee Exporters Association (AEKI) and from the Ministry of Agriculture were included with ICCRI staff in the training. The project counterpart agencies in all countries must take a lead pursuing continued collaboration with other concerned agencies in developing and implementing training.

The importance of collaboration between the public and private sectors is underlined when we consider the overwhelming impact of marketing influences of farmers' and traders' behaviour and the limited opportunities for introducing price incentives to encourage better handling of coffee at all stages of the chain.

It is pertinent to recall the finding of the socio-economic study on the feasibility of farmers improving their handling practices in accordance with GHP. The study concluded that with present approaches to enforcing hygiene requirements internationally in the mainstream coffee trade the feasibility of improving practices depends upon how much costs can be reduced along the supply chain, how much of this cost reduction is passed on to growers, and whether this increase in income makes it financially beneficial to upgrade coffee. Finding solutions along these lines will require good interaction between public and private spheres and strong private sector involvement in training.

1.3.2 Effective training: Focusing on key issues and consideration of the 'wider picture'

It has already been highlighted that governmental institutions charged with providing training and technical support to the coffee sector are being down-sized precisely at a time when they need to add a new dimension to their training programmes – that of food hygiene.

There is a tendency for the coffee institutions to continue to handle their training responsibilities as they have in the past: it is much easier to continue to work in a familiar way then to re-think and re-organize training, particularly at a time when staffing levels have been greatly reduced and remaining staff are over-worked.

However the situation – new training needs, reduced resources for carrying out training programmes and institutional restructuring – obliges the concerned institutions to make drastic changes in their approaches to training if they are to make a real contribution to the viability of the national coffee sector.

This project developed a CD-Rom to support concerned local institutions in their efforts to provide effective technical support and training to the sector. The CD-Rom includes a component that provides practical and detailed guidance to educators and extensionists in the development of effective training programmes. This emphasises the need to understand the 'wider picture' as a pre-requisite to planning and preparing for effective and efficient training programmes. Failure to do this can, and most likely will, result in inappropriate training programmes that have no impact and waste already limited financial and staff resources.

It has been noted on several occasions that training alone is unlikely to bring about changes in practices by operators along the coffee chain. There have been several examples of this within the project:

- The ICCRI's linking of training in good practices with assistance in strengthening farmers' groups and identifying market opportunities that offer price incentives for improved quality;
- The finding of the socio-economic study in Lampung, Indonesia that the limited market for 'quality coffee' means that the application of good practices by farmers has no impact on their level of remuneration or their access to local markets. Seeing that there are many other activities competing for farmers' time, it would make no sense for them to apply good practices in the present marketing context (see Annex E.4);
- The recommendation coming out the socio-economic study of the Uganda coffee chain that strengthened farmers groups are necessary to improve the negotiating power of farmers to achieve price incentives for improved quality (see Annex E.1);
- The observed failure of a private sector initiative for improved quality in Uganda due to unethical practices of traders that was possible given weak regulation of the sector (See Annex E.10).

Addressing some of these constraints will require broad involvement of stakeholders from both public and private sectors. This task is facilitated in countries where there is a clearly articulated government policy on coffee quality and safety. Concerned

authorities must also be up-to-date and in tune with issues affecting the sector. In some countries strong links have been or are being developed between these institutions and important stakeholder groups - this will facilitate effective information flow and facilitate pro-active programming. It is an approach that should be more widely followed.

The socio-economic studies and market chain surveys carried out under the project (see Part E of this report) have provided essential information to be used by national bodies in the formulation of relevant and effective training. These studies have identified common malpractices within the sector as well as the factors – social, economic and technological – that have to be addressed if adoption of improved practices is to be achieved.

1.4 Regulatory Frameworks for the Control of Coffee Quality and Safety

Regulatory control of the coffee sector, by definition, requires an adequate legal basis that establishes the 'rules' to be followed by operators within the sector, defines the authorities empowered to take actions to see that the rules are respected, specifies the 'powers' of the authorities, procedures to be followed in assessing compliance and the penalties that can be applied to operators who do not comply with established rules.

Food safety regulation has a cost. Measures have to be carefully considered in light of:

- Existing production, storage and handling practices in the food chain;
- The feasibility and economic consequences of their application;
- Their advantages and disadvantages as compared with other options that may be available for managing any particular risk.

Control of hygiene in the green coffee production chain has, until recently, received little attention. If governments are to include regulatory measures among the 'tools' that they use to ensure that coffee is handled according to hygiene requirements then they must first ensure that an adequate legal base is in place to support such. Once the legal base is in place enforcement of regulatory measures requires that adequate human and financial resources be deployed.

Reviews of the national systems for the management and control of coffee quality and safety were commissioned in three of the project countries: India, Kenya and Uganda. These reports assessed the legal basis for the control and made recommendations to the governments for updating this in line with modern conceptions of food control. The full text of these reports can be found in Annexes G.1 and G.2 on the enclosed CD-Rom.

Before going on to outline the key findings of the review of regulatory systems, it should be emphasised that modern food safety management and control depends very much on cooperation between producers/processors and government. The mainstay of the system has to be the full cooperation of the industry in carrying out their food handling operations in an acceptable manner. They should be fully

involved in the establishment of regulations which should be practicable and feasible in the context in which they have to operate. The main role of government is in helping them to conform to requirements.

1.4.1 Control of coffee quality and safety in India

1.4.1.1 The role of the Coffee Board of India

The main institution involved in the control of coffee quality and safety in the coffee production/marketing chain is the Coffee Board of India (CBI) with its head quarters in Bangalore. The Coffee Board was constituted under Section 4 of the Indian Coffee Market Expansion Ordinance, 1940 and the subsequent amendments of The Coffee Market Expansion Acts during 1943 to 1994. It also derives it powers from the Coffee Act No VII of 1942 to control the coffee industry.

Despite the existence of the Coffee Act and rules, which provides for the functioning of the Coffee Board, there are no enabling provisions in the act and rules to enforce them. It has prescribed quality specifications to be followed on a voluntary basis by the industry. The Board has tried in the past to establish the means to enforce mandatory controls but has not been successful. The Board has drafted a new comprehensive Coffee Act which is still under discussion and not yet in the public domain.

As is the general case in the 'coffee world' the CBI has focused on quality parameters and until recently has not been involved in the management of hygiene issues.

In relation to improving hygiene controls, the Coffee Board circulated a proposal on a *'Voluntary Regulatory Scheme on Quality of Green Coffee for Exports from India'* to stakeholders for comments in July 2005. The proposal is based on the *'OTA Risk Management Guidelines for Buying Green Coffee'* produced by the European Coffee Federation, and the process to check green coffee with the help of a decision tree.

The salient features of the revised proposal are:

- Submission by exporters of a report on cup quality (as per original proposal assessment of moisture, visual appearance, smell, cup quality & defect norms), along with application for export permits;
- Submission by exporters of a report on ochratoxin A analysis, if required by the buyer.

The CBI's explicit commitment to an overall programme of quality improvement is directly supportive to the prevention of mould and OTA contamination.

1.4.1.2 Organization of food control nationally and its implications for control of coffee

Food control responsibilities in India are presently spread over a number of agencies and some of these are potentially relevant to the coffee sector.

State governments and local health authorities implement the Prevention of Food Adulteration Act (PFA). This Act defines 'coffee': green, raw or unroasted; roasted,

ground coffee, soluble coffee powder. It also prescribes analytical methods for various compositional determinations – these do not presently include an official method for the determination of OTA in coffee. The provisions directly relating to coffee were developed in consolation with the Coffee Board.

A maximum limit of ochratoxin in coffee has not been fixed under the PFA rules. However the Central Committee on Food Standard is currently discussing the establishment of a national maximum limit for ochratoxin in wheat, barley and rye. No discussions on ochratoxin in coffee have yet been initiated. The CBI would be expected to lead any such initiative and the first step would be the preparation of a detailed note to the Ministry of Health outlining the background of the problem from the health perspective, the international trade perspective as well as relevant data generated within the country on consumption patterns of affected foods and on levels of contamination in the local marketing chain.

The Directorate of Marketing and Inspection of Ministry of Agriculture implements the Agriculture Produce (Grading & Marketing) Act 1937 (AGMARK). Grading and Marking under AGMARK is voluntary for domestic market. For the export market, AGMARK has been made compulsory under the provisions of the Export (Inspection & Quality) Act 1963. As of February 2005, over 150 food products grade standards have been prescribed under the Act, but these do not include coffee.

The Agricultural and Processes Food Products Export Development Authority (APEDA), of the Ministry of Commerce and Industry, was established in 1986 under the APEDA Act. The authority is charged with the development and promotion of exports of certain agriculture and processed food products and for matters connected therewith. It has power to prohibit or control imports and exports of scheduled products. Coffee is not included among these scheduled products.

The Bureau of Indian Standards (BIS) derives its power from the Bureau of Indian Standards Act of 1986. It provides quality certification of food products and services, on the basis of national standards and official test methods. Its certification schemes include Quality System certification against ISO 9000 accredited by Raad Voor Accrediate (RVA) Netherlands and Hazards Analysis and Critical Control Points (HACCP) Scheme against national standard IS 15000. The BIS scheme is voluntary in nature but compulsory for items meant for mass consumption.

At present the BIS has no specific standards for coffee. There is already some collaboration between the CBI and BIS with the Chairman of the Coffee Board chairing the BIS Stimulant Food Sectional Subcommittee (FAD-6-BIS). The two organizations are also collaborating on the proposed voluntary scheme for regulating exports of coffee, as mentioned above.

The Export Inspection Council, set up under the Export (Quality Control and Inspection) Act of 1963 is an advisory body to Central Government which is empowered under the act to notify commodities which will be subjected to quality control and inspection prior to export; establish standards and quality for such notified commodities; specify types of Quality Control/or inspection to be applied to such commodities. Coffee is not among the commodities that it presently regulates.

The institutional and supporting legal frameworks for food control in India described above are fragmented and confusing. However, a proposed Food Safety and Standard Bill, placed before parliament in August 2005, is expected to establish a single national Food Safety and Standards Authority. According to the review carried out by the national consultant the scope of this Bill covers all the conditions and measures necessary for the manufacture, processing, sale, storage and distribution of food designed to ensure safe and wholesome food for human consumption. This includes food safety management system as may be notified by the Food Authority, for application by food businesses.

The review of the national system for the control and management of coffee quality and safety reveals a major weakness in terms of the inability of CBI to establish mandatory requirements for the sector. The CBI continues to address quality and safety issues facing the sector through the development of voluntary programmes that they implement and through collaboration with the agencies that have established roles in mandatory food control activities.

Despite the fragmented and apparently confusing designation of responsibilities for food control activities, there seems to be widespread recognition of the central role of the CBI in deciding on regulatory actions to be applied to the coffee sector. Thus overlaps in the management of coffee quality and safety are avoided. CBI already has formal collaboration with some of these agencies on issues of interest to the coffee sector.

1.4.1.3 Conclusions on aspects of coffee regulation

The representatives of the CBI at the final project meeting in September 2005 agreed to take steps towards the development of national code of practice for the prevention of mould in coffee, based on the guidelines developed under this project. Their role in leading such initiatives is widely established and they are already well-positioned to ensure broad stakeholder participation – covering both the private and public sectors - in the development of such a national code. Formal mechanisms for finalising a code are unclear, especially in light of the possible establishment of a new national Food Safety Authority. The history of engagement with regulatory bodies suggests that CBI will be able to find the required pathway.

Apart from the development of the national code of practice for coffee, CBI can consider what other legal instruments are necessary for effective management of coffee along the entire chain. Some relevant issues are outlined below:

- Consideration might be given to the inclusion of hygiene considerations in licensing and registration procedures for coffee processing/handling establishments;
- Consideration might also be given to the establishment of mandatory moisture limits for coffee in the marketing chain. Present CBI voluntary guidelines specify a maximum moisture content of 11.0 % (wb) with a tolerance of 0.5%. There is much information that has come out of the global project that could inform deliberations on this issue including: description of practices of moisture measurement in the market chain and moisture levels of coffee in local marketing from the market chain survey (see Annex E.2); assessment of moisture

measurement methods (Part C, Section 3 of this report); and the relationship between A_w and moisture content of coffee (also Part C, Section 3);
- There may be some interest in evaluating the need for national maximum OTA limits, particularly given the growing national consumption and the observed practice revealed in the market chain survey of including 'gleanings' in the mainstream coffee marketing. There is evidence of increased OTA risks associated with such coffee (see Part C, Sections 6 and 9).

Naturally any consideration of regulation for the sector must involve broad stakeholder participation and must evaluate the practicability of enforcement. The CBI already has in place the necessary mechanisms to facilitate this participation.

1.4.2 Control of coffee quality and safety in Uganda

1.4.2.1 The role of the UCDA

The Uganda Coffee Development Authority (UCDA) was established by the Uganda Coffee Development Authority Statute, 1991, to be the apex body for promoting, overseeing and regulating the coffee sub-sector, including control of quality and safety.

UCDA is currently undergoing reform to make it solely regulatory, and the line Ministry to which it is responsible has changed to the Ministry of Agriculture. The UCDA Statute needs to be updated to reflect these changes as well as to embrace changes brought about by global developments, and changes in national macro-policies of liberalization, privatization and decentralization.

The UCDA at present does not have regulations in place to support the application of modern concepts of quality and safety management and control in coffee, especially with regard to the prevention of mould growth and contamination by OTA.

However, UCDA is committed to coffee quality improvement and has initiated several innovative voluntary programmes to achieve this. Measures include certification of mills and experimenting with a warehouse receipt system, which has been initiated at two sites, one in the west and another in the east of the country.

Under the warehouse receipt system, only coffee of high quality is accepted and it is then sold through auction at a negotiated price that adequately rewards the farmers. The UCDA is also encouraging wet-processing of robusta (details are provided in Part E, Section 1.3.5 of this report and in Annex E.10). Furthermore, there are efforts to create a pool of out growers around large-scale nucleus farms. The NUCAFE is also being encouraged to assist farmers to have direct sale of their coffee to the ultimate international buyers rather than middlemen, for better remuneration.

These voluntary initiatives would benefit from the existence of clear regulations defining 'acceptable practice' and 'acceptable quality'.

Some regulations do exist. The maximum coffee moisture content is defined by law (13% to 14% for hulling) and controlled by UCDA, but it is rarely enforced. Furthermore, the regulation does not address moisture abuse earlier in the chain. The

market chain study (see Annex E.1) revealed moisture contents ranging from between 14% – 28% at various stages of marketing.

In collaboration with concerned stakeholders (in particular, the local authorities), UCDA has tried to address the need for enforcement of moisture limits. Each year strategically placed road blocks are set up to check the moisture content of coffee being transported through the Masaka district on the way to Kampala. If the moisture content is above 13% the exporter is fined and made to re-dry the coffee immediately.

1.4.2.2 Other concerned agencies

The Uganda National Bureau of Standards (UNBS) is mandated to formulate Ugandan national standards and codes of practice for implementation by various agencies. The UNBS mandate is derived from the Uganda National Bureau of Standards Act of 1983. While this mandate is recognized and there is some cooperation between UNBS and UCDA, there are no standards or codes of practice elaborated through UNBS for use in the coffee sub sector.

It is incumbent on the sector concerned, to propose to the national standards body the standards needed by the sector so that they are prioritized for action in the work plans. The need for strong collaboration between UCDA and UNBS cannot be over emphasized.

Decentralization of service delivery is government policy and is enshrined in the Local Governments Act, 1997 (LGA). Under the LGA, certain functions and services previously implemented by the central government have been divested to the districts and lower governments. The functions and services divested include agricultural extension and advisory services for crops and licensing of produce buying.

Although the Ministry of Local Government has no specific legal mandate for control of coffee quality, its district officials in the Local Councils (LCs) and Resident District Commissioners (RDCs) in some districts are providing significant assistance to UCDA in ensuring that coffee in the marketing chain is dried properly. The districts and lower governments constitute a key component of the institutional framework for the coffee sub-sector.

The National Phytosanitary Service operates under the MAAIF. Officers of the National Phytosanitary Service are stationed at UCDA to routinely carry out phytosanitary certification of coffee for export.

The National Agricultural Research Organization (NARO) was established by the NARO Statute, 1992 as the main agricultural research organization in Uganda. It undertakes, coordinates and disseminates all research for improvement of crops, livestock, fisheries and forestry. NARO is the institution mandated to carry out strategic research on coffee, through one of its institutes, the Coffee Research Institute (CORI). They could make a valuable input into the process of developing science-based systems of food safety management for the coffee sector.

National Agricultural Advisory Services (NAADS) derives its mandate from the National Agricultural Advisory Services Act, 2001 and is the apex institution responsible for agricultural extension or advisory services in Uganda. With the changed mandate of the UCDA, they will have a crucial role in getting key messages about OTA-prevention to farmers.

1.4.2.3 Conclusions regarding regulation of coffee quality and safety

Uganda, along with the other project countries, has agreed to establish a national code of practice for the prevention of mould contamination of coffee. This is in line with international recommendations for the prevention of OTA contamination. Section 1.2 above states the commitment of the project countries concerning steps to be taken towards the establishment of national codes of practice. In Uganda, UCDA must take the lead and pursue close collaboration with the UNBS if this goal is to be realised. This is an essential step towards promoting self-regulation within the sector.

The restructuring of the coffee sub-sector in Uganda, including downsizing have left UCDA with limited capacity and, therefore, reduced effectiveness in coffee quality and safety management and control.

There are a number of agencies that have potential to augment the level of effectiveness of regulation and control, for example NAADS, UNBS, NARO and local governments, but due to the low level of collaboration and coordination between them and UCDA they have not made a contribution. It is incumbent on UCDA to proactively enhance the degree of cooperation.

There are no by-laws to assist the local authorities in implementing quality/safety management and control in their areas of jurisdiction. Plans should also be initiated for broad stakeholder discussion of the need to establish such by-laws and establish new standards and regulations to ensure adequate enforcement of hygiene requirements.

UCDA's efforts in working with private sector groups representing farmers and exporters are highly significant. Strong interactions between the regulators and the private sector are essential in the development of practicable regulations and mutually beneficial collaboration. This base that UCDA has developed should be immediately utilised in the work of developing a national code of practice and establishing new regulations as required to support the national OTA prevention and coffee quality improvement programmes.

Uganda's activities in relation to regulation of good hygiene practice in the coffee chain are not isolated from the wider international scene. The example of the seizure of a 'wet' consignment of coffee in the Masaka District in July 2004 by UCDA for having a moisture content (17%) well over the national limit (13%) nicely illustrates this point. At a meeting with concerned stakeholders to explain its action the UCDA pointed out that non-compliance with basic quality requirements would harm the medium-term reputation of Ugandan coffee and could lead to the loss of its quality premium on the London market. The exporters, on the other hand, pointed to the fact that on the international market, there was no distinction made between coffee re-dried just before exporting and coffee dried to less than 13% earlier in the marketing chain and therefore there was no risk of negative impact on the Uganda coffee

premium. Furthermore they pointed to the fact that the current market structure forces them to deal with purchases on volume terms and 'wet' coffee is what is most commonly available.

UCDA represents the Uganda coffee sector at various international fora, in particular, the ICO. It is essential that UCDA use these opportunities to:

- Influence international policies regarding the regulation of the international coffee trade;
- Ensure that its guidance of the national coffee sector is informed by its understanding of international practice and emerging international trends.

The report prepared by LMC International Ltd. (see Annex E.12 on the enclosed CD-Rom) on the feasibility of measures to improve hygiene practices along the chain, notes that with current practices in the international marketing of coffee (i.e. limited enforcement of Good Hygiene Practices), individual countries would actually be penalised if they strictly enforced good practice. This would not be the case if there were strict enforcement of Good Hygiene Practices by all concerned.

1.4.3 Control of coffee quality and safety in Kenya

1.4.3.1 Institutional framework for the regulation of coffee quality and safety

The Coffee Board of Kenya (CBK) is the apex body for regulation of the coffee sector, including the control of coffee quality and safety. Recent policy reforms have made CBK's role strictly regulatory. The CBK derives its new mandate from the Coffee Act, 2001, and carries out its role in conjunction with Coffee Research Foundation (CRF).

The Coffee Act, 2001, is out-of-date and has to be amended to reflect changes brought about by global developments, new national macro-policies on liberalization, decentralization, privatization and reforms in the coffee sub-sector. While the CBK is charged with quality control, at present it has no established capacity to enforce or oversee quality criteria other than the traditional cup quality, and other parameters like defects, maximum moisture content and grades.

The CBK has the mandate to licence operators involved in the growing, roasting, milling and movement of coffee. Through licensing, CBK can exercise regulation and control over the private sector players to comply with quality requirements. However, at present, the criteria for licensing do not take into account the issue of quality and safety management and control, particularly through the prevention of mould growth and contamination of OTA.

The CRF also derives its mandate from the Coffee Act, 2001, which empowers CRF to conduct research, including research on quality factors, to provide advisory services including recommendations regarding the quality of inputs like chemicals and seeds, to train and to produce technical publications to guide the sector.

While the districts do not have a direct legal mandate for regulation of the coffee sector they can control quality of coffee in their jurisdiction, through if appropriate

by-laws are established. The districts can assist by using society field committees to monitor production and processing practices.

The Kenya Bureau of Standards (KEBS) is mandated to formulate Kenya national standards and Codes of Practice, for implementation by various agencies. The KEBS operates under the Kenya Bureau of Standards Act, 2004, and the line Ministry, which exercises oversight on KEBS, is the Ministry of Trade and Industry. Although this is recognized and there is good cooperation between KEBS and CRF as well as CBK, there are few national standards elaborated by KEBS for use in the coffee production to marketing chain: specifications for instant (soluble) coffee (1978); specifications for roasted coffee beans and roasted ground coffee (1993); and specifications for green coffee beans (1987).

The consultant's review noted that these standards were all inconsistent with prevailing requirements for control of quality and safety in coffee with respect to moisture content and omission of mycotoxins, particularly OTA. Furthermore, the KEBS agreed that the standards needed review. KEBS further acknowledged the urgency to formulate standards that address control of coffee quality and safety and prevention of contamination by OTA. The importance of cooperation between CBK, CRF and KEBS in the formulation of a code of practice that takes a modern approach to preventing OTA contamination at all stages of the chain cannot be over-emphasised.

Gaps exist in legislation with respect to delineating clear responsibilities for institutions involved in regulation of quality control and implementation of extension services, for example among CBK, CRF, the Department of Agriculture and decentralised authorities. By-laws that are necessary to expedite implementation are absent and need to be developed, for example, by CBK and the district local governments. Where the responsibilities are clear in the legal framework there is still the problem of shortage of human resource and facilities to carry out the work required.

1.4.3.2 Conclusions regarding regulation of coffee quality and safety

The greatly reduced staffing levels at CRF and CBK have already been highlighted and this factor must be taken into consideration in planning feasible approaches to the management and control of the coffee sector.

The private sector players in the coffee chain already have a collaborative relationship with the CBK and CRF, and it is important for the CBK and CRF to build on this and optimally harness and maximize the capacity of the private sector entities along the coffee chain to practice self-regulation to comply with quality and safety requirements and produce safe coffee of premium quality.

In keeping with project recommendations, and in line with international trends, efforts to address the problem of OTA contamination of coffee should focus on prevention of contamination at all stages of production and handling. This underlines the importance of the development of a code of practice based on project guidelines as agreed by all participating countries at the final project meeting in Salvador, Brazil in September 2005. The project counterparts, CRF, should spearhead

efforts, in collaboration with CBK and KEBS, to meet the timelines established for the elaboration of a national code.

Plans should also be initiated to discuss the need to update existing standards and establish new standards as required to ensure adequate attention to hygiene issues. Subsidiary legislation and regulations required to allow enforcement of the requirements must also be addressed with broad stakeholder participation.

1.5 Technical and Scientific Support to National Programmes for Improved Coffee Quality and Safety

1.5.1 Monitoring

Routine monitoring, or targeted monitoring, of OTA in coffee can allow authorities to pick up on trends that can trigger proactive programming. It can also lead to the identification of sub-sectors or specific practices that are associated with greater risk and where greater attention could be focused.

Monitoring data directly guides decision-making on regulations at national and international levels. Collection of monitoring data at this time is especially important given upcoming deliberations on maximum limits on green coffee within the EU, and the discussion of a possible international code of practice for OTA prevention within the Codex Alimentarius Commission.

Building capacity for OTA analysis received considerable attention during the project, and this capacity should be put to immediate use and further developed by the responsible authorities (OTA capacity building is discussed in Part F, Section 3 of this report).

1.5.2 Scientific support to decision-making

The project introduced a scientific approach to addressing the problem of OTA contamination. Trials carried out, as reported in Part C of this report, have made a significant contribution to our understanding of the problem, but questions remain to be resolved and new questions have arisen. Perhaps the two most urgent of these are:

- Understanding the contribution of contamination during primary production and how it could be controlled;
- Understanding the association of certain visible defects with contamination of OTA.

There are limited human and financial resources available for carrying out such work in most coffee-producing countries. The lead coffee institutions need to be proactive in leading the coordination of efforts - nationally, regionally and internationally - to get the information that is most pertinent to decisions that must be taken.

The reviews carried out in Uganda, Kenya and India have pointed to other government research institutes that could collaborate in the overall OTA prevention

effort. There is also scope for including universities in this area of work. The key point to be made is that the main coffee institutions should coordinate this effort.

1.5.3 Models of food safety management for application by different stakeholder groups

It has already been noted that the guidelines for the prevention of mould formation produced under this project encourage the application of safety management systems by operators along the coffee chain rather than the non-verifiable application of isolated actions.

It has further been stated that there is general agreement that national codes of practice should be developed on the basis of these project guidelines but tailored to the national situation. These national codes represent the next step towards improved practices along the coffee chain, but are not the ultimate step.

It must be realised that in the vast majority of operators within the coffee sector are small-scale farmers and traders who will need assistance in 'translating' the national code of practice into day-to-day actions that provide assurance of good safety management. This assistance must come from the institutions – governmental and non-governmental – that are mandated to support the sector.

The task of developing models for the day-to-day management of hygiene by different groups of operators in the coffee chain will require considerable effort and will likely need to be the subject of review and improvement over a period of years. The main challenge is to develop models that match the level and the capacity of the operators who must apply them. It is not enough for the technical experts to simply identify hazards and outline principles of control and leave operators to figure out solutions for themselves.

The process of developing a programme of quality and safety assurance has already begun at the CRF, and will support the work of cooperative factory managers.

1.5.4 Coffee processing equipment

Good local capacity for design and construction of coffee processing equipment – both commercial and non-commercial – has been a significant factor in the implementation of this project and associated FAO supported projects on coffee quality improvement.

Where this capacity is well developed there is a greater chance for the sector to find affordable solutions to new challenges. In most of the project countries this was not the case. In countries where there are various options for the production of equipment, it would be useful to encourage dialogue between the users and producers of the equipment to ensure that the supply fits well with the demand. This 'dialogue' did not seem to exist in some countries where there significant equipment manufacturing occurs.

There was also considerable interest among the project counterparts for collated information on commercially available coffee-processing equipment in other countries so that they could more readily access available technologies.

Part H

Overall Conclusions and Recommendations

Cupping during a ToT course, Rwanda

Part H
Overall Conclusions and Recommendations

1.1 Understanding of Mould and Mycotoxin Contamination of Coffee

1.1.1 Conclusions

The goal of the field trials carried out under the project was to better characterise the conditions that lead to OTA contamination of coffee so that acceptable process controls could be more clearly defined and points of greatest risk identified.

The trials all contributed to an improved understanding of mould and OTA contamination of coffee and are an essential input to the development of science-based recommendations on measures for improving coffee hygiene that are commensurate with the food safety risks. However, the ultimate goal of establishing experimentally supported critical limits for identified critical control points was largely not met. Summaries of the findings of experimental trials are contained in Part C of this report.

The findings of the trials have also led to the formulation of priority areas for investigation that could further enhance our understanding of the problem of OTA contamination of coffee and help us to refine food safety management systems for its effective control. Accumulation of OTA in coffee beans during primary production and association of defects with OTA contamination are two areas of investigation that should be of wide interest. Other specific issues may be of interest to particular countries according to the systems of processing and marketing that are in place.

The guidance provided in Part C, Section 12 should promote efficient use of resources to tackle information gaps, and the results and findings of the trials conducted under this project will inform the design of future trials and surveys.

1.1.2 Recommendations

- Coffee research institutions should focus efforts on improving understanding of OTA accumulation in coffee beans during primary production. Planning of such research should take into consideration the advice provided in Part C, Sections 1, 2 and 12 of this report;

- Coffee producing countries should give serious consideration to the investigation of associations between coffee bean defects that are common in local coffee sectors and OTA contamination;

- Project collaborating institutes (or lead coffee institutes in non-project countries) should take a leading role in discussing with other concerned stakeholders the need for specific investigations to support good practice recommendations for the local sector;

- National coffee institutes should take the lead in coordinating work on coffee safety and quality undertaken at concerned technical and research facilities in the country;

- Regional and international coffee organizations should:

  - Promote collaboration among coffee producing countries in planning coffee research;
  - Provide support for mobilisation of funding for agreed priority areas;
  - Promote opportunities for countries to share findings of investigations carried out in individual countries.

1.2 Guidelines for the Prevention of Mould Contamination

1.2.1 Conclusions

The project developed '*Guidelines for the Prevention of Mould Formation in Coffee*' based on assessments of the coffee chain in several producing countries, expert opinion of associated risks of mould growth and mycotoxin contamination at the various stages of the chain as well as on the findings from experimental trials.

The guidelines are not intended for the direct use by every stakeholder, rather they aim to provide national authorities with concrete guidance for developing national guidelines or codes of practice specifically tuned to their respective sector given the diversity of practice in each producing country.

At the closing project meeting held in Salvador, Brazil these guidelines were discussed and all project collaborators agreed that they would be used as the basis for elaborating national codes of practice in an inclusive process involving other government agencies concerned with food safety regulation and all of the stakeholders of the national coffee sector.

These guidelines, and national guidelines or codes of practice that should be derived from them, will form the basis of national programmes for the reduction of OTA contamination in coffee. Concerned institutions must develop effective programmes of training to support the implementation of national guidelines that promote modern systems of food safety management as opposed to simply informing on good and bad practice. Technical support institutes will have to put a lot of thought and effort into developing such programmes that provide for essential hygiene controls without overburdening small-scale operators with unnecessary tasks.

1.2.2 Recommendations

- The project collaborating institutions and the major coffee institutions in other coffee-producing countries should use the guidelines produced under this project as the basis for wide stakeholder input into the elaboration of national codes of practice for the coffee sector;

- National coffee institutions and other relevant partners concerned with food hygiene regulation should complete the first draft of the national code of practice

for stakeholder comment within one year of dissemination of this report (within June 2007);

- National coffee institutions and other relevant partners concerned with food hygiene regulation should seek to finalise national codes of practice for the coffee sector by June 2008.

1.3 Training in Good Hygiene Practices along the Coffee Chain

1.3.1 Conclusions

The situation at the start of the project was a general lack of awareness of about food hygiene among professionals in the main technical institutes supporting the coffee sector. In most countries the coffee sector has evolved quite separately from the rest of the food sector and the coffee sector institutions were largely uninformed about the handling of food safety issues at national and international levels.

Formal and informal feedback at the end of the Training of Trainers' (ToT) courses confirmed that the participants learned a lot from the courses which was directly useful in the execution of their duties.

The counterparts have all, to varying degrees, reported on follow-up training activities. The nature of the follow-up activities depended very much on the existing mechanisms for training and information dissemination at each of the collaborating centres.

In many cases emphasis was placed on training of extension staff in coffee-growing regions who then ensure the passage of guidance on improved hygiene practices along with other guidance required by the coffee farmers. In several cases, funding provided through the project has also been used in the printing of brochures and posters, targeting mainly small holder farmers, to convey simple messages about recommended improvements in the national coffee chain that have been identified as problematic. Some of the collaborators have noted that the absence of price incentives in much of the mainstream coffee market hinders uptake of good practices.

More elaborated guidance to several key stakeholders is still required. The '*Guidelines for the Prevention of Mould Formation in Coffee*' that were developed under the project advocate the implementation of systems of quality and safety management – as appropriate to the situation – at coffee processing and handling facilities. Support in the design of suitable quality and safety assurance programmes, and training to small-scale operators to apply them, should be provided by the technical institutions whose mandate it is to support the sector. This is not a simple job and it is the next step that should be taken within coffee-producing countries by the main coffee institutes.

1.3.2 Recommendations

- The collaborating institutions in the seven project countries and relevant institutions from other coffee producing countries should develop training programmes for key target groups following the guidance provided in the '*Good Hygiene Practices along the Coffee Chain*' CD-Rom (see below);

- Evaluation of training programmes should be routinely carried out and feedback from these used to improve training;

- Collaborating coffee institutes should take the lead in coordinating training activities on coffee hygiene carried out by other governmental and non-governmental institutions and should provide them with materials to support sound hygiene training programmes.

1.4 CD-Rom Based Coffee Hygiene Resource Package

1.4.1 Conclusions

A CD-Rom based coffee hygiene resource tool has been developed under the project to assist coffee institutes develop appropriate hygiene programmes. The tri-lingual CD-Rom (English, French and Spanish) has been widely distributed to concerned institutions in all coffee producing countries and is available from the project website (www.coffee-ota.org).

The guidance that it provides on training programme development will help training institutes take adequate consideration of factors influencing the 'coffee system' in planning and delivering training.

This is particularly important given the fact that the experience of the project clearly demonstrates that technical know-how alone will not lead to improved practices along the chain. If relevant issues are not known and considered before-hand training resources will be inefficiently used if not totally wasted. There are several examples during the course of the project of the responsible institutions being unaware of behaviours and trends affecting coffee handling in the local chain.

The CD-Rom will also guide trainers to redefine training objectives and training course content in relation to the new skills and approaches required for modern food safety and quality management in the sector.

Collaborating project institutes realise that their role goes beyond the training of coffee operators and extends to providing advice to decision-makers on policies affecting hygiene and food safety in the sector. The coffee hygiene CD-Rom provides information to support them in this role.

1.4.2 Recommendations

- The CD-Rom should be distributed widely to government and non-governmental institutions that support the improvement of coffee quality and safety;

- Users of the CD-Rom should be encouraged to provide feedback to national coffee institutes and to the PEA, the Food Quality and Standards Service of FAO (via coffee-ota@fao.org) on additional information required that could be used to develop an expanded second version.

1.5 Capacity for Mycological Analysis

1.5.1 Conclusions

At the start of the project, collaborating countries had varying technical capacity in the area of mycological analysis. This ranged from no research experience and no laboratory facilities, to scattered university and governmental facilities through to equipped and experienced, publicly funded, coffee research institutes working in the area of coffee mycology.

The project successfully upgraded the capacity of collaborating institutes to carry out the mycological work essential for completing the field activities. The capacity building included formal training and informal one-on-one training by the international consultant in mycology. It also included advice and financial support for modifications to working areas and provision of materials and equipment.

This capacity building will allow strengthened scientific support from collaborating institutions to the coffee sector in future.

The handbook of mycological methods and the checklists of materials and equipment required to do such work will serve, not only the continued activity of collaborating centres, but should also help launch similar programmes in other countries interested in strengthening their mycological capacity as a means of delivering concrete guidance on quality assurance and hygiene controls to their coffee sectors.

Commitment from management will be necessary to overcome institutional and financial constraints to strengthening scientific support to the sector.

1.5.2 Recommendations

- Management of the project collaborating institutes should commit adequate resources for mycological work that must be carried out in support of agreed field trials and surveys;

- Management of project collaborating coffee institutes should seek opportunities for the staff to receive further training in coffee mycology either through collaboration with other academic institutions in the country or through external training.

1.6 Capacity for OTA analysis

1.6.1 Conclusions

The project focussed considerable attention and resources on building capacity at the collaborating centres for OTA analysis in coffee. The capacity building activities included provision of equipment and materials for OTA analysis of coffee, regional and national training courses, study tours to well-established laboratories working in OTA analysis and participation of all collaborators in a series of proficiency testing rounds.

OTA analysis laboratories at all collaborating institutes are now functional utilising official methods of OTA analysis based on TLC and HPLC techniques. Ability to use both techniques gives the institutions flexibility in designing programmes of analysis according to technical requirements and cost considerations.

The project has emphasised the need for a system of laboratory management that allows accurate results to be reliably obtained and that promotes acceptance of analytical results in the international arena. OTA analysis data from monitoring programmes provide essential feedback on the efficacy of prevention measures as well as highlighting problem areas to be addressed. These data can also play a crucial role in deliberations on the need for an International Code of Practice for prevention of OTA contamination, and will be key in any future decisions on OTA limits in green coffee whether at national or international level.

The model manual on quality assurance for OTA analysis of coffee that was developed under this project by LACQSA - the reference laboratory for mycotoxin analysis of the Ministry of Agriculture, Livestock and Supply of Brazil - is available as Annex H.1 on the enclosed CD-Rom. The manual is based on ISO 17025 - '*General requirements for the competence of calibration and testing laboratories*' - and on the existing Quality Assurance System at LACQSA. This manual is a model that is being adapted by each of the collaborating centres according to the specificities of their own situation. The manual is available for use by laboratories in any coffee producing country interested in improving the functioning of their analytical services.

Proficiency testing has demonstrated a growing competence among the participating labs although improvements are still required.

Sustainable impact of the OTA analysis capacity building requires that:

- The management of the collaborating institutes make the necessary budgetary provisions to implement the facility improvements recommended by the project and by the subsequent laboratory assessment funded by FAO;
- The laboratory staff regularly undertake OTA analyses so as to maintain and improve their skill levels;
- The management ensures that adequate funds are available for replacement of laboratory consumables needed for OTA analyses.

1.6.2 Recommendations

- Management and senior technical staff of the collaborating project institutes should decide on priorities for OTA analysis programmes;
- According to agreed priorities, senior technical staff should prepare an annual plan of work and budget including provisions for equipment upkeep, replacement of consumables and adequate staff to carry out the analyses;
- Management should ensure that adequate resources are available for the programme of analysis;
- Management should encourage opportunities for further development of laboratory staff skills, for inter-laboratory comparisons with other labs in the country and, where strategic, pursue accreditation of the laboratory for the OTA analyses that they carry out;
- Planning of programmes of monitoring and analysis should be informed by updated knowledge of current practices and trends in the market.

1.7 Participation in International Food Safety Decisions Concerning Coffee

1.7.1 Conclusions

The project has made key stakeholders in the coffee sector aware of the World Trade Organization (WTO) and its role in enforcing rules of international trade. Producing countries are now equipped to utilise the mechanisms provided through WTO membership to get information on other members' sanitary requirements and to question measures that they consider to be unjustified. Project counterparts and collaborators are now aware of the importance of Codex Alimentarius texts and how to contribute to the formulation of national positions on Codex issues that are relevant to the coffee sector.

The ongoing discussions in the Codex Committee on Food Additives and Contaminants (CCFAC) to decide upon the need for an international Codex Code of Practice for the prevention of OTA contamination in coffee is an important opportunity for coffee producing countries to influence the rules that will govern the sector.

The International Coffee Organization (ICO) is an ideal forum for developing common positions on issues affecting the coffee sector. The fact that the ICO has observer status in Codex enhances its potential role in following up on hygiene issues that are relevant to the sector at its regular sessions.

The ICO obtained observer status in Codex on 20th January 2006 as a direct result of the awareness-raising activities carried out under this project.

1.7.2 Recommendations

- The project counterpart institutions should participate in national technical Codex sub-committees, where they exist, or liaise with national Codex Contact Points to contribute to the preparation of the discussion paper on OTA contamination in coffee which is to be considered at the next session of the Codex Committee of Food Additives and Contaminants in 2007;

- Coffee institutes in producing countries should remain informed of all future deliberations within the Codex Alimentarius Commission that are relevant to the coffee sector. The ICO could play an important role of bringing such issues to the attention of members;

- Policy-makers within the coffee sector in all coffee producing countries must follow any developments on maximum limits being considered for contaminants and residues in coffee by WTO members, in particular the pending decision of the EU on maximum OTA limits for green coffee.

1.8 Improving the Regulatory and Policy Frameworks for Control of Coffee Quality and Safety

1.8.1 Conclusions

Market liberalization, which took place in many coffee-producing in the early 1990s, brought about a profound change in the running of the coffee sector. Many in the coffee sector view with great suspicion any form of control which is considered as being antithetical to principles of market liberalization.

This project has played an important role in ensuring that decision-makers within the coffee sector understand that food safety regulation is not a departure from free market principles but rather a necessary complement to free trade if public health is to be protected.

The project has emphasised non-regulatory measures to promote Good Hygiene Practices but some investigations undertaken during the project have shown the need for clear regulations and the means for their enforcement.

The review of national systems for the control of coffee quality and safety in three of the project countries revealed many weaknesses in the institutional and legal frameworks that underpin such control. These will need to be addressed if national authorities intend to improve and enforce relevant regulations.

Targeted studies and surveys were conducted in some of the project countries in order to respond to the need for additional information to support decision-making on specific policy and programme issues. Work in Uganda and Indonesia on initiatives for coffee quality improvement pointed to the importance of well-functioning farmers' groups in achieving sustainable programmes. The studies also identified a number of other factors influencing the feasibility of the coffee quality improvement programmes that should be addressed (various reports on studies in Indonesia and Uganda are available as Annexes E.4 through to E.10).

The report of the survey of the coffee sector in the Northern Rift Valley in Kenya (Annex E.3) has provided information on the impact of recently introduced regulations on extent of coffee production in that zone and on the implications for coffee quality and hygiene. Policy makers need to consider the findings take the measures necessary to resolve the apparent conflict between the new regulation and national policy on coffee quality improvement.

1.8.2 Recommendations

- Coffee institutes in coffee producing countries should work with national food safety authorities to carry out a review of national systems for the control and management of coffee quality and safety;
- According to the weaknesses identified in the system for regulating coffee quality and safety, responsible authorities should encourage broad-based stakeholder input in updating regulations and standards;
- The project collaborating centres in Kenya, Uganda and India should carefully consider the recommendations made in the reports of the reviews of their regulatory systems contained in Annexes G.1 and G.2 and take action as required;
- In developing OTA prevention and coffee quality improvement initiative, policy makers need to holistically examine factors that could affect the outcome of such programmes and ensure a coherent policy environment to facilitate achievement of the programmes' goals;
- Proposed regulations and other policy instruments need to be carefully considered to ensure coherence with overall national strategy and goals for coffee quality improvement.

This document relates the principal technical findings of a 5-year global project: 'Enhancement of Coffee Quality through the Prevention of Mould Formation'.

In the late 1990s the occurrence of a fungal mycotoxin, ochratoxin A (OTA), in coffee from various origins was reported. This project was designed to address the concerns of coffee-producing countries in building their capacity to reduce mould and OTA contamination in coffee, and the consumer heath concerns of regulatory bodies worldwide.

The project focused on assisting coffee-producing countries to develop and implement national prevention programmes through field investigations and training in relevant disciplines. Seven major coffee-producing countries were directly involved in the project: Brazil, Colombia, Côte d'Ivoire, India, Indonesia, Kenya and Uganda, covering all major coffee-producing regions, and commercially traded varieties.

The '*Guidelines for the Prevention of Mould Formation in Coffee*', developed by the project, form part of this report. These guidelines are based on assessments of the coffee chain in several producing countries, expert opinion of associated risks of mould growth and mycotoxin contamination at the various stages of the chain, as well as on the findings from extensive experimental trials carried out under the project.

The project was funded with grants from the Common Fund for Commodities and the Government of the Netherlands, and support from the European coffee industry. It was implemented under the supervision of the International Coffee Organization, and executed on behalf of the above by the Food Quality and Standards Service of the Food and Agriculture Organization of the United Nations.

An enclosed CD-Rom contains electronic versions of this report, the Final Management report, and various third-party reports and studies commissioned by the project are included in over 30 separate Annexes.

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