FunShield4Med

SHIELDING FOOD SAFETY AND SECURITY BY ENABLING THE FORESIGHT OF FUNGAL SPOILAGE AND MYCOTOXINS THREATS IN THE MEDITERRANEAN REGION UNDER CLIMATE CHANGE CONDITIONS

D4.3 - Summer Schools for ESRs & ECSs

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DELIVERABLE TITLE	**Summer Schools for ESRs & ECSs**
RESPONSIBLE AUTHOR	**Pantelis Natskoulis (ELGO-ITAP)**

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ACRONYMS LIST

ITAP	Institute of Technology of Agricultural Products
ELGO	Ellinikos Georgikos Organismos (Hellenic Agricultural Organisation)
ESR(s)	Early-Stage Researcher(s)
ECS(s)	Early Career Scientist(s)
HE	Horizon Europe EU Framework Programme for Research and Innovation
WIDERA	Widening Participation and Spreading Excellence
SO(s)	Specific Objective(s)
CO_2	Carbon di-oxide
QMEA	Quantitative Mycotoxin Exposure Assessment
ELISA	Enzyme Linked Immunosorbent Assay
MS	Mass Spectrometry
UNIPR	University of Parma
AUA	Agricultural University of Athens
TOF/MS	Time-Of-Flight Mass Spectrometry
QQQ-MS/MS	Triple Quadrupole Tandem Mass Spectrometry
QuEChERs	Quick Easy Cheap Effective Rugged Safe
DOI	Digital Object Identifier
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography
EC	European Commission
3-MCPD	3-monochloropropane diol
UCSC	Università Cattolica del Sacro Cuore
GAPs	Good Agricultural Practices
CC	Climate Change
UTh	University of Thessaly
UniWA	University of West Attica
INRAE	Institut National de la Recherche Agronomique
UCO	University of Cordoba
ELMEPA	Hellenic Mediterranean University
NTNU	Norwegian University of Science and Technology
OTA	Ochratoxin A
FUM(s)	Fumonisin(s)
DON	Deoxynivalenol

AOH	Alternariol
AME	Alternariol Methyl Eether
ZEN	Zearalenone
ACN	Acetonitril
IAC	Immuno-Affinity Column
MRLs	Maximum Residue Levels
BCGs	Biosynthetic Gene Clusters
WTO	World Trade Organization
PCR	Polymerase Chain Reaction
BLAST	Basic Local Alignment Search Tool

EXECUTIVE SUMMARY

The present "Deliverable 4.3: Summer Schools for Early-Stage Researchers (ESRs) and Early Career Scientists (ECSs)" reports on the outputs and significance of the 2 summer schools organised under the FunShield4Med project. The target group of these events are apart from the ELGO-ITAP personnel, also the ESRs of project's partners, as well as ESRs and ECSs from academia and private sector. Contributors in these events were apart from project's advanced partners, also invited speakers from academia with a relevant and renowned expertise background in toxigenic fungi and mycotoxins. FunShield4Med Summer School 1 and 2 are addressing both Specific Objectives of the project and of the call (WIDERA, Twinning): SO2, To increase knowledge, expertise, and skills of ELGO-ITAP research staff in the field of spoilage mycotoxigenic fungi and mycotoxins prevalence for the further development of quantitative mycological exposure assessment (QMEA) considering the current climate change context, and SO3, To develop educational/training activities for academia and industry aiming at development of professional skills.

The document presents an introduction to the subjects addressed under the 2 Summer Schools organised by the project, and their relation to the SOs of the call under Chapter 1, and the 2 Summer Schools with their programs and lectures from tutors, participation metrics, and relevant materials (photos, etc), under Chapters 2 & 3, respectively.

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1. Introduction

FunShield4Med project to address the specific objectives of the project and of the relevant HE WIDERA call (Twinning), on increasing knowledge, expertise, and skills of ELGO-ITAP research staff in the field of spoilage mycotoxigenic fungi and mycotoxins prevalence under the current climate change conditions, and on development of educational/training activities for academia and industry for the development of professional skills, has foreseen and realised the organisation of two Summer Schools, with the contribution of advanced partners' and other universities experts' Short-Term Expert Visits.

Contamination of food with mycotoxins represents a huge global challenge which cannot be faced with a universal solution. Awareness, proper analytical tools, and special measures need to be implemented from all sides of the food supply chain to achieve a considerable control of this hazard. In addition, the rising of global temperatures (especially in the Mediterranean area), extreme weather phenomena, increase of atmospheric CO_2, and unusual rainfall variations, are all factors promoting growth opportunities of toxigenic fungi, and thus create an extra awareness to the scientific community, and an increased vigilance for agrifood stakeholders. FunShield4Med, through the organisation of these summer schools is leveraging the knowledge and expertise of ELGO-ITAP personnel to efficiently understand these phenomena, offering solutions to tackle the problem. Also, a special focus is given on training of ESRs, and private sector's staff like ECSs, and to provide this way the young professionals with key knowledge and new competencies. The 2 Summer Schools, as all training activities of FunShield4Med, are open access and without fees, while all training and new e-learning (i.e. webinars and e-tutorials) material developed will be provided freely to the public. Participating Universities, all higher-level educational institutions, have involved their BSc, MSc, and PhD students with the project to acquire advanced competencies. Additionally, ELGO-ITAP hosts many under- and post-graduate students originating either from several Greek or foreign universities, enabling thus a wider dissemination of these educational/training activities.

According to the foreseen actions of FunShield4Med under WP4 "Enhancement of mobility and scientific potential of young scientist" are included the organisation of the two Summer Schools. The first one realised at UNIPR, Parma, during M7 of the project, with title "Mycotoxins under Climate Change" addressed the basics on fungal biology, food mycology, toxigenic fungi and mycotoxins, and some more advanced subjects on computational toxicology, quantitative mycotoxin exposure assessment (QMEA) and modern analytical techniques (ELISA, MS analysis, food sampling protocols). The second Summer School entitled "Tools & Strategies to manage the mycotoxins menace", was realised during M17 at AUA, Athens, and included broader and more advanced thematic matters, including multi-toxins detections protocols and their co-occurrence in foods, state-of-the-art techniques on fungal chemo-diversity, transcriptomics, molecular biology, rapid immunoassays, smart diagnostics for mycotoxigenic fungi, importance of insects presence in food security and their interactions with toxigenic fungi and mycotoxins, among other. A more thorough presentation of the subjects addressed within the 2 Summer Schools are given under the dedicated chapters 2 & 3 following.

2. Summer School 1: Mycotoxins under Climate Change

The first Summer School of FunShield4Med project took place during 3-7 July 2023 at UNIPR, Parma, Italy. Tutors were professors and researchers of the advanced partners of the project, UNIPR, CU and NKUA (see Table 2.1). The 24 participants were members of the ELGO-ITAP personnel (4) and post-graduate students (20) from UNIPR and other Universities, while almost all were ESRs (21). Analytical metrics on participation are presented under Table 2.2.

Table 2.1 Tutors and contribution of FunShield4Med Summer School 1

Name	Organisation	Lecture(s) title
Prof. C. Dall'Asta	UNIPR	"Welcoming: introduction, & presentation of FS4M 1st Summer School program", & "Presentation of teamwork"
Prof. L. Dellafiora	UNIPR	"Computational methods in toxicology"
Dr O. Mihalache	UNIPR	"Case studies on exposure assessment"
Prof. A. Medina	CU	"Fungal Biology", & "Intro to Mycotoxins & Climate Change"
Dr Carol Verheecke-Vaessen	CU	"Toxigenic fungi & mycotoxins", & "Mycotoxin mitigation - Case studies"
Dr A. Patriarca	CU	"Introduction to food mycology", & "Mycotoxin mitigation - Case studies"
Dr C. Cervini	CU	"Mycotoxin mitigation strategies", & "Mycotoxin mitigation - Case studies"
Prof. M. Dasenaki	NKUA	"MS analysis of mycotoxins – target & non-target screening approaches"
Prof. C. Proestos	NKUA	"ELISA technique for mycotoxins", & "Food sampling: basic principles, protocols, techniques, EU Regulation and international standards"
Dr E. Kollia	NKUA	"Risk assessment"

Table 2.2 Participation metrics of FunShield4Med Summer School 1

Organisation	Total	ESRs
ELGO-ITAP	4	2
UNIPR	15	15
University of Valencia	1	1
NKUA	4	3
Total	**24**	**21**

Following is presented the social media release of the event (Figure 2.1) and its detailed Agenda per day (Figure 2.2). A small deviation took place regarding the contribution of Prof. Paola Battilani from Università Cattolica del Sacro Cuore, who was invited to give a lecture on "Mycotoxigenic fungi biocontrol", but due to a last-minute illness she did not manage to participate. To cover her absence her spot was dedicated to more time on Teamwork.

Figure 2.1 Social media release on FunShield4Med Summer School 1

SUMMER SCHOOL PROGRAM

DAY 1: MONDAY 3 JULY 2023

TIME	SUBJECT	SPEAKER
9:15-10:00	Welcoming: introduction, & presentation of program	Chiara Dall'Asta (UNIPR)
10:15-11:00	Presentation of teamwork	
11:15-12:00	Presentation of teamwork	
12:15-13:15	BREAK	
13:30-14:15	Fungal biology	Angel Medina (CU)
14:30-15.15	Introduction to food mycology	Andrea Patriarca (CU)
15:30-16:15	Toxigenic fungi & mycotoxins	Carol Verheecke-Vaessen (CU)

DAY 2: TUESDAY 4 JULY 2023

TIME	SUBJECT	SPEAKER
9:15-10:00	MS analysis of mycotoxins – target & non-target screening approaches	Marilena Dasenaki (NKUA)
10:15-11:00	MS analysis of mycotoxins – target & non-target screening approaches	Marilena Dasenaki (NKUA)
11:15-12:00	Computational methods in toxicology	Luca Dellafiora (UNIPR)
12:15-13:15	BREAK	
13:30-14:15	Computational methods in toxicology	Luca Dellafiora (UNIPR)
14:30-15.15	Case studies on exposure assessment	Octavian Mihalache (UNIPR)
15:30-16:15	Visit of facilities	

DAY 3: WEDNESDAY 5 JULY 2023

TIME	SUBJECT	SPEAKER
9:15-10:00	ELISA technique for mycotoxins	Charalambos Proestos (NKUA)
10:15-11:00	ELISA technique for mycotoxins	Charalambos Proestos (NKUA)
11:15-12:00	Food sampling: basic principles, protocols, techniques, EU Reg. and International standards	Charalambos Proestos (NKUA)
12:15-13:15	BREAK	
13:30-14:15	Food sampling: basic principles, protocols, techniques, EU Reg. and International standards	Charalambos Proestos (NKUA)
14:30-15.15	Risk assessment	Eleni Kollia (NKUA)
15:30-16:15	Risk assessment	Eleni Kollia (NKUA)

DAY 4: THURSDAY 6 JULY 2023

TIME	SUBJECT	SPEAKER
9:15-10:00	Mycotoxigenic fungi biocontrol	Paola Battilani (UCSC)
10:15-11:00	Mycotoxin mitigation strategies	Carla Cervini (CU)
11:15-12:00	Mycotoxin mitigation - Case study	Carla Cervini, Carol Verheecke-Vaessen & Andrea Patriarca
12:15-13:15	BREAK	
13:30-14:15	Teamwork	
14:30-15.15	Teamwork	
15:30-16:15	Teamwork	

DAY 5: FRIDAY 7 JULY 2023		
TIME	SUBJECT	SPEAKER
9:15-10:00	Presentations of RA by the groups	
10:15-11:00	Presentations of RA by the groups	
11:15-12:00	Presentations of RA by the groups	
12:15-13:15	BREAK	

Figure 2.2 FunShield4Med Summer School 1 Program

Next follows a brief description of each lecture subject and its relevance to FunShield4Med objectives:

"Fungal biology" (Prof. A. Medina, CU)

Among the numerous different species present in nature, fungi play a very important role, as through their widespread presence challenges about the risks they pose for humans and animals. Fungal species metabolize and produce broad range organic compounds, from simple ones to very complex. Most of these present certain biological activities, a characteristic one being the production of toxins. Mycotoxins are low-molecular weight, colourless, odourless, and tasteless, secondary metabolic products that affect the exposed humans and animals in a variety of ways. As they are not necessary for fungal growth are considered secondary metabolites most probably produced to play a role in eliminating other microorganisms competing in the same environment. The lecture sets the fundamentals of mycotoxins production by the relative fungal species, as well as the biological processes of their production, and describes the basic factors affecting both the responsible fungus growth and its toxins production.

"Introduction to Food Mycology" (Dr A. Patriarca, CU)

This lecture delved into the classification, identification, and roles of fungi in food spoilage and safety. It covered the main fungal genera responsible for food spoilage and their relationship with susceptible crops, such as fruits, vegetables, and grains. Fungal spoilage of processed food was also explored through the description of key groups and their characteristics enabling their growth under different conditions, like xerophilic, heat-resistant, and preservative-resistant fungi. Methods for fungal detection and enumeration in foods, e.g., direct examination, direct plating, and dilution plating, were explained and exemplified. Their application and limitations were discussed, as well as sources of error. Finally, the characteristics of culture media for determining and quantifying fungi in food were exposed.

"Toxigenic fungi & mycotoxins" (Dr C. Verheecke-Vaessen, CU)

This presentation introduced mycotoxins and put them back into their history context. An overview of the main mycotoxins and their associated toxicity was presented, followed by introduction to the key environmental factors

leading to mycotoxins accumulation. These were later put back into a food supply chain context. Examples of mycotoxins challenges in wheat, peanuts, oats, and maize were introduced with practical examples given.

"MS analysis of mycotoxins – target & non-target screening approaches" (Prof. M. Dasenaki, NKUA)

Mass spectrometry has been the analytical technique of choice for the qualitative and quantitative determination of mycotoxins in highly complex food and feed matrices, offering paramount selectivity and sensitivity. In this lecture the main principles of mass spectrometry were summarized and the most utilized methods for mycotoxin analysis were described, such as triple quadrupole mass spectrometry (QQQ-MS/MS) and time of flight mass spectrometry (TOF/MS). Both target and non-target screening workflows and protocols were presented to address the challenges of detecting extremely low mycotoxin concentrations, as well as masked and emerging mycotoxins. Moreover, the most important sample preparation methodologies were mentioned, such as solid phase extraction and QuEChERs methodology, preceding mass spectrometric analysis.

"Computational methods in toxicology" (Prof. L. Dellafiora, UNIPR)

The two-module presentation about the use of computational methods in toxicology provided the participants with an introduction of molecular modelling techniques applied to toxicology assessment, which made proficient following to the e-learning part. Taking advantage of selected case studies targeting ochratoxin A, participants learned how in silico methods can be broadly applied to investigate the toxicodynamics and toxicokinetic of mycotoxins supporting the early stage of risk assessment (i.e. the hazard identification and characterization). Based on the two modules mentioned above, e-learning materials were made available on Zenodo (DOI: 10.5281/zenodo.10931842) explaining in depth and step-by-step the procedure used and the underpinning rationale and base of knowledge to make participants virtually able to reproduce such kind of analysis.

"Case studies on exposure assessment" (Dr O. Mihalache, UNIPR)

The presentation of "Case studies on exposure assessment" provided the participants with an in-depth understanding of the methodologies used in dietary exposure to mycotoxins and risk characterization. The participants learned how food consumption patterns and occurrence data impact risk assessment. The attendees also performed hands-on activities by working on case studies related to consumers' dietary exposure to mycotoxins and potential health risks. Overall, the seminar helped participants learn the principles of risk assessment, how regulatory standards are set, the importance of health-based guidance values for protecting public health, and how to perform risk assessment using several case studies.

"ELISA technique for mycotoxins" (Prof. C. Proestos, NKUA)

The enzyme linked immuno-sorbent assay (ELISA) is a laboratory-based immunoassay procedure which has been used over a decade around the world to either screen or detect specific mycotoxins, while it is a procedure that offers accuracy and precision. One of the main advantages of this laboratory immunoassay method is to provide a rapid means of analysis to eliminate negative samples and therefore reduce the overall analysis number. This technique is a popular laboratory-based chemical experiment that relies on the ability of specific antibodies which can distinguish the three-dimensional structure of certain mycotoxins. This immunoassay is easy to use, highly specific, and of high sensitivity, which makes it very accurate and precise laboratory test to carry out. At present, the majority commercially available ELISA kits that can be used for detecting mycotoxins are working in the kinetic phase of antibody-antigen binding, which has the added advantage of reducing the incubation times into minutes rather than hours. Other advantages offered by ELISA methods are the favoured options for high throughput

analysis since this procedure requires low sample volumes, and the potential of less sample extract clean up when compared to other conventional methods such as HPLC and TLC.

"Food sampling: basic principles, protocols, techniques, EU Regulation and International standards" (Prof. C. Proestos, NKUA)

The presentation contained information on the specific content and specific arrangements for the performance of official controls as regards contaminants in food. Guidance on the implementation of the rules and practical arrangements for the performance of the official controls as regards contaminants in food. Provisions for the sampling and analysis for the official control of the maximum levels for contaminants have been laid down as follows:

- For the control of levels of mycotoxins: Commission Regulation (EC) 401/2006.
- For the control of levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs: Commission Regulation (EC) No 333/2007.
- For the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs: Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014.
- For the control of levels of nitrates: Commission Regulation (EC) 1882/2006.
- For the control of perfluoroalkyl substances: Commission Regulation (EU) 2022/1428 Guidance document on identification of mycotoxins and plant toxins in food and feed.

Finally, instructions on the report on the relationship between analytical results, measurement uncertainty, recovery factors, and the provisions of EU Food and Feed legislation were presented.

"Risk assessment" (Dr E. Kollia, NKUA)

Risk assessment of mycotoxins plays a crucial role in safeguarding food safety and public health. Recent years have witnessed notable progress in the methodologies and procedures employed for this purpose. The main objective of risk assessment is to assess the potential health hazards associated with mycotoxin exposure, thereby enabling well-informed decision-making and regulatory measures. A significant benefit of modern risk assessment methodologies lies in their capacity to offer comprehensive assessments that consider various factors such as exposure levels, toxicity, and susceptibility of the population. These approaches are meticulously structured and methodical, ensuring that all pertinent data is taken into consideration during the evaluation. Modern risk assessment methodologies now utilize various analytical tools and models to predict mycotoxins' potential health impacts with precision and detail. By integrating qualitative and quantitative data, these approaches deliver reliable results crucial for identifying and managing mycotoxin risks accurately.

"Mycotoxigenic fungi biocontrol" (Prof. P. Battilani, UCSC)

Cancelled. Replaced by dedicating her slot to Teamwork.

"Mycotoxin Mitigation Strategies" (Dr C. Cervini, CU)

This lecture focused on presenting mycotoxin mitigation strategies on grains/nuts. It started with introducing what mycotoxins are, their implication on human health and what is the EU strategy to protect consumers from being exposed to them. Then, mitigation strategies were explored at different stages of grains/nuts supply chain. During

pre-harvest stage attention was given to the adherence to Good Agricultural Practices (GAPs) and adoption of biocontrol agents to prevent mycotoxin occurrence while crops are in the field. At the harvest stage, visual inspection and sorting were recommended followed by adequate drying process to safe moisture content before storage. Finally, methods to mitigate mycotoxin occurrence during transport and storage were presented. Overall, this lecture included examples of different approaches to be used in various contexts from smallholders' farmers to industrial scale.

"Intro to Mycotoxins & Climate Change" (Prof. A. Medina, CU)

Climate change (CC) is expected to have a significant role on the security of food commodities. CC impact can accelerate the infection of such crops by mycotoxigenic moulds and their subsequent contamination with mycotoxins. The lecture presents the basic examination under interaction of elevated CO_2, temperature increase, and drought stress on growth/mycotoxin production by key spoilage fungi. It also examines the available evidence of this impact on growth and mycotoxin production by key mycotoxigenic fungi including *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* species. In addition, gives the predictive modelling approaches available to help identify regions where maximum impact may occur in terms of infection by mycotoxigenic fungi and toxin contamination. The present available knowledge is discussed in the context of the resilience of staple food chains and the impact that interacting CC factors may have on the availability of food in the future.

"Mycotoxin Mitigation - Case study" (Dr C. Cervini, Dr C. Verheecke-Vaessen, & Dr A. Patriarca, CU)

A mycotoxin mitigation case study was conducted based on three food commodities and their main associated mycotoxins. The case studies proposed were sesame seeds and aflatoxins, raisins and ochratoxin A, and peanuts and aflatoxins. The participants were divided into groups and were randomly assigned a case study. They were provided with the corresponding food supply chain schemes and were requested to identify critical stages for mycotoxin development or accumulation. Once the risks were identified, the participants should propose mitigation strategies and debate their implementation. The discussion outcomes were showcased in 10-minute oral presentations from the participants on the final day of Summer School, to open a debate with the rest of the participants and tutors.

Finally, some photos are following from this successful event of the project.

Participants and tutors outside lectures room


Prof. M. Dasenaki with participants during lectures

Participants during the UNIPR facilities tour

Prof. A. Medina and participants during a lecture

Prof. L. Dellafiora and participants during a lecture

Figure 2.3 Photos from FunShield4Med Summer School 1

3. Summer School 2: Tools & Strategies to manage the mycotoxins menace

The second Summer School of FunShield4Med project took place during 10-14 June 2024 at AUA, Athens, Greece. Tutors were professors and researchers of the advanced partners of the project, UNIPR, CU and NKUA, as well as invited speakers from University of Thessaly (UTh), Agricultural University of Athens (AUA), University of West Attica (UniWA), and French National Institute for Agricultural Research (INRAE) (see Table 3.1). The event was characterised by a very high participation (76 participants) even though the week realized had an enormous heat wave for the period, reaching some days of the event almost the 40°C! The 76 participants were members of the ELGO-ITAP personnel (15) and post-graduate students from UNIPR (3), NKUA (12), AUA (19), UniWA (23), University of Tuscany - UoT (1), Norwegian University of Science and Technology - NTNU (1), University of Cordoba - UCO (1), and Hellenic Mediterranean University - ELMEPA (1), while almost all were ESRs (70). Analytical metrics on participation are presented under Table 3.2.

Table 3.1 Tutors and contribution of FunShield4Med Summer School 2

Name	Organisation	Lecture(s) title
Dr P. Natskoulis	ELGO-ITAP	Welcome
Dr D. Miliordos	ELGO-ITAP	Ochratoxin A and Climate Change related to wine produced in Greek territory
Mr. G. Froutis	ELGO-ITAP	Assessment of Ochratoxin A & Aflatoxins B1, B2, G1, and G2 contamination in maize-based products from Greece
Dr A. Panara	NKUA	Multi-toxin detection in dried grapes
Prof. V. Valdramidis	NKUA	Training on QMEA: case study on bread products – Hands-on
Dr C. Verheecke-Vaessen	CU	Transcriptomics techniques for fungi – pros and cons
Dr C. Cervini	CU	Molecular biology techniques for fungi identification: special focus on *Aspergillus* spp.
Mrs I. Picicci	UNIPR	Exploring Fungal Chemo-diversity: The Fusarium genus
Mrs H. Gjoni	UNIPR	Regulatory Frameworks for Mycotoxins: Ensuring Food Safety and Compliance
Dr R. Torrijos	UNIPR	Occurrence and co-occurrence of regulated and emerging mycotoxins in plant-based beverages from Italy
Prof. A. Batrinou	UniWA	Detection of mycotoxins with a rapid immunoassay, & Hands-on with lateral flow test strips for AFs detection
Prof. C. Athanasiou	UTh	Importance of stored product insects for global food security, & Use of stored product insects for food and feed: The EU perspective
Prof. M. Kakagianni	UTh	Linking dietary exposure and risk assessment of mycotoxins, & Training on QMEA: case study on bread products – Hands-on
Prof. D. Tsitsigiannis	AUA	Smart diagnosis of plant diseases caused by mycotoxigenic fungi and novel mycotoxin detection systems & Biological and chemical management of mycotoxigenic fungi and mycotoxins
Dr J.-M. Membre	INRAE	Quantitative Microbiological Exposure Assessment – QMEA, & Practical examples of QMEA – Hands-On

Table 3.2 Participation metrics of FunShield4Med Summer School 2

Organisation	Total	ESRs
ELGO-ITAP	15	9
UNIPR	3	3
NKUA	12	12
AUA	19	19
UniWA	23	23
NTNU	1	1
UCO	1	1
ELMEPA	1	1
UoT	1	1
Total	**76**	**70**

Following is presented the social media release of the event (Figure 3.1) and its detailed Agenda per day (Figure 3.2). A small deviation took place regarding the programmed tour to AUA facilities and laboratories, which were cancelled due to the great heat wave in Athens during the days of the Summer School. After a decision of AUA Deanery, all university facilities were closed, while organisers of the event also decided to cancel tours for safety reasons.

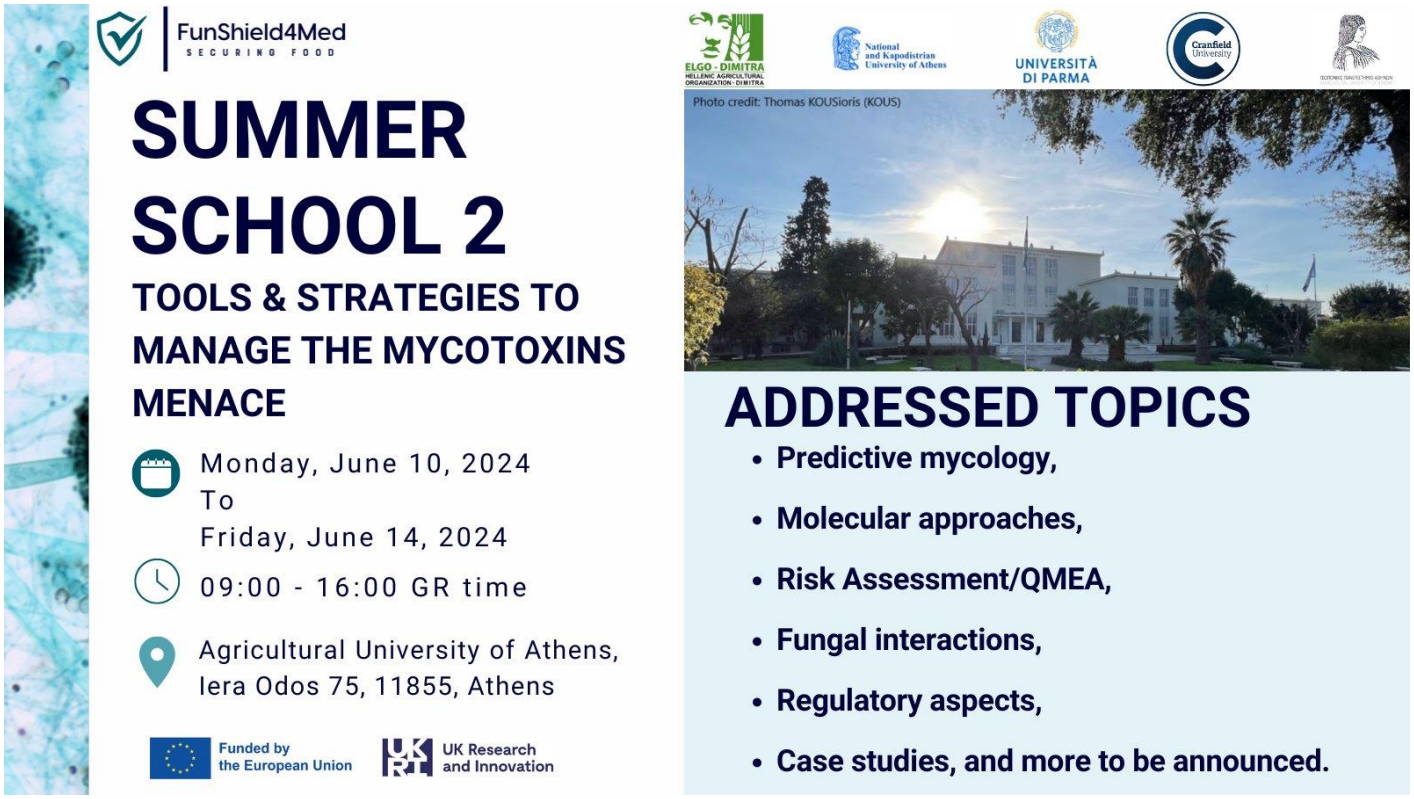

Figure 3.1 Social media release on FunShield4Med Summer School 2

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FunShield4Med
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HORIZON-WIDERA-2021-ACCESS-03-01
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FunShield4Med Summer School 2
"Tools & Strategies to manage the mycotoxins menace"

Date
10 - 14 June 2024 (09:15-15:30 GR time)

Location
Conference Amphitheatre, Agricultural University of Athens, Iera Odos 75, 11855, Athens

INRAE · UNIVERSITÀ DI PARMA · National and Kapodistrian University of Athens · Cranfield University

Important Notes:
- ✓ All participants must register for the event and have their PCs with them for the hands-on sessions.
- ✓ Participation is required all days to receive your certificate of attendance.
- ✓ No fees are charged.

FunShield4Med Summer School 2 - Day 1 Program
Monday 10 June 2024

TIME	SUBJECT	SPEAKER
09:15-09:30	Welcome	Dr Pantelis Natskoulis (ELGO-ITAP, FunShield4Med COO)
09:30-10:15	Ochratoxin A and Climate Change related to wine produced in Greek territory	Dr. Dimitris Miliordos, (ELGO-ITAP)
10:15-10:45	Break	
10:45–11:30	Multi-toxin detection in dried grapes	Anthi Panara (NKUA)
11:30-12:15	Assessment of ochratoxin A & Aflatoxins B1, B2, G1, and G2 contamination in maize-based products from Greece	Giorgos Froutis (AUA/ELGO-ITAP)
12:30-13:30	Break & Networking	
13:45-14:30	Tour to AUA research facilities	All
14:30-15:30	Tour to AUA research facilities	All

FunShield4Med Summer School 2 - Day 2 Program
Tuesday 11 June 2024

TIME	SUBJECT	SPEAKER
09:30-10:15	Exploring Fungal Chemo-diversity: State of the Art Technologies	Irene Piccci (UNIPR)
10:15-10:45	Break	
10:45–11:30	Regulatory Frameworks for Mycotoxins: Ensuring Food Safety and Compliance	Hilva Gjoni (UNIPR)
11:30-12:15	Transcriptomics techniques for fungi – pros and cons	Carol Verheecke-Vaessen (CU)
12:30-13:30	Break & Networking	
13:45-14:30	Occurrence and co-occurrence of regulated and emerging mycotoxins in plant-based beverages from Italy	Raquel Torrijos (UNIPR)
14:30-15:15	Detection of mycotoxins with a rapid immunoassay	Anthimia Batrinou (UniWA)

FunShield4Med Summer School 2 - Day 3 Program
Wednesday 12 June 2024

TIME	SUBJECT	SPEAKER
09:30-10:15	Molecular biology techniques for fungi identification: special focus on Aspergilli	Carla Cervini (CU)
10:15-10:45	Break	
10:45–11:30	Smart diagnosis of plant diseases caused by mycotoxigenic fungi and novel mycotoxin detection systems	Dimitris Tsitsigiannis (AUA)
11:30-12:15	Biological and chemical management of mycotoxigenic fungi and mycotoxins	Dimitris Tsitsigiannis (AUA)
12:30-13:30	Break & Networking	
13:45-14:30	Quantitative Microbiological Exposure Assessment – QMEA	Jeanne-Marie Membre (INRAE)
14:30-15:30	Practical examples of QMEA – Hands-On	Jeanne-Marie Membre (INRAE)

FunShield4Med Summer School 4 - Day 2 Program
Thursday 13 June 2024

TIME	SUBJECT	SPEAKER
09:30-10:15	Importance of stored product insects for global food security	Christos Athanasiou (UTh)
10:15-10:45	Break	
10:45–11:30	Use of stored product insects for food and feed: The EU perspective	Christos Athanasiou (UTh)
11:30-12:15	Linking dietary exposure and risk assessment of mycotoxins	Myrsini Kakagianni (UTh)
12:30-13:30	Break & Networking	
13:45-14:30	Training on quantitative mycotoxin exposure assessment: case study on bread products – Hands-on	Myrsini Kakagianni (Uth) / Vasilis Valdramidis (NKUA)
14:30-15:30	Training on quantitative mycotoxin exposure assessment: case study on bread products – Hands-on	Myrsini Kakagianni (Uth) / Vasilis Valdramidis (NKUA)

FunShield4Med Summer School 2 - Day 5 Program
Friday 14 June 2024

TIME	SUBJECT	SPEAKER
09:30-10:15	Presentations of case studies from hands-on	Students
10:15-10:45	Break	
10:45–11:30	Presentations of case studies from hands-on	Students
11:30-12:15	Visit to labs/ Demonstrations	All
12:30-13:30	Break & Networking	

Figure 3.2 FunShield4Med Summer School 2 Program

Next follows a brief description of each lecture subject and its relevance to FunShield4Med objectives:

"Ochratoxin A and Climate Change related to wine produced in Greek territory" (Dr D. Miliordos, ELGO-ITAP)

The lecture serves as a comprehensive elucidation of the intricate interplay between Climate Change and the occurrence of Ochratoxin A (OTA) in wine, originating from diverse geographical areas within Greece. Wine displays a significant role in economic development of several countries, yet it can face mycotoxin contamination from specific fungi. Greek wine samples were analysed, of different types and from several regions, using HPLC determination after a modified QuEChERS extraction. Climate change's impact on vines and mycotoxigenic fungi, and subsequent OTA presence in wine, requires urgent attention through modelling studies and data interpretation. Evaluating OTA presence in grape products from different cultivars and regions is crucial to assess human exposure risk. Under the lecture was also presented a novel and refined methodology pertaining to the extraction and identification of OTA from wine samples. The significance of this work regarding the summer school context and the FunShield4Med project, aligns with the overarching strategic areas outlined for enhancing OTA awareness and regulatory measures across European MS. The dissemination of knowledge concerning OTA not only fosters heightened consciousness surrounding this mycotoxin across various societal domains, as well as encompasses a wide array of stakeholders such as viticulturists, agronomists, traders, and governmental bodies. Moreover, offers a better description of the prevalence and magnitude of OTA mycotoxin contamination in the Mediterranean region, which stands out as particularly susceptible, laying down the foundation for prioritizing mitigation strategies.

"Multi-toxin detection in dried grapes" (Dr A. Panara, NKUA)

The method development and optimization for the determination of mycotoxins in dried fruits was presented through the lecture "Multi-toxin detection in dried fruits. The presentation showcased the conditions used for the

sample preparation (IAC columns and QuEChERS), while chromatography (mobile phases, elution programmes), and mass spectrometry (source temperature, curtain gas) were explained. Two instrumental methods developed were presented: one for fumonisins (FUM B1, FUM B2), T-2, HT-2, and deoxynivalenol (DON), and another for aflatoxins (B1, B2, G1, G2), ochratoxin A (OTA), alternariol & alternariol methyl ether (AOH & AME), and zearalenone (ZEN). Additionally, the optimal parameters for the QuEChERS approach were described, which included acidification by ACN with 0.01% acetic acid, and reconstitution volumes of 400 µL for dried fruits and 800 µL for fresh fruits. Furthermore, a comparison was made between the two sample preparation methods (QuEChERS, and IAC). QuEChERS was utilised to determine AME, AOH, HT-2, ZEN, and DON, while IAC was employed to determine aflatoxins, OTA, T-2, FUMs, and DON. For each of the mycotoxins, the methods' detection limits were significantly lower than the Maximum Residue Limit. Each mycotoxin that was examined for this project on both substrates (fresh and dried fruits) was found to be below the legislation limits (MRLs). The presentation was noteworthy, since it explains the methodologies that were performed to develop and optimize appropriate methods for the determination of mycotoxins, which are a diverse structural compound that are restricted by rigorous regulations in complicated matrices (fresh and dried fruits).

"Assessment of ochratoxin A & Aflatoxins B1, B2, G1, and G2 contamination in maize-based products from Greece" (Mr. G. Froutis, ELGO-ITAP)

The presentation, titled "Assessment of Ochratoxin A and Aflatoxins B1, B2, G1, and G2 Contamination in Maize-Based Products from Greece: HPLC and Molecular Analysis of Fungal Isolates", focused on the occurrence of these mycotoxins in commercially available maize-based products in the Greek market. The study which was conducted at the Institute of Technology of Agricultural Products (ELGO-DIMITRA), highlighted the increased vulnerability of maize to mycotoxins due to climate change. The occurrence of these mycotoxins is of significant concern to the scientific community because of their potential adverse effects on human health. This research is crucial within the FunShield4Med project, as it addresses the impact of mycotoxins presence in widely consumed food products and underscores the need for effective strategies to manage mycotoxin contamination in agricultural products, particularly in the Mediterranean area, where climate change is especially pronounced.

"Exploring Fungal Chemo-Diversity: State-of-Art Techniques" (Mrs I. Picicci, UNIPR)

Toxigenic filamentous fungi are characterized by astonishing biodiversity and chemo-diversity. The main source of chemical diversity is their secondary metabolism. Secondary metabolites are structurally heterogeneous, low molecular mass molecules mainly involved in the interaction with the environment, the host, or the microbial community in which the fungus lives. Among these metabolites are mycotoxins, which are toxic to humans and livestock and thus major threats to food safety. Genes involved in the production of secondary metabolites are grouped in clusters called biosynthetic gene clusters (BCGs). In recent years, different innovative approaches called "omics" (e.g., genomics, transcriptomics, proteomics, metabolomics) have been developed to fully understand these organisms, the toxins they produce, and the stimuli involved in the modulation of their biosynthetic genes. Currently, the aim is to merge the information retrieved by each different technique into a single multi-omics approach to obtain a comprehensive and in-depth profiling of the biological processes involved in mycotoxin production, and to act against toxigenic fungi, especially in the present climate change scenario.

"Regulatory Frameworks for Mycotoxins: Ensuring Food Safety and Compliance" (Mrs H. Gjoni, UNIPR)

Several reasons make food regulation important, the main one being consumer protection. Without a safe food product, human, theoretically, wouldn't have food at all. The presence of maximum limits guarantees safe food for

all, of course this is ideal. Sometimes, especially in underdeveloped countries, there isn't this luxury. However, when considering international trade there needs to be a common starting point. Mycotoxins remain a leading food safety concern. Each country has its own maximum limits based on exposure, which is exactly what makes these thresholds different. Even if the hazard (mycotoxins) is the same, exposure data is different for each country, which leads to different maximum limits. So, how are international aspects dealt with? It's not easy to be open to trade and at the same time have at best interest consumer protection. Nevertheless, through international organizations such as the World Trade Organization (WTO) and international documents such as the Codex Alimentarius trade can be facilitated and promoted.

"Transcriptomics techniques for fungi – pros and cons" (Dr C. Verheecke-Vaessen, CU)

This presentation intended learning outcomes included: (1) Understand and identify what are the different transcriptomics techniques available for mycotoxigenic fungi, (2) critically assess how transcriptomic techniques can help to understand the fungal physiology versus mycotoxin production, (3) reflect on which experiment to put in place to solve food supply-chain challenges. The PowerPoint was divided into sections from reminder of cellular biology to a deeper presentation on what are the current transcriptomic techniques and what outcome can be generated out of them. With the use of interactive tools, the audience interacted and shared their own experience and knowledge.

"Occurrence and co-occurrence of regulated and emerging mycotoxins in plant-based beverages from Italy" (Dr R. Torrijos, UNIPR)

In recent years, the market for plant-based beverages has increased in popularity, mainly due to environmental and health concerns. Nevertheless, the raw materials used in their production can be contaminated with mycotoxins. In this context, new methodologies for identifying and quantifying mycotoxins in plant-based beverages are necessary to assess the occurrence and evaluate the exposure. The lecture described the development of a multi-toxin analytical method for the determination of 19 mycotoxins in plant-based beverages. Participants learned the basis of method development and optimization, with a particular emphasis on the analytical techniques used (UHPLC-MS/MS) and the method validation. Moreover, the results obtained after applying the developed methodology in commercial samples from Italy regarding the occurrence and co-occurrence of mycotoxins were presented to the participants.

"Detection of mycotoxins with a rapid immunoassay" (Dr A. Batrinou, UniWA)

Aflatoxin is a toxic and carcinogenic substance produced by certain strains of the moulds *Aspergillus flavus* and *A. parasiticus*. There are four principal types of aflatoxin: B1, B2, G1 and G2. The accurate determination of the mycotoxin's presence is of major importance to those monitoring the quality of food and feed in which aflatoxin may occur. Moreover, many countries have set maximum allowable levels of aflatoxin in food and feed.

Hands-on with lateral flow test strips for AFs detection (Dr A. Batrinou, UniWA)

In this session, a workshop was performed in which we used a rapid immunoassay kit (Reveal Q+, Neogen) for the detection of total aflatoxins with a single step lateral flow immunochromatographic assay based on a competitive immunoassay format. Twenty samples were processed (nuts, breakfast cereals, oat, and corn-based products, feed) and analysed on the spot (in the amphitheatre where the lectures were performed). The students formed groups of 4-5 persons and each group treated one sample, to detect whether the sample was positive or negative.

This workshop was important for the students to participate in a hands-on rapid analysis of mycotoxins that can be applied as a screening tool in the industry or in any food processing environment.

"Molecular biology techniques for fungi identification: special focus on Aspergilli" (Dr C. Cervini, CU)

This lecture summarised the main steps to identify through a molecular approach Aspergillus species isolated from food samples. It started with introducing the Aspergillus genus, its economic and medical importance and latest updates in taxonomy. The polyphasic approach for Aspergillus identification was introduced based on Samson et al., 2014. An overview on each of the stage to conduct molecular identification was provided, including examples of different DNA extraction protocols, PCR analysis with Aspergillus species recommended primers, Sanger's sequencing and interpretation of BLAST results.

"Smart diagnosis of plant diseases caused by mycotoxigenic fungi and novel mycotoxin detection systems" (Prof. D. Tsitsigiannis, AUA)

Prof. Tsitsigiannis gave an overview of mycotoxins as one of the major threats to food and feed safety and quality worldwide, especially considering recent estimates that they contaminate 60–80% of the food produced worldwide. The development of methods of classical and artificial smart diagnosis of diseases caused by mycotoxigenic fungi and the detection of mycotoxins using molecular analyses, spectral and thermal imaging techniques, sensors, drones and satellites were presented.

"Biological and chemical management of mycotoxigenic fungi and mycotoxins" (Prof. D. Tsitsigiannis, AUA)

The lecture presented that the ever-increasing demands of international markets for safe food have led to the development of digital holistic integrated plant protection strategies for a more robust control system for agricultural products. Several research approaches were presented such as (a) the prognosis of mycotoxin outbreaks through early warning systems using IoT sensors and prediction/forecasting models and (b) the discovery and evaluation of novel biocontrol agents, plant resistance inducers and chemical substances to control mycotoxigenic fungi/mycotoxins of crops at pre- and post-harvest level. Decision Support Systems were also presented that are based on computer-based knowledge systems and enable the mycotoxin risk prediction and monitoring and determine the critical stages of various plant protection spray interventions.

"Quantitative Microbiological Exposure Assessment – QMEA" & "Practical examples of QMEA – Hands-On" (Dr J.-M. Membre, INRAE)

Food safety is the guarantee that food is harmless, in other words that its consumption will have no adverse consequences for health. Beside foodborne bacteria, moulds and their potential production of mycotoxins are definitely hazards to give priority consideration in food safety. Food safety encompasses all the measures taken to provide food that is as safe as possible, and hence the policies and measures applied must relate to the entire food chain, from production to consumption. The development of food safety standards has been based on a formal process called "risk analysis". Risk analysis includes risk assessment and risk management as well as risk communication. The morning lecture was dedicated to risk assessment with a focus on exposure assessment, illustrated with spoilage of bakery products by moulds. In the afternoon, during a hands-on session, students could learn and practise Monte Calo simulation, probability density, uncertainty and sensitivity analysis. Together, morning and afternoon sessions allowed students to learn or to consolidate their skills in quantitative exposure assessment. It is necessary, indeed essential, in a context of changing global food production patterns, climate

change, the emergence of new technologies and many other factors that have created an increasingly demanding environment in which food safety must operate.

"Importance of stored product insects for global food security" (Prof. C. Athanasiou, UTh)

Stored product insects and their infestations are considered as a key element in global food security, due to mass infestations and qualitative degradations. In this context, there are species that are also important for public health, through direct contact or contamination of food/feed. At the same time, most stored product insects are able to spread mycotoxigenic fungi, given that some of these species are very effective "fungus feeders". This talk was based upon the potential of certain stored product insects to be carriers of fungi and toxins, and effectively negatively affect grains and other durable commodities under storage.

"Use of stored product insects for food and feed: The EU perspective" (Prof. C. Athanasiou, UTh)

Insects have been authorized as food and feed by the EU in 2017, while their utilization for this purpose is continuously updated until today. Apart from being important nutrient sources, insect can be also effective waste management agents, for different types of agricultural by-products, especially grains and other durable commodities at their post-harvest stages. Moreover, these insects can also reduce the number of mycotoxins on grains and related products (flour etc.), through a unique mechanism that deals with their microbiota traits. This presentation provided a viable overview of this topic, based on the use of insects for this purpose.

"Linking dietary exposure and risk assessment of mycotoxins" (Prof. M. Kakagianni, UTh)

Mycotoxins, toxic compounds produced by certain fungi, can contaminate crops and subsequently enter the food supply, posing serious health hazards. This lecture focused on understanding the relationship between the consumption of food contaminated with mycotoxins and the potential health risks posed to humans. It also highlighted the methods for assessing dietary exposure to these toxins and the risk assessment frameworks used to evaluate their impact on public health. This topic was critical as it helped in developing strategies to minimize mycotoxin contamination, ensuring food safety, and protecting consumer health.

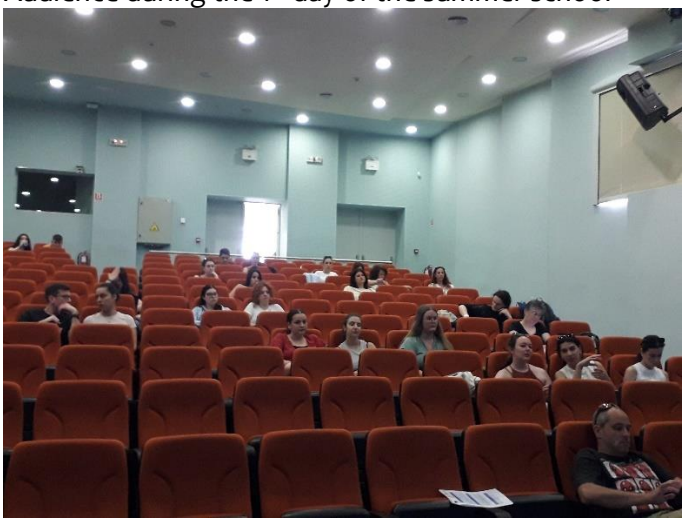
"Training on quantitative mycotoxin exposure assessment: case study on bread products – Hands-on" (Prof. M. Kakagianni, UTh; Prof. V. Valdramidis, NKUA)

This lecture focused on practical training in assessing the exposure to mycotoxins through bread and flatbread consumption, as case studies, with literature-based occurrence data. With the use of interactive application tools, the participants interacted and learned quantitative methods for measuring mycotoxin levels in bread and flatbread products, analysing data, and interpreting the results to understand potential health risks. This training was crucial because it offered to food safety researchers with the skills needed to quantitatively assess and manage mycotoxin contamination, ensuring safer food products and protecting public health.

Finally, in the next pages are presented some photos of this event of the project.

Audience during the 1st day of the summer school

Audience during the 2nd day of the summer school



Audience during the 3rd day of the summer school

Organisers, Dr O. Papadopoulou, Dr D. Miliordos, & Dr P. Natskoulis with invited speaker Dr A Panara (NKUA)

Participants and invited speaker Prof. A. Batrinou (UniWA) during hands-on session

From left, invited speaker Prof. M. Kakagianni (UTh) with Dr P. Natskoulis, and invited speaker Prof. C. Athanassiou (UTh) with Dr D. Miliordos

Invited speakers from left, Dr C. Cervini and Dr C. Verheecke-Vaessen (CU), Dr R. Torrijos, Mrs H. Gjoni, & Mrs I. Picicci (UNIPR), Dr J.-M. Membre (INRAE), with Dr E. Kolia (NKUA) and Dr D. Miliordos & Dr P. Natskoulis (ELGO-ITAP, organisers)

Participants and tutors during the last day of the Summer School

Figure 3.3 Photos from FunShield4Med Summer School 2

FunShield4Med

SHIELDING FOOD SAFETY AND SECURITY BY ENABLING THE FORESIGHT OF FUNGAL SPOILAGE AND MYCOTOXINS THREATS IN THE MEDITERRANEAN REGION UNDER CLIMATE CHANGE CONDITIONS

D4.3 - Summer Schools for ESRs and ECSs

Funded by
the European Union

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