



*A decade
inspiring science*

X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025

Book of Abstracts

Draft

www.eedaa2025.uevora.pt



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH



REPÚBLICA
PORTUGUESA

Title: X PhD Students Meeting in Environment and Agriculture

Editors:

Marta Laranjo

Nuno Pedroso

Bruno Medronho

Sofia Eufrázio

Cláudia Marques

Teresa Pinto Correia

Address:

Universidade de Évora,

Largo dos Colegiais, 2

7004-516 Évora

Published:

UE – Universidade de Évora

Dear participants,

Welcome to the X edition of the PhD Students Meeting in Environment and Agriculture (Encontro de Estudantes de Doutoramento em Ambiente e Agricultura–EEDAA)!

This year marks a special milestone: a decade of EEDAA. Over the past ten years, this meeting has grown into a dynamic forum where PhD students come together to exchange ideas, present their research, and build networks across disciplines and between institutions. EEDAA is now more than a scientific event – is a space for inspiration, where early-career researchers contribute to shaping the future of science in the fields of agriculture, environment and sustainability.

The X EEDAA will take place over two days, on December 10-11, 2025, at the at the University of Évora – Mitra Campus (Conference Room). The official language of the Meeting is English.

This event is particularly designed for PhD students developing their theses in Agricultural Sciences, Biochemistry and Biotechnology, Biology and Ecology, Environment, Sustainability and Socioeconomic Development, Food Sciences, Veterinary Sciences and Animal Production, and related subjects.

Only abstracts submitted by PhD students will be considered for oral communications. However, all other researchers working in the thematic areas of the meeting are invited to participate and submit an abstract for poster presentation.

This event is organised by MED – Mediterranean Institute for Environment, Agriculture and Development and IIFA – Institute for Research and Advanced Training, of the University of Évora, with the support of the Food & Water subnetwork of UNIMED – Mediterranean Universities Union and the Associate Laboratory CHANGE.

The meeting also offers a great opportunity to explore the charming city of Évora, a UNESCO World Heritage Site full of history and culture.

Welcome to Évora for this special edition!

The Organising Committee,
Marta Laranjo, MED|CHANGE
Nuno Pedroso, MED|CHANGE
Bruno Medronho, MED|CHANGE
Cláudia Marques, IIFA
Teresa Pinto Correia, MED|CHANGE
Sofia Eufrázio, MED|CHANGE

COMMITTEES

Organising Committee

Marta Laranjo, Universidade de Évora - MED|CHANGE
Nuno Pedroso, Universidade de Évora - MED|CHANGE
Bruno Medronho, Universidade do Algarve - MED|CHANGE
Cláudia Marques, Universidade de Évora - IIFA
Teresa Pinto Correia, Universidade de Évora -MED|CHANGE
Sofia Eufrázio, Universidade de Évora -MED|CHANGE

Scientific Committee

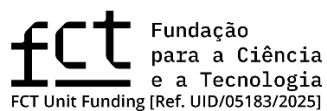
Ana Teresa Caldeira, Universidade de Évora – HERCULES|IN2PAST
Célia Antunes, Universidade de Évora – ICT
Luís Martins, Universidade de Évora – MED|CHANGE
Maria Rosário Martins, Universidade de Évora – HERCULES|MED|CHANGE
António Mira, Universidade de Évora – MED|CHANGE
Ana Catarina Sousa, Universidade de Évora – CHRC
Carla Pinto Cruz, Universidade de Évora – MED|CHANGE
Pedro R. Almeida, Universidade de Évora – MARE-UE
Elsa Leclerc Duarte, Universidade de Évora – MED|CHANGE
Ana Faustino, Universidade de Évora – CHRC
Helder Cortes, Universidade de Évora – MED|CHANGE
Luís Leopoldo Silva, Universidade de Évora – MED|CHANGE
António Pedro Santos, Universidade de Évora – MED|CHANGE
Maria João Cabrita, Universidade de Évora – MED|CHANGE
Raquel Lucas, Universidade de Évora – CEFAGE|MED|CHANGE
Pedro Henriques, Universidade de Évora – CEFAGE|MED|CHANGE
Teresa Pinto Correia, Universidade de Évora – MED|CHANGE
Maria Helena Guimarães, Universidade de Évora – MED|CHANGE
Raquel Garcia, Universidade de Évora – MED|CHANGE
Cristina Barrocas Dias, Universidade de Évora – HERCULES

Organised by:



UNIVERSIDADE DE ÉVORA
INSTITUTO DE INVESTIGAÇÃO
E FORMAÇÃO AVANÇADA

Supported by:





*A decade
inspiring science*

X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025

PROGRAMME

www.eedaa2025.uevora.pt



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH



REPÚBLICA
PORTUGUESA

X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



INSTITUTE FOR ADVANCED STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



X PhD Students Meeting in Environment and Agriculture



December 10-11, 2025

UEVORA | Mitra Campus



WIRELESS ACCESS FWUE

User name: EEDAA25

Password: EEDAA25

Instructions to connect



PT | EN

GENERAL PROGRAMME

Wednesday, December 10

09:00

Registration

09:45

Opening session

10:00

Oral Communications

11:00

Coffee break and poster session

11:30

Solange Oliveira Plenary Session

12:30

Lunch break

14:30

Oral Communications

15:45

Coffee break and poster session

16:15

Oral Communications

17:45

End of the first day

Thursday, December 11

09:30

Oral Communications

10:45

Coffee break and poster session

11:30

Plenary Session

12:30

Lunch break

14:30

Oral Communications

15:30

Coffee break and poster session

16:00

Oral Communications

17:00

Closing Session

- Best Poster Prize
- Solange Oliveira Prize (MED)



X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



FULL PROGRAMME

Wednesday, December 10

09:00 Registration

09:45 Opening session

Hermínia Vilar | Universidade de Évora | Rector

Rui Salgado | IIFA-Institute for Advanced Studies and Research | Director

Fátima Baptista | MED-Mediterranean Institute for Agriculture, Environment and Development | Director

10:00 Oral Communications | Moderator: Hugo Duarte, Universidade do Algarve, MED & CHANGE

- 1** *Irrigation effects on the volatile profile of traditional grapevine varieties: Analysis by HS-SPME-GC×GC-ToFMS*
Daniela Fonseca
Universidade de Évora, MED & CHANGE
- 2** *Characterization of functional protein ingredients from farmed catfish coproducts for food formulations*
Busenur Özkan
University of Lisbon, Faculty of Veterinary Medicine, CIISA, AL4Animals
- 3** *Extraction of phenolic antioxidants from kiwiberry (*Actinidia arguta*) using ultrasound technology: optimization and bioactivity evaluation*
Catarina Pinheiro Macedo
REQUIMTE/LAQV, ISEP, Polytechnic of Porto; REQUIMTE/UCIBIO, University of Porto; i4HB —Institute for Health and Bioeconomy
- 4** *Volatile Organic Compound Profiles of Young Monovarietal Portuguese Red Wines by HS-SPME/GC×GC-ToFMS*
Sousa Gastão Muchecha
Universidade de Évora, MED & CHANGE



X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



11:00 - 11:30

Coffee break and poster session

Wednesday, December 10

11:30

Plenary Lecture Solange Oliveira

Beneficial soil microorganisms: different roads to promote plant growth

Ana Alexandre Universidade de Évora, Dep. Biologia, ECT, MED & CHANGE

12:30 - 14:30

Lunch break [1st group: 12:30-13:30 | 2nd group: 13:30 - 14:30]

14:30

Oral Communications | Moderator: Daniela Rosa, CEBAL, MED&CHANGE

5

Unravelling the molecular mechanisms behind floral diversity and development in the Fagaceae family

Hugo Afonso

Universidade do Minho, CBMA

6

Magnetic resonance mass spectrometry for metabolomics-guided design of synthetic microbial communities for agriculture

João Luz

Faculdade de Ciências da Universidade de Lisboa, FT-ICR and Structural Mass Spectrometry Laboratory

7

Influence of grazing management practices on gastrointestinal nematode burden and clinical parameters in sheep

Maria Rebelo Braz

Universidade de Évora, MED & CHANGE

8

Ensuring safe food waste upcycling: pesticide bioaccumulation in black soldier fly larvae reared on food waste

Joana Oliveira

Egas Moniz School of Health & Science - CiiEM

9

A systems approach to the tick-host immunobiology – A starting pipeline

Sara Zúquete

University of Évora, MED & CHANGE; Universidade Lusófona, FMV; University of Lisbon, CIISA



X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



15:45 - 16:15

Coffee break and poster session

Wednesday, December 10

16:15

Oral Communications | Moderator: Daniela Rosa, CEBAL, MED&CHANGE

10

Tomato brown rugose fruit virus in Portugal: first report providing molecular and phylogenetic characterization

Joana Amaro Ribeiro

Universidade de Évora, MED & CHANGE; Instituto Politécnico de Santarém, CERAS

11

New methodologies to study the influence of oak crown cover in the Montado, a traditional agro-silvopastoral system of the Iberian Peninsula

Eva Gouveia-Barrocas

Universidade de Évora, MED & CHANGE

12

IAP2/ERF-mediated transcriptional response of cowpea to CABMV and CPSMV infection

Cínthia Nunes

Federal Rural University of Pernambuco, Laboratório de Expressão Gênica

13

*Evaluation of Biochar Application in Cabbage (*Brassica oleracea* var. *capitata*): Effects on Germination and Plant Growth*

Maria João Caldinhas

Universidade de Évora, MED & CHANGE

14

*Population Behavior of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae): in Response to Different Trapping Strategies*

Silvio Junior

Universidade do Algarve, MED & CHANGE

15

Optimizing Water Use: Regulated Deficit Irrigation Effects on Citrus and Avocado Yields

Ana Rita Trindade

Universidade do Algarve, Faculdade de Ciências e Tecnologia, MED & CHANGE

17:45

End of the first day of the X PhD Students Meeting in Environment and Agriculture



X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025

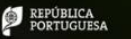


A decade inspiring science



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



Thursday, December 11

9:30

Oral Communications | Moderator: Diogo Costa, Universidade de Évora, MED & CHANGE

16

Pathogen sharing associated with the introduction of non-native turtles: A global review

João Rato

Universidade de Évora, MARE & ARNET

17

Eating the invaders: plant preferences and seed ingestion by captive ungulates

Mónica Roldão Almeida

Universidade de Coimbra, CFE & TERRA / Instituto Politécnico de Coimbra, CERNAS

18

Do covercrops shape the arthropod communities in almond and olive superintensive groves? Insights of the first monitoring season

Laura Machado

Universidade de Évora, MED & CHANGE

19

Correlation Between PM10-Bound Organic Compounds and Cellular Responses in A549 Lung Cells

Marlene Soares

Universidade de Aveiro, CESAM

20

Effects of Artificial Canopy Gaps on Holm Oak Regeneration in Dryland Afforestations

João Rosa

Universidade do Porto, CIBIO & InBIO, BIOPOLIS; University of Natural Resources and Life Sciences, BOKU; Biological Station of Mértola; Smithsonian Conservation Biology Institute, Migratory Bird Center

10:45 - 11:30

Coffee break and poster session



X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



Thursday, December 11

11:30

Plenary Lecture

Overcoming three planetary challenges for the 21st century

Francisco Ferreira Universidade Nova de Lisboa, CENSE & CHANGE, ZERO

12:30 - 14:30

Lunch break [1st group: 12:30-13:30 | 2nd group: 13:30 - 14:30]

14:30

Oral Communications | Moderator: Susana Filipe, Universidade de Évora, MED & CHANGE

21

Justice by Design: Mapping Mindsets in Energy Transitions

Beste Gün Aslan

Universidade Nova de Lisboa, CENSE & CHANGE

22

Local perspectives on a rural Renewable Energy Community surrounded by solar farms: Insights from Comenda (Alentejo, Portugal)

Carolina Cruz de Castro

Universidade Nova de Lisboa, CENSE & CHANGE

23

Fire regime effects on soil habitat and carbon balance in Mediterranean landscapes


João Canedo

University of Évora, MED & CHANGE

15:30 - 16:00

Coffee break and poster session





X PhD Students Meeting in Environment and Agriculture

University of Évora | December 10 -11, 2025



A decade inspiring science



UNIVERSITY OF ÉVORA
INSTITUTE FOR ADVANCED
STUDIES AND RESEARCH

CHANGE
Global Change and Sustainability Institute



Thursday, December 11

16:00

**Oral Communications | Moderator: Susana Filipe,
Universidade de Évora, MED & CHANGE**

24

*Arbuscular mycorrhizal fungi inoculation as sustainable tool to improve yield and phytochemical value of *Cynara cardunculus* L.*

Claudia Formenti

University of Catania

25

The Role of Urban Green Space Vegetation in Carbon Sequestration and Storage: The Case Study of Jardim das Comunidades, Loulé

Pedro Matias

University of Algarve, MED & CHANGE; CIMA& ARNET

26

The social stigmas surrounding Neglected and Underutilized Species (NUS): a narrative review

Débora Paulino

UTAD

27

Assessing Fungi in tomato greenhouses: a One Health concern

Daniela Simões

INIAV; Faculty of Sciences, University of Lisbon, CE3C

17:00

Closing Session

Best Poster Prize

Solange Oliveira Prize (MED)

Note: Posters will be displayed throughout the whole Meeting.



Plenary Lectures

Plenary Lectures

Plenary Lecture Solange Oliveira

Beneficial soil microorganisms: different roads to promote plant growth

Ana Alexandre

MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Department of Biology, School of Science and Technology, Universidade de Évora, Portugal

Email: anaalex@uevora.pt

Soil is a living and dynamic matrix that typically comprises high microbial diversity. In addition to the more general roles of soil microorganisms in the decomposition of organic matter and soil structure, some of these microorganisms interact closely with plants, mainly through external and/or internal colonization of roots. These interactions can be beneficial for plant growth and also contribute to the plant's tolerance to biotic and abiotic stresses. In this context, the role of soil microorganisms in agroecosystems and natural ecosystems may be addressed from different perspectives.

The plant-microbe interaction might be very complex and include the formation specialized structures resulting from the association between soil bacteria and plants, as is the case of the symbiotic mutualism between rhizobia and legumes, which leads to the development of nodules on the roots or shoots. On other cases, the interaction might be as simple as the plant benefiting from the uptake of products resulting from bacteria metabolism, as for example the case of phosphate-solubilizing bacteria.

On a broader approach, focusing on populations and not on a single interaction, microbes might be investigated using metagenomics tools, for the study of bacterial and fungi communities on soil or root samples. On the other hand, and more focused on the functional aspects of soil microorganisms, other tools, such as soil enzymatic activities can be used to evaluate microbial activity.

Overall, these different dimensions of the plant-microbe interaction analysis allow us to investigate the impact of different agronomic practices, or the effect of different ecological contexts, may have on the soil microbiome. This type of studies contribute to improve our knowledge on which actions/conditions will potentiate the benefits of soil microorganisms.

This work was funded by Fundação “la Caixa”, BPI and FCT- Fundação para a Ciência e a Tecnologia (Promove 2022, PD21-00019), Agenda para a transformação digital das cadeias de valor florestais numa economia portuguesa mais resiliente e hipocarbónica TRANSFORM (C644865735-00000007) and by National Funds through FCT under the Project UIDB/05183.

Overcoming three planetary challenges for the 21st century

Francisco Ferreira

CENSE – Center for Environmental and Sustainability Research & CHANGE - Global Change and Sustainability Institute, Departamento de Ciências e Engenharia do Ambiente, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; ZERO – Associação Sistema Terrestre Sustentável, 1600-241 Lisboa, Portugal.

Email: ff@fct.unl.pt

Planetary Health and the One Health approach are key to humanity and ecosystems: human wellbeing is inseparable from the integrity of natural systems (climate, water, soils, biodiversity), so preventing health and social crises requires integrated, cross-sector governance, systems thinking, and a strong focus on equity and justice for the most exposed communities.

We are facing three interconnected global crises: the climate crisis (driven mainly by fossil-fuel combustion), the biodiversity crisis (accelerating extinction risk), and the resources/pollution crisis (human ecological footprint exceeding what nature can regenerate). We have escalating evidence and impacts—record heat, ocean heat uptake, intensifying storms and disrupted water cycles that amplify floods, droughts, wildfire risk, displacement, economic damage, and rising health threats (including the shifting geography and emergence of infectious diseases).

But we have solutions that are known and largely available and science and technology play a major role: countries have committed under the Paris Agreement to work toward net-zero by 2050, and major biodiversity objectives are set under the Kunming–Montreal Global Biodiversity Framework (including protecting and restoring significant ecosystem areas by 2030). To unlock the future, we have to redefine the human–nature relationship, ensuring durable prosperity beyond GDP (making sustainable choices the easiest choices), and investing at scale—while guarding against greenwashing and prioritizing transparency, smarter decision-making, and action by institutions, companies, and individuals.

Agricultural Sciences

***Tomato brown rugose fruit virus* in Portugal: first report providing molecular and phylogenetic characterization**

J. A. Ribeiro^{1,2*}, A. Albuquerque¹, M. D. Campos³, M. Basaloco², M. Patanita², F. Santos³, C. Varanda², P. Materatski¹ and M. R. Félix³

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal

²CERNAS – Research Centre for Natural Resources, Environment and Society, ESAS - Escola Superior Agrária de Santarém Instituto Politécnico de Santarém, Quinta do Galinheiro, S. Pedro, 2001-904 Santarém, Portugal

³MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

*Email: joanaar@uevora.pt

Plant viral diseases have intensified globally in recent decades, and the facilitated introduction and rapid spread of new viral threats make disease management increasingly challenging. *Tomato brown rugose fruit virus* (TBRFV) has emerged as a major concern for tomato production and is now reported across all continents, in more than 45 countries. In Portugal, detections have been limited to a few nurseries and one processing tomato field, all subjected to eradication measures. Consequently, TBRFV is currently classified nationally as transient - detected but not considered established - although in recent years many informal reports of symptomatic plants have circulated among growers and technicians. The urgency of understanding this virus arises from its rapid global spread, and the considerable economic losses associated with its outbreaks. Our main objective was to obtain a Portuguese isolate and characterize it molecularly and phylogenetically, to provide the first confirmed official report of the presence of this virus in the country. We detected TBRFV in symptomatic plants from a processing tomato field in Torres Vedras by RT-PCR. Genome sequencing was carried out using RNA-seq analysis and Sanger sequencing for molecular and phylogenetic characterization. For Sanger sequencing, we designed primer sets that allowed amplification of the entire TBRFV genome in parts fragments. Most nucleotide substitutions were located in the replicase subunits and were predominantly non-synonymous, suggesting adaptative selective pressure that may contribute to the virus's evolutionary success and its ability to spread and persist in diverse environments. Phylogenetic analysis revealed that the Portuguese isolates were closely related to a Chinese isolate, with most of the isolates in the same clade originating outside of Europe. These findings contribute to the broader knowledge of TBRFV genetic diversity and evolution and support efforts to the protection of tomato crops, ensuring the sustainability of the tomato industry in Portugal and beyond.

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UIDB/05183/2025 and under PhD scholarship 2022.13638.BD, attributed to Joana Amaro Ribeiro, <https://doi.org/10.54499/2022.13638.BD>.

**New methodologies to study the influence of oak crown cover in the
Montado, a traditional agro-silvopastoral system of the Iberian Peninsula**

E. Gouveia-Barrocas¹, A. Gonçalves²

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: eva.barrocas@uevora.pt

The *Montado* is a traditional High Nature Value Farming (HNV) agro-silvopastoral system of the South of the Iberian Peninsula. The *Montado* is characterised by the presence of long-lived oak trees, the cork oak (*Quercus suber*) and the holm oak (*Quercus ilex*). Signs of the oak crown cover decline have been reported. Managing such a complex and long-lived agro-silvopastoral system requires integrated knowledge of forest structure, regeneration dynamics, and soil conditions. This study adopted an integrative approach to investigate how crown cover influences stand structure, natural regeneration, soil properties, and litter layer in holm oak stands. During this research, the oak population was stratified by crown cover, and a random stratified sampling was applied on a 180-hectare *Montado* property in Ferreira do Alentejo, grazed by a flock of 600 sheep. A total of 40 plots (2000m²) were selected. A forest inventory was designed that included dendrometric measurements, such as oak diameter (cm), total height (m), crown radius (m), height to live-crown base (m), and a natural regeneration evaluation. The soil parameters sampled were litter layer (LL, kg/m²), soil organic carbon (SOC, %), and pH in H₂O and KCL. Two new methodologies were created: the STRUX Index which facilitates stand structure classification and it can be calculated using only the diameter, and the Natural Regeneration (NR) Classification, in order to assess natural regeneration viability and quality. The results showed that a higher crown cover was linked to an uneven-aged stand structure, a higher number of established natural regeneration, and a tendency for higher values of soil organic carbon and litter layer. There was a relevant negative correlation between crown cover and soil pH(H₂O). Furthermore, according to our results of the NR Classification, 85% of the plots, with a crown cover higher than 30%, had good quality saplings suited to be recruited. A crown cover higher than 10% was linked to an uneven-aged structure. These findings provide a positive outlook on the resilience and regenerative potential of holm oak stands within the *Montado*, offering valuable insights for sustainable management and conservation of this landscape.

Keywords: Montado, agroforestry systems, organic carbon, natural regeneration

AP2/ERF-mediated transcriptional response of cowpea to CABMV and CPSMV infection

C. C. C. G. Nunes¹, A. A. G. Barros², F. L. B. Medeiros², W. D. Oliveira², M. D. Campos³, C. Campos⁴, R. Carvalho¹, R. L. O. Silva⁵, A. M. Benko-Iseppon²

¹ Laboratório de Expressão gênica, Agronomy Department, Federal Rural University of Pernambuco, Rua Dom Manuel de Medeiros, s/n, Recife, 52171-900, PE, Brazil.

² Laboratório de Genética e Biotecnologia Vegetal, Center of Biosciences, Genetics Department, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235, Recife 50670-901, PE, Brazil.

³ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁵ Laboratório de Análises Genéticas, Department of Natural and Earth Sciences, State University of Minas Gerais – Divinópolis Unit, Avenida Paraná, 3001, Bairro Belvedere, Divinópolis, CEP: 35501-170, Minas Gerais, Brazil.

Email: cinthia.carla@ufrpe.br

Cowpea (*Vigna unguiculata*) is a legume strategically cultivated in semi-arid regions due to its high drought tolerance and efficiency in biological nitrogen fixation. However, the incidence of viral diseases such as CABMV (cowpea aphid-borne mosaic virus) and CPSMV (cowpea severe mosaic virus) can severely reduce productivity. Understanding the molecular defense mechanisms is therefore crucial for the development of more resilient genotypes. In this context, this study aimed to identify and evaluate the expression profile of AP2/ERF genes in cowpea under viral infection. The genes were identified using the HMM profile and renamed according to their chromosomal location, and duplication mechanisms were inferred through MCScanX. Plant expression profiles were analyzed following viral inoculation assays with CABMV and CPSMV, at 60 minutes post-inoculation. A total of 189 genes containing a complete AP2 domain were identified, showing a heterogeneous distribution across the chromosomes, except for chromosomes 4 and 11, which contained 6 and 9 genes, respectively, and chromosome 7, which harbored 31. Both tandem and segmental duplications were identified, suggesting that the expansion of the gene family occurred through multiple gene duplication mechanisms. The syntenic relationships among different *loci* revealed a strong structural correlation, indicating that these events contributed to the functional diversification of the AP2/ERF genes and their adaptation to various environmental stresses. A total of 22 differentially expressed AP2/ERF genes (DEGs) were identified under CABMV infection, 5 upregulated (UR) and 17 downregulated (DR), while CPSMV infection induced 12 DEGs (4 UR and 8 DR), indicating a predominantly repressive transcriptional response during early viral infection. The predominance of down-regulated AP2/ERF genes under CABMV infection suggests that the virus may suppress key regulatory defense pathways, whereas the more balanced up- and down-regulation under CPSMV implies a subtler reprogramming of the transcriptional network rather than outright repression. In conclusion, this study identified 189 AP2/ERF genes in *V. unguiculata*, expanded by tandem and segmental duplications. The distinct responses to CABMV and CPSMV highlight the key role of this family in early antiviral defense and provide a basis for improving cowpea resilience through molecular breeding.

Evaluation of Biochar Application in Cabbage (*Brassica oleracea* var. *capitata*): Effects on Germination and Plant Growth

Maria Caldinhas¹, José Marques da Silva², Paulo Mourão^{3,4,5,6*}

¹ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, University of Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal

² MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Rural Engineering, School of Sciences and Technologies, University of Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal

³ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Chemistry and Biochemistry, School of Sciences and Technologies, University of Évora, Rua Romão Ramalho nº 59, 7000-671 Évora, Portugal

⁴ VALORIZA – Research Center for Endogenous Resource Valorization, Polytechnic Institute of Portalegre, Campus Politécnico 10, 7300-555 Portalegre, Portugal

⁵ LAQV-REQUIMTE – Associated Laboratory for Green Chemistry, Portugal

⁶ Research Chair in Material Science, University of Namibia, Windhoek, Namibia

Email: maria.caldinhas@uevora.com

This study evaluated the effects of two distinct types of biochar, produced from Acacia species biomass under different pyrolysis conditions, on the germination and early growth of *Brassica oleracea* L. var. *capitata* (Scirocco F1). The biochar's differed in production temperature, residence time and resulting physicochemical properties. Their impact was assessed using varying application rates and particle sizes, with the aim of identifying conditions that optimise plant development while avoiding potential inhibitory effects.

The achieved results demonstrated that biochar type, particle size and application rate significantly influenced germination rate, plant biomass and health indicators. The BPA biochar produced at higher temperature and shorter residence time exhibited a more developed porous structure and a higher total apparent surface area (90 m²/g), which likely led to competition with plants for available nutrients. This resulted in a decline in plant health indicators, including reduced chlorophyll content and increased incidence of leaf damage. Conversely, the biochar produced at lower temperature and longer residence time displayed lower porosity (35 m²/g) and did not exhibit the same degree of nutrient competition, leading to more favourable plant health outcomes. High application rates, particularly at or above 50% biochar content, substantially reduced germination success and inhibited early seedling development. The most pronounced negative effects were observed at 100% biochar, where growth was almost completely suppressed. Larger particle sizes were associated with lower biomass accumulation, likely due to reduced substrate homogeneity and nutrient distribution. The most effective conditions for promoting germination and early growth were identified at low application rates (1% to 3%) and smaller particle sizes (2.0 to 9.5 mm).

These findings highlight the importance of tailoring biochar production parameters and application strategies to specific agronomic contexts. Optimised biochar use has the potential to enhance soil quality and crop performance, contribute to sustainable agricultural practices, and support environmental goals by promoting carbon sequestration and resource efficiency.

Keywords: Biochar, Sustainable agriculture, Soil amendment, Crop productivity, Climate resilience.

Population Behavior of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae): in Response to Different Trapping Strategies

Silvio Lisboa de Souza Junior^{1,2}, Ana Rita Trindade^{1,2}, Pedro Miguel Inácio Matias^{1,2}, Amílcar Manuel Marreiros Duarte², Luís Miguel Mascarenhas Neto², José Manuel Herrera Vega³

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²Faculty of Science and Technology, University of the Algarve, Gambelas Campus, 8005-139, Faro, Portugal

³Instituto de Investigación Vitivinícola y Agroalimentaria (IVAGRO), Universidad de Cádiz, Spain

Email: a91166@ualg.pt

Ceratitis capitata is one of the main citrus pests around the world, causing direct and indirect damage to fruits, with consequences to the citrus production chain. The control of this pest has been carried out mainly using synthetic chemical insecticides however, the intensity and continuity of use of these products negatively affects the environment and leads to the development of resistance to the main molecules available. Particularly in Mediterranean regions, such as Portugal and Spain, where there is a limited number of chemical active ingredients against this pest, alternatives for chemical control of this species is necessary, as mass trapping or attract-and-kill strategies. The objective of this work was to evaluate the capture of *Ceratitis capitata* using attractants, with and without insecticide, and to analyze the capture pattern over the evaluation period. The study was carried out in an orchard plot in the municipality of Faro, Portugal, using McPhail-type traps. The experimental design adopted was a randomized block design (RBD), with 4 treatments: Trypack (T1), Pheromone (T2), Trypack + Pheromone + Insecticide (T3), with 20 replications and three evaluation times: 7, 15, and 21 days. At each evaluation data, the traps were taken to the laboratory for counting the captured insects. The results obtained indicate a statistically significant difference between treatments, reflecting different levels of attractiveness. Traps containing only Trypack showed greater efficiency for the capture of *C. capitata* in all evaluations and insecticide traps have been shown to have the least effectiveness. The results indicate that the type of attractive directly influenced the capture of the pest insect. Thus, we conclude that the choice of attractants for capture is fundamental for the correct monitoring or mass trapping of insects in the field. Also, the insecticides used with deltamethrin as the active ingredient, together with the attractants, in this work induce a drastic decrease in insect capture. Therefore, the choice of a killing device can also be a key factor to maximizing catches.

Keywords: Integrated pest management; Fruit protection; Mediterranean fruit fly.

The author would like to thank Universidade do Algarve and the European University of the Seas (SEA-EU) for their support within the framework of the *Partnerships for Excellence GA 101089757 - SEA-EU 2.0*.

Optimizing Water Use: Regulated Deficit Irrigation Effects on Citrus and Avocado Yields

Ana Rita Trindade^{1,2*}, Luísa Coelho^{3,4}, Luís Cabrita⁵, Sérgio Prats^{4,6} & Amílcar Duarte^{1,2}

¹ MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

² Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal

³ GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, Ed. 2, Gab. 2.1, 8005-139 Faro.

⁴ MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora. Núcleo da Mitra Gab. 206 Edifício dos Regentes Agrícolas, Apartado 94, 7006-554 Évora, Portugal.

⁵ Comissão de Coordenação e Desenvolvimento Regional do Algarve (CCDR Algarve), Patação, 8001-904, Faro

⁶ Misión Biológica de Calicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

Email: artrindade@ualg.pt

The Algarve region (Portugal) has a diverse agricultural sector where irrigation is crucial. As climate change intensifies water scarcity, improving irrigation efficiency is essential. Regulated deficit irrigation (RDI) consists of reducing irrigation during phenological phases in which crops are less sensitive to water stress. Citrus and avocado trees, which require similar and relatively high amounts of water, are suitable candidates for evaluating this technique. Studying RDI under Algarve soil and climate conditions aims to achieve substantial water savings while preserving both yield and fruit quality.

During the summer of 2024, conventional irrigation (100%-ETC) was compared to three deficit irrigation treatments, reducing water use by 10%, 20%, and 30% for both citrus and avocado trials. Throughout this period, fruit growth was monitored. At harvest, yield (kg/tree) and average fruit weight (g) were assessed, and water productivity (kg/m³) was calculated.

Due to delays in the installation of the irrigation control system, the deficit irrigation period was relatively short in both the citrus and avocado trials. Nevertheless, water savings were achieved: 71 L per tree for the RDC-10 treatment, 135 L per tree for RDC-20, and 203 L per tree for RDC-30, in citrus; and 518.4 L per tree for the RDI-10 treatment, 1,036.8 L per tree for RDI-20, and 1,555.2 L per tree for RDI-30, in avocado.

In the first year of the trial, although a slight reduction in yield was observed under deficit-irrigation treatments in both citrus and avocado trials, these differences were not statistically significant. The lower yields were mainly due to a reduced number of fruits set in these treatments, as average fruit weight was very similar across all treatments. This indicates that RDI did not negatively affect fruit growth. The differences in yield are therefore attributed to a lower fruit set, which could not have been influenced by deficit irrigation, as RDI began well after the fruit-setting period.

Although these results reflect only the first year of RDI application in these orchards, they are encouraging, particularly considering the substantial water savings achieved.

Agricultural Sciences

This research was funded by the European Union, PRR, AGRO+EFICIENTE (PRR-C05-i03-I-000010). Additional funding was received from the Foundation for Science and Technology (FCT) for MED UIDB/05183/2020 (doi: 10.54499/UIDB/05183/2020) and CHANGE LA/P/0121/2020 (doi:10.54499/LA/P/0121/2020).

Assessing Fungi in tomato greenhouses: a One Health concern

Daniela Simões^{1,2} & Eugénia de Andrade¹

¹National Institute for Agricultural and Veterinarian Research (INIAV), Public Institute (P.I.), Av. da República, Quinta do Marquês, 2780-159 Oeiras, Portugal

²Centre for Ecology, Evolution and Environmental Changes, Faculty of Sciences, University of Lisbon, Campo Grande 016, 1749-016 Lisbon, Portugal

Email: dsofia.simoies@gmail.com

Tomato (*Lycopersicon esculentum* L.) significantly contributes to the global food supply, being consumed both fresh and processed. However, various fungal species, from the genera *Alternaria*, *Fusarium*, and *Aspergillus*, can impact tomato plants' health and fruit quality, causing serious food security and food safety challenges. They can cause destructive pre- and/or post-harvesting diseases and contaminate tomatoes with a wide range of mycotoxins.

In recent years, *Alternaria* mycotoxins have been increasingly reported in tomatoes and tomato-based products, with severe effects on human health by consumption. Since regulations for these compounds are still under development, monitoring their occurrence and studying/characterizing the mycotoxigenic strains responsible is a priority. In addition, the presence of *Fusarium* and *Aspergillus* species, which may be potentially pathogenic to humans, can also be a concern, as they represent an occupational health risk for greenhouse workers. Greenhouses are closed environments, with high temperature and humidity, low ventilation, and dense plant material, all of which favoring fungal proliferation and increasing exposure.

This work aims to screen the fungal genera present in the air of six tomato greenhouses using traditional mycological methods and to characterize *Alternaria*, *Fusarium* and *Aspergillus* species using molecular tools. Two greenhouses, one of soil and one of hydroponic, from each of three regions (North, West and South) were sampled in order to evaluate whether the production system and/or the geographic region influence the occupational exposure risk of the farmers and the potential for *Alternaria* mycotoxins contamination in tomatoes.

This work was funded by National Funds through FCT under the fellowship: UI/BD/154444/2022, <https://doi.org/10.54499/UI/BD/154444/2022>.

Effect of Flowering Intensity on Fruit Production in Sweet Orange

A.Z. Khan^{1,2*}, F. Nobre³, M. Mota^{3,4}, E. Attard⁵ & A. Duarte^{1,2}

¹MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE – Global Change and Sustainability Institute, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

² Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

³Institute of Agronomy, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon.

⁴LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Institute of Agronomy, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon.

⁵Institute of Earth Systems, Division of Rural Sciences and Food Systems, University of Malta, Msida MSD2080, Malta.

Email: azkhan@ualg.pt

During the 2025, an experiment was conducted to assess the relationship between flowering intensity and fruit yield in two major sweet orange (*Citrus × sinensis*) cultivars ‘Navelina’ and ‘Dom João’ across 14 orchards in the Algarve region of Portugal. Seven orchards were selected for each variety, with 20 plants chosen per orchard (totaling 140 plants per cultivar) – 10 low-flowering and 10 high-flowering trees. The spring flush was characterized and the number of flowers per 100 nodes was calculated during the flowering period in March-April 2025, and the corresponding fruit set per plant was counted in September 2025. Scatter plot analyses with trendlines revealed a weak positive or negative correlation between the number of flowers per 100 nodes and the number of fruits per plant in all orchards for both cultivars with very low coefficients of determination (R^2). These findings suggest that flowering intensity alone did not significantly influence fruit yield in the studied orchards. Instead, orchard management practice and canopy size, other internal physiological or external environmental factors may play a more decisive role in determining fruit production. This study highlights critical research gaps and provides a foundation for future investigations into the factors influencing citrus yield performance in the Algarve region under changing climatic conditions.

The author Ahmad Zia Khan would like to thank Universidade do Algarve and the European University of the Seas (SEA-EU) for their support within the framework of the Partnerships for Excellence GA 101089757 - SEA-EU 2.0. This research was partially funded by the European Union, project AGRO+EFICIENTE (PRR-C05-i03-I-000010). Additional funding was received from the Foundation for Science and Technology (FCT) for MED UIDB/05183/2020 (doi: 10.54499/UIDB/05183/2020) and CHANGE LA/P/0121/2020 (doi:10.54499/LA/P/0121/2020).

From cork oak to almond: *Biscogniauxia mediterranea*, a new pathogenic agent of almond trees

A. Faustino^{1,2,3}, C. Marinho^{1,2}, M.M. Oliveira⁴, M.R. Félix⁵, L. Marum^{1,2}

¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Universidade Politécnica de Beja (IPBeja), 7801-908 Beja, Portugal.

²MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal.

³MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Plant Functional Genomics Laboratory, 2780-157 Oeiras, Portugal.

⁵MED & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Apartado 94, 7002-554 Évora, Portugal.

Email: ana.faustino@cebal.pt

In recent years, almond production has tripled due to the introduction of new commercial almond orchards in the Alentejo region. The increased water availability through the Alqueva dam and the possibility of mechanical harvest allowed the introduction of this crop in a region known before for its dynamic ecosystem, “Montado”. *Biscogniauxia mediterranea* is known as the causal agent of charcoal disease in *Quercus* species such as *Quercus suber*, the main species of “Montado”. Although the disease is more frequent in the genus *Quercus*, some reports indicated *B. mediterranea* as responsible for the appearance of canker in other species. The identification of the fungal community of symptomatic diseased trees from Soleta and Vairo cultivars in Beja demonstrates the presence of *B. mediterranea* in all analyzed almond trees, using both molecular and morphological techniques. Furthermore, *B. mediterranea* was identified in a separate study in Soleta almond trees from two additional orchards located in Mora and Ferreira do Alentejo. To confirm the potential of *B. mediterranea* to trigger disease symptoms in almond trees, pathogenicity tests were performed on *in vitro* plants and potted plants. All the *in vitro* plants inoculated with *B. mediterranea* developed superficial and internal brown-black discoloration after one week. In addition, *B. mediterranea* was successfully reisolated from lesions, confirming Koch’s postulates. On the other hand, 90% of the potted almond trees inoculated showed internal wood discoloration, with a mean lesion size of 2.18 cm after one month. The control plants in both assays present healed wounds without any wood discoloration. The microbiome of “Montado”, together with climatic changes, mechanization, and the introduction of new species, may have made *B. mediterranea* a new potential agent of disease.

This study was carried out in the scope of Inov-Amendo-AL: Microenxertia in vitro de amendoeiras selecionadas para a promoção do amendoal no Alentejo (ALT20-03-0246-FEDER-000068), supported by Program Alentejo 2020 through the European Fund for Regional Development (ERDF), within the scope of the Collective Action Support System – Transfer of scientific and technological knowledge – Domain of Competitiveness and Internationalization. The authors also acknowledge FCT for funding the R&D Units UID/5183/2025 to the Mediterranean Institute for Agriculture, Environment and Development (MED), and UIDB/04551/2020 (DOI:10.54499/UIDB/04551/2020) to GREEN-IT Bioresources for Sustainability, as well as LA/P/0121/2020 to Global Change and Sustainability Institute (CHANGE), the Contrato – Programa of L. Marum (CEEC-INST/00131/2018), and a PhD study grant of A. Faustino (UI/BD/153511/2022, DOI:10.54499/UI/BD/153511/2022).

Balancing biodiversity and carbon dynamics through improved wetland management in the Paul da Gouxa lowland peatland reserve, Southern Europe

Ana Trindade¹, Miguel Potes², João Canedo¹, Tiago Reis³, João Galante⁴, Filipe Galante⁴, Diogo Gomes⁴, Rita Ribeiro⁴, Tatiana Moreira^{4, 5, 6}, Daniela Farrôpo⁷, Ana Cerveira^{4, 7}, João Carvalho⁷, Bruna Chaves⁴, Samuel Barias², Isaac Okiti⁸, Mihkle Pindus⁸, Kadir Yildiz⁸ and Kuno Kasak^{8, 9}, Ana Mendes¹

¹ University of Évora, MED - Mediterranean Institute for Agriculture, Environment and Development, Portugal

² University of Évora, CREATE, Portugal

³ Escola Superior Agrária de Santarém, Instituto Politécnico de Santarém, Portugal

⁴ cE3c - Centre for Ecology, Evolution and Environmental Change, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

⁵ MNHNC - Museu Nacional de História Natural e da Ciência, Lisbon, Portugal

⁶ Butterfly Lab of Stockholm, Faculty of Science – Stockholm University, Sweden.

⁷ CESAM - Centro de Estudos do Mar e do Ambiente, universidade de Aveiro, Departamento de Biologia, Aveiro, Portugal

⁸ Department of Geography, Institute of Ecology & Earth Sciences, University of Tartu

⁹ University of California, Berkeley, USA

Contact: aimendes@uevora.pt

The expansion of Nature Reserve networks across Europe—supported by the EU Biodiversity Strategy for 2030 and the global goal of protecting 30% of land and sea—emphasises the need for management strategies that simultaneously safeguard biodiversity and regulate greenhouse gas (GHG) emissions. Peatlands are relevant due to their ecological importance and high carbon storage capacity. To identify effective management options for the Paul da Gouxa peat bog (140 ha; peat deposits up to 9 m), we evaluated biodiversity patterns and GHG fluxes across ten sites representing contrasting habitat uses: temporary pasture, cow pasture, horse pasture, four wetland areas, one 20-year restored site and one non-restored site.

Biodiversity was assessed across mammals, birds, beetles, butterflies and bees using standard field methods. Three clear patterns emerged: restored areas, horse pasture and grassland showed the highest species richness; non-restored and reed/pasture plots consistently supported fewer species; and bird and insect communities were strongly influenced by fine-scale habitat structure, especially along habitat edges.

Soil-level GHG fluxes (CO₂, CH₄ and N₂O) were measured in spring and autumn using static chambers with LI-COR analysers (LI-7810, LI-7820). Ecosystem-scale CO₂ and CH₄ exchanges were quantified through eddy covariance (LI-7500DS and LI-7700) mounted on a 10-m tower. Seasonal variability was marked: maximum CH₄ emissions occurred in summer, while peak CO₂ uptake was recorded from late spring to mid-July. Chamber data showed that the peatland and permanent horse pasture acted as net CH₄ sources (247.2 ± 510.1 and $6.4 \pm 17.6 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$), whereas the restored area, temporary pasture and reed/cow pasture were weak CH₄ sinks (-10.5 ± 4.3 , -9.6 ± 9.6 and $-12.0 \pm 15.5 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$). Measurements from willow stems indicated substantial CH₄ emissions during spring flooding.

Our results show that habitat mosaics at the reserve edges support higher biodiversity, while the inner wetland hosts fewer but specialised species. Seasonal water levels strongly shape carbon fluxes, and early data indicate that the wetland is currently a net carbon source. These patterns underline a key need: rewetting is essential to restore carbon sequestration and return Paul da Gouxa to a functioning carbon sink.



Funded by the
European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor the granting authority can be held responsible for them.

**Exploring the evolution and stress-induced regulation of chitinase genes in
Olea europaea L.**

C. Campos¹, A. Albuquerque¹, M.R. Félix², H. Cardoso³, L. Rodrigues¹, M. Patanita¹, J. A. Ribeiro¹, M. D. Campos²

¹ Universidade de Évora, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Largo dos Colegiais 2, 7004-516, Évora, Portugal

² Universidade de Évora, Escola de Ciência e Tecnologia, Departamento de Fitotecnia, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Largo dos Colegiais 2, 7004-516, Évora, Portugal

³ Universidade de Évora, Escola de Ciência e Tecnologia, Departamento de Biologia, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Largo dos Colegiais 2, 7004-516, Évora, Portugal

Email: mccampos@uevora.pt

Chitinases (EC 3.2.1.14) are glycosyl hydrolases (GHs) that catalyze the hydrolysis of glycosidic bonds in chitin. In plants, these enzymes play crucial roles in responses to both biotic and abiotic stresses, particularly in defense against fungal pathogens through the degradation of fungal chitin. The olive tree (*Olea europaea* subsp. *europaea*), a key fruit species in Mediterranean ecosystems, is highly susceptible to several pathogenic fungi that can severely impact yield. Investigating the chitinase gene family (*OeChi*) in the olive tree may therefore contribute to the development of sustainable disease management strategies.

A total of 33 chitinase genes were identified in the olive genome. Phylogenetic analysis grouped *OeChi* genes into two major subfamilies (GH18 and GH19), further divided into eight classes, each displaying distinct motif compositions and functional domains. The exon–intron organization was generally conserved within each class. Evidence of tandem duplication was detected among seven GH18 genes, most of which appear to be evolving under purifying selection. Comparative genomic analysis revealed a weakly conserved collinearity pattern between *O. europaea*, its wild relative (*O. europaea* subsp. *europaea* var. *sylvestris*), and *Sesamum indicum*, suggesting evolutionary divergence within this gene family.

Expression profiling under fungal infection indicated substantial variation in transcriptional responses among *OeChi* members, with several genes showing strong induction. Promoter analysis uncovered a broad array of *cis*-regulatory elements linked to stress responses and hormone signaling pathways, reinforcing their likely involvement in plant defense mechanisms. Therefore, screening chitinase enzymes with high antifungal activity is important to meet the need to improve *O. europaea* resistance against phytopathogens. Overall, our findings provide a valuable foundation for future strategies aimed at improving fungal disease resistance in olive tree.

This work is funded by the project 'AltF4 - From Lab to Field: Understanding and managing *Alternaria* disease in olive orchards' (COMPETE2030-FEDER-00905300). J.A.R. is supported by the Foundation for Science and Technology (FCT) through the PhD scholarship 2022.13638.BD. This work is also funded by National Funds through FCT under the Project UID/05183/2025.

Assessment of Key Indicators for Determining the Soil Quality Index in a Mediterranean Agroforestry System

G.M.R. Ferreira¹, I. Brito², T. Pinto-Correia¹

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: gustavo.ferreira@uevora.pt

The *Montado* is a complex Mediterranean agroforestry system characteristic of the Alentejo region in Portugal. It integrates Cork oak (*Quercus suber*) and Holm oak (*Q. rotundifolia*) woodlands with extensive livestock grazing. This system plays a crucial role in carbon sequestration, biodiversity conservation, and landscape sustainability. However, the Mediterranean climate is predominantly semi-arid to dry sub-humid, characterized by low rainfall and high summer temperatures, which exert strong environmental stress. In recent decades, *Montado* areas have undergone intensive use without proper management, resulting in progressive ecosystem degradation. Soils in this system are typically shallow, stony, acidic, low in nutrients and organic matter, and may present manganese toxicity. The Soil Quality Index (SQI) is defined as the soil's ability to support plant and animal productivity while maintaining environmental integrity and human health. Establishing a standardized SQI across large areas is challenging because each environment exhibits distinct characteristics. Therefore, identifying locally sensitive soil indicators is essential for effectively assessing *Montado* soils. This study aimed to identify key indicators for developing a Minimum Data Set (MDS) for SQI calculation in the *Montado*. Soil samples were collected from nine farms under six management types, totalling 276 samples from the 0–10 cm layer. Physical (bulk density and stoniness), chemical (pH, Soil Organic Carbon (SOC), P₂O₅, K₂O, CEC, GSB, Al%, EC, Mg, Mn, N, B, Zn, Cu, and Fe), and biological indicators (dehydrogenase, β -glucosidase, arylsulfatase, and phosphatase) were analysed. To select the MDS, Spearman correlation analysis ($p < 0.05$, $r > 0.6$) was used to remove redundant indicators, followed by Principal Component Analysis (eigenvalues > 1 ; loadings > 0.7). The key indicators identified for *Montado* soils were pH, SOC, K₂O, EC, arylsulfatase, β -glucosidase, and phosphatase. The selected chemical indicators reflect nutrient availability and soil fertility, whereas the biological indicators represent soil biogeochemical functionality, including processes essential for microbial energy supply and nutrient cycling for plant uptake.

This work was funded by Plano de Recuperação e Resiliência - PRR under the Project Montados Net Zero (PRR-C05-i03-I-000236).

Evaluation of Olive Pollen Viability Using Epifluorescence Microscopy

C. Inês¹, A. Galveias², A. R. Carlos³, A. R. Costa², C. M. Antunes², L. Alho⁴, A. Cordeiro¹, I. Velada⁴

¹ INIAV - Instituto Nacional de Investigação Agrária e Veterinária, I.P. - UEISBRG, Pólo de Elvas, Olivicultura, Estrada de Gil Vaz, Apartado 6, 7350-404 Elvas, Portugal;

² CREATE - Centro de Investigação em Ciência e Tecnologia para o Sistema Terra e Energia, Instituto de Investigação e Formação Avançada, Departamento de Ciências Médicas e da Saúde, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora, Rua Romão Ramalho, 59, 7000-671 Évora, Portugal;

³ CE3C - Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande 1749-016 Lisboa, Portugal;

⁴ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: ivelada@uevora.pt

INTRODUCTION: Climate change is leading to more frequent and severe extreme temperature events, negatively impacting agricultural productivity and threatening global food security. Plant reproduction, the process fundamental to crop yield, is highly susceptible to heatwaves, which disrupt pollen development and ultimately affect seed-set and crop yields. The male gametophyte (pollen) is particularly sensitive to elevated temperatures through all phases of its development. A better understanding of the molecular mechanisms that underlie the temperature stress response in pollen will be a significant step towards developing effective breeding strategies for high and stable production in crop plants.

AIM: To implement a reliable method to evaluate pollen viability in olive tree species to be used in research for improving reproductive heat stress tolerance.

METHODS: Anther harvesting was done on 8 olive varieties installed in the Olive Reference Collection of Portugal (ORCP) (INIAV, Elvas). Seven Portuguese varieties were used: 'Azeitoneira', 'Galega Vulgar', 'Cobrancosa', 'Verdeal de Tras-os-Montes', 'Cordovil de Serpa', 'Carrasquenha de Elvas', 'Verdeal de Serpa', as well as the Spanish variety 'Arbequina'. When the BBCH 65 flowering stage (at least 50% of the flowers open) was dominant in the olive tree canopy, the anthers were collected into a 2 mL tube containing liquid pollen viability solution and gently crushed. The pollen suspension was stained with dichlorodihydrofluorescein diacetate (H₂DCFDA), a reliable dye for cell viability analysis that functions as an indicator of reactive oxygen species (ROS). Pollen grains were imaged using a fluorescence microscope (Leica DM6000B). The H₂DCFDA signal was detected using a green emission filter, and the fluorescence intensity of pollen grains was quantified with Fiji/ImageJ.

RESULTS: Two distinct subpopulations of pollen, characterized by high and low ROS levels, were observed in all varieties examined. The 'Galega Vulgar' and 'Verdeal de Tras-os-Montes' varieties exhibited a higher proportion of the high-ROS subpopulation compared to the 'Arbequina' variety, suggesting a higher percentage of viable pollen at dehiscence in these varieties.

CONCLUSION: A method for evaluating olive pollen viability using H₂DCFDA staining coupled with epifluorescence microscopy was implemented. Understanding how the distribution of these subpopulations is affected by heatwaves during pollen development can provide valuable insights into reproductive heat stress tolerance.

Physiological Responses of Seeds to Microbial and Plant-Based Biostimulants

L. Rodrigues¹, L. Sousa², M. Mendes², C. Campos¹, R. Amante³, P. Monreal⁴, A. García⁴, R. Illán⁴,
A. Barradas⁵, H. Cardoso⁶

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²School of Science and Technology. Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³Alltech Crop Science Iberia. Polígono Industrial La Estrella/C/Saturno, 170, 30500 Molina de Segura, Murcia, Spain.

⁴Ideagro. Castillo de Monteagudo 13 Oficina 4, 30564 Lorquí, Murcia, Spain.

⁵Fertiprado – Sementes e Nutrientes LDA. Herdade dos Esquerdos, 7450-250, Vaiamonte, Portugal.

⁶MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

Email: lenia.rodrigues@uevora.pt

In the current context of climate change, agricultural crops are increasingly exposed to biotic and abiotic stresses, leading to global agricultural yield losses. To mitigate these effects, farmers often resort to the excessive use of chemical fertilizers and synthetic pesticides, which negatively impacts agricultural sustainability. The use of biostimulants (BSTs) has therefore emerged as a promising and environmentally sustainable alternative in modern agricultural production systems. BSTs, which include compounds, microorganisms, or combinations of both, can activate a wide range of molecular pathways that modulate plant physiology, thereby enhancing nutrient uptake, crop yield, and fruit quality, while also strengthening antioxidant defenses and improving stress tolerance.

Although most studies on BSTs focus on their application at later stages of plant development, information on their application on seeds remains scarce. Seed germination is a vital stage in plant development and a key determinant of crop establishment and productivity. The application of BSTs to seeds represents a promising and sustainable strategy to enhance germination, seedling vigor, and early plant establishment. This work aims to present a new line of research focused on the application of different categories of biostimulants to seeds, namely microbial-based biostimulants and those derived from plant extracts. So far, a concentrate of plant growth-promoting bacteria and leaf-derived extract from a Mediterranean species have been tested on clover (*Trifolium suaveolens*), ryegrass (*Lolium multiflorum*), and wheat (*Triticum aestivum*). Germination assays were conducted to evaluate different methods of BST application to seeds, yielding very promising results in terms of germination percentage, root development, and shoot growth. Future work will involve pot experiments to monitor plant development throughout the entire life cycle, including physiological assessments, collection of plant material, and biochemical and molecular analyses. These findings provide new insights into the potential of seed-applied biostimulants and their role in enhancing early plant development.

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UID/05183/2025.

Phytotoxicity and fungitoxicity of biochar-amended substrates: implications for seedling development and disease suppression

Horst, L.¹; Canedo, JNGV²; Coelho, L.^{2,3}; Silva, V.¹; Silva, C.²; Prats, S.^{2,4}

¹ Wageningen University and Research. Droevendaalsesteeg 4, 6708 PB Wageningen, The Netherlands.

² MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora. Núcleo da Mitra Gab. 206 Edifício dos Regentes Agrícolas, Apartado 94, 7006-554 Évora, Portugal.

³ GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, Ed. 2, Gab. 2.1, 8005-139 Faro.

⁴ Misión Biológica de Calicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

Email: lieke.vanderhorst@wur.nl

Biochar has emerged as a promising soil amendment capable of improving physical and chemical soil properties, enhancing water retention, and promoting microbial diversity—all while contributing to a more sustainable, circular bioeconomy. However, despite these well-documented benefits, key uncertainties remain regarding the potential phytotoxic effects of biochar on plant development and the influence of biochar aging within terrestrial ecosystems. Moreover, the potential of biochar to suppress fungal diseases represents an innovative and relatively unexplored avenue for its application in sustainable agriculture. This study, conducted within the framework of the SOLVO project, aims to 1) evaluate the effects of biochar-amended substrates on seedling development and potential phytotoxic responses, and 2) assess the fungitoxic and disease-suppressive properties of biochar against key fungal pathogens.

Phytotoxicity tests were conducted using *Lactuca sativa* L. seeds incubated in darkness at 25 °C for 72 h. Soil–biochar mixtures were prepared with biochar concentrations ranging from 1% to 100%. Tests were performed both at the start and after a three-month pot experiment developed within the scope of the project to evaluate the effects of biochar aging. Fungitoxicity assays targeting anthracnose (*Colletotrichum accutatum*) were performed using tomato seeds, using the samples after a three-month pot experiment. *In vitro*, in Petri dishes with PDA medium, biochar concentrations between 0.5% and 5% were tested against *C. accutatum*, to determine the inhibition percentage.

Preliminary results indicate that biochar concentrations between 1.5% and 10% promoted seed germination and seedling growth. Phytotoxic effects became evident at concentrations $\geq 20\%$, with significant reductions in root and shoot elongation. Complete inhibition of seed germination occurred at 50% biochar concentration and above. Biochar aging for one three months did not alter its physicochemical properties (except for a slight decrease in pH) or its biological effects. Anthracnose incidence was significantly reduced at biochar concentrations of 3–5%. Overall, biochar levels between 3% and 10% enhanced plant development and demonstrated strong anthracnose-suppressive activity.

This work was funded by National Funds through FCT under the Project SOLVO (2022.06004.PTDC) and PRR Project - Vine and Wine Portugal (Solvit).

Olive response to biostimulants application: potential genes involved in strengthening the plant immune system

C. Côdea¹, L. Rodrigues², A. Albuquerque², M.R. Félix³, C. Campos², M.I. Gafanhoto², M. Patanita², J.A. Ribeiro², M. Basaloco², F. Santos³, M.D. Campos³

¹Master student in Agronomic Engineering, Universidade de Évora, Portugal.

² MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

³ MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

Email: mdcc@uevora.pt

The use of biostimulants as a strategic tool to increase crop resilience in the context of climate change has been receiving increasing attention. These compounds are a sustainable alternative for agriculture because they improve crop growth and productivity, increasing plant resistance to various stress situations, and reduce dependence on synthetic agrochemicals. However, the specific role these products play in plant defense systems remain unclear. In this context, the identification of suitable biostimulants for strengthening the olive immune system is of great interest.

The present study reports transcriptional changes in genes that encode enzymes involved in the olive plants' defence response, following application of three biostimulants. The selected products were horsetail extract (*Equisetum arvense*), *Ascophyllum nodosum* algae extract, and chitosan, which were applied in young olive potted plants once a week, for a six-week period. Leaves samples were collected before product application and at three defined timepoints after the final application. The selected target genes included plant chitinases, known to be implicated in plant defense against fungal pathogens, and genes involved in reactive oxygen species (ROS) metabolism. Additionally, ROS were evaluated by quantification of H₂O₂, a signalling molecule responsible for activating various stress response pathways.

Our results revealed that chitosan caused a significantly up-regulation of a chitinase *GH19*-classI gene, as measured by real-time qPCR. However, a product specificity was observed, since this gene was downregulated when horsetail or algae extracts were applied. The application of the *A. nodosum* and horsetail extracts were responsible for the significantly overexpression of *Superoxide dismutase (SOD)* gene. SOD is one of the most important antioxidant enzymes, with a primary function of detoxify superoxide radicals, a highly reactive and damaging form of ROS. In parallel, H₂O₂ quantification revealed significantly lower values when horsetail extract and chitosan were applied. Plants respond to stress conditions by increasing ROS levels, particularly H₂O₂, and the observed decrease of ROS after biostimulants application may indicate more resilient olive plants.

Although preliminary, these results shed light on the effects of biostimulants on the molecular mechanisms underlying induced resistance and may contribute to the development of more resilient and environmentally sustainable monitoring strategies.

This work is funded by the project 'AltF4 - From Lab to Field: Understanding and managing *Alternaria* disease in olive orchards' (COMPETE2030-FEDER-00905300). J.A.R. is supported by the Foundation for Science and Technology (FCT) through the PhD scholarship 2022.13638.BD. This work is also funded by National Funds through FCT under the Project UID/05183/2025.

Impact of biostimulant application on olive tree resilience: a physiological and molecular approach

M.I. Gafanhoto¹, L. Rodrigues¹, M.R. Félix², A. Albuquerque¹, M. Patanita¹, J.A. Ribeiro¹, M. Basaloco¹, F. Santos², M.D. Campos²

¹ MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

² MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

Email: maria.ines.gafanhoto@uevora.pt

The olive tree (*Olea europaea* L.) is one of the most representative crops in the Portuguese agricultural sector, constituting an economically and culturally significant pillar, particularly in the Alentejo region. However, olive groves face serious challenges arising from climate change and agricultural intensification, namely exposure to biotic and abiotic stresses that compromise productivity and olive oil quality. The increasing restrictions on the use of synthetic plant protection products under European environmental policies further emphasize the need for sustainable alternatives that ensure both plant protection and the economic viability of olive farming.

In this context, biostimulants emerge as promising tools to enhance crop resilience by promoting plant growth, optimizing nutrient uptake, and strengthening natural defense mechanisms. However, knowledge about the underlying mechanisms of their action remains limited, especially under combined biotic and abiotic stress conditions.

The goal of the PhD project here presented is to evaluate the impact of different biostimulants on olive tree resilience through an integrated physiological, transcriptomic, proteomic, and metabolomic approach. Commercial products with distinct modes of action — chitosan, sunflower oil with nettle extract, and a mineral fertilizer based on P₂O₅ and K₂O — will be tested on 'Galega vulgar' olive plants under controlled conditions. The research will be conducted in three complementary phases: (i) selection of the most effective biostimulants through monitoring of physiological parameters and multi-omics analyses; (ii) assessment of olive tree responses to biotic and abiotic stresses, namely inoculation with *Colletotrichum nymphae* and heat exposure (42 °C); and (iii) validation of the most promising products under field conditions, with evaluation of physiological and productive parameters.

Through this multidisciplinary approach, the project seeks to identify genes, proteins, and metabolites associated with induced resistance and stress adaptation, contributing to the knowledge of the molecular and physiological mechanisms underlying biostimulant efficacy and supporting the development of more sustainable agricultural practices.

This work is funded by the project 'AltF4 - From Lab to Field: Understanding and managing Alternaria disease in olive orchards' (COMPETE2030-FEDER-00905300). J.A.R. is supported by the Foundation for Science and Technology (FCT) through the PhD scholarship 2022.13638.BD. This work is also funded by National Funds through FCT under the Project UID/05183/2025.

Mulch and Biochar as Soil Amendments in Alentejo Olive Groves: preliminary study

M. Custódio^{1,2}; A. Catarino^{1,2}; N. Semedo¹; L. Coelho³; J. Canedo³; S. Prats^{3,4}; P. Palma^{1,2,5,6}

¹Polytechnic Institute of Beja, Agrarian School of Beja, IPBeja Campus, Rua Pedro Soares, 7800-295 Beja, Portugal.

²Center for Sci-Tech Research in Earth System and Energy (CREATE)—Pole of the 9 Polytechnic Institute of Beja, 7800-295 Beja, Portugal.

³Mediterranean Institute for Agriculture, Environment and Development (MED) & Global Change and Sustainability Institute (CHANGE), University of Évora, Núcleo da Mitra, Apartado 94, 7006-554 Évora, Portugal.

⁴MBG-CSIC, Misión Biológica de Galicia – National Spanish Research Council, 36143 Salcedo, Pontevedra, Spain.

⁵GeoBioTec, NOVA School of Science and Technology, Campus da Caparica, 2829-516 Caparica, Portugal.

⁶Public Waters of Alentejo, Water Management Company, Rua Dr. Aresta Branco nº 51, 16 7800-310, Beja, Portugal.

Email: mariana.custodio@uevora.pt

Although organic amendments are known to improve soil properties, their combined effects and performance under irrigated conditions remain poorly understood. This study evaluates the effects of the application of two organic amendments on soil properties of Alentejo olive groves. Sampling was conducted in two olive groves with different management agricultural practices: MOU (rainfed, biologic) and VFO (irrigated, intensive). In two campaigns (January (T1) and May (T2) of 2024), composite soil samples were collected across three sites with different slopes (MOU1, MOU2, MOU3 and VFO1, VFO2, VFO3). In each site the collection was performed in three olive lines: (i) control (C; without amendment); M (line with mulch produced from olive leaves; 1 t/ha), and MB (line where was applied mulch + biochar; obtained by 1 t/ha leaves + 10 t/ha acacia wood biochar). Subsamples were collected from three microenvironments: underneath the tree (ST), wheel track area (SR), and inter-row vegetation (SV). Soil agronomic parameters analyzed included pH, electrical conductivity (EC), organic matter (OM), and total nitrogen (N).

In MOU, the effectiveness of the treatments was strongly conditioned by slope and microenvironment, with the MB amendment improving OM (C:1.94-2.28%; MB:2.28-2.95%), N (C:0.13-0.16%; MB:0.16-0.20%), and EC (C:137.65-179.40 $\mu\text{m cm}^{-1}$; MB:163.53-228 $\mu\text{m cm}^{-1}$) mainly in the steeper slope sites, while M was more effective in the lower-slope site, namely on OM (C:1.32-1.69%; M:1.43-1.73%) and EC (C:87.83-135.35 $\mu\text{m cm}^{-1}$; M:97.07-247.80 $\mu\text{m cm}^{-1}$). In the irrigated VFO olive grove, M consistently improved OM (C:1.40-4.03%; M:1.98-4.02%), and N (C:0.14-0.32%; M:0.18-0.48%) across all microenvironments, results more evident in sites with lower slope. The effects of MB occurred primarily in less compacted microenvironments (SV and ST), where biochar incorporation into the soil was facilitated.

In general, both amendments improved soil properties, although their effects varied according to site-specific factors such as slope, management practices, and microenvironment. At the MOU farm, the ideal amendment depended on the slope, with MB showing the greatest benefits on steeper slopes. In contrast, at the VFO farm, M produced more consistent improvements in organic matter across all microenvironments, while MB provided more localized improvements.

This work was funded by National Funds through FCT under the Projects SOLVO (2022.06004.PTDC), CREATE (UID/06107/2023), and GeoBioTec (UID/04035/2023).

Use of remote sensing data to identify the presence of diseases and assess fruit quality in tomato

M. Serrano¹, A. Rato^{1,2}, A. Sousa^{1,3}, A. Alexandre^{1,4}, I. Brito^{1,4}

¹ MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Universidade de Évora, Portugal

² Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

³ Departamento de Engenharia Rural, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

⁴ Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: miguel.serrano@uevora.pt

The industrial production and export of tomato holds significant economic importance in Portugal. However, tomato plants are currently facing severe phytosanitary challenges, particularly fungal infections caused by *Verticillium dahliae*, *Phytophthora infestans*, and *Fusarium oxysporum*. These issues are further exacerbated by climate change, as well as by potential nutritional imbalances to which these plants may be exposed. Consequently, the development of monitoring tools for tomato fields is of great relevance, as it may contribute to prevent yield losses and promote a more sustainable use of the resources.

This project aims to develop computational models enabling remote and large-scale monitoring of the sanitary and quality status of tomato plants and fruits, through the design of classification algorithms based on high-resolution multispectral imagery (drone and Sentinel-2). The construction of these models is based on parameters intrinsic to tomato plants and soil microbial activity assessed both in the field and by laboratory analysis. These parameters included leaf's chemical composition, chlorophyll and fluorescence levels, tomato water and lycopene content, as well as pH and soluble solids determination. Moreover, taking in consideration that soil microbial activity strongly affects plant health and development, an analysis of soil microbial activity was also performed, encompassing measurements of soil basal respiration, estimation of arbuscular mycorrhiza colonization rate, and quantification of soil microbial enzyme activity.

Sample collection for this study was conducted during 2023 and 2024 campaigns, across distinct tomato fields belonging to the project's partner producers. Two samplings were performed each year in every field, and, before each sampling event, multispectral images of the field were collected by drone flight. To date, all laboratory data have been acquired and the multispectral images treated for the subsequent phase, where a multivariate analysis will be performed to construct predictive models of tomato plant sanitary condition and fruit quality. In the next cropping season (2026) these models will be validated under field conditions.

Research concerning the use of models associated to remote monitoring for tomato fields remains scarce, and to the best of current knowledge, still non-existent at the national level. Therefore, this project also presents a distinctly innovative character.

This work was funded by Fundação "la Caixa", BPI and FCT- Fundação para a Ciência e a Tecnologia (Promove 2022, PD21-00019).

A multi-omic approach to understanding the role of secreted biomolecules in modulating the somatic embryogenic response

R. Pires¹, L. Rodrigues¹, F. Milhano², S. Ciordia³, I. Duarte⁴, A. Peixe⁵, H. Cardoso⁵

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidade Autónoma de Madrid (IIS-FJD, UAM), 28040 Madrid, Espanha.

³Functional Proteomics Laboratory, National Center for Biotechnology (CNB-CSIC), Darwin 3, 28049 Madrid, Espanha.

⁴Department of Chemistry, LAQV-REQUIMTE & CICECO—Aveiro Institute of Materials, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal.

⁵MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

Email: rnpires@uevora.pt

Somatic embryogenesis (SE) represents a unique developmental pathway in which somatic cells acquire embryogenic competence through extensive molecular reprogramming. Recognised as one of the most efficient systems for plant regeneration, SE supports advanced breeding strategies and enables research integrating genetic engineering and multi-omics analyses. Its efficiency is tightly regulated by complex interactions between endogenous and environmental cues, including secreted biomolecules into the culture medium. The use of that culture medium (conditioned media) from highly embryogenic lines to stimulate SE in recalcitrant genotypes, commonly known as nurse cell cultures, has long been documented. However, despite their clear modulatory effects, the nature and functional roles of these secreted biomolecules remain largely unknown.

Given the strong recalcitrance exhibited by most olive cultivars when adult tissues are used as explants, a major limitation for genetic engineering and genome-editing approaches, a research line was established to identify the biomolecules secreted by high-efficiency embryogenic lines and their potential role in modulating SE in recalcitrant genotypes. To induce SE, zygotic embryos of *Olea europaea* cv. 'Arbequina' were used to induce SE [1]. Embryogenic calli were maintained by repeated subcultures in liquid olive embryogenesis cyclic (ECO) medium, allowing the selection of high- and low-embryogenic competence lines [1]. Extracellular proteomic (secretome) and metabolomic analyses of both lines were performed using LC–MS/MS and NMR spectroscopy, respectively [2]. The secretome revealed distinct molecular signatures between high- and low-efficiency lines, including extracellular-vesicle-associated proteins and several known embryogenesis biomarkers such as kinases and peroxidases. The metabolomic profile highlighted an active primary carbon metabolism involving glycolysis, fermentation pathways and the tricarboxylic acid cycle.

Together, these findings provide novel insights into the pivotal role of extracellular biomolecules in regulating SE in *O. europaea* and open new perspectives for enhancing embryogenic efficiency in recalcitrant cultivars.

This research was funded by FCT–Fundação para a Ciência e Tecnologia, I.P. under the project reference UI/BD/153509/2022 (<https://doi.org/10.54499/UI/BD/153509/2022>) and through MED under the project UIDB/05183/2020 (MED exploratory project ID 10_2021). MED and CHANGE research institutes are funded by FCT with project references MED (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>) and CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>).

References

- [1] Pires, R., Cardoso, H., Rodrigues, L., Peixe, A. (2025). *Plants*, 2025, 14, 2881.
- [2] Pires, R., Rodrigues, L., Santos, F.M., Duarte, I.F., Ciordia, S., Peixe, A., Cardoso, H. (2025). *Horticulturae*, 11, 331.

Effects of the application of mulch and biochar on soil organic matter and carbon in Mediterranean agricultural soils stocks

Novado, R.¹, Canedo, J.N.G.V.¹, Trindade, A.², Castillo, R.², Coelho, L.^{1,3}, Prats, S.^{1,4}

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora. Núcleo da Mitra Gab. 206, Edifício dos Regentes Agrícolas, Apartado 94, 7006-554 Évora, Portugal.

²Universidade do Estado de Amapá, Av. Pres. Vargas, 650 – Central, Macapá, -AP, 68901-258, Brasil.

³GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, Ed. 2, Gab. 2.1, 8005-139 Faro.

⁴Misión Biológica de Galicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

*Email: ruinojado@uevora.pt

Soil organic matter (SOM) is a key indicator of soil quality, influencing fertility, structure, and water retention, while serving as a major carbon reservoir. In Mediterranean agricultural systems, intensive practices such as frequent tillage and bare-soil management accelerate runoff and erosion, leading to a progressive decline in SOM. Testing and implementing sustainable soil management practices is therefore essential to enhance soil resilience and carbon sequestration.

This study evaluated the effects of mulch and biochar on SOM and soil organic carbon (SOC) dynamics over two years in four sandy loam soils (two olive groves and two vineyards) located in Alentejo, the Portuguese region with the most pronounced Mediterranean climate. Three treatments were applied: control, mulch (2 Mg ha⁻¹), and mulch + biochar (2 + 10 Mg ha⁻¹). Litter and soil samples from three depths (0–2, 2–5 and 5–10 cm) were collected at the start of the experiment, and after 18 months. Organic matter content was quantified by loss-on-ignition at 560 °C for three hours. Organic carbon was estimated using the Van Bemmelen factor, and total organic carbon (TOC) analyses are ongoing to validate these estimations.

Olive groves exhibited higher mean SOM than vineyards, likely due to reduced soil disturbance and natural ground cover. In both land uses, the mulch + biochar treatment produced the greatest increases in SOM (increases by 7 to 15%), particularly in the litter and the 0–2 cm layer (increases by 11 to 17%). The control consistently showed the lowest values, and no differences were observed at 5–10 cm depth.

These findings demonstrate that mulch and biochar applications can enhance and stabilize SOM and SOC accumulation in Mediterranean agroecosystems. Ongoing analyses will quantify these effects more precisely and assess the long-term potential of such practices to promote carbon sequestration and improve soil quality under Mediterranean conditions.

This work was funded by National Funds through FCT under the Project SOLVO (2022.06004.PTDC) and SOLVIT (PRR Project - Vine and Wine Portugal (C644866286-00000011)).

Influence of phytohormones on *in vitro* rooting of Carrizo citrange rootstock for alternative micrografting protocols

T. Magalhães¹, I. Matos², B. Calado², A. Duarte^{1,2}, J.C. Tomás³, L. Cabrita³, N. Marques^{1,2}

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro

³CCDR Algarve – Agricultura e Pescas, 8005-511 Faro, Portugal.

Email: nmarques@ualg.pt

Successful citriculture relies on the cultivation of healthy plants of elite genotypes. The *in vitro* micrografting technique allows the production of virus- and viroid-free plants. The employment of this technique improves phytosanitary standards. Conventional micrografting, which employs a paper bridge in liquid Murashige and Skoog medium, frequently exhibits a low success rate in terms of achieving a union between the stem apex and the rootstock. Within the AGRO+EFICIENTE project, an alternative micrografting protocol was developed. This protocol involved cutting the Carrizo citrange rootstock into four to eight segments (1.3-1.5 cm each), followed by micrografting and culturing in Petri dishes containing semi-solid full MS medium. The scion shoot tip was then placed on the apical end of each rootstock segment. This method enabled the processing of a large number of micrographs in a single session. Union between the meristem and the rootstock was achieved in 52% of cases. However, the spontaneous rooting rate for segments cultured in a phytohormone-free medium was only 6%. A rooting assay was performed to address this issue, evaluating the effect of different concentrations and combinations of naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indol-3-butyric acid (IBA) and gibberellic acid (GA₃) on root induction in the rootstock segments, using 15 rooting media formulations. Preliminary results indicate that a medium supplemented with NAA (0.5-1.0 mg/L) and IAA (0.2 mg/L), promoted root initiation in Carrizo citrange segments more than threefold. However, the rooting rate remained below 65%, indicating the need for additional enhancements to ensure the viability of this protocol.

This work was funded by National Funds through Projectos PRR-C05-i03- I-000010-“Valorização de Recursos Genéticos Tradicionais”: PRR-C05-i03-I-000010-LA4.5 “Recursos Genéticos Vegetais” and PRR-C05-i03-I-000010-LA4.7 “Promoção de ações de divulgação”.

Biochemistry & Biotechnology

Unravelling the molecular mechanisms behind floral diversity and development in the Fagaceae family

Hugo Ricardo Afonso¹, Mariana Macedo¹, Ana Teresa Alinho¹, Helena Silva¹, José Gomes-Laranjo², Rómulo Sobral¹, Maria Manuela Ribeiro Costa¹

¹ Centre of Molecular and Environmental Biology (CBMA), University of Minho, Campus de Gualtar, Braga, Portugal

² Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Email: hugoricardoafonso@gmail.com

The development of unisexual flowers is a highly-adaptative trait that enhances cross-breeding and gene flow. This trait has emerged several times throughout evolution, being observed in 10% of flowering plants, and is often concomitant with a temporal separation of male and female flowering events. In the predominantly monoecious Fagaceae, a family comprising over 900 species of great economic and ecological importance, this separation can range from one week to over two months. The molecular pathways that induce flowering are well characterized in model species, with most flower-promoting cues being integrated in the expression of *FLOWERING LOCUS T (FT)* in the leaves. The FT protein then migrates to the meristems, leading to the conversion from a vegetative to a reproductive state. Following floral initiation, the identity of the different floral organs is specified by different combinations of transcription factors, as outlined by the ABCDE-model. However, despite the importance of the Fagaceae, studies on the processes underlying flower development in this family are scarce.

In this work, gene expression analysis of *FT* has revealed that *Quercus suber* displays two annual expression peaks, likely responsible for the separate induction of male and female flowers. By contrast, in *Castanea sativa* only one expression peak was observed, thought to simultaneously induce both sexes. Yeast-2-hybrid analysis of floral homeotic genes showed novel protein-protein interactions, suggesting functional changes in the canonical ABCDE-model dynamics. A differential expression of B- and C-class genes in male and female flowers was detected, with B-class genes being predominantly expressed in male flowers. The B-class gene *PISTILLATA* is exclusively expressed in male flowers of *Q. suber*, *Q. ilex* and *Q. orocantabrica*, while its *C. sativa* homologue appears to be partially active in female flowers, possibly related with the presence of underdeveloped staminodes in these flowers. In addition, the C-class gene *SHATTERPROOF* is expressed in both flowers of *Q. suber* and *Q. ilex*, but is not detected in *Q. orocantabrica* male flowers, despite the absence of significant morphological differences between the flowers of these species. Collectively, these results suggest that the development of unisexual flowers in the Fagaceae may rely on distinct regulatory processes.

Funding: This work was supported by the European Regional Development Fund (ERDF), through the Operational Programme for Competitiveness and Internationalization (COMPETE 2020), under Portugal 2020, and by the Fundação para a Ciência e a Tecnologia – FCT I.P. (National Agency for Science and Technology) with a project grant PTDC/ASP-SIL/27980/2017, and “Contrato-Programa” UIDB/04050/2020. A.T.A was funded by the grant SFRH/BD/136834/2018. H.R.A. was funded by the grant 2024.04723.BD.

Magnetic resonance mass spectrometry for metabolomics-guided design of synthetic microbial communities for agriculture

João Luz¹, Luís Carvalho², Marta Sousa Silva¹, Carlos Cordeiro¹

¹FT-ICR and Structural Mass Spectrometry Laboratory, Faculdade de Ciências da Universidade de Lisboa, Portugal

²Bioscale Lda., Portugal

Email: jmluz@fc.ul.pt

Agriculture is under increasing pressure to phase out the use of chemical pesticides to minimize risks to human health and the environment. For this purpose, the use of microorganisms as biological control agents to reduce plant disease, promote growth and increase yield has been gaining traction as a sustainable, pesticide-free agriculture. Specifically, the use of synthetic microbial communities (SynComs) containing two or more species is the most promising approach, harnessing microbial interactions to facilitate colonization and enhance anti-pathogenic activity. However, design of microbial communities fit to purpose remains a challenging task, owing to the great complexity of these interactions. A metabolomics-guided approach based on magnetic resonance mass spectrometry (MRMS), an extreme resolution analytical technique, can be used to investigate global metabolic changes in microbial species as part of multi-species consortia. Providing by far the highest resolution and mass accuracy currently available for metabolomics research, MRMS has several advantages over other MS techniques, including its ability to analyse samples without chromatographic separation, leading to a greater conservation of biological information, and its ability to resolve the isotopic fine structure of individual molecules in complex spectra, translating into unambiguous attribution of molecular formulas. Taken together, these advantages allow for far more complete and informative snapshots of microbial metabolomes and meta-metabolomes, thus providing a powerful tool for the development of new biocontrol agents. Here we show an example application of our approach to a three-species consortium of *Priestia megaterium*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. Far more than the sum of its parts, the consortium was found to possess a highly distinct metabolic profile, as evidenced by multivariate statistical analysis and the presence of around 400 unique metabolites not found in any of the species when grown individually nor in the original growth media.

This work was funded by the Fundação para a Ciência e a Tecnologia (Portugal) through the PhD grant 2023.05150.BDANA to J. Luz and the Portuguese Mass Spectrometry Network project RNEM-LVT (Lisboa 2030/FCT, LISBOA2030-FEDER-01319300). We also acknowledge the support from the research center BioISI, grant UIDB/04046/2020 (DOI 10.54499/UIDB/04046/2020). This work was conducted in the framework of the Research Infrastructure project European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers (EU FT-ICR-MS) funded by the European Union's Horizon 2020 research and innovation programme (Grant Agreement Nr 731077).

Low-voltage anion-exchange electrodialysis: application for removing acetic acid from olive pomace hydrolysates

A. I. Arroyo-Escoto^{1,2,3*}; S. de Souza Queiroz⁴; B. Li⁵; S. I. Mussatto⁴; M. C. Fernandes^{1,2}; Y. Zhang⁵

¹Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL), Polytechnic Institute of Beja (IPBeja), Apartado 6158, 7801-908 Beja, Portugal.

²Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, CEBAL—Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, 7801-908 Beja, Portugal.

³Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research (IIFA), University of Évora, Polo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴Department of Biotechnology and Biomedicine, Section for Synthetic Biology, Biomass Conversion Bioprocess Technology, Technical University of Denmark (DTU), Søtofts Plads 223, 2800 Kgs. Lyngby, Denmark.

⁵Department of Environmental and Resource Engineering (DTU Sustain), Water Technology & Processes, Technical University of Denmark (DTU), Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark.

Email: alonso.escoto@cebal.pt

Detoxifying lignocellulosic hydrolysates while preserving fermentable sugars is essential for cost-effective bioconversions and integrated biorefineries. Here, we evaluate an anion-exchange membrane (AEM) electrodialysis step operated at low voltage (1.0–2.5 V) to selectively recover acetate from olive-pomace hydrolysates without compromising monosaccharides. A factorial design assessed the effects of (i) membrane history (pristine vs reused), (ii) receiving-phase chemistry (phosphate vs citrate buffers), and (iii) applied potential, with an antifouling pre-filtration upstream. HPLC monitored process performance for organic acids and sugars, pH tracking, and qualitative indicators of fouling and polarization. The AEM configuration achieved acetate/sugar separation, with glucose, xylose, and arabinose remaining within analytical variability, indicating negligible sugar drag. Increasing the potential from 1.0 to 2.5 V did not yield a proportional increase in acetate flux; notably, no visible gas evolution occurred despite operating near the typical water-electrolysis onset, suggesting limitations in electrode kinetics and cell architecture rather than thermodynamic thresholds. Membrane history significantly influenced transport: reused membranes showed earlier polarization and reduced acetate flux relative to pristine membranes, consistent with fouling or partial functional loss. Receiving-phase composition was decisive; phosphate buffers provided higher acetate capture and more stable pH control than citrate, indicating more favorable ionic strength and buffering conditions. Overall, acetate removal was incomplete under the tested conditions, but the trends identify clear levers for improvement. We conclude that low-voltage AEM electrodialysis is a viable pretreatment to attenuate acetate while conserving fermentable sugars in olive-pomace hydrolysates. Priority next steps include (1) membrane cleaning/conditioning and life-cycle control, (2) optimization of receiving-phase composition and conductivity, and (3) refinement of electrode materials and cell geometry to mitigate polarization and increase stable acetate flux without sugar carryover, thereby strengthening integrated biorefinery schemes and enabling acetate valorization.

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) by CHANGE (DOI: <https://doi.org/10.54499/LA/P/0121/2020>). The authors also acknowledge funding from MED (DOI: <https://doi.org/10.54499/UIDB/05183/2020> and DOI: <https://doi.org/10.54499/UIDP/05183/2020>). A.I. Arroyo-Escoto acknowledges support from the PhD grant UI/BD/153579/2022. M.C.Fernandes acknowledges BIOALENTEC (ALT2030-FEDER-01951700) from the Regional Programme ALENTEJO2030, co-financed by the European Regional Development Funds.

XYLEMOLIVE – Anatomical and functional xylem traits and defense priming responses in olive (*Olea europaea* L.) varieties for identifying Portuguese cultivars potentially tolerant to the xylem-limited pathogen *Xylella fastidiosa*

R. Azedo¹, C. Inês², R. Teixeira³, A. Poeiras⁴, A. Penha⁴, T. Oliveira⁵, A. Cordeiro², M. Vaz⁴, I. Velada¹

¹ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Dep. Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² INIAV - Instituto Nacional de Investigação Agrária e Veterinária, I.P. - UEISBRG, Pólo de Elvas, Olivicultura, Estrada de Gil Vaz, Apartado 6, 7350-404 Elvas, Portugal;

³ BioISI, Faculdade de Ciências de Lisboa, Universidade de Lisboa, 1749-016, Lisboa, Portugal;

⁴ CREATE - Center for Sci-Tech Research in Earth System and Energy, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁵ CHRC - Comprehensive Health Research Centre, Department of Veterinary Medicine, School of Science and Technology, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: ivelada@uevora.pt

Agricultural crops are increasingly threatened by *Xylella fastidiosa* (*Xf*), an emerging and highly destructive plant pathogen that poses a major global threat to plant health. In olive trees, the bacterium causes the Olive Quick Decline Syndrome (OQDS) which, ultimately, leads to the death of infected plants by obstructing the xylem vessels and disrupting water transport within the host. This xylem-limited bacterium is regulated as quarantine pest within the European Union (EU) and has been detected in Italy, France, Spain, and Portugal. The potential economic losses associated with the full spread of *Xf* across the EU are estimated to reach an average of €5.5 billion per year. As the world's sixth-largest producer of olive oil, Portugal faces a particularly serious risk, with 18 *Xf*-demarcated zones already identified in the country. Currently, there is no effective strategy to fully protect or cure infected plants. One of the EU's recommended approaches involves the replanting of resistant/tolerant olive cultivars in infected areas. To date, only three such cultivars—Leccino, Lecciana, and FS17 (Favolosa)—have been identified. The XYLEMOLIVE research project (COMPETE2030-FEDER-00747900) aims to identify Portuguese olive cultivars that are potentially tolerant/resistant to *Xf*. The study will characterize economically relevant Portuguese cultivars in terms of their xylem anatomical traits and their responses to *Xf*-derived immune elicitors. The project seeks to investigate the activation of immune defense responses in olive varieties after *priming* treatment to find suitable markers that can be used to confer resistance to *Xf* in susceptible varieties.

This work is funded by COMPETE2030-FEDER-00747900 and by National Funds through FCT – Foundation for Science and Technology under the Project UIDB/05183/2025.

Salivary and emotional responses mediating anticipation to foods

Carla Simões^{1,2*}, Anita Ramos¹, María Botia Gonzalez³, Maeva Wendremaire⁴ Fabrice Neiers⁴,
Frédéric Lirussi⁵, Ana Isabel Costa², Asta Tvarijonaviciute³, Elsa Lamy¹

¹MED&CHANGE, Institute for Advanced Studies and Research (IIFA), Universidade de Évora, Portugal

²Food Behaviour Lab, CATÓLICA LISBON School of Business & Economics, Universidade Católica Portuguesa, Portugal

³Interdisciplinary Laboratory of Clinical Analysis of the University of Murcia (Interlab-UMU), Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Spain

⁴Université Bourgogne Europe, Dijon, France

⁵PACE, Plateau d'Analyses Chromatographiques et Élémentaires, Department of Pharmacology-Toxicology & Metabolomics, University Hospital of Besançon (CHU), Besançon, France

Email: [*d51413@alunos.uevora.pt](mailto:d51413@alunos.uevora.pt)

One of the most well-recognized physiological adaptations in anticipation of food stimuli is the “mouth-watering” sensation we felt when seeing or smelling our favorite food. Combined with other cephalic phase responses, such as increase of secretion of gastric acid or food-related hormones (e.g, insulin, ghrelin), it serves the purpose of preparing the body to receive and process food.

Cues associated with food, such as smell, sight or even touch, may trigger these anticipatory responses and have a key role influencing ingestive behavior, promoting appetite and eventually encouraging consumption.

Although the increase in the amount of saliva secreted in response to pre-ingestive sensory food stimulation is well described in the literature, it remains to clarify whether saliva composition is changed by such stimulation. To address this aspect, we are investigating potential changes in human salivary biochemical composition in response to: 1) the visualization of food pictures, 2) smell of food odorants; 3) and to real-life condition of exposure to the presence of real-food products.

Results get so far strength the existence of salivary cephalic phase response with changes at proteome level. Here, we will present the key findings, obtained until the moment and discuss the importance of collecting emotional arousal data and affective responses while studying cephalic-phase salivary responses, using both implicit and explicit measurements. Ultimately, together with a presentation of the on-going analysis, we will emphasize the importance of an in-deep understanding of cephalic-phase salivary responses to shed light on individual differences in food preferences and choices and support research on development of multisensory interventions targeting eating-disorders and increase the consumption of healthy diets.

This work was funded by National Funds through Fundação para a Ciência e Tecnologia under the UID Project (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>), the Associate Laboratory CHANGE – Global Change and Sustainability Institute (<https://doi.org/10.54499/LA/P/0121/2020>) and the PhD fellowship awarded to Carla Simões (reference number 2021.06485.BD).

Ethical statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Commission of the University of Évora for human studies (no.GD/29823/2022).

Transcriptional Tales of Drought: Messages from Thirsty Portuguese Grapevines

C. Estêvão¹, C. Campos¹, L. Rodrigues¹, M. Marques¹, R. Pires¹, J. L. Silva², A. C. Neto², R. Flores²,
M. J. Cabrita³, H. Cardoso^{4*}

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal

²Esporão S.A., Herdade do Esporão, Reguengos de Monsaraz, Portugal

³MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

⁴MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

*Email: hcardoso@uevora.pt

Vitis vinifera L. is one of the most economically important crops in Mediterranean regions, such as Portugal, where increasing water scarcity poses a major threat to grapevine productivity and sustainability. Understanding the molecular mechanisms underlying drought stress response and acclimation is crucial for developing management strategies to cope with climate change scenarios.

This study aims to identify key genes/pathways involved in drought tolerance by following a transcriptomic approach.

Leaf samples of six Portuguese *V. vinifera* cultivars were collected at the end of the ripening period, from grapevines grown under field conditions, where they were subjected to two irrigation regimes (irrigated and non-irrigated). Three biological replicates were considered per regime and cultivar. Total RNA was sequenced using an Illumina platform at a specialized sequencing company (Novogene), and reads were further analyzed using standard RNA-seq pipelines. Gene expression analysis was conducted to evaluate global transcriptional variation among samples and conditions. Differentially expressed genes (DEGs) and functional enrichment of Gene Ontology (GO) terms and pathways were then assessed.

Initial results revealed clear differences between irrigated and non-irrigated plants, as well as pronounced cultivar-specific responses. The six cultivars exhibited highly variable responses to water deficit, with the number of DEGs ranging from 3,924 in 'Tinta Francisca' to 94 in 'Tinta Caiada'. In all cultivars, most DEGs were down-regulated under non-irrigated conditions, suggesting a general reduction of transcriptional activity under drought. Only 10 DEGs were shared among all cultivars, highlighting the diversity of molecular strategies involved in drought adaptation. Functional enrichment analyses revealed that DEGs were mainly associated with stress response, transmembrane transport, regulation of DNA-templated transcription, protein metabolism, and signal transduction. Processes related to hormone signaling and photosynthesis were also detected.

These preliminary findings demonstrate that Portuguese grapevine cultivars display markedly distinct molecular responses to water deficit. The ongoing analyses will further elucidate the mechanisms underlying cultivar drought tolerance, contributing to a better understanding of adaptive traits relevant for sustainable viticulture under climate change.

This work is financially supported by national funds through FCT under the PhD scholarship 2024.00814.BDANA and the project UID/05183/2025, and by the project BioGrapeSustain, Vine&Wine – SubProject Proposal – Project 81 Agenda Mobilizadora: Driving Sustainable Growth Through Smart Innovation (Reference C644866286-00000011).

Influence of arbuscular mycorrhizal fungi on the expression of *PPR* genes and on the physiological response of wheat to manganese toxicity

C. Barbas¹, H. Cardoso², L. Rodrigues³, C. Campos³

¹ Escola de Ciência e Tecnologia, Universidade de Évora, Largo dos Colegiais 2, 7004-516, Évora, Portugal

² Universidade de Évora, Escola de Ciência e Tecnologia, Departamento de Biologia, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Largo dos Colegiais 2, 7004-516, Évora, Portugal

³ Universidade de Évora, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Largo dos Colegiais 2, 7004-516, Évora, Portugal

Email: mccampos@uevora.pt

The relation between plant's genotype and phenotype is strongly influenced by its symbiotic microbiota, specifically arbuscular mycorrhizal fungi (AMF). AMF are disseminated throughout the soil via extraradical mycelial networks, thereby enhancing nutrient acquisition and contributing to the plant's phenotypic plasticity. These interactions also increase plant resilience to adverse conditions.

Manganese (Mn) is an essential element for plant development and growth, serving as an enzymatic cofactor and a constituent of photosystem II. However, in excess, it can replace magnesium as a cofactor and inhibit enzymatic reactions, causing chlorosis, necrosis, and drastically reducing the plant development, a scenario frequently encountered in acidic soils or soils subject to flooding.

While previous studies have established that distinct communities of AMF can confer differential protection to wheat plants (*Triticum aestivum* L.) under conditions of Mn toxicity, the mechanisms responsive to their interaction with specific plant genetic pathways remain largely unknown.

This work seeks to investigate the role of Pentatricopeptide Repeat Proteins (*PPRs*) genes in mediating the response of wheat to Mn toxicity. This gene family, with over 300 members in plants, regulates mitochondrial and chloroplast gene expression, including RNA stability, editing, splicing, and translation. *PPRs* are also involved in plant responses to biotic and abiotic stresses and in the production of reactive oxygen species (ROS).

It is hypothesised that different species of AMF differentially influence the expression and DNA methylation level of *PPR* genes in two wheat genotypes subjected to high levels of manganese, one of which is known to be tolerant to Mn excess. Given that *PPRs* are involved in the plant's response to stress, the production of ROS, particularly H₂O₂, the expression of genes involved in the response to oxidative stress and several physiological parameters will be also evaluated. Thus, this study aims to deepen the understanding of the molecular and epigenetic mechanisms underlying the AMF-wheat interaction in the context of manganese toxicity, contributing to the development of sustainable strategies that promote plant resilience in soils with this problem.

This work is funded by National Funds through FCT under the Project UID/05183/2025.

Impact of Appetitive Food Visualization on Salivary Biochemical Responses: Variations by Time of Day and Age Group

C.Mendes¹, C.Simões¹, C.Raposo², M.Perez-Jimenez¹, F.Capela e Silva^{1,3}, E.Lamy¹

¹MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Portugal.

²Student from Biochemistry degree, Universidade de Évora, Portugal

³Department of Medical and Health Sciences, School of Health and Human Development, Universidade de Évora, Portugal

Email: Cristina.mendes@uevora.pt

Exposure to visual food stimuli shapes preferences, consumption decisions, and triggers neurophysiological mechanisms that prepare the body for ingestion, including anticipatory changes in salivation. While increases in saliva volume are well established, recent findings show that saliva composition also changes when food is anticipated through smell or visualization. Our team previously demonstrated that food images can affect the salivary proteome. However, observing an image may differ from seeing the real food, with the possibility of sensory assessment. Individual factors such as age, sex, eating habits, physiological state, emotional context, and time of day can modulate these anticipatory responses, and several of these factors are also known to influence saliva composition.

This study aimed to analyze how visual exposure to a real chocolate cake affects salivary biochemical composition, and whether these effects vary according to time of day and/or age group. Saliva was collected before and during exposure to the cake (4 minutes each) in adults tested in the morning (N=21) and afternoon (N=21), and in children tested in the morning (N=19). Participants reported the subjective sensations elicited by viewing the cake and tasted it after the exposure period. Salivary flow rate, total protein concentration, and α -amylase activity were analyzed. Results showed distinct response patterns in children and adults. In children, changes in salivary secretion were more closely associated with sweetness, whereas in adults these changes were more related to self-reported sensations of hunger or thirst. Notably, in children, the feeling of mouthwatering positively correlated with the sweetness perceived when the cake was later tasted. Regarding biochemical parameters, exposure to the cake increased salivary flow rate, while total protein concentration decreased. α -Amylase activity increased during anticipation of chocolate cake ingestion, with a tendency for a stronger effect in males. Overall, the findings indicate that viewing an appetitive food modulates saliva composition, but the magnitude and nature of the response depend on the subjective feelings evoked by visualization and on the individual's age.

The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for the PhD grant 2021.06485.BD. Acknowledgements also to the R&D unit MED – Mediterranean Institute for Agriculture, Environment and Development (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>), and the Associate Laboratory CHANGE – Global Change and Sustainability Institute (<https://doi.org/10.54499/LA/P/0121/2020>).

Ethical statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Commission of the University of Évora for human studies (no.GD/29823/2022).

Enzymatic Activities as Indicators of Grape Quality in Berry and Leaf Extracts of *Vitis vinifera* L. cv. Touriga Nacional' under different foliar leaf protectors application

G. Carneiro Murta¹, A.L.M Carvalho², M.J Cabrita³, A.E Rato³, M.R Martins²

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²Departamento de Ciências Médicas e da Saúde, Escola de Saúde e Desenvolvimento Humano; Laboratório HERCULES & IN2PAST, Universidade de Évora, Rua Romão Ramalho, 59, 7002-671 Évora, Portugal

³MED (Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento), Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: gabriela.murta@fa.uevora.pt

Viticulture in the Alentejo region, characterized by high temperatures, intense solar radiation, and limited water availability, is increasingly challenged by oxidative stress, which compromises grape quality and vine productivity. This study aimed to evaluate the effects of different foliar protectors on the phenolic composition and antioxidant enzyme activity of *Vitis vinifera* L. cv. 'Touriga Nacional'. Seven treatments were applied: silicon (Si) at 4% and 8%, kaolin (Ka) at 2%, two Si–Ka combinations (Si 4% + Ka 2% and Si 8% + Ka 2%), an extract of *Opuntia ficus-indica* (OFI), and a control, without treatment was also used. Grapes and leaves were collected at two stages of ripening (mid-veraison and harvest), and the extracts were analysed for total phenolics, flavonoids, and anthocyanins using spectrophotometry and HPLC-DAD. Antioxidant capacity was assessed by the DPPH method, and berry enzymatic activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were evaluated by UV/Vis spectrophotometric assays. Results showed that combined treatment Si 4% + Ka 2% promoted the highest accumulation of total phenolics and flavonoids, as well as higher antioxidant anti-radicalar activity at harvest. Silicon at 8% significantly enhanced anthocyanin synthesis, particularly malvidin-3-glucoside, a major pigment determinant of red wine color stability. The OFI extract promoted a stabilizing effect on flavonoid content and antioxidant capacity during ripening, highlighting its potential as a natural biostimulant. Enzymatic assays revealed low CAT, SOD, and GPx activities at harvest, suggesting minimal oxidative stress and confirming the physiological balance and sanitary quality of the grapes. These findings indicate that foliar protectors—especially silicon combined with kaolin or OFI—can strengthen the vine's defense mechanisms, enhance phenolic composition, and contribute to sustainable vinicultural practices suited to Mediterranean climates. Overall, the integration of natural and mineral-based treatments represents a promising strategy to mitigate climate-induced stress, improve grape quality, and preserve the enological potential of Touriga Nacional under warm, dry conditions typical of southern Portugal.

This work was funded by National Funds through FCT under Project "Vine & Wine — Driving Sustainable Growth Through Smart Innovation" (sub-project-Miks4Vine) with the reference "C644866286", funded by the "PRR — Recovery and Resilience Plan" based on Component 5 - Capitalization and Business Innovation, integrated in the Resilience Dimension.

Exploring Microbial Diversity and Fungal Control in Heritage Paintings from the MNAA: An Interdisciplinary Approach

Inês Silva¹, Cátia Salvador^{1,2}, Ana Z. Miller^{3,1}, Luís Dias¹, Sara Valadas^{1,4}, António Candeias^{1,2,4}, Ana Teresa Caldeira^{1,2,4(*)}

¹ HERCULES Laboratory - Cultural heritage, Studies and Safeguarding & IN2PAST — Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory & Institute for Research and Advanced Training (IIFA), University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

² Chemistry and Biochemistry Department, School of Sciences and Technology, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

³ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain

⁴ City University Macau Chair in Sustainable Heritage, Institute for Advanced Studies and Research, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

(*) atc@uevora.pt | Email (IS): d51300@alunos.uevora.pt

Paintings are complex artworks made of both organic and inorganic materials, which makes them especially sensitive to microbial attack and biodeterioration. Fungi, in particular, can degrade binders, pigments, and varnishes, leading to irreversible structural and aesthetic damage.

This study investigated microbial contamination in four paintings preserved in the reserves of the Museu Nacional de Arte Antiga (MNAA), which holds one of Portugal's most important collections of historical artworks. The paintings showed visible signs of deterioration, such as dark, brownish, and whitish stains, suggesting active fungal growth.

To characterize the microorganisms involved, we used both culture-based and molecular methods, specifically Next-Generation Sequencing (NGS), together with Scanning Electron Microscopy (SEM). SEM analysis revealed extensive fungal colonization with visible spores and hyphae, while NGS results showed a dominance of Ascomycota, mainly *Aspergillus*. In culture, *Cladosporium* and *Paralloneottiosporina* were the most frequently isolated genera.

Antifungal tests showed variable effectiveness among the biocides evaluated, with Biotin-T demonstrating the highest efficacy and consistent results across all samples. These findings highlight the importance of selecting treatments based on scientific validation and compatibility with heritage materials. Overall, this study highlights the value of interdisciplinary collaboration - combining microbiology, conservation science, and art history - to develop safer and more sustainable preservation strategies for cultural heritage paintings.

Acknowledgments: This work was funded by national funds through the FCT—Foundation for Science and Technology, I.P. —under the projects UI/BD/153582/2022 (I. Silva PhD Grant), HERCULESLab (DOI: 10.54499/UIDB/04449/2025) and IN2PAST (DOI: 10.54499/LA/P/0132/2020). The authors gratefully acknowledge the City University of Macau endowment to the Sustainable Heritage Chair & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science supported by the Belt and Road Initiative. The authors also wish to acknowledge MNAA for allowing them to perform this study.

From SALIVA to SENSES: *Ex-vivo* assay of XEROSTOMIA and FOOD PERCEPTION

Laura Carreira¹, Anita Ramos¹, Maria Perez¹, Nuno Martins¹, Maria João Cabrita¹, Jonas Nunes², Carlos Cordeiro³, Elsa Lamy^{1,*}

¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal;

²Dry mouth institute, Teknon Medical Center, 08022 Spain;

³FT-IRC and Structural Mass Spectrometry Laboratory, Faculty of Science, University of Lisbon, Campo Grande 1749-016, Lisbon, Portugal;

*Email: ecsl@uevora.pt

Xerostomia, a sensation of dry mouth, significantly impacts oral functions such as swallowing, mastication and flavour perception. Its etiology is multifactorial, including autoimmune diseases, radiotherapy in the head and neck region, polypharmacy, or idiopathic factors. Saliva, composed primarily of water, proteins (e.g., mucins, proline-rich proteins), and enzymes (e.g., α -amylase), plays a crucial role in oral lubrication, taste, aroma and texture perception. Alterations in the flow rate and composition of this fluid influence sensory attributes such as astringency and aroma release, which together determine food acceptance and enjoyment.

However, the effect of changes in saliva from xerostomic patients in these sensations is not well known. This research aims to evaluate the impact of the salivary biochemical parameters of xerostomic patients in astringency, focusing on protein–polyphenol interactions, and in retronasal aroma, through volatile compound release. Two complementary *ex vivo* studies were conducted using saliva from xerostomic patients before and after six months of pilocarpine treatment, compared to healthy controls. In the first study, saliva samples (N=20, 10 xerostomic and 10 healthy individuals) were incubated with enological tannins (1.5 g/L) to assess protein–tannin binding capacity. Following incubation and centrifugation, SDS-PAGE analysis revealed differences in salivary protein precipitation patterns between patients and controls, suggesting altered binding affinity, both in xerostomia and after treatment, indicating potential changes in perceived astringency in these patients. In parallel, a second study addressed how saliva from xerostomic affect volatile release. A micro-volume protocol, with non-xerostomic saliva (<500 μ L) was optimized to evaluate saliva–aroma interactions using a sweet orange aroma through HS-SPME-GC \times GC-ToFMS analysis. Preliminary results indicate that saliva addition modifies the volatile release profile, with xerostomic saliva showing reduced modulation capacity compared to healthy samples, partially restored after pilocarpine therapy. Overall, these findings provide an innovative *ex-vivo* approach to study how xerostomia modifies the salivary proteome and functional properties related to oral food perception. Understanding these mechanisms may support the design of targeted dietary strategies and therapeutic interventions to improve sensory experience and quality of life in xerostomic patients.

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UIDB/05183, and also through the PhD scholarship granted to Laura Carreira under the reference SFRH/BD/06273/2021.

DNA Barcoding of Parchment: Tracing Animal Origins from Fragmented DNA

M. J. Penetra¹, A. Candeias^{1,2,3}, C. Miguel^{1,3}, A. T. Caldeira^{1,2,3} (*)

¹HERCULES Laboratory -Cultural heritage, Studies and Safeguarding & IN2PAST – Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Évora University, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

²Chemistry Department, School of Sciences and Technology, Évora University, Rua Romão Ramalho 59, 7000- 671 Évora, Portugal

³City University of Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, University of Évora, Casa Cordovil, 7000-651 Évora, Portugal

*Email: atc@uevora.pt

Parchment, an age-old writing material derived from animal skin, mainly sheep, calf and goat, has been instrumental in preserving human knowledge and cultural heritage for centuries. However, the recovery and analysis of DNA remain challenging due to the harsh chemical and physical treatments used during manufacture, as well as subsequent degradation caused by handling, microbial activity, and environmental exposure.

In this study, we aimed to develop molecular tools capable of retrieving species information from even the most degraded parchment samples. We designed and evaluated 38 primers—17 universal and 21 species-specific—targeting short mitochondrial DNA (mtDNA) fragments from the 12S rRNA, 16S rRNA, and Cytochrome b genes. All primers underwent *in silico* screening for specificity and amplicon size, followed by *in vitro* validation using reference animal tissues. Seventeen primers demonstrated high performance and were selected for testing in both modern and historical parchment samples obtained through diverse sampling strategies. Of the universal primers, thirteen successfully amplified mtDNA, and six showed consistent results in degraded parchment. Among the species-specific primers, eleven proved highly accurate in identifying their respective targets. Application to parchment samples confirmed strong amplification success and reliable species-level identification across both recent and ancient materials.

The results highlight the effectiveness of this targeted mtDNA barcoding approach, offering a robust, sensitive, and practical toolkit for determining the biological origin of parchments. This method enhances the genetic analysis of cultural heritage materials and expands the possibilities for species identification in archaeological, archival, and conservation contexts.

The authors wish to acknowledge the financial support of FCT – Foundation for Science and Technology, I.P.-within the scope of UI/BD/154922/2023 (Penetra, M. J. PhD Grant), the project “ROADMAP - Research On Antonio De Holanda Miniatures Artistic Production” (PTDC/ART-HIS/0985/2021), the HERCULES Lab (UIDB/04449/2025) and City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural, and IN2PAST (DOI:10.54499/LA/P/0132/2020).

In vitro establishment of Portuguese *Ceratonía siliqua* L. traditional genotypes

M. Marques¹, R. Pires¹, L. Rodrigues¹, C. Campos¹, C. Estêvão¹, A. Peixe², H. Cardoso³

¹MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE – Global Change and Sustainability Institute, IIFA (Institute for Research and Advanced Training), University of Évora, Mitra Campus, Ap. 94, 7002-554 Évora, Portugal.

²MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE - Global Change and Sustainability Institute, Department of Crop Science, University of Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE – Global Change and Sustainability Institute, Department of Biology, University of Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: d63881@alunos.uevora.pt

The growing demand for resilient plant species capable of contributing to ecosystem restoration and sustainable agricultural development has renewed interest in the carob tree (*Ceratonía siliqua* L.), a Mediterranean species valued for its remarkable tolerance to drought, pests, and diseases. Beyond its ecological relevance, *C. siliqua* plays an important role in soil recovery, reforestation, and the regional bioeconomy through its multipurpose use in food and industrial applications. However, the increasing demand for high-quality planting material cannot be fulfilled by conventional propagation methods, which often provide insufficient material due to the recalcitrant behavior to the development of adventitious roots when propagated through semi hardwood cuttings. Within the framework of the ALCAROB project, special emphasis has been placed on the micropropagation of Portuguese traditional genotypes, aiming to establish reliable in vitro systems for large-scale multiplication. The current work focuses on the in vitro establishment of ten selected Portuguese genotypes, integrating nine traditional varieties (female plants): ‘Lamy’, ‘Mulata de Sousa’, ‘Mulata do Espargal’, ‘Galhosa’, ‘Canela’, ‘Cavi’, ‘Aida’, ‘Brava de Lagoa’, and ‘Lami’, and a hermaphrodite genotype. Several optimization studies have been conducted regarding culture medium composition and environmental conditions. These optimizations have enabled the successful initiation and maintenance of uninodal segment cultures, providing the basis for consistent shoot multiplication. This work will present the results obtained so far, demonstrate the successful establishment of elite Portuguese genotypes, marking an important milestone toward the development of integrated micropropagation protocols for *Ceratonía siliqua* L., and contributing to the sustainable valorization of this species within Mediterranean ecosystems and agroforestry systems.

This research was funded by National Funds through FCT - Foundation for Science and Technology under the project UIDB/05183/2020, and by FCT and Fundação “la Caixa” under the Project ALCAROB – Applicability of carob tree on sustainability of Alentejo region (Promove – Projetos I&D Mobilizadores, ref. PD24-00024). Authors would thank to Microplant Lda. and to CCDR-Algarve for providing the plant material for establishment of in vitro cultures.

Protein Extracts from Pristine Microorganisms: Towards Sustainable Cultural Heritage Protection

Patrícia Gatinho^{1,2,3}, Cátia Salvador^{1,4}, Ana Zélia Miller^{3,1}, Ana Teresa Caldeira^{1,4,5}

¹ HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, 7000-809, Évora, Portugal.

² Institute for Research and Advanced Training (IIFA), University of Évora, 7000-809, Évora, Portugal.

³ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), 41012, Sevilla, Spain.

⁴ Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000-809, Évora, Portugal.

⁵ City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, Institute for Advanced Studies and Research, University of Évora, 7000-809 Évora, Portugal.

Email: patricia.gatinho@uevora.pt

Biodeterioration caused by microbial colonization poses a significant threat to the conservation of Cultural Heritage, leading to the progressive degradation of artworks, monuments and historical sites. Although conventional biocidal treatments are often effective in reducing microbial growth, they are associated with relevant drawbacks, including the development of microbial resistance, limited specificity, and environmental and toxicological risks. These concerns highlight the urgent need for alternative and eco-friendly strategies. One promising avenue is the exploitation of bioactive metabolites produced by microorganisms isolated from pristine environments, which have evolved unique adaptive mechanisms under extreme conditions and may provide selective antimicrobial activity with a reduced ecological impact. In this study, bacterial strains from pristine environments, belonging to the genera *Agrococcus*, *Brevibacillus*, *Evansella* and *Sulfitobacter*, were cultured under laboratory conditions. Concentrated Protein Extracts (CPEs) were obtained via sequential ultrafiltration and systematically screened for antimicrobial activity against biodeteriogenic microorganisms. Preliminary results suggest that certain CPEs exhibit significant inhibitory effects, although notable variability in antimicrobial activity was observed across different molecular weight fractions and among bacterial extracts. These findings demonstrate the potential of pristine bacteria as an untapped reservoir of bioactive compounds with promising applications in the sustainable preservation of Cultural Heritage.

The authors acknowledge the FCT, Portuguese Foundation for Science and Technology, I P within the scope of the projects, MICROCENO (DOI:10.54499/PTDC/CTA AMB/0608 2020), ART3mis (DOI:10.54499/2022.07303.PTDC), R&D Units HERCULES Lab (DOI:10.54499/UIDB/04449/2025) and IN2PAST-Associate Lab (DOI:10.54499/LA/P/0132/2020), and P. Gatinho FCT PhD Grant (2024.00316.BD). The authors acknowledge the City University of Macau endowment to the Sustainable Heritage Chair & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science supported by the Belt and Road Initiative.

Calmodulin gene family in *Juglans regia* L.: Genome-wide identification and expression patterns during adventitious root development

Rafaela Santos¹, Rita Pires¹, Mónica Marques¹, Lénia Rodrigues¹, Catarina Campos¹, Augusto Peixe², Hélia Cardoso³

¹MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

³MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

*Email: hcardoso@uevora.pt

Micropropagation of the common walnut (*Juglans regia* L.) provides an efficient alternative to conventional propagation methods, allowing large-scale multiplication of selected genotypes through overcoming recalcitrant behavior to adventitious root formation (ARF). Nevertheless, the molecular mechanisms underlying ARF in *J. regia* remain poorly explored. This study aimed to investigate the molecular basis of ARF in two genotypes with contrasting rooting behaviors: the recalcitrant cultivar 'Chandler' and the easy-rooting hybrid 'Paradox'. Calcium ions (Ca²⁺) act as key secondary messengers involved in abiotic stress response, and particular emphasis was placed on Calmodulin (CaM) genes, encoding Ca²⁺-activated proteins, putatively involved in ARF. *In silico* analysis identified six *CaMs* in the *J. regia* genome (*JrCaMs*), each comprising 149 amino acids and four Ca²⁺-binding *EF-Hand Motifs*, consistent with canonical CaM properties. The *JrCaMs* were classified into four subfamilies (*JrCaM1* to *JrCaM4*), with *JrCaM1* and *JrCaM3* each comprising two members (*JrCaM1a*, *JrCaM1b*, *JrCaM3a*, *JrCaM3b*).

To determine the involvement of *JrCaM* genes on ARF, a rooting experiment was established using both genotypes, 'Chandler' and 'Paradox'. For rooting induction plantlets were cultivated in a DKW medium supplemented with 0,01 mg L⁻¹ IBA for 7 days. Simultaneously, the same procedure was applied to plantlets cultured in medium lacking the rooting inducer. Gene expression analysis revealed differential expression only for *JrCaM1*, *JrCaM2* and *JrCaM4*, with a strong genotype effect. *JrCaM1* exhibited similar temporal patterns in both genotypes but reached significantly higher levels in 'Chandler' under IBA treatment, indicating a putative role in ARF. *JrCaM2* and *JrCaM4* showed largely stable expression patterns in 'Paradox': *JrCaM2* displayed significant induction only at 7 days, while *JrCaM4* showed no IBA-associated differences, suggesting limited involvement in ARF in this genotype. In contrast, both genes were overexpressed in 'Chandler' under IBA treatment, with a putative co-expression pattern that suggests they may share similar functions and relevance during ARF.

These divergent expression patterns highlight genotype-specific regulatory dynamics and direct future investigation toward the CaM gene repertoire of *J. hindsii*, one of the progenitors of 'Paradox', to identify additional CaM genes potentially associated with the easy-to-root behaviour of this genotype.

Exploring Microbially Induced Calcium Carbonate Precipitation (MICP) in Bacteria Associated with Oyster Shells

R. Estevinho¹, S. M. Arantes¹, P. Barrulas^{1,2}, A. Candeias^{1,2,3}, A.T. Caldeira^{1,2,3}

¹HERCULES Laboratory - Cultural heritage, Studies and Safeguarding & IN2PAST — Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

²Chemistry and Biochemistry Department, School of Sciences and Technology, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

³City University Macau Chair in Sustainable Heritage, Institute for Advanced Studies and Research, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

Email: ricardo.estevinho@uevora.pt

Marine shells are natural biocomposites formed through biomineralization processes, during which microorganisms may play a crucial role. Understanding the interactions between microbes and minerals can lead to the development of sustainable strategies for environmental applications, such as biocementation, heavy metal sequestration, and soil stabilization. In this study, we examined bacterial isolates obtained from oyster shells to identify potential biomineralizing strains capable of inducing calcium carbonate precipitation through enzymatic activity. Cultures were grown on YPDuc medium, which had been previously optimized for promoting microbial calcification, and then purified on solid media. Ureolytic potential was first assessed using Christensen's urea agar, where a color shift indicated ammonia release and consequent pH increase. A total of 13 isolates were obtained from oyster shells. One granular/fine-sand isolate showed very slow growth and was excluded from further analysis. The remaining 12 isolates displayed either milky (n = 7) or crystalline (n = 5) morphotypes. Both morphotypes showed consistent growth and positive urease activity and were selected for 16S rRNA identification. Sequence analysis revealed that the dominant ureolytic isolates belong to the *Bacillus* and *Lysinibacillus* genera, both of which are widely recognized for their roles in MICP due to their robust urease activity and resilience in high pH and salinity conditions. High-throughput sequencing through Next Generation Sequencing (NGS) of the oyster shell microbiome was also conducted to characterize the total microbial diversity and to establish a link between cultivable ureolytic strains and the broader microbial community structure. These data will guide subsequent biomineralization assays under controlled laboratory conditions to quantify urease and carbonic anhydrase activities, elucidating their synergistic roles in carbonate precipitation. This study lays the foundation for exploring natural biomineralization as an inspiration for sustainable biotechnological solutions. By studying bacteria associated with naturally mineralizing environments, this research aims to connect microbial ecology with environmental engineering approaches for green material development.

This work was funded by National Funds through FCT/MCTES under the Project ROCKinBIO (2022.04830.PTDC)

Pollen analysis and quantification within the AirBiD project: preliminary results

Rita V Teixeira^{1,3}, Ana Galveias¹, Alexandra M Penha^{1,2}, Ana R Costa^{1,2,3}, e Célia M Antunes^{1,2,3}

¹Center for sci-tech Research in EArth sysTem and Energy (CREATE), University of Évora; ² Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal, ³Centro Académico Clínico do Alentejo – C-TRAIL, Évora, Portugal.

Corresponding author: d61919@alunos.uevora.pt

The AirBiD project* aims to explore the links between urban green spaces, airborne biodiversity, and human health. This study focused on optimizing the AirBiD methodology for air sampling and eDNA extraction and analysis.

Sampling was conducted in Évora city center over four weeks in spring. Samples were collected weekly: 1 sample/week from 3 samplers for OM analysis (12 samples in total), and 1 sample/week from the same 3 samplers for DNA extraction (analysed over 3 weeks, in this case, making a total of 9 samples). Were used three Sigma-2 passive samplers installed at two different locations, separated by 1.1 km. In one of the locations, devices were disposed at two heights, 2m and 10m, the last one adjacent to Hirst-type device (gold standard for pollen monitorization - EN16868:2019). Each Sigma-2 collected particles in two slides per week: one for optical microscopy (OM) and the other for DNA extraction. Total pollen and the major expected taxa (*Olea* sp., Poaceae, *Quercus* sp.) were quantified by OM. DNA was extracted by different methodologies, and Poaceae presence was analysed by digital-PCR using specific primers.

Pollen was detected by OM in all the Sigma-2 analysed samples. The highest pollen sedimentation rate, over the four weeks, occurred at 10m, with 2764 pollen/cm², while at 2m did not exceed 2000 pollen/cm².

Daily data from Hirst show that, during the analysed period, occurs the beginning of the Poaceae and *Olea* pollen season, with maximum daily values observed on 26th May (1251 pollen/m³ and 447 pollen/m³, respectively). Accordingly, in the passive samplers located in Hirst's geographical area, higher pollen values were observed in the last week. The pollen detected at 2m in the urban Sigma-2, reach the highest values in the previous week (13th to 20th May), which points to differences relating to the urban context.

Relatively to DNA, one week slide exposure was enough, in this period, to collect and quantify DNA from every sample, ranging from 188.2 – 1609.8 ng DNA/cm². Poaceae DNA was detected in these samples by digital-PCR, highlighting the relevance of this technique for environmental DNA (eDNA) analysis.

In conclusion, these results show that it was possible to consistently collect pollen and extract DNA, using the passive sampler Sigma-2. Moreover, it was shown that eDNA might be used as proxy for airborne pollen, supporting the next phases of AirBiD project.

* <https://sites.google.com/fmach.it/airbid/home>

Quantificação de fenóis em sumos e bebidas fermentadas comerciais e contributo para a saúde oral

R. S. Tajra¹, L. I. C. Lopes², F. Capela e Silva³, M. C. D. Pereira⁴, A. S. M. Batista⁵, M. do R. Martins⁶

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal

²Departamento de Química e Bioquímica, Universidade de Évora, Évora, Portugal

³MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora, Évora, Portugal

⁴ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Departamento de Paisagem, Ambiente e Ordenamento, Universidade de Évora, Évora, Portugal

⁵Departamento de Zootecnia, Universidade Estadual Vale do Acaraú, Sobral, Brasil

⁶Escola de Saúde e Desenvolvimento Humano

Laboratório HERCULES, Departamento de Ciências Médicas, Universidade de Évora, Évora, Portugal

Email: rosanastajra@gmail.com / rosana_solon@uvanet.br

Phenolic compounds, key pillars of the Mediterranean Diet, are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties, with documented benefits for systemic health. For oral health, these compounds are particularly relevant, as they can contribute to the control of periodontal disease through their anti-inflammatory activity, to the prevention of caries through antimicrobial action, and to protection against oxidative stress in oral tissues. The preservation of these compounds in fruit-based products, including fermented beverages that are gaining market traction, is a crucial indicator of nutritional quality. In this study, we quantified the total phenolic content in different categories of juices and beverages commercialized in Portugal (grape, apple, orange, blueberry + raspberry, Detox, and Kombucha) to evaluate the levels of these compounds, using Brazilian red propolis as a reference standard for high phenolic concentration. The quantification of total phenolic compounds, after extraction, was performed using the Folin-Ciocalteu method, allowing for a robust comparison among samples, including the fermented beverage (Kombucha), fruit juices, and propolis. The results reveal a well-defined hierarchy: red propolis showed a phenolic compound content per 5 mL in the same order of magnitude as the juices per 100 mL, followed by this descending order: 20% propolis > 11% propolis > blueberry + raspberry juice > grape juice > Detox > Kombucha > orange juice > apple juice. The inclusion of Kombucha introduces an interesting variable, allowing us to explore the effect of fermentation and carbonation on the availability of these compounds, in contrast with the profiles of traditional and functional juices. The results demonstrate that the nature of the product decisively influences the content of bioactive compounds. The study provides a quantitative basis that highlights the variable potential of these beverages as functional juices and dietary sources of phenols, guiding future investigations into their specific biological efficacy in promoting oral health.

Biology & Ecology

Pathogen sharing associated with the introduction of non-native turtles: A global review

Rato J.^{1,2}, Rocha R.³, Brandão P.^{1,2}, Xavier R.⁴, Harris D.J.⁴, Banha F.^{1,2}, Anastácio P.^{1,5}

¹MARE- Marine and Environmental Sciences Centre; ARNET- Aquatic Research Network;

²IIFA- Institute for advanced Studies and Research, University of Évora, Portugal;

³Veterinary Hospital of the University of Évora;

⁴BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO-InBIO, Campus Agrário de Vairão, Universidade do Porto, Rua Padre Armando Quintas, 4485-661 Vairão, Portugal;

⁵Department of Landscape, Environment and Planning, School of Sciences and Technology, University of Évora Portugal

Email: joao.rato@uevora.pt

Freshwater turtles are increasingly recognized as important models in invasion biology and disease ecology. Their popularity in the exotic pet trade, combined with the frequent release of unwanted individuals into natural environments, raises concerns regarding their role as reservoirs and vectors of infectious pathogens. However, the extent to which these species contribute to pathogen transmission remains insufficiently understood. In this study, we conducted a systematic review of bacterial, viral, and fungal pathogens reported in freshwater turtles worldwide. From 163 peer-reviewed publications, we compiled 815 pathogen occurrence records spanning 188 taxonomic genera. The species most commonly identified as hosts were *Trachemys scripta*, *Pelodiscus sinensis*, and *Emys orbicularis*. Notably, *T. scripta*, an invasive species in many regions, was associated with a high number of zoonotic pathogens—particularly *Salmonella* spp.—reinforcing its relevance as a potential disease vector. We also found evidence of pathogen sharing between native and introduced species, even among distantly related taxa, suggesting that environmental overlap and human-mediated processes play a key role in transmission dynamics. Bacterial pathogens dominated the dataset, viral records highlighted intercontinental links between Asia and North America, and fungal pathogens appeared more geographically constrained. Despite this, clinical impacts on turtles themselves are rarely reported, and introduced species remain underrepresented in health monitoring programs. Overall, our results emphasize the need to integrate invasion biology with public health, disease ecology, and conservation strategies. Recognizing non-native freshwater turtles as active participants in pathogen transmission is essential for effective wildlife management and for advancing One Health approaches.

This study had the support of FCT through the strategic project UIDB/04292/2020 awarded to MARE, through project LA/P/0069/2020 granted to the Associated Laboratory ARNET and through the Fundo Ambiental under the project “Erradicação e controlo de espécies de tartarugas exóticas dos géneros *Graptemys*, *Pseudemys* e *Trachemys* nos parques naturais a sul do Tejo”. Filipe Banha is supported by the Foundation for Science and Technology through an individual contract (CEEC/01896/2021). João Rato holds a Ph.D. grant from FCT (2024/01395/BD). Raquel Xavier was supported by FCT under the Programa Operacional Potencial Humano—Quadro de Referência Estratégico, national funds from the European Social Fund and Portuguese Ministério da Educação e Ciência (2020.00854.CEECIND/CP1601/CT0001).

Eating the invaders: plant preferences and seed ingestion by captive ungulates

Mónica Roldão Almeida^{1,2}, Elizabete Marchante¹, Hélia Marchante², Joana Alves¹

¹Centre for Functional Ecology (CFE), TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

²CERNAS, Polytechnic Institute of Coimbra, Coimbra Agriculture School, Bencanta, 3045-601 Coimbra, Portugal

Email: monica.almeida@student.uc.pt

Invasive alien plants (IAPs) are one of the main threats to biodiversity, profoundly modifying ecosystems, and negatively impacting economic activities, food security and human health. In Portugal, the number of IAPs exceeds 100 species and continues to rise. Ungulates, acting as ecosystem engineers, may contribute to the expansion and/ or control of some IAPs, but in European Mediterranean ecosystems, the interactions between common ungulates and widespread IAPs needs further clarification. This study investigates these interactions through controlled trials with captive ungulates at the Serra da Lousã Biological Park. Working with captive ungulates in controlled conditions allows for precise monitoring of individuals, diet and behaviour, replication of trials, and accurate quantification of key variables, while reducing environmental variability. The study comprised two main experiments. Foraging preferences of red deer (*Cervus elaphus*) and domestic goat (*Capra hircus*) were assessed using multi-choice preference tests involving four IAPs (silver wattle, *Acacia dealbata*; Australian blackwood, *Acacia melanoxylon*; bushy needlewood, *Hakea decurrens*; willow-leaved hakea, *Hakea salicifolia*) and two native species (pedunculate oak, *Quercus robur*; prickly broom, *Pterospartum tridentatum*). Additionally, silver wattle seed ingestion and germination were evaluated for red deer, domestic goat and wild boar (*Sus scrofa*). The foraging preference tests revealed that both deer and goats strongly preferred the native oak and the invasive silver wattle. Bushy needlewood was the least consumed species, with other plants showing intermediate levels of consumption. Intact and fragmented silver wattle seeds were found in all ungulates' faeces. Germination trials confirmed that a proportion of the intact seeds remained viable and capable of germination. These results demonstrate the complex dual role of ungulates in IAPs dynamics, a key mechanism in plant invasion that managers may be currently overlooking. Through selective browsing on palatable IAPs and seed destruction, ungulates can exert a top-down control pressure. Conversely, by dispersing viable seeds via endozoochory, they can act as vectors for IAPs spread, though *Acacia* spp. and *Hakea* spp. seed ingestion in the wild is unlikely. Our results underline that the role of ungulates needs to be considered when developing effective management strategies against IAPs in Mediterranean ecosystems.

This thesis is funded by National Funds through FCT under the PhD scholarship 2022.09958.BD.

Do covercrops shape the arthropod communities in almond and olive superintensive groves? Insights of the first monitoring season

L. Machado¹, R. Azedo¹, P. Matono¹, C. Pinto-Cruz¹

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: d61164@alunos.uevora.pt

In Portugal, super-intensive permanent crops, namely olive groves and almond orchards, have expanded, with notable environmental impacts. Given their economic and cultural importance, research on their environmental sustainability is needed. Although the olive grove ecosystem has been studied, for the almond orchard they remain scarce, especially regarding how these systems influence the ecosystem functions provided by the arthropod insect community, such as pest controllers. Due to the sharp decline of pollinators and, within the framework of the European Restoration Law, we are studying the contribution of herbaceous cover to the conservation and increase of specific and functional diversity of arthropods in two agroecosystems (olive grove and almond orchard) in the Alentejo region. Four seed mixtures were selected and planted in November of 2024 in superintensive crops located near Torrão (Alcácer do Sal municipality). The plant species composition was chosen with specific purposes, such as promoting pollinators, self-seeding capacity and soil health improvement.

In May of 2025 we collected soil arthropods with pitfall traps, pollinators with entomological nets in transects, following the SPRING protocol, and we registered floristic diversity and abundance with quadrats. The present work aimed to monitor the evolution of the seed mixtures and its impacts on the arthropofauna, based on success indicators. The results from the spring season shows a great success of the seed mixture germinations and a wide range of insect groups and species. The ants were dominant on both crops and in all of the mixtures. Success indicators, such as number of plant species from the mixture that germinated, diversity of ants, diversity of pollinators and richness and diversity of plants, allowed us to identify, for the first year, the best seed mixture in each crop, for each indicator. The almond crops showed a best mixture for all indicators, while the olive crops still show different results for each indicator in this short-term and preliminary analysis.

This work was funded by Bolschare Agriculture scientific patronage.

Correlation Between PM₁₀-Bound Organic Compounds and Cellular Responses in A549 Lung Cells

Marlene Soares¹, Leonardo Furst¹, Yago Cipoli¹, Alan Silva¹, Margarita Evtugina¹, Helena Oliveira², Célia Alves¹

¹Department of Environment and Planning, CESAM — Centre for Environmental and Marine Studies, University of Aveiro, 3810-193 Aveiro, Portugal

²Department of Biology, CESAM — Centre for Environmental and Marine Studies, University of Aveiro, 3810-193 Aveiro, Portugal

Email: marlene98@ua.pt

Particulate matter is a significant environmental and public health concern due to its ability to penetrate deep into the respiratory tract, with the finer particles reaching the alveoli. Particulate matter lower than 10 µm (PM₁₀) possesses genotoxic, mutagenic, and carcinogenic properties, making it a major contributor to adverse health effects such as chronic obstructive pulmonary disease (COPD), and lung cancer. Its toxic effects are largely associated with the production and heightening of reactive oxygen species (ROS), DNA damage, and cell death. Despite satellite data indicating that many African cities are among the most polluted, there remains a lack of studies linking air quality to specific health outcomes across the continent. This study aims to relate specific components of PM₁₀ collected in Luanda, Angola, with their biological effects on A549 lung cells. For these purpose PM₁₀ sampling was carried out in central Luanda, Angola, near a major road with heavy traffic, representing a typical urban pollution environment. A high-volume air sampler was used, operating at a flow rate of 500 L min⁻¹, equipped with 150 mm quartz fibre filters for the collection of PM₁₀ intended for subsequent chemical and toxicological analyses. PM₁₀ organic extracts were analysed by GC-MS and A549 cells were exposed to the same extracts at 150 µg mL⁻¹. Cell viability, cell cycle alterations and ROS production levels were studied after 72h of exposure. A bivariate Spearman correlation analysis evaluated the relationships between the PM₁₀-bound organic compounds and biological responses in the cells. Although no significant correlations with cell viability were found, saccharides and phenolics displayed moderate negative relashinships. Steroids, plasticisers, and carboxylic acids were positively correlated with cell viability, indicating lower toxicity. Regarding cell cycle effects, aliphatic alcohols and hydroxy acids displayed strong positive correlations with the G₀/G₁ phase and negative correlations with the S and G₂ phases, suggesting cell cycle arrest before DNA replication. In oxidative stress responses, quinones and PAHs showed strong and moderate positive correlations with ROS, respectively. Overall, PM₁₀ compounds exhibited class-specific toxicological behaviours, highlighting the need for composition-based risk assessment in air pollution studies and implementation of air monitoring stations in Luanda.

The Portuguese Foundation for Science and Technology (FCT) is acknowledged for the PhD fellowships to M. Soares (2020.04826.BD), A. Silva (2023.02059.BD), L. Furst (SRFH/BD/08461/ 2020) and Y. Cipoli (SFRH/BD/04992/2021). FCT is also acknowledged for the research contracts under Scientific Employment Stimulus to H. Oliveira (DOI: 10.54499/CEECIND/04050/2017/CP1459/CT0023). The financial support to CESAM by FCT/MCTES UID/50006 + LA/P/0094/2020 (doi.org/10.54499/LA/P/0094/2020), through national funds, is also acknowledged. This work was developed within the project “Air Pollution in an African Megacity: Source Apportionment and Health Implications – APAM” (DOI: 10.54499/2022.04240.PTDC), financially supported by national funds (OE), through FCT/MCTES.

Effects of Artificial Canopy Gaps on Holm Oak Regeneration in Dryland Afforestations

J. N. Rosa¹³⁴⁵⁶, R. S. Ceia¹²⁴⁶, M. B. Pesendorfer⁵⁷, M. Rodrigues¹⁴⁶, F. Moreira¹²⁴⁶

¹CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associated Laboratory, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

²CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associated Laboratory, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

³Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal;

⁴BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

⁵BOKU, University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33, 1180 Vienna, Austria

⁶Biological Station of Mértola, Rua Professor Baptista nº 1, 7750-360 Mértola, Portugal

⁷Migratory Bird Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington, DC 20008, USA

Email: jnunorosa@hotmail.com

Holm oak (*Quercus rotundifolia*) naturally regenerates in the understory of stone pine (*Pinus pinea*) and eucalypt (*Eucalyptus camaldulensis*) plantations in southern Portugal, offering an opportunity to test the creation of canopy gaps as a technique for assisting natural regeneration in degraded forest patches. The responses of holm oak plants have been explored in a manipulative experiment, started in 2023, using a Before-After Control-Impact (BACI) design involving replicates of 20x20m plots for each of two restoration treatments (logging and thinning) and a control implemented in both pine and eucalypt plantations. It is expected that canopy-thinning optimizes light availability for the growth of holm oak recruits, while protecting them from environmental extremes that can risk their development. Over three years, we monitored the survival and growth of holm oak individuals under varying light conditions. Preliminary results show distinct regeneration patterns between the two plantation types. In stone pine stands, moderate thinning yielded the highest survival rate of holm oak individuals (98%), though differences were not statistically significant. However, this treatment showed significantly lower growth rates among seedlings, while saplings exhibited the opposite trend. In eucalypt plantations, the survival and growth of seedlings and saplings showed no significant differences among treatments. In 2024, an additional trial was established in the same experimental areas, where 100 acorns were cached per plot to assess the influence of treatments on germination. In the germination trials, moderate thinning resulted in the highest seedling emergence (50%) in pine areas with high significance. While increased canopy openness also had a positive effect on germination in eucalyptus stands, the differences were not significant. Our results demonstrate how holm oak regeneration responds to the creation of canopy openings in different contexts and how gap size influences its dynamics. These findings inform thinning operations that can optimize light conditions while reducing environmental stress, providing insights into assisted natural regeneration as a viable strategy for restoring afforested landscapes in semi-arid regions.

This work has been supported by National Funds through FCT-Fundação para a Ciência e a Tecnologia in the scope of the scholarship 2023.03601.BD and the project POCI-07-62G4-FEDER-181664 under the Compete2020 program. J. N. Rosa acknowledges Biological Station of Mértola for their support.

Exploring Genomic Resources for *Cynara cardunculus*: From DNA Sequencing to Bioinformatics

A. Paulino^{1,2,3}, A. Usié^{1,3}, D. Rosa^{1,3}, O.S. Paulo², M.F. Duarte^{1,3} and L. Marum^{1,3}

¹Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Instituto Politécnico de Beja (IPBeja), Beja, Portugal

²cE3c – Centre for Ecology, Evolution and Environmental Changes, Change - Global Change and Sustainability Institute, Departamento de Biologia, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

³MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, Beja, Portugal

Email: ana.paulino@cebal.pt

Cynara cardunculus is a perennial species of the Asteraceae family with increasing importance in agriculture, biotechnology, and bioenergy. Its capacity for high biomass production, resistance to adverse environments, and production of bioactive compounds make it a valuable resource for numerous applications. Although this potential, genomic resources for *C. cardunculus* remain reduced, and more studies on its genetic variability are required to support breeding and biotechnological approaches.

This study aims to explore the genomic diversity and population structure of *C. cardunculus* through whole-genome resequencing (WGRS) of 23 offspring from 13 mother plants from northern and southern Alentejo populations. Furthermore, this group of 23 offspring plants displays three distinct cynaropicrin production profiles (high, medium, and low), which were analysed previously using RNA-Seq to study the differential expression of their genes [1]. In a previous study, SSRs of the mother plants (121 plants) were used, which provided a broad view of genetic diversity and population structure and, in the present study, serve to compare and validate patterns [2].

In the present work, a bioinformatics workflow was implemented to process and analyse the sequencing data, including quality assessment, read alignment to the *C. cardunculus* reference genome, post-processing, and variant calling and filtering. Downstream analyses comprise SNP-based population structure, phylogenetic inference, and association of genetic variation with cynaropicrin biosynthesis profiles.

This integrative approach combines next-generation sequencing and computational genomics to expand available resources for *C. cardunculus* and to provide insights into its genetic diversity.

References:

[1] Paulino et al., Acta Horticulturae, International Society for Horticultural Science, pp 351–358, 2023.

[2] Castro et al., PLoS One, 16, 2021.

Authors acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), and A. Usié (CEECINST/00100/2021/CP2774/CT0001), Ph.D. grant to A. Paulino ((SFRH/BD/145383/2019) (<https://doi.org/10.54499/SFRH/BD/145383/2019>)), Project UIDB/05183 to MED (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>);, and Project LA/P/0121/2020 to CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>).

Conservation of Remnant Habitats in Montado: the Role of Management Practices

E. Almeida¹, P. Matono², C. Pinto-Cruz²

¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, IIFA (The Institute for Advanced Studies and Research), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: erikaroldao@gmail.com | d49668@alunos.uevora.pt

The Montado, a traditional agro-silvo-pastoral system, dominated by cork oak (*Quercus suber*) and holm oak (*Q. rotundifolia*), represents a High Nature Value farming system, supporting high levels of biodiversity within a production matrix. Its ecological value depends on maintaining structural heterogeneity and low-intensity management. Natural Remnant Habitats (NRHs) - small, heterogeneous patches of native vegetation - are essential for ecosystem resilience and biodiversity conservation, providing unique ecological niches for species that cannot persist in the surrounding matrix. However, land-use changes and management intensification threaten these habitats.

This study investigates how land management intensity shapes NRHs abundance, diversity and conservation status in Montado. We considered six NRHs types: forest/ shrubs patches, rocky outcrops, temporary/ permanent ponds, and riparian galleries. Fieldwork covered 36 sampling areas on 21 Montado estates in Central Alentejo (spring seasons 2021–2023), covering a gradient of management intensification. In total, 226 NRHs were recorded and their conservation status assessed by a specialist. We characterized the surrounding matrix by recording variables related to management, such as canopy and litter cover, indicators of woody plants natural regeneration and signs of grazing pressure.

Multivariate hierarchical and ordination analyses were used to identify montado matrix types with distinct management characteristics and to relate these to NRH attributes, including total and specific richness, density, relative area and conservation status.

Three matrix type management-related groups were identified: group *a*, dominated by dense tree canopy, well-developed litter layer, and lower intervention (less grazing signs, greater natural regeneration); group *c*, combines sparse tree canopy, higher bare soil cover, and intensive management (strong signs of grazing, reduced natural regeneration); and group *b*, exhibits intermediate features. Along this land use intensity gradient, the HNRs revealed clear ecological contrasts. Less intervened matrices support the highest overall HNR density, relative area, and conservation status, while intensive management reduces HNRs, leading to landscape simplification and habitat /biodiversity loss.

These findings show the influence of land management practices on landscape heterogeneity, NRHs conservation, and thereby biodiversity safeguard, ensuring the long-term resilience and sustainability of the Montado.

This work is funded by National Funds through FCT under the Ph.D. scholarship 2020.07109.BD. and Project UID/05183/2025.

Bats and ponds: tracking seasonal patternsJoana Ribeiro-Silva^{1,2}, Carla Pinto-Cruz^{1,3}, Neftalí Sillero², João Tiago Marques^{1,3}

¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, Évora 7006-554, Portugal

²CICGE, Centro de Investigação em Ciências Geo-Espaciais, Faculdade de Ciências da Universidade do Porto, Alameda do Monte da Virgem, Vila Nova de Gaia 4430-146, Portugal

³Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Évora 7002-554, Portugal

Email: joanasilva.ctx@gmail.com

Water availability is declining across the Mediterranean region, posing a serious threat to biodiversity. Mediterranean Temporary Ponds are shallow water bodies that alternate annually between flooded and dry periods and occur widely in the region. However, they are highly vulnerable and face numerous pressures, increasing competition for water resources, which affects water-dependent species. Bats are particularly sensitive, using ponds as essential sources of drinking and foraging. We investigated how environmental variables and insect availability influence bats throughout the year - summer and autumn of 2023, and winter and spring of 2024 - at 20 temporary ponds, each paired with a control site at least 100 m from the pond edge, in Montado landscapes. At each site, we recorded bat acoustic activity for three nights, along with air temperature and water presence. However, our models only include data from the night when prey availability was also sampled. Ponds had about twice the species richness, 31% higher bat activity, and 145% higher feeding activity than control sites without water. Insect suborders influenced species richness, insect availability influenced bat activity, and insect biomass influenced feeding buzzes, indicating that insect activity and diversity are key drivers of bat use of ponds. Water surface area also affected all three response variables, suggesting that bats preferentially use smaller ponds. Bats were significantly more active and fed more frequently in ponds during warmer nights, highlighting the importance of these habitats during summer and autumn. Maintaining small temporary ponds in Montado year-round is essential to sustain bat activity and species richness, as they serve primarily as feeding sites, reinforcing their role in bat conservation.

This work is funded by National Funds through FCT - Foundation for Science and Technology under the Project UID/05183/2025 and a FCT grant 10.54499/2021.04651.BD (<https://doi.org/10.54499/2021.04651.BD>).

Functional characterization of a key transcription factor regulating parasitism-related genes in the plant-parasitic nematode, *Bursaphelenchus xylophilus*

Madalena Mendonça¹, Cláudia S. L. Vicente¹, Sebastian Eves-van den Akker², Margarida Espada¹

¹ MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies, and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² The Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom.

Email: maria.silva@uevora.pt

Plant-parasitic nematodes (PPN) are major economic and ecological threats to crops and forest species worldwide. The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a quarantine organism responsible for the pine wilt disease (PWD), which leads to the decline of conifer species in Europe and East Asia. The lack of effective control measures for PWD highlights the urgent need for innovative and sustainable strategies targeting the parasite, including a deeper understanding of the molecular mechanisms of PWN parasitism. Interactions between the PWN and the host are mediated by parasitism proteins, primarily produced in specialized cells, known as gland cells (GC), that play a crucial role in nematode migration, defense and host immunity response. Based on a nematode transcriptomic comparative analysis (Espada et al., 2016, 2018), we were able to select seven predicted genes encoding for transcription factors (TF) that were putatively more expressed in the GC tissues, compared to the whole-nematode body. Therefore, we hypothesised that these molecules may be involved in the regulation of the expression of parasitism-related genes. All seven candidate genes are expressed in the GCs and were validated by in situ hybridisation (ISH). One of the candidate TF, BXY_079, was successfully knocked down by RNA interference. Transcriptomic data from the silenced TF revealed that several parasitism-related genes were also significantly downregulated. When analyzed together with PWN life-stage transcriptomic data (Tanaka et al., 2019), these genes were found to be highly expressed during parasitic juvenile stages of PWN and are predicted to encode lytic functions that are associated with nematode feeding and migration during host interaction. Moreover, yeast one-hybrid functional analysis showed that BXY_079 binds to the promoters of genes involved in both lytic functions and cell signaling regulation, proving the direct regulation of the predicted parasitism-related genes. We suggest that silencing this regulatory element could simultaneously disrupt the expression of several parasitism-related genes and potentially reduce PWN infection. Exploring regulatory molecules in PPN could have a major biotechnological impact on the forestry system, as these regulators represent promising targets for genetic editing to achieve effective nematode resistance in plants.

This work was funded by FCT under the Project NemaWAARS - 10.54499/PTDC/ASP-PLA/1108/2021 and individual funding for M. Mendonça (2024.00901.BD).

Movement ecology as a holistic approach to test the efficacy of conservation measures in a ground-nesting steppe raptor

S. E. Moreno-Fernández^{1, 2, 3, 4}, J. Gameiro^{1, 2, 5}, A. T. Marques^{1, 2, 5}, B. Arroyo⁶ & J. P. Silva^{1, 2, 4, 5}

¹ Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO-InBio), Laboratório Associado, Universidade do Porto, Portugal

² BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Portugal

³ Department of Biology, Faculty of Sciences, Universidade do Porto, Portugal

⁴ Estação Biológica de Mértola (EBM), CIBIO, Portugal

⁵ CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBio Laboratório Associado, Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal

⁶ Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain

Email: semfmoreno@gmail.com

Changes in agricultural practices, climate warming, and the increasing frequency of extreme weather events are now acting synergistically, posing significant threats to biodiversity. The Montagu's harrier (*Circus pygargus*) is a ground-nesting steppe bird and one of the most threatened species in the Iberian Peninsula, relying largely on extensive cereal farming and, in the northern regions of the country, on areas with natural vegetation such as shrublands. In Iberia, despite holding one of the most important populations in Europe, the species is highly threatened, primarily due to nest destruction caused by heavy machinery and early harvesting. In Portugal, a major shift in agricultural policy at the start of the millennium, ending cereal-crop subsidies while promoting livestock grazing, led to a dramatic population decline of approximately 80% within 10–15 years. Currently, emergency conservation actions are being implemented with the "SOS Pygargus" LIFE project to halt the decline, and the PhD project now beginning aims to identify new conservation opportunities and evaluate the effectiveness of ongoing measures. Specifically, we aim to address the following questions: 1) What are the behavioural responses to breeding unsucccess and disturbances during reproductive period? 2) Is food availability limiting productivity? and 3) Is hacking an effective conservation strategy aiming to increase productivity? To address these questions, we will tag a considerable number of adult and juvenile individuals with high-resolution, long-lasting GPS and accelerometer devices, allowing us to infer population-level responses. In parallel, we will monitor nesting events and collect pellets to study the species' diet. Our study area covers the species' distribution in Portugal, from Alentejo to the northeast, as well as relevant transborder *C. pygargus* populations in Galicia, Castilla y León, and Extremadura. Overall, this study will provide a deeper understanding of the effectiveness of conservation measures applied to ground-nesting birds and how these measures can be improved to better preserve steppe birds in agricultural ecosystems.

This work was funded by National Funds through FCT under the Project EBM_2025 scholarship and LIFE SOS Pygargus project.

Satellite-derived spectral indices as early indicators of ecological degradation in Mediterranean ponds

Rita Inácio ¹, Sofia Augusto ^{2,3}, Diogo Alagador ⁴, Maria José Viñals ⁵

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²Escola Superior de Saúde de Lisboa (ESSL).

³Centro de Ecologia, Evolução e Alterações Ambientais (CE3C).

⁴“Rui Nabeiro” Biodiversity Chair, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Portugal.

⁵Centro de Investigación en Arquitectura, Patrimonio y Gestión para el Desarrollo Sostenible (PEGASO), Universitat Politècnica de València, España.

E-mail de contacto: d54213@alunos.uevora.pt

Mediterranean wetlands deliver key ecosystem services, including hydrological regulation, biodiversity support and cultural or recreational benefits. However, these small freshwater systems are highly sensitive to climatic variability and local anthropogenic pressures. This study evaluates the potential of satellite-derived vegetation and water indices to act as early warning signals (EWS) of ecological degradation, and thus of potential loss of ecosystem services. Five Mediterranean freshwater ponds (< 60 ha) were analysed using monthly Sentinel-2 imagery from 2018–2025, processed on Google Earth Engine. Vegetation (e.g. EVI, NDVI, NDMI) and water indices (e.g. MNDWI, AWEIsh, AWEInsh) were extracted and assessed using temporal autocorrelation (AC1) and variance (VAR), with trends tested through the Mann–Kendall method.

The Enhanced Vegetation Index (EVI) showed a consistent increase in variance across all ponds, indicating rising instability in riparian vegetation. However, EVI did not exhibit the simultaneous rise in autocorrelation expected for classical critical slowing down. In contrast, hydrological indices (AWEIsh/AWEInsh) displayed concurrent increases in AC1 and VAR in three of the five ponds, aligning with theoretical predictions for EWS of approaching ecological transitions.

To contextualise and support these patterns, we integrated climatic analyses (precipitation and temperature anomalies) and land-use metrics (constructed surfaces, agricultural area, bare soil). These external drivers helped explain the spatial variation in EWS strength, particularly the links between hydrological instability, regional drying trends and local human pressure.

We propose a hierarchical alert framework in which AWEIsh/AWEInsh function as primary hydrological EWS, complemented by EVI as an indicator of riparian stress. This structure enhances the capacity for early detection and supports proactive management of Mediterranean pond ecosystems.

Environment, Sustainability and Socioeconomic Development

Justice by Design: Mapping Mindsets in Energy Transitions

Beste Gün Aslan¹; Patrícia Fortes¹; Nuno Videira¹

CENSE - Center for Environmental and Sustainability Research & CHANGE - Global Change and Sustainability Institute, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Caparica, 2829-516, Portugal

Just energy transitions involve multiple contested values, uncertain futures, and a diverse set of stakeholders with divergent perspectives. Traditional, expert-driven transition planning often overlooks local knowledge and obscures the lived experiences of communities, resulting in limited inclusiveness and reinforcing inequities. Despite growing interest in participatory and reflexive approaches, there remain significant gaps in transition research regarding the methods used to capture and analyze stakeholder mental models. In particular, there is a lack of systematic tools to document how actors perceive problems, attribute causality, and conceptualize justice, as well as methods that integrate complexity and enable the co-construction of shared problem understanding across multiple stakeholders. This paper responds to these gaps by presenting a multi-stage causal mapping methodology to explore stakeholder reasoning and justice framings in just energy transitions, applied to the coal phase-out in Portugal, focusing on the Pego region in the Médio Tejo. Mental models—personal, internal representations of external reality (Jones et al., 2011)—shape how stakeholders define problems, prioritize solutions, and perceive fairness. Making these models explicit is critical to identify misaligned perceptions, surface systemic leverage points, and build a coherent understanding of transition dynamics. The methodology integrates desktop research and semi-structured interviews to elicit and synthesize diverse mental models, while optionally incorporating participatory systems mapping (PSM) to enable co-production of knowledge and visualize system interdependencies. By combining qualitative inquiry with systems thinking, this approach captures both causal reasoning and systemic perspectives, providing tools to represent complexity and compare stakeholder viewpoints. The application of this methodology highlights tensions, blind spots, and alignments across institutional, social, and economic dimensions, revealing areas where shared understanding is lacking and where targeted intervention may enhance justice-oriented governance. The resulting system maps serve both analytical and practical purposes: analytically, they provide a structured framework to assess justice across distributive, procedural, and recognition dimensions; practically, they facilitate dialogue among diverse actors, bridging technical and experiential knowledge. Overall, this work advances methodological transparency, epistemic plurality, and deliberative engagement in sustainability research, offering a replicable toolkit to study socio-ecological transitions while centering stakeholder perspectives, causality, and justice in the analysis.

This work is supported by FCT through the scholarship UI/BD/153685/2022.

Local perspectives on a rural Renewable Energy Community surrounded by solar farms: Insights from Comenda (Alentejo, Portugal)

C. C. Castro¹, R. Lopes¹, J. P. Gouveia¹

¹CENSE – Center for Environmental and Sustainability Research & CHANGE - Global Change and Sustainability Institute, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, 2829-516, Caparica, (PORTUGAL).

Email: cc.castro@campus.fct.unl.pt

A just energy transition must avoid replicating the extractive and exclusionary dynamics of fossil fuel regimes. Instead, it must prioritize the lived experiences, needs, and aspirations of local communities by fostering inclusive, accountable, and context-sensitive energy governance. Rural areas have become central to Europe's energy transition, serving both as sites of renewable energy production and as territories disproportionately affected by energy poverty and land-use conflict. While the European Union promotes Renewable Energy Communities (RECs) as tools for citizen empowerment, energy poverty mitigation, and a fair transition, their implementation in rural contexts remains limited due to barriers such as insufficient participation, regulatory complexity, infrastructural deficits, and a lack of local awareness.

This study examines local perceptions of the “CER da Comenda – LESTE II”, a REC being developed in the rural parish of Comenda (Gavião, Alentejo, Portugal), a territory increasingly surrounded by large-scale solar farms and characterised by contested land-use dynamics. Drawing on twenty-three semi-structured exploratory interviews conducted in March 2025, the research examines how motivations, knowledge levels, and types of involvement shape perceptions of the REC and broader renewable energy developments. Findings indicate that REC participation is associated with higher energy literacy and a more comprehensive understanding of REC implementation challenges, including bureaucratic obstacles and community coordination issues. Motivations (economic, social, or environmental) influence how individuals perceive the barriers to implementing the REC. Additionally, REC participants tend to frame their concerns about nearby solar parks in ecological and collective terms, while non-members focus more on individual or financial risks.

These results contribute to the growing scholarship on rural energy transitions by situating energy justice in the lived experiences of local residents navigating territorial tensions. They point to the role of RECs in shaping knowledge, perceptions, and community agency in rural energy transitions. They also emphasise the need for inclusive communication strategies, tailored support mechanisms, and participatory governance models that reflect the complexity of rural areas and the contested nature of development contexts.

This work is supported by FCT I.P through the scholarship 2024.06070.BD

Integrating Climate Change Adaptation into Water Law: Challenges and Perspectives for Sustainable Development

H. Hatat¹, A. Zine-dine²

¹Laboratoire des Etudes Juridiques et Judiciaires, Université Moulay Ismail, Meknes MAROC.

²Laboratoire des Etudes Juridiques et Judiciaires, Université Moulay Ismail, Meknes MAROC.

Email: h.hatat@edu.umi.ac.ma

Climate change is profoundly transforming the hydrological cycle, intensifying droughts, floods, and water scarcity. These shifts challenge the adequacy of existing legal frameworks governing water resources, which were largely designed under conditions of climatic stability. This paper explores the integration of climate change adaptation principles, as reflected in international instruments such as the Helsinki Water Convention (1992) (UNECE, 2024), the UN Watercourses Convention (1997) (UN General Assembly, 1997), and the Paris Agreement (2015) (UNFCCC, 2015), into water law as a critical pathway to achieving sustainable development. It specifically analyzes the Moroccan water law framework, highlighting the need for its evolution towards resilience and adaptive capacity amid climatic uncertainties. Morocco's recent Water Law No. 36-15 of 2016 emphasizes decentralized, integrated management and incorporates climate adaptation components aligned with the National Water Plan and Green Morocco Plan (World Bank, 2017; Ministry of Environment, 2016).

Through legal and doctrinal analysis, it argues that water legislation must transition from a static regulatory model to a dynamic framework capable of addressing uncertainty, variability, and resilience. At the national level, Morocco faces barriers such as institutional fragmentation, enforcement difficulties, and limited resources, which constrain the effective incorporation of adaptation measures into its water governance system (Bensaid, 2022).

By examining comparative experiences, theoretical perspectives, and Morocco's legal context on principles such as precaution, flexibility, and intergenerational equity (Cambridge University Press, 2025; UNECE, 2024), this paper highlights gaps between environmental norms and adaptive governance demands. It emphasizes that integrating adaptation into Moroccan water law requires strengthening stakeholder participation and multi-level governance mechanisms. The study concludes that a coherent, adaptive water law is essential to secure both environmental sustainability and socioeconomic resilience in Morocco's face of climate change.

Fire regime effects on soil habitat and carbon balance in Mediterranean landscapes

Canedo, JNGV¹; Fernández, C²; Mendes, A¹; Silva, C¹; Prats, S^{1,2}

¹ MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora. Núcleo da Mitra Gab. 206 Edifício dos Regentes Agrícolas, Apartado 94, 7006-554 Évora, Portugal.

² Misión Biológica de Calicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

E-mail: joao.canedo@uevora.pt

Wildfire severity and frequency in European Mediterranean regions are projected to intensify under climate change, exacerbating soil degradation and disrupting carbon sequestration. Moreover, these events pose serious risks to human life, infrastructure, and local economies. Although prescribed burns are advocated to mitigate wildfire risk, EU fire policies remain reactive, prioritizing short-term social stability over long-term forest resilience—a critical gap given conflicting evidence on fire impacts. While some studies suggest wildfires reduce carbon stocks, others argue they create more stable carbon forms. Additionally, some researchers suggest impacts on soil carbon dynamics are highly dependent on fire regime (severity and frequency).

This PhD project seeks to align fire management with broader climate mitigation goals, by comparing fire regime effects (high vs. low severity; high vs. low frequency) on Mediterranean soil: 1) habitats, through laboratory-based assays to assess plant development and microbiota, and 2) carbon balance, through field analysis of carbon stocks/forms (Total Organic Carbon, Thermogravimetry) and carbon flux measurements, using a Trace Gas Analyzer.

Preliminary results showed that the fire residues (char+ashes) produced from high-severity wildfires are more water-repellent than those from low-severity wildfires and prescribed burns - a factor that is positively correlated with runoff and soil erosion. Residues from high-severity wildfires showed higher pH (8.79 ± 0.76) but lower organic-matter content ($5 \pm 2\%$) compared to residues from low-severity wildfires and prescribed burns (pH = 7.83 ± 0.66 and organic-matter = $60 \pm 10\%$). High-severity, high-frequency wildfires exhibited the highest pH values (9.44 ± 0.45), whereas high-severity, low-frequency wildfires showed the lowest organic-matter contents ($4 \pm 2\%$). This increased soil alkalinity and lower organic-matter content are factors that may contribute to phytotoxicity. Ongoing pot assays with *Lolium perenne* and germination/root elongation tests using *Lactuca sativa* are evaluating the effects of the residues produced from different fire regimes on soil habitat (plant development, soil and leachate phytotoxicity, water retention capacity and microbial community). Ultimately, this research will clarify how contrasting fire regimes reshape the functional capacity of Mediterranean soils, providing the scientific basis for fire management strategies that safeguard both ecosystem resilience and the net carbon balance of Mediterranean landscapes.

This work was funded by National Funds through FCT under (2025.00523.BD), by the Galician Innovation Agency-funded GAIN Tipo C Project MULCHAR (IN607D 2025/08) - Restauración de solos queimados co uso combinado de MULch e bioCHAR: estudo multiescala dos impactos nos servizos dos ecosistema solo: erosión, formas de carbono, fertilidade e habitat and by the Spanish Ministry of Science, Innovation and Universities through the research contract RYC2022-035489-I.

Arbuscular mycorrhizal fungi inoculation as sustainable tool to improve yield and phytochemical value of *Cynara cardunculus* L.

Claudia Formenti, Gaetano Pandino, Sara Lombardo

Department of Agriculture, Food and Environment (Di3A), University of Catania, Italy

Email: claudia.formenti@phd.unict.it

Cynara cardunculus L., a species of Mediterranean region, includes globe artichoke (var. scolymus) and cultivated cardoon (var. altilis), crops of rising interest for their capacity to produce biomass and phytochemical compound. Due to their high content of polyphenols, flavonoids and sesquiterpene, these species are increasingly investigated as multipurpose crops for the nutraceutical, pharmaceutical, and bio-based sectors. However, under Mediterranean agroecosystems, the sustainability of their cultivation is constrained by limited water availability, soil nutrient depletion, and the demand for reduced agrochemical inputs. Arbuscular mycorrhizal fungi (AMF) play a key role in enhancing nutrient uptake, water use efficiency (WUE), and plant tolerance to abiotic stresses. The PhD project examines the AMF symbiosis to sustainable cultivation strategies to improving yield and phytochemical quality in *C. cardunculus* under typical edaphoclimatic conditions of eastern Sicily. Field trials were established in the experimental at an organically managed farm located in the Gela area (CL). In globe artichoke (cv. Romanesco G1), a split-plot design was adopted to evaluate the effect of three AMF inoculation treatments Rizocore®, AEGIS IRRIGA®, and control on plant performance and soil water. In cultivated cardoon ('Altilis 41'), a sustainable cropping model combining AMF inoculation (AEGIS IRRIGA®) and two planting densities (2 vs. 4 plants m⁻²) was tested under repeated shoot cuttings. Physiological traits, biomass production, AMF root colonization and biochemical parameters (phenolics, flavonoids and sesquiterpenes) were assessed. This research clarifies the role of AMF to improving the sustainability and functional value of *C. Cardunculus* under field conditions. In globe artichoke, different AMF treatments may influence soil water dynamics and secondary metabolite production, particularly under conditions of WUE. In cultivated cardoon, the combination of AMF and optimized plant density use to stimulate vegetative regrowth and enhance biomass and phytochemical yield, particularly under repeated cutting regimes. the analyses will provide insights into the AMF colonization patterns and their association with morphological traits and antioxidant profiles, contribute to development of agroecological practices aimed at enhancing water use efficiency and the accumulation of natural bioactive compounds in *C. cardunculus* species. The results will contribute to understanding how AMF symbiosis can improve the sustainability, resilience, and functional output of *C. Cardunculus* cropping systems in Mediterranean agroecosystems.

This work was funded by National Funds through FCT under the Project UID/AGR/00115/2013 and InAlentejo ALENT-07-0262-FEDER-001871. J. R-S. acknowledges CAPES, Brasil (BEX 1254138) and A. A. acknowledges FCT (SFRH/BPD/73243/2010).

The Role of Urban Green Space Vegetation in Carbon Sequestration and Storage: The Case Study of Jardim das Comunidades, Loulé

P. Matias^{1,2,3,4}, V. Simões⁴, S. Lisboa^{1,2}, A.R. Trindade^{1,2}, A. Duarte^{1,2} & M. Moreira da Silva^{3,4,5}

¹Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

²MED – Instituto Mediterrânico para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Instituto para a Mudança Global e Sustentabilidade, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

³CIMA/ARNET – Centro de Investigação Marinha e Ambiental / Rede de Investigação Aquática, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

⁴Instituto Superior de Engenharia, Universidade do Algarve, Campus da Penha, 8005-139 Faro, Portugal

⁵CEiiA – Centro de Engenharia e Desenvolvimento, Av. D. Afonso Henriques 1825, 4450-017 Matosinhos, Portugal.

*Correspondence: pmmatias@ualg.pt

Carbon dioxide (CO₂) is the main driver of increased radiative forcing and, consequently, of global warming. Cities, where more than half of the world's population lives, are particularly vulnerable due to the concentration of infrastructure, buildings and services. Extreme heat is intensified by the urban heat island effect, driven by impervious surfaces and limited vegetation. Urban green spaces mitigate heat through shading, plant transpiration, soil evaporation, and reduced impervious cover. Vegetation also absorbs atmospheric CO₂ through photosynthesis, storing part of it in plant tissues. To assess the role of urban vegetation in carbon sequestration and storage, we used Jardim das Comunidades (Loulé) as a case study, quantifying carbon captured and stored with the i-Tree Eco v6 model, supported by meteorological data from IPMA. The urban park contains 94 trees belonging to 15 species, 1 palm tree from 1 species, 85 shrubs from 8 species, and 3700 m² of lawn. *Olea europaea*, *Jacaranda mimosifolia*, *Pinus pinea*, *Populus nigra*, and *Ceratonia siliqua* are the five most abundant tree species, while *Nerium oleander*, *Punica granatum*, and *Laurus nobilis* are the three most common shrub species. The lawn is composed mainly of *Lolium perenne*, *Festuca arundinacea*, *Poa pratensis* and *P. annua*. We found that, annually, the trees are responsible for sequestering 4284 kg of CO₂, while the shrubs contribute less, at approximately 1114 kg. *P. nigra* is the species that sequesters the most CO₂ on average (76.0 kg yr⁻¹), followed by *Casuarina equisetifolia* (69.6 kg yr⁻¹), which appears to be related to the fact that these are the tallest trees in the park (14.8 m and 12.3 m on average, respectively) and are fast-growing species. In the biomass of tree and shrub vegetation, the park stores 47432 kg of carbon, with trees accounting for 95% of this total and shrubs contributing the remaining 5%.

We conclude that urban green spaces can contribute to carbon sequestration and storage, depending on species composition, size, and growth rate. Trees are the main carbon reservoirs, with greater tree abundance increasing storage. This vegetation annually absorbs the emissions of one resident in Portugal plus 35% of the emissions of another.

The social stigmas surrounding Neglected and Underutilized Species (NUS): a narrative review

Débora Paulino, Ana Paula Rodrigues, Octávio José Rio do Sacramento

Universidade de Trás-os-Montes e Alto Douro

Email: paulino.deb@gmail.com

Neglected and Underutilized Species (NUS) represent vast and largely untapped resources with significant potential to concurrently address the interconnected challenges of food insecurity and agricultural vulnerability in a changing climate. The inherent potential of these species contrasts sharply with their current limited participation in mainstream agricultural systems, research agendas, and consumer markets. Among the factors for this underutilization is a complex set of socio-cultural barriers, of which social stigma plays a significant and often overlooked part. This narrative review addresses this crucial area, identifying and analyzing the most prevalent stigmas surrounding NUS through a detailed synthesis of the existing literature. Stigma theory (Goffman, 1963) serves as the primary analytical lens. The review synthesized studies identified through comprehensive searches of the Scopus and Web of Science databases, with a primary focus on literature published between 2010 and 2024. The review identifies the primary stigmas associated with NUS, which include: (1) Status Stigma, wherein they are perceived as "food for the poor" lacking the prestige of mainstream commodities. These perceptions are frequently internalized and perpetuated by younger generations, who increasingly favor mainstream, "prestigious" globalized diets. (2) Risk Stigma, rooted in fears of toxicity and associations with being "wild" or unregulated; and (3) Practice Stigma, stemming from the perception of NUS as "old-fashioned" and associated with "backwardness" particularly concerning traditional agricultural methods and culinary practices. In order to counter negative perceptions, the literature suggests a multi-faceted approach, including targeted educational and communication campaigns to dispel myths of toxicity, strategic market repositioning and culinary rebranding to overcome the "food for the poor" stigma, and product innovation linked to modern food trends to challenge their "old-fashioned" image. The review identifies key research gaps, calling for future studies to evaluate the prevalence and effectiveness of these stigmas across stakeholder groups, assess the impact of existing communication and education strategies, and explore how media narratives influence public perceptions.

Keywords: Neglected and Underutilized Species (NUS), social stigma, food systems.

Integrating Ecotoxicological Assessment to Evaluate Agricultural Impacts on Aquatic Ecosystems: A Case Study of the Lage Reservoir (Mediterranean Region)

A. Catarino^{1,2,3}, C. Mourinha^{1,2}, M. Custódio^{1,2}, P. Anastácio³, P. Palma^{1,2,4,5}

¹Instituto Politécnico de Beja, Escola Superior Agrária de Beja, Rua Pedro Soares, 7800-295 Beja, Portugal;

²Center for Sci-Tech Research in Earth System and Energy (CREATE)—Pole of the Polytechnic Institute of Beja, 7800-295 Beja, Portugal

³MARE—Marine and Environmental Sciences Centre, ARNET—Aquatic Research Network, Department of Landscape, Environment and Planning, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal;

⁴GeoBioTec, NOVA School of Sciences and Technology, Campus da Caparica, 2829-516 Caparica, Portugal;

⁵Águas Públicas do Alentejo, Grupo Águas de Portugal, Rua Dr. Aresta Branco nº51, 7800-310 Beja, Portugal

Email: adriana.catarino@ipbeja.pt

This research evaluated the application of a comprehensive set of tools to determine how agricultural practices influence the water quality classification of the Lage hydro-agricultural reservoir in Southern Portugal. The methodology combined the analysis of 51 pesticides with ecotoxicological tests on organisms representing different trophic levels: the bacterium *Aliivibrio fischeri*, the microalgae *Pseudokirchneriella subcapitata*, and the crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Samples were collected from two locations within the reservoir—Lage (L) and Lage S (LS)—between 2018 and 2020. Throughout the study, 36 of the 51 target pesticides were identified in the reservoir. The total pesticide concentration increased successively each year, from 0.95 µg L⁻¹ to 1.99 and finally 2.66 µg L⁻¹. Terbutylazine and metolachlor were the most frequently detected compounds, present in 100% and 83% of the samples, respectively, and reaching maximum concentrations of 115.6 and 85.5 µg L⁻¹. Ecotoxicological results indicated greater toxicity at the LS site, where *A. fischeri* exhibited 30-min EC₅₀ values of 39–51%. Consistent inhibition of microalgae growth was observed, which correlated with periods of agricultural activity, particularly herbicide and insecticide application. In contrast, tests on *D. magna* feeding rates showed no inhibitory effects in the Lage samples. A key finding was that, although the measured concentrations of each pesticide remained below official regulatory limits, they collectively induced significant toxic effects on the organisms tested. Based on this evidence, the potential ecological status of the reservoir was classified as "moderate." The integrated toolbox provided a more detailed assessment, refining the water status classification beyond what standard methods would allow. The study concludes that this multifaceted approach, combining chemical and ecotoxicological data, creates a more robust framework for water quality assessment. It serves as a powerful complementary tool to the Water Framework Directive (WFD) methodology. This proposed system not only identifies existing pollution impacts but also enables: (1) early detection of toxic effects of emerging contaminants to prevent ecological damage; (2) proactive management through targeted actions to restore water status; and (3) the promotion of more sustainable water use.

This work is a contribution to the projects CREATE (UIDB/06107/2023) and GeoBioTec (UIDB/04035/2023), both funded by FCT—Fundação para a Ciência e a Tecnologia, Portugal—and to the FitoFarmGest Operational Group (PDR2020-101-030926). This work is part of Adriana Catarino's PhD with the grant 2023.04004.BD of Fundação para a Ciência e a Tecnologia, Portugal.

Remote sensing and hydrological modeling to optimize soil water storage and minimize diffused pollution – Decision Support Tool for water governance

A. Alexandre¹, D. Costa²

¹MED Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Portugal.

²MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change, Universidade de Évora, Portugal

Email: d70317@alunos.uevora.pt

Soil water storage is a key component of agroecosystem resilience, especially under the increasing intensity of droughts and floods in Mediterranean climates. In this context, a solution-oriented model was initially developed to help optimize soil water storage in the Mondego River Basin, combining remote sensing data, biogeophysical variables, and spatial analysis. Just by focusing on the Critical Zones for water infiltration and storage (headwater systems, riparian margins, and recurrent floods, ca. 1/3 of the total area), the framework aimed to inform best management practices. Several nature-based solutions were discussed, from buffer restoration to improved species selection, also referencing pioneering studies monitoring Natural Infrastructure in Dryland Streams (NIDS). The latter links hydrological function with land-use planning and ecosystem service enhancement, with results showing that rock detention structures, like small beaver dams, can increase both soil water and carbon storage in arid climates, with significant improvements in water quality.

Now, under the ENGAGE project, focus is given to the Guadiana River Basin, alongside two additional transnational watersheds in Europe - Gauja-Koiva in Latvia and Estonia and Hobøelva in Norway, each facing distinct climatic and governance challenges. The objective is to deploy nested large-scale models of hydrology, hydrodynamics, and water quality to support the co-development of a decision-support tool. In order to understand and improve water governance at a landscape level, regional stakeholders from different study areas will collaborate to identify key common solutions for managing diffuse nutrient pollution. Beyond helping to advance large-scale biophysical modelling capacity, the project seeks to integrate socio-economic, governance, and policy elements for a more informed decision process. The partners of the project include University of Évora, Polytechnic Institute of Beja, Baltic Studies Center, Mykolas Romeris University, Norwegian Institute of Bioeconomy Research and Swedish University of Agricultural Sciences, together we aim to deliver a replicable framework to guide evidence-based water governance across Europe.

This work was funded by FCT – Fundação para a Ciência e a Tecnologia through the projects Water4All/0003/2023 (MED). A. Alexandre acknowledges FCT for doctoral support within the ENGAGE project (PhD in Earth and Space Sciences, University of Évora).

Understanding the sustainability of Angolan consumers: a study of cassava and cassava product consumption.

A. Gonalo¹, P.D. Henriques², M.R. Lucas³

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, IIFA (Instituto de Investigaão e Formaão Avanada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Economia, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora, Portugal.

³MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gesto, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora, Portugal.

Email: rosanvunda11@hotmail.com

In the early stages of humankind's existence on Earth, humanity depended solely on gathering fruits and hunting. As humanity evolved, it acquired knowledge, allowing it to better intervene in and exploit natural resources to satisfy needs, profits, and the well-being of nations. This path contributed to the degradation of ecosystems and the environment, causing disasters and destruction. To prevent these problems, the United Nations held the Stockholm Conference in 1972, addressing environmental education as the basis for environmental protection and improvement. In 1975, UNESCO and UNEP held the Belgrade Conference, systematizing the concept of Environmental Education. The Tbilisi Conference of 1977, Georgia (former USSR), defined environmental policies and the model for implementing environmental education worldwide. The Brundtland Report (1987) added the concept of Sustainability to "Development." Angola faces enormous environmental challenges, compounded by the ineffective implementation of the Basic Law on the Environment, Law No. 5/89 of June 19th. Article 22 establishes environmental education as a right for all Angolan citizens. We assume that basic instruments to raise citizens' knowledge levels on sustainability and environmental issues do not exist. The consumption of food products, and in particular cassava, in Angola, at approximately 984.1 kg/per capita/year—a very significant value—calls for consumer education towards ecological awareness to be fundamental at present. The objective is to evaluate the levels of knowledge of Angolan consumers regarding sustainability. The research is descriptive and exploratory, with both qualitative and quantitative elements. Data collection involved administering 416 questionnaires to consumers in the provinces of Malanje and Luanda and conducting interviews with teachers in different educational subsystems. Data analysis and interpretation were performed using the SPSS program. The results demonstrate a lack of knowledge about environmental issues, an absence of environmental education programs in the different education subsystems in Angola, a need to raise the levels of awareness and knowledge of citizens related to environmental problems, and that environmental education should encompass two models: formal and informal.

Keywords: Sustainability; Knowledge; Consumers; Environmental Education; Cassava; Angola.

This work was funded by National Funds through FCT (Portuguese Foundation for Science and Technology) under the Project UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/LA/P/0121/2020>).

Paying for Nature: Building a Biodiversity Economy in EU Farmlands

A.R. Almeida^{1,2}, L. Madureira^{1,3}, J.R. Marques da Silva^{2,4,5,6}

¹ Department of Economics, Sociology and Management, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

² Institute of Research and Advanced Training, University of Évora, Portugal

³ CETRAD - Centre for Transdisciplinary Development Studies, UTAD, Vila Real, Portugal

⁴ MED - Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Portugal

⁵ CHANGE – Global Change and Sustainable Institute, University of Évora, Portugal

⁶ Agroinsider, Lda (agroinsider.com).

Email: ana.raquel.ca@gmail.com

Across more than nine million agricultural holdings in the European Union (EU), farm diversity reflects climatic, geographic, and demographic variations. Yet, the decline of small-scale farms and the growing dominance of crop specialization remain widespread trends. In this context, the erosion of traditional and regenerative systems, combined with rural depopulation, has accelerated ecosystem degradation and reshaped agricultural landscapes. These systems play a dual role: (i) as drivers of global environmental change and (ii) as potential allies in biodiversity conservation. However, engaging farmers into a Nature-based Agriculture (NbA) transition requires the economic valuation of ecosystem services and biodiversity, ensuring fair remuneration for their positive environmental impacts. This study positions the EU within the international landscape of agri-environmental payment schemes, comparing its approach with more mature voluntary credit markets located within similar latitudes and sharing comparable climatic and economic patterns — namely the United States, the United Kingdom, Canada, and China — as well as intra-EU initiatives. Through a critical review, it identifies, analyses, and systematizes existing Payment for Ecosystem Services (PES) schemes, providing a comprehensive overview with particular focus on agriculture as a provider of ecosystem services and biodiversity. The review encompasses both established and pilot schemes, structured through an analytical framework that characterizes each case according to its objectives, governance structure, indicators, assessment tools, payment mechanisms, timelines, stakeholders, geographical scope, and targeted ecosystems, linking these dimensions to the priorities set by Regulation (EU) 2024/1991 on Nature Restoration. The discussion integrates the funding challenges of PES schemes, exploring the interface between the EU's Common Agricultural Policy (CAP) and emerging private-sector financing, to identify innovative mechanisms that can support the transition toward NbA. By embedding the specificities and socio-economic characteristics of traditional agroforestry systems within this framework, the research proposes an inclusive and viable pathway toward a more sustainable agricultural model, positioning traditional systems at the forefront of future PES and biodiversity credit developments.

**Environmental impact of metal nanoparticle synthesis: comparative study
between biosynthesis and alternative methods**

A. Carrapiço

HERCULES Laboratory, University of Évora, Portugal

IN2PAST - Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Portugal

Institute for Research and Advanced Training (IIFA), University of Évora, Portugal

Email: antonio.carrapico@fa.uevora.pt

The synthesis of metal nanoparticles (MNPs) is a growing field due to the unique properties of nanoparticles and their broad application in fields such as medicine, agriculture, and environmental remediation. However, conventional MNP synthesis often involves chemical processes that carry significant environmental risks due to the use of toxic reagents and high energy requirements. An alternative approach, biosynthesis, has emerged as a promising, eco-friendly method, using biological organisms or molecules to produce nanoparticles under milder conditions and without so many hazardous chemicals. This study aims to provide a comprehensive, comparative analysis of the environmental impacts of metal nanoparticle biosynthesis relative to conventional and other emerging synthesis methods. Drawing on a review of current literature, the research assesses multiple dimensions of environmental impact, including resource consumption, energy efficiency, waste production, and ecological toxicity. The study takes a prospective approach, synthesizing and comparing data to highlight the potential of biosynthesis to reduce environmental impact without compromising yield or nanoparticle quality. The insights gained from this comparative analysis will contribute to understanding the feasibility of biosynthesis as a sustainable alternative and may support the development of guidelines for selecting environmentally responsible methods for nanoparticle production. Additionally, it aligns with broader sustainability goals, seeking to inform future research directions and encourages the adoption of greener synthesis techniques in nanotechnology and related fields.

This work was funded by Fundação para a Ciência e Tecnologia (FCT) under the multi-annual financing projects (2020–2023) UIDB/04449/2020 and UIDP/04449/2020, with research grant number UI/BD/153583/2022, under the collaboration protocol agreed between the FCT and HERCULES Laboratory for the financing of student doctorate research.

A Narrative Review to Support the Contextualization of the SAFA Framework for Sustainability Assessment of Alentejo Olive Groves

A. R. Martins¹, P. D. Henriques², A. Marta-Costa³

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Economia, Escola de Ciências Sociais, Universidade de Évora, Portugal.

³CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Departamento de Economia, Sociologia e Gestão, Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-801 Vila Real, Portugal.

E-mail: antonio.rico.martins@uevora.pt

In the context of olive growing in Alentejo, there has been a significant expansion of intensive olive groves with the aim of producing olive oil. The turnover momentum was the inauguration of Alqueva dam in 2002. In 2011, the combined area of high-density and intensive groves, did not reach 5.000 ha while in 2024 campaign, the areas with high-density olive groves amount to 46.489 ha, intensive olive groves, 20.061ha, and traditionally olive groves 659 ha. Although the agriculture intensification offers productivity gains, the sustainability of olive groves is strongly affected by certain practices that undermine their environmental performance. However, the concept of sustainability assumes a wide range of themes, approaches and meanings. These conceptual discrepancies highlight its inherently interdisciplinary nature, particularly within agriculture, thereby reinforcing the needed for robust frameworks to support sustainability assessment. Moreover, there is a notable lack of information regarding the sustainability evaluation of intensive and high-density olive groves across the triple bottom line dimensions (economic, social, and environmental). Studies conducted in Spain addressing environmental sustainability further emphasize the need to reassess the current commitment to intensification, given its significant impacts when compared with conventional or traditional systems. The Sustainability Assessment of Food and Agriculture Systems (SAFA) framework stands out as one of the most widely adopted methodologies for sustainability assessment in scientific research, owing to its comprehensive structure and broad applicability across diverse geographic regions and normative contexts. The study aims to assess the sustainability of olive groves in the Alentejo region using the SAFA framework, focusing on environmental integrity, economic resilience and social well-being dimensions. At this stage, a narrative review was conducted to explore how sustainability indicators can be assessed and contextualized within SAFA dimensions for olive cultivation. The review facilitated the refinement of the data collection strategy, ensuring that twenty-three environmental, two economic, and four social indicators were effectively addressed. This process supports clearer indicator assessment through farmers' practices and enhances the efficiency of subsequent primary data collection.

This work is supported by national funds through the FCT (Portuguese Foundation for Science and Technology) under the projects UIDB/04011/2025 and UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/LA/P/0121/2020>).

Can a mulch-biochar combination mitigate catchment-scale erosion and nutrient export in Mediterranean agriculture?

Hartgers, J.B.¹; Nunes, J.P.¹; Canedo, J.N.G.V.²; Coelho, L.^{2,5}; Kok, S.^{1,4}; Ning, Y.¹; Prats, S.A.^{2,3}

¹Wageningen University and Research. Droevendaalsesteeg 4, 6708 PB Wageningen, The Netherlands.

²MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora. Núcleo da Mitra Gab. 206 Edifício dos Regentes Agrícolas, Apartado 94, 7006-554 Évora, Portugal.

³Misión Biológica de Calicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

⁴Deltares Utrecht. Daltonlaan 600, 3584 BK Utrecht, The Netherlands, P.O. Box 85467 3508 AL Utrecht.

⁵GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, Ed. 2, Gab. 2.1, 8005-139 Faro.

Email: bart-jan.hartgers@wur.nl

In the Mediterranean Alentejo region of southern Portugal, agricultural intensification is rapidly replacing the traditional landscape with intensive olive orchards. While this shift boosts economic productivity, it can accelerate soil erosion and nutrient export to water bodies, decreasing soil fertility and promoting the eutrophication of critical reservoirs for irrigation and drinking water. Although plot-scale studies demonstrate that a combined mulch-biochar application can effectively mitigate runoff, erosion, and nutrient loss, its catchment-scale effectiveness remains unquantified.

This study aims to assess the impact of agricultural intensification and evaluate the potential of a mulch-biochar combination as a conservation measure in the Maranhão reservoir catchment, in Alentejo. Using the Soil and Water Assessment Tool (SWAT), we will simulate runoff, sediment yield, and nutrient (specifically phosphorus) dynamics under three scenarios: (1) a baseline representing current conditions, (2) an intensification scenario reflecting the ongoing expansion of olive groves, and (3) a conservation scenario applying a mulch-biochar combination.

The model will be calibrated and validated using a multi-source data approach. Field-scale data from experimental plots will be used to parameterize both the baseline and the conservation scenario. The intensification scenario will be developed using COS1990 and COS2023 land use maps (Direção-Geral do Território) to project the ongoing expansion of olive groves. Catchment-scale streamflow, sediment and phosphorus calibration will rely on data from hydrometric stations (SNIRH - Sistema Nacional de Informação de Recursos Hídricos) and wastewater treatment plants (EEA - European Environment Agency).

The outputs will include model performance metrics, time-series comparisons of sediment and phosphorus loads, and spatial maps identifying erosion hotspots. Ultimately, this research will quantify the catchment-scale consequences of land-use change and determine the effectiveness of a promising conservation practice, providing a scientific foundation for sustainable agricultural policy and management in the region.

This work was funded by National Funds through FCT under the Project SOLVO (2022.06004.PTDC) and PRR Project - Vine and Wine Portugal (C644866286-00000011).

The legal regulation of the Moroccan agriculture as a measure to mitigate environmental and climate changes

B. BEN MESSAOUD, A. ZINEDINE

Research Center for Law and Judicial Studies, Faculty of Law Economic and social sciences, Moulay Ismail University, Meknes, Morocco

Email: btissam.benmessaoud@gmail.com

Morocco is known by its rich biodiversity in terms of fauna, flora and ecosystems. This richness reflects its geographical characteristics, the diversity of its bioclimatic models as well as the variety of natural ecosystems. All of those parameters make the Moroccan kingdom one of the highest endemism rates in the world and place its agriculture as one of the most important issues for its economic social and territorial plans. Effectively, the agricultural sector has stood out in recent decades and has impacted many other activities and sectors exacerbating the impact of climate change, leading to the scarcity of water resources.

In addition to the above, the agricultural sector accounts for a large proportion of greenhouse gas emissions at the national level with 24.6%. The average annual growth rate of emissions from this sector between 2010 and 2016 was 1.5%.

Once the climate and environmental situation becomes alarming, the Moroccan institutions become involved in order to mitigate the negative impacts and to evaluate the positive ones. One of the most important institutions involved is the legislator. The agricultural sector was regulated for longtime with endemics and local customs and traditions that were amended with news positive rules introduced by the famous "Agricultural investment code" in 1969. Then, the agriculture sector were amended with many policies like the dam policy introduced by the King Hassan the second in order to fight against the scarcity of water, followed by the green Morocco plan and finally the green generation. Every policy was undertaken during a specific period and regulated with specific rules. The most important observations are related to the variation of the agricultural situation due to the involvement of many and various stakeholders, the diversity of the land structure, and mainly the necessity to reform the legal arsenal in order to ensure the legal regulation of the agricultural sector.

Finally, the legal regulation of the Moroccan agriculture may constitute a very important measure that could strengthen the national economy and consequently the social sector.

Decarbonization Through Retrofitting: Auxiliary Engines and Low-Emission Strategies for Container Ports

C. Vázquez¹, L. Rosa¹, L. Marinho¹, and, T. Batista¹

¹ MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Institute for Advanced and Research, University of Évora, 7004-516 Évora, Portugal

Email: carmen.vasquez@uevora.pt

The need to reduce emissions and mitigate severe environmental and public health impacts from maritime activities in port cities is urgent, driven by strict International Maritime Organization (IMO) guidelines to reduce emissions of greenhouse gases (GHG), sulphur oxides (SOx), nitrogen oxides (NOx), and other particulate matter (PM). The use of traditional marine fuels, especially diesel, is a major source of these emissions, with SOx causing acid rain and severe respiratory illnesses, NOx contributing to smog and harmful tropospheric ozone formation, and GHG driving global warming. Furthermore, the emissions include Black Carbon (BC)—a component of PM that is a powerful short-lived climate forcer contributing to the melting of polar ice—and Volatile Organic Compounds (VOC), which are toxic and play a key role in the formation of ground-level ozone. This complex pollution mix results in a significant impact on the air quality of port cities and other effect. This work presents the results of a semi-systematic literature review focused on Auxiliary Engine Retrofitting strategies in the port sector as a key tool for the energy transition. The study specifically focuses on container terminals because they represent the type of infrastructure that international regulations, including the IMO, have targeted for the promotion of conversion to electric systems (onshore power supply) in auxiliary engines during berthing, aiming to eliminate direct emissions and maximize environmental benefits. The research demonstrates that retrofitting offers immediate and scalable solutions for existing fleets, achieving substantial emission reductions through three main avenues: 1) Conversion to dual or alternative fuel (e.g., LNG or methanol) reduces CO₂ y SOx; 2) Installation of cleaning systems (e.g., scrubbers or Selective Catalytic Reduction, SCR) effectively addresses SOx and NOx contaminants, ensuring regulatory compliance; and, finally, 3) Conversion to hybrid or electric systems enables zero-emission at berth and improves energy efficiency, which is critical for reducing local environmental impacts. These findings validate retrofitting as a fundamental pillar in Low-Emission Ports strategies and underscore its indispensable role in helping maritime terminals meet established sustainability goals.

The contents of this article were produced within the scope of the Agenda “NEXUS—Pacto de Inovação— Transição Verde e Digital para Transportes, Logística e Mobilidade”, financed by the Portuguese Recovery and Resilience Plan (PRR), with no. C645112083-00000059 (investment project no. 53).

Family Farming in São Tomé and Príncipe: Between Multifunctionality and Ethnographic Modelling for Rural Sustainability

Gilkson Tiny^{1,3}, Maria Raquel Lucas², Ana Alexandra Marta-Costa³, Pedro Damião Henriques⁴

¹MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute

²MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, and Department of Management, University of Évora, Portugal

³CETRAD-Center for Transdisciplinary Studies for Development, and Department of Economics, Sociology and Management, University of Trás-os-Montes e Alto Douro, Portugal

⁴MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, and Department of Economics, University of Évora, Portugal

Emails: gilkson.tiny@uevora.pt; mrlucas@uevora.pt; amarta@utad.pt; pdamiao@uevora.pt

Family farming plays a central role in the economy of São Tomé and Príncipe, contributing to food security, poverty reduction, socioeconomic inclusion, and the preservation of agroforestry systems. Its multifunctional nature ensures not only the subsistence of rural households but also the maintenance of traditional sociocultural practices and the sustainable management of natural resources. However, a deeper understanding of these systems requires analytical approaches that integrate both productive complexity and the social and cultural dynamics of communities.

This study combines reflections on the characterization of Santomean family farming with the application of Ethnographic Linear Programming a methodological tool that integrates quantitative optimization models with ethnographic research. ELP emerges as a viable alternative to conventional microeconomic models, whose application in the country is hindered by the lack of consistent databases. This methodology makes it possible to represent agricultural activities, resource constraints, seasonality, land use, family labour, and well-being objectives, while incorporating sociocultural practices, gender- and age-based division of tasks, community relations, and social reproduction strategies.

The analysis is supported by secondary and primary data derived from participant observation and the application of a structured questionnaire, allowing for the capture of the country's agroecological and socioeconomic diversity and the recognition of different forms of organization among rural households. Furthermore, the review of international studies shows that ELP is effective in simulating production scenarios, assessing public policies, analysing agricultural technologies, and forecasting the impacts of climate change, revealing trade-offs between income, labour time, food security, community participation, and land-use decisions.

In the Santomean context, adopting this approach has the potential to strengthen agricultural planning, improve food security, support sustainable agroforestry systems, analyse gender inequalities, and inform context-specific government policies. However, its implementation depends on technical capacity-building, systematic data collection, and adaptation to the country's cultural and territorial specificities.

It is concluded that integrating family farming characterization with Ethnographic Linear Programming constitutes a robust pathway for understanding the complexity of rural systems in São Tomé and Príncipe, contributing to more informed decision-making, effective policies, and sustainable rural development strategies.

Keywords: Family farming; Ethnographic Linear Programming; Production systems; Sustainability; São Tomé and Príncipe; Rural development.

Legal Regulation of Sustainable Investment in Developing Economies: Challenges and Opportunities

I. Amrhar¹, M.Mounir²

¹Laboratoire d'Etudes et de Recherches Juridiques et Politiques, Université Mohamed V, Rabat MAROC.

² Laboratoire d'Etudes et de Recherches Juridiques et Politiques, Université Mohamed V, Rabat MAROC.

Email: imane.amrhar@um5r.ac.ma

Legal regulation of sustainable investment in developing economies presents both complex challenges and critical opportunities to promote long-term economic, social, and environmental benefits. Regulatory fragmentation, inconsistent ESG (Environmental, Social, and Governance) standards, limited enforcement capacity, and transparency deficits frequently hinder sustainable investment flows and diminish investor confidence. These challenges are further compounded by limited institutional resources and competing development priorities as countries strive to balance environmental protection with economic growth (World Bank, 2024). This study examines these regulatory challenges while identifying pathways toward more effective legal governance that aligns investment with sustainable development goals (SDGs). Harmonizing ESG criteria, mandating transparent disclosures, and strengthening institutional capabilities offer significant opportunities for reform. Embedding principles of international human rights, labor standards, and environmental protections within national investment laws and treaties can ensure investments contribute positively to local communities and ecosystems (UNCTAD, 2025).

International investment agreements are increasingly evolving to incorporate sustainable development objectives and reinforce state regulatory space, thus balancing investor protection with public welfare considerations. Requirements for social and environmental impact assessments and integrating sustainability clauses create a more predictable and accountable investment environment. These mechanisms facilitate green finance, economic transitions, and innovative financing tailored to sustainable outcomes (Columbia Center on Sustainable Investment, 2025).

By fostering robust and coherent legal frameworks, developing countries can mobilize private capital toward sustainable projects, mitigate risks of exploitation, and promote resilient economic growth aligned with social equity and environmental stewardship. This study contributes actionable insights intended to guide policymakers, investors, and scholars in strengthening sustainable investment governance as a crucial mechanism for achieving sustainable development and climate goals in emerging economies.

Potential Freshwater Impacts of Polymer-Surfactant Interactions

Ana Santos^{1,2}, Luís Alves¹, Isabel Lopes², Cátia Venâncio²

¹University of Coimbra, CERES, Department of Chemical Engineering, Pólo II – R. Silvio Lima, 3030-790 Coimbra, Portugal

²Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal

E-mail: anar.santos@ua.pt

Environmental safety requirements are increasingly driving the development of advanced materials, particularly for widely used personal care products (PCP). Their continuous release into wastewater systems leads to accumulation in the environment and these compounds can become pseudo-persistent pollutants. While regulatory assessments focus on individual compounds, commercial formulations are complex mixtures whose component interactions on aquatic toxicity remain poorly understood. This work is based on industry-academia partnership aiming at investigating the ecotoxicological behavior of three quaternized hydroxyethyl cellulose polymers commonly used in PCP, SoftCAT™ SK-M, SK-MH, and SL-5, both alone and in combination with three surfactants: Sodium Lauryl Ether Sulfate (SLE2S), Alkyl Amido Propyl Betaine (APB), and Tallow Ester Quat (EQ). Testing concentrations for polymers were based on pre-established HC₅ values (Hazardous Concentration for 5% of species) determined in prior work: 0.00354 mg/L (SK-M), 0.00127 mg/L (SK-MH), and 95.5 mg/L (SL-5). Surfactants were set at 33-fold higher concentrations, maintaining a 1:33 polymer-to-surfactant ratio typical of commercial formulations. The experimental design comprised eight treatments per polymer: control, individual compounds (polymer at HC₅; surfactants at 33 × HC₅), and binary mixtures. Ecotoxicological testing was carried out using a battery of assays with freshwater organisms covering different trophic levels: the macrophyte *Lemna minor* (producer), the rotifer *Brachionus calyciflorus* and the cladoceran *Daphnia magna* (both primary consumers), and the fish *Danio rerio* (secondary consumer). Both lethal and sublethal endpoints were assessed. Toxicity patterns were polymer-specific and species-specific. Exposure to SK-M and surfactants (alone and combined) altered cardiac function in *D. rerio*, with combined exposures producing differing responses compared to individual compounds. SK-MH altered cardiac function only in combination with APB, where the mixture response differed from both the polymer and surfactant alone. No significant effects were detected for *L. minor*, *D. magna* or *B. calyciflorus* for SK-M and SK-MH. Conversely, SL-5 resulted in high mortality in *D. rerio* and significant growth inhibition in *L. minor*, with SLE2S and APB (alone and combined with SL-5) producing the most severe effects. These results highlight that component interactions can substantially alter toxicity profiles, suggesting that single-compound evaluations may underestimate real-world environmental risks posed by PCPs ingredients.

Thanks are due for the financial support to This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia I.P., under the project CESAM-Centro de Estudos do Ambiente e do Mar, references UID/50017/2025 (doi.org/10.54499/UID/50017/2025) and LA/P/0094/2020 (doi.org/10.54499/LA/P/0094/2020), and for the PhD scholarship attributed to Ana Santos (2024.05527.BD). FCT is also acknowledged by the financial support under the project CERES, reference UID/00102/2025 (<https://doi.org/10.54499/UID/00102/2025>) and UID/PRR/00102/2025 (<https://doi.org/10.54499/UID/PRR/00102/2025>).

A critical application of the Agroecology Criteria Tool to evaluate agri-food initiatives in Portugal

L. Duarte¹, J. Muñoz-Rojas², L. Horstink³

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Institute for Advanced Studies and Research, University of Évora, Portugal.

²MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Department of Landscape, Environment and Planning, University of Évora, Portugal

³CERNAS-IPV Research Centre, Polytechnic University of Viseu, Viseu, Portugal

Email: luis.duarte@uevora.pt

Agroecology has quickly become a promising solution for addressing the challenges faced by today's industrialized and globalized agri-food systems. However, because agroecology encompasses a systemic and holistic approach that includes environmental, economic, and societal factors, accurately measuring the adequacy and success of agroecology initiatives presents several difficulties. As a result, various frameworks have recently been created to evaluate agri-food initiatives for compliance with the agroecological principles and their performance. At the same time, there is still a limited understanding of agroecological efforts on larger scales, especially at the national levels. This paper aims to describe agroecological initiatives in Portugal by assessing 34 initiatives using a promising evaluation tool—the Agroecology Criteria Tool (ACT). To the best of our knowledge, this constitutes the first attempt to assess agroecology with the ACT on a national scale. Our findings show that, although multiple systemic initiatives already exist, most of the projects analyzed demonstrate only limited integration of agroecological principles and knowledge. We observed that these initiatives mainly align with a few agroecological values, acting more as social enablers or supporters of healthier agroecosystems, but not promoting a systemic agroecological transition. Additionally, we suggest improvements for the ACT tool, such as merging certain criteria and reorganizing them within different elements, to better serve future applications. Using an assessment tool is a procedure we fully recommend to avoiding co-optation (conscious or unconscious) of agroecology concept by corporate power and to ensure the stakeholders involved internalize the fully transformative and systemic benefits of agroecology. We concluded that using an assessment tool is a very effective method for evaluating the genuine agroecological transformative character of the analyzed initiatives. We emphasize the importance of effective agroecology assessment tools to guide farmers, other food sector actors, policymakers, and investors while also allowing them to monitor evolution over time. This leads us to recommend integrating these assessment tools in policymaking, particularly in the design, conduct, and evaluation of policies and programs. Strong performance assessment tools are essential for facilitating the agroecological transition we aim to achieve.

This work was funded by National Funds through FCT under the scholarship grant 2023.0279.BD. Authors also acknowledge the support from the R&D unit MED, under the project UIDP/05183.

Perception Dynamics in Sustainable Land Management: A Multi-Actor Project Study among Wine and Olive Oil Farmers in Portugal

Maria Rivera¹, Catarina Esgalhado¹, Paola Hernandez¹, Rui Novado¹, Luísa Coelho¹ and Sergio Prats^{2,1}

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal.

²Misión Biológica de Galicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain

Email: mrmendez@uevora.pt

The advanced state of global land degradation—currently affecting more than 3.2 billion people—raises critical concerns for the sustainability of social-ecological systems and calls for the large-scale adoption of contextualized Sustainable Land Management (SLM) practices. Despite decades of policy effort and scientific advancement, farmers' adoption of SLM remains a persistent challenge. This is largely due to the complexity and diversity of influencing factors, including differences in assets, ambitions, values, and local contexts, as well as agronomic, financial, market, and policy barriers and opportunities. Moreover, farmland characteristics, knowledge availability, and social networks significantly shape how SLM practices are perceived, adapted, and implemented on the ground.

Social learning has been increasingly recognized as a key process in facilitating SLM adoption and driving environmental management transitions. Farmers' mental models and perceptions play a decisive role in their management choices and responsiveness to innovation. In this regard, multi-actor, interactive projects that foster iterative knowledge exchange and joint experimentation represent promising approaches for stimulating perception change and innovation uptake. Knowledge exchange in these collaborative environments enables participants to re-evaluate their assumptions, integrate new insights, and collectively reframe challenges such as soil erosion, fertility loss, and ecosystem degradation.

This study examines how the perceptions of Wine and Olive oil farmers in Portugal evolve through their engagement in a multi-actor project of experimental nature—SOLVO—focused on the development and testing of sustainable farming practices that protect soils from erosion and restore fertility. SOLVO integrates innovative nature-based solutions combining mulch and biochar derived from olive mill residues, designed to improve soil organic matter, control erosion, enhance water retention, and promote soil-mediated ecosystem services. The project thus creates a rich setting for co-learning, where science, practice, and policy meet around shared sustainability goals.

The central research question guiding this work is: Do stakeholders' perceptions of land degradation and soil restoration practices change after being engaged from the beginning in a multi-actor interactive project? To address this, we employ Fuzzy Cognitive Mapping (FCM)—a participatory, semiquantitative systems-mapping tool—to capture and compare participants' cognitive representations of land degradation drivers, soil management practices, and their perceived impacts on ecosystem services. Individual interviews (n=10) are conducted to elicit

Environment, Sustainability and Socioeconomic Development

stakeholders' views on key dimensions of soil management, guided by questions addressing (1) factors influencing land degradation, (2) factors influencing crop production, and (3) perceived impacts of soil restoration practices (e.g., mulching, biochar, compost, manure, chemical fertilization, fertirrigation) on soil-mediated ecosystem services—namely erosion control, water storage, carbon storage, soil fertility, and soil habitat.

Through longitudinal comparison of individual and collective FCMs before and after participation in SOLVO activities, we aim to reveal the extent and direction of perception change among farmers and other stakeholders. This approach enables the identification of evolving mental models and potential leverage points for facilitating broader transitions toward sustainable soil management in Mediterranean agroecosystems.

By integrating participatory social learning and system mapping within a real-world innovation setting, this study contributes to the growing body of evidence on how multi-actor approaches can accelerate the uptake of sustainable soil management practices. The findings are expected to enhance understanding of how perception change—driven by co-creation, dialogue, and experimentation—supports the protection and sustainability of soils, aligning directly with the goals of the Healthy Soils, Healthy Planet agenda.

This work was funded by National Funds through FCT under the Project SOLVO (2022.06004.PTDC) and SOLVIT PRR Project - Vine and Wine Portugal (C644866286-00000011) and by a research contract (RYC2022-035489-I) the Spanish Ministry of Science, Innovation and Universities through the research contract RYC2022-035489-I.

Food Sciences

**Irrigation effects on the volatile profile of traditional grapevine varieties:
Analysis by HS-SPME-GC×GC-ToFMS**

Daniela Fonseca¹, Nuno Martins², Raquel Garcia^{2,3} and Maria João Cabrita^{2,3}

¹ MED - Mediterranean Institute for Agriculture, Environment and Development & IIFA - Institute of Research and Advanced Training, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³ Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: daniela.fonseca@uevora.pt

Adapting to climate change without losing wine quality and typicity is the main challenge to many wine producers. Irrigation management can be a short-term adaptation measure, but with limited water resources, it is also important to assess plant resilience to water scarcity. Using varieties adapted to warmer and drier conditions can mitigate the risks of crop loss and potentiate more stable yields, but might also represent a threat to wine typicity, as autochthonous varieties are integral to the identity of regional wines. Understanding the impacts of climate change in the grape quality from autochthonous varieties is key to find a balance between the need for climate resilience, safeguarding water resources, and the desire to preserve wine quality and authenticity.^{1,2}

This work aims to investigate the volatile composition of minority grapes varieties Diagalves, Manteúdo, Perrum and Rabo de Ovelha under different irrigation regimes: water comfort, moderate water deficit and rainfed, by HS-SPME-GC×GC-ToFMS.³

A total of seventy-six volatile compounds, of which fifty-one were varietal volatiles, were identified and semi-quantified in the varieties under study. The results reveal that irrigation influences the volatile profile and, consequently, the odor type of grape varieties, with varying responses across the cultivars. While Diagalves exhibited no significant differences between irrigation regimes, Manteúdo and Perrum demonstrated higher levels of varietal volatile compounds, particularly terpenes and norisoprenoids, under rainfed conditions. Rabo de Ovelha exhibits high levels of terpenes under rainfed conditions, while no significant differences are observed between irrigation regimes for norisoprenoids. Conversely, higher concentrations of other compounds are found under water comfort conditions. Manteúdo and Perrum emerged as the most promising varieties in terms of adaptation to water scarcity, showing statistical significance in its volatile composition. Principal Component Analysis shows the differences between the irrigation regimes for each variety.

These results underscore the importance of selecting resilient autochthonous grape varieties as a strategy to mitigate the effects of climate change while preserving the aromatic typicity of wines.

This research was funded by “Vine&Wine-Driving Sustainable Growth Through Smart Innovation” project (sub-project-BioGrapeSustain), “Mobilizing Agendas for Business Innovation” under the Recovery and Resilience Program. Authors also acknowledge MED <https://doi.org/10.54499/UIDB/05183/2020> and CHANGE <https://doi.org/10.54499/LA/P/0121/2020>. We gratefully acknowledge Herdade do Esporão for kindly providing the grape samples used in this study.

References

1. Fraga, H., García de Cortázar Atauri, I., Malheiro, A. C., Moutinho-Pereira, J., Santos, J. A. *OENO One* 51 (2017) 61–69.
2. Agostinelli, F., Caldeira, I., Ricardo-da-Silva, J.M., Damásio, M., Egipto, R., Silvestre, J. *Plants* 12 (2023) 2063-2085.
3. Fonseca, D., Martins, N., Garcia, R., Cabrita, M. J. *Molecules*, 29 (2024) 1989-2005.

Characterization of functional protein ingredients from farmed catfish coproducts for food formulations

Busenur Özkan^{1†}, Alícia Pereira^{2†}, Marta Dias², M. Leonor Nunes², António Marques^{2,3}, M. João Fraqueza¹, Carla Pires^{2,3*}

¹ CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, AL4AnimalS - Associate Laboratory for Animal and Science, University of Lisbon

² CIIMAR/CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto

³ IPMA, IP - Portuguese Institute for the Sea and Atmosphere, Division of Aquaculture, Upgrading and Bioprospection

† Equally contributed authors

*Corresponding author

Coproducts from freshwater fish farming processing industry can be valued by transforming them into functional ingredients, thereby contributing to reducing waste and improving the efficiency of its use. Heads, bones and skins of catfish (*Clarias gariepinus*) remain underutilized, despite their high protein content and potential for preparing products with added-value applications. In this study, fish protein hydrolysates (FPHs) were produced from catfish coproducts using Alcalase (A) and Alcalase+Protana (AP), and their composition, functionality, bioactivity and microbiological quality were evaluated for potential use in food formulations. Hydrolysis of catfish coproducts was carried out using A (60 °C, pH 8.5) and AP (55 °C, pH 7) with 1% (w/v) enzyme for 3 h, followed by thermal inactivation (90 °C, 10 min). FPHs were centrifuged, supernatants filtered, freeze-dried, and the dried product stored at -80 °C. All hydrolysates presented low fat (<3%) and high protein (74-82%) contents, with AP generally providing higher protein values. AP resulted in a significantly higher degree of hydrolysis than A (49% vs 29%; $p < 0.001$). Colour appeared to differ depending on the enzyme, producing lighter and less red hydrolysates (L^* and a^* , both $p < 0.001$) with AP with a slightly lower yellow tone (b^* , $p < 0.001$). Enzyme treatment affected foaming capacity ($p = 0.003$) and emulsifying stability (emulsifying activity index (EAI) $p = 0.003$; emulsion stability index (ESI) $p < 0.001$), with A showing superior performance, while foaming stability and water-holding capacity did not differ significantly ($p > 0.05$). Antioxidant activity was also influenced by the enzyme: both 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were significantly different ($p < 0.001$), consistent with stronger activity in A FPHs (EC₅₀, half maximal effective concentration: DPPH 11 mg/mL; ABTS 3.76 mg/mL). Angiotensin-converting enzyme (ACE) inhibition was stronger in A FPH, which had a lower EC₅₀ value (1.15 mg/mL) compared to AP (3.14 mg/mL). *Listeria monocytogenes* and sulfite-reducing *Clostridium* were <1 log CFU/g. No significant differences were found between A and AP for hygiene indicators. Antimicrobial testing showed no inhibition at 200 mg/mL tested. These results indicate that catfish coproduct hydrolysates combine safety, high protein and useful functionality, making them practical ingredients for new formulations and better use of coproducts.

This work was developed under the frame of the Project Grant Agreement No. 101136739 INNOECOFOOD, financed by Horizon Europe and UK Research and Innovation, and it comprises an experienced multi-actor consortium of 20 partners in Europe and Africa and rural communities. FCT - Fundação para a Ciência e a Tecnologia, I.P., support this research under projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS). This work was also funded by national funds through FCT - and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (<https://doi.org/10.54499/UID/04423/2025>), and LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>).

Extraction of phenolic antioxidants from kiwiberry (*Actinidia arguta*) using ultrasound technology: optimization and bioactivity evaluation

Catarina Macedo ^{1,2,3,*}, Ana Margarida Silva ¹, Ana Sofia Ferreira ¹, María De La Luz Cádiz-Gurrea ⁴, Álvaro Fernández-Ochoa ⁴, Antonio Segura-Carretero ⁴, Cristina Delerue-Matos ², Paulo Costa ^{2,3}, and Francisca Rodrigues ¹

¹REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4249-015 Porto, Portugal

²REQUIMTE/UCIBIO, MedTech-Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira nº. 228, 4050-313 Porto, Portugal

³Associate Laboratory i4HB—Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

⁴Department of Analytical Chemistry and Food Science and Technology, University of Castilla-La Mancha, Ronda de Calatrava 7, 13071 Ciudad Real, Spain

Email: up202112258@edu.med.up.pt

Kiwiberry (*Actinidia arguta*) is a nutrient-dense fruit notable for its content of phenolic compounds with antioxidant and health-promoting properties. Within sustainable agri-food systems, valorizing fruits unsuitable for commercial sale due to non-conforming size or minor aesthetic defects is a promising route to reduce post-harvest waste and increase resource efficiency. Recovering high-value bioactive compounds from such underutilized fruit using green technologies supports circular economy goals and the development of eco-innovative ingredients for food, nutraceutical and cosmetic applications. This study optimized polyphenol extraction from discarded kiwiberry using ultrasound-assisted extraction (UAE) combined with Response Surface Methodology (RSM). Solvent composition (water:ethanol), sonication time and probe amplitude were evaluated. Optimal conditions were 50% aqueous ethanol, 17.5 min, and 50% amplitude. The optimized extract exhibited high total phenolic content (18.705 mg GAE/g dw) and marked antioxidant/antiradical activity (FRAP = 186.876 μ mol FSE/g dw; ABTS = 16.334 mg AAE/g dw). Additionally, it also showed efficacy in the superoxide anion (O₂⁻), hypochlorous acid (HOCl), and peroxy radical (ROO⁻) assays, highlighting its antiradical and antioxidant activities. HPLC-ESI-QTOF-MS profiling annotated 22 compounds, including six flavonoids, five phenolic acids and seven organic acids. Importantly, *in vitro* cytotoxicity assays were performed using two human oral carcinoma-derived buccal cell lines, TR146 and HSC-3, to assess the effect of the extract on cell viability. While exposure of TR146 cells to increasing concentrations of the optimal extract did not significantly reduce cell viability, the extract markedly decreased HSC-3 viability at concentrations above 500 μ g/mL, suggesting a concentration-dependent effect. These findings indicate potential bioactivity against carcinoma cells at relatively high concentrations. Overall, results reinforce kiwiberry as a valuable source of natural antioxidants and demonstrate how green extraction strategies can transform low-value fruit fractions into functional ingredients, contributing to sustainable valorization and circular bioeconomy development.

This work received financial support from project EXPL/BAA-GR/0663/2021 – Kiwi4Health – Exploring the Eco-Innovative Re-Use of Kiwiberry, supported by national funds by FCT/MCTES and by the projects UIDB/50006/2020 and UIDP/50006/2020 through national funds. This work was also financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

Volatile Organic Compound Profiles of Young Monovarietal Portuguese Red Wines by HS-SPME/GC×GC-ToFMS

S. Gastão -Muchechea¹, N. Martins², R. Garcia^{2,3}, M.J. Cabrita^{2,3}

¹ Mediterranean Institute for Agriculture, Environment and Development & Institute of Research and Advanced Training, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² Mediterranean Institute for Agriculture, Environment and Development & Global Change and Sustainability Institute, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³ Department of Crop Science, School of Science and Technology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: sousa.gastao@uevora.pt ; mjbc@uevora.pt

Wine aroma results from a complex mixture of volatile organic compounds (VOCs), whose composition is influenced by grape variety and harvest year. This study systematically examined the volatile compounds found in young Portuguese red wines produced at the University of Évora winery. The research focused on three monovarietal cultivars, Alicante Bouschet, Touriga Nacional, and Trincadeira, across five consecutive vintages from 2020 to 2024.

Headspace solid-phase microextraction (HS-SPME) with a CAR/DVB/PDMS fiber was combined with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-ToFMS), providing high chromatographic resolution and sensitivity. Furthermore, the solvent-free extraction combined with comprehensive chromatography illustrates sustainable and innovative strategies.

The identification of a wide range of volatile and semi-volatile compounds, including esters, higher alcohols, volatile fatty acids, norisoprenoids, and terpenes, provided a comprehensive chemical basis for characterizing the wine's aromatic profiles. Statistical analyses revealed significant effects of both variety and harvest year on VOC distribution. Alicante Bouschet wines showed higher relative contributions of fermentative esters, whereas Touriga Nacional and Trincadeira exhibited distinctive terpenic and norisoprenoid markers. These results confirm the suitability of HS-SPME/GC×GC-ToFMS for varietal discrimination studies, providing a robust chemical fingerprint that supports the valorization of Portuguese cultivars.

The authors acknowledge the R&D unit MED – Mediterranean Institute for Agriculture, Environment and Development (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>), as well as the Associated Laboratory CHANGE – Global Institute for Change and Sustainability (<https://doi.org/10.54499/LA/P/0121/2020>).

This work was funded by National Funds through FCT within the scope of the Consortium of Schools of Agricultural Science (CECA), under the FCT Research Grants Regulation (RBI)- PRT/BD/154325/2022 (S. Gastão -Muchechea)

Is it safe to consume fish from the Tagus estuary? POPs and heavy metals in the edible portion of meagre: a case study.

André F. Jorge^{1,2}, Bernardo Quintella^{3,4}, Marco G. Silva², M. João Lança⁵

¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²LAQV, REQUIMTE, Departamento de Química, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

³MARE - Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Institute for Research and Advanced Training (IIFA), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

⁴Departamento de Biologia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal

⁵MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: d52876@uevora.pt

Fish is a nutritional source that forms part of a balanced diet and is a key element in the Mediterranean diet. However, high anthropogenic pressure, climate change, and other factors increasingly require the monitoring of pollutants in the aquatic environment to ensure consumer safety (One Wealth). The Tagus estuary has banks that cover industrial, agricultural, and densely urbanized areas [1], which increases the possibility of anthropogenic pollution in its ecosystem. However, the estuary also serves as a nursery habitat for several fish species [2,3] of great economic importance, such as meagre (*Argyrosomus regius*). Using minimal sample preparation (microwave-assisted digestion and liquid extraction under pressure) and robust analytical techniques (ICP-OES and GC-QqQ-MS/MS), it was possible to detect mercury (Hg) and arsenic (As) in several meagre samples and also a new technique for analyzing organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) is currently under development, with the aim of quantifying a broad spectrum of these compounds using meagre as an example matrix for fish. Preliminary results show the need to monitor pollutants in meagre from the Tagus estuary and, possibly, in other species, to ensure consumer safety.

References

- [1] Tavares, A.O.; dos Santos, P.P.; Freire, P.; Fortunato, A.B.; Rilo, A.; Sá, L. Flooding Hazard in the Tagus Estuarine Area: The Challenge of Scale in Vulnerability Assessments. *Environ Sci Policy* 2015, 51, 238–255, doi:10.1016/J.ENVSCI.2015.04.010.
- [2] França, S.; Costa, M.J.; Cabral, H.N. Assessing Habitat Specific Fish Assemblages in Estuaries along the Portuguese Coast. *Estuar Coast Shelf Sci* 2009, 83, 1–12, doi:10.1016/J.ECSS.2009.03.013.
- [3] Vasconcelos, R.P.; Reis-Santos, P.; Maia, A.; Fonseca, V.; França, S.; Wouters, N.; Costa, M.J.; Cabral, H.N. Nursery Use Patterns of Commercially Important Marine Fish Species in Estuarine Systems along the Portuguese Coast. *Estuar Coast Shelf Sci* 2010, 86, 613–624, doi:10.1016/J.ECSS.2009.11.029.

This work was funded by National Funds through FCT under the Project 153381/2022 and <https://doi.org/10.54499/UI/BD/153381/2022> (PhD Grant), also through project LA/P/0008/2020 and <http://doi.org/10.54499/LA/P/0008/2020>, UIDB/50006/2023 (LAQV-REQUIMTE), also through project LA/P/0121/2020 and <https://doi.org/10.54499/LA/P/0121/2020>, UIDP/05183/2020 and <https://doi.org/10.54499/UIDP/05183/2020>, UIDB/05183/2020 and <https://doi.org/10.54499/UIDB/05183/2020> (MED-CHANGE), and project PTDC/BIA-BMA/030517/2017, and through the multi-annual funding program contract of the UID MARE (Marine and Environmental Sciences Centre) via project UIDB/04292/2020 and <https://doi.org/10.54499/UIDB/04292/2020> and under the project LA/P/0069/2020 and <https://doi.org/10.54499/LA/P/0069/2020> granted to the Associate Laboratory ARNET.

Screening of the volatile profile of several flours produced locally in São Tomé and Príncipe

Anísio P. Quintas¹, Nuno Martins², Maria João Cabrita^{2,3}, Maria Paula Duarte⁴,
Raquel Garcia^{2,3}

¹MED - Mediterranean Institute for Agriculture, Environment and Development & IIFA - Institute of Research and Advanced Training, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³ Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴The Mechanical Engineering and Resource Sustainability Center (MEtRICs), Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.

Email: anisio.quintas@uevora.pt

According to the data from the Food and Agriculture Organization of the United Nations (FAO), global wheat production is insufficient to meet consumption needs.¹ The use of alternative flours could be a promising approach to mitigate this scarcity. Thus, anticipating a reduction in wheat flour imports in São Tomé and Príncipe and attempting to take advantage of local agricultural production, small industries and food cooperatives have been producing flours from fruits and tubers, namely taro (locally known as matabala), cassava, sweet potato, breadfruit, plantain, and cooking banana. However, knowledge of the physicochemical and nutritional properties of these flours is very limited, including the volatile composition that could influence the consumer's acceptability of these products. Therefore, this study focuses on the screening of the volatile profile of the produced flours using SPME-GC×GC-ToFMS and moreover the correlation with sensory analyses of various bakery and pastry products made from these flours. In fact, the production of flours using local agricultural resources will allow the development of new products with potential nutritive increment, and that will contribute to the economic sustainability of the country, avoiding waste caused by seasonality and rapid perishability.

This work was funded by National Funds through FCT within the scope of the Consortium of Schools of Agricultural Science (CECA), under the FCT Research Grants Regulation (RBI)- PRT/BD/154431/2021 (A.P. Quintas). Authors also acknowledge MED <https://doi.org/10.54499/UIDB/05183/2020> and CHANGE <https://doi.org/10.54499/LA/P/0121/2020>.

References

1. FAO. Cereal Supply and Demand Brief- Cereal markets remain well supplied, with a positive outlook for the short term. October 2025. <https://www.fao.org/worldfoodsituation/csdb/en/>

The relevance of saliva in the perception of tannin-induced astringencyAnita Ramos¹, Laura Carreira¹, Carla Simões^{1,2}, María Pérez-Jiménez¹, Elsa Lamy¹¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, University of Évora²Food Behaviour Lab, CATÓLICA LISBON School of Business & Economics, University Católica Portuguesa

Astringency is the sensation of dryness or roughness in the mouth, often described as a "corky" feeling and which modulates the preference for beverages, as wines, or plant-based foods in general. This sensation results from the interaction between astringent compounds (mostly tannins in plant-based beverages and foods) and salivary proteins, forming stable complexes that precipitate, compromising the lubrication of the oral mucosa. Despite this knowledge, the exact mechanism of astringency development, namely the specific salivary proteins and the particular chemical characteristics of the oral medium, is not fully clear. As well, there is a need for clarification about to what extent the differences in saliva composition, from different consumers, influence their acceptance and choices of astringent products.

This study aimed at identifying the non-mucin salivary proteins that are more associated with the astringency sensed in solutions of tannins. We hypothesized that: 1) the salivary proteome of "baseline" saliva is associated with the intensity of astringency sensed; 2) the level of particular salivary proteins, precipitated by tannins, is associated with the astringency sensed; 3) different chemical types of tannins (condensed vs. hydrolysable) result in an astringency in which different salivary proteins are involved. To test these hypothesis, 6 women went to the lab on 6 different days and evaluated the astringency intensity of condensed and hydrolysable tannin solutions, 3 times each. The protein profiles of saliva samples collected before (baseline), during exposure to the stimulus, and immediately after were compared and correlated with the perceived astringency intensity. Furthermore, the different saliva samples collected on baseline (6 individuals * 3 test days * 2 tannin types) were *ex-vivo* incubated with each tannin solution, at 37 °C, for 5 minutes, to assess the capacity of different proteins to precipitate the different tannins.

Data from the present study can aid in understanding the mechanisms of astringency development, and in the salivary proteins more directly correlated with astringency perception, with potential for use, in the future, in helping predicting and correcting astringency intensity from different products, allowing higher acceptance.

Keywords: astringency, tannins, saliva, proteome.

Effect of the recipe ingredients composition on the properties of acorn-based functional bread

Hugo Duarte^{1,2}, Margarida Oliveira¹, Isabela dos Anjos², Solange Magalhães^{1,3}, Carla Varela¹, Maria da Graça Rasteiro¹, Bruno Medronho^{2,3}, Marta Laranjo⁴ and Luís Alves^{1,*}

¹ University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal.

² MED—Mediterranean Institute for Agriculture, Environment and Development, CHANGE—Global Change and Sustainability Institute, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal.

³FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden.

⁴MED-Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*Correspondence: luislalves@ci.uc.pt

Bread is one of the most widespread and earliest foods in the world, with a strong presence in the daily lives of populations due to its easy access and nutritional value of this food. More recently, the consumers' conscientization of the need to maintain high health standards has led to the development of the so-called “functional foods”. In this sense, the role of essential oils, probiotic microorganisms and acorn flour is seen as essential, because of their good biological activities and beneficial impact on human health.

The use of gluten-free flour has gained increasing attention in the last years. The acorn flower is an excellent alternative to conventional flowers, yet little explored. The integrated use of the endogenous resources from the Alentejo region intends to contribute to the development of an innovative concept of functional bread with significant positive results in human health, economic valorisation of resources, environmental and social impact, due to the maintenance of Montado, from where is obtained the acorn and aromatic and medicinal plants are obtained. The incorporation of acorn flour in bread recipes, in combination with other flours, resulted in bread of good texture and taste. Recipes with only acorn flour led to bread with a highly compact texture. On the other hand, the incorporation of acorn flour, even in low concentrations, resulted in a significant increase in the antioxidant activity of the functional bread.

The authors want to acknowledge Fundação la Caixa and FCT for the financial support through the project “Plantas aromáticas do Alentejo, probióticos e farinha de bolota no desenvolvimento de pão funcional” (PD23-0025). FCT is also acknowledged for its financial support through the Strategic Research Centre Project of CERES (<https://doi.org/10.54499/UID/00102/2025>) and MED (<https://doi.org/10.54499/UIDB/05183/2020>).

Acorn flour and essential oils for a functional bread from the Montado

**Hugo Duarte^{1,2*}, Isabela dos Anjos¹, Margarida Oliveira¹, Solange Magalhães¹, Marta Laranjo³,
Anabela Romano², Bruno Medronho^{2,4}, Luís Alves¹**

¹University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal.

²MED (Mediterranean Institute for Agriculture, Environment and Development) CHANGE (Global Change and Sustainability Institute), Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal.

³MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE – Global Change and Sustainability Institute, IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

⁴FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

*Correspondence: uc49007@uc.pt

As one of the most popular and ancient foods worldwide, bread has a strong presence in many populations due to its accessibility. The growing consumer awareness towards health and nutrition has led to the rise of functional foods, by incorporating bioactive ingredients such as essential oils and probiotic microorganisms. In addition, the increasing interest in gluten-free alternatives called attention to the potential of acorn flour. This promising yet underutilized resource from the Montado ecosystem can be merged with essential oils derived from medicinal and aromatic plants, towards the development of an innovative functional bread [1]. Essential oils (EOs) are known to possess valuable properties which led to their widespread use in food technology. Therefore, it is determinant to comprehensively characterize these plant extracts by examining their extraction methods, the underexplored influence of their geographical origin on physicochemical characteristics, and consequently suitable applications [2,3]. EOs were analysed by GC-MS, as significant compositional variations were identified. Among the tested oils, a Rosemary and a Thyme extract were chosen and used to supplement a standard bread recipe. The antioxidant capacity of the dough was measured before and after baking, showing there was a slight increase in antioxidant capacity after the baking process. Furthermore, essential oils holding antifungal properties are being tested to retard mould from growing in bread, aiming to extend its shelf life. While the addition of acorn flour showed a significant increase in antioxidant capacity, its higher amount changed the bread's structure due to the lower gluten content. To overcome this, several food grade biopolymers and proteins are being tested, accompanied by a rheological characterization of the dough. This project aims not only to improve health benefits but also promoting economic valorization, environmental stability, and the conservation of the Montado ecosystem, providing both acorns, as medicinal and aromatic plants.

[1] Zocchi, D.M.; Bondioli, C.; Hamzeh Hosseini, S.; Miara, M.D.; Musarella, C.M.; Mohammadi, D.; Khan Manduzai, A.; Dilawer Issa, K.; Sulaiman, N.; Khatib, C.; et al. Food Security beyond Cereals: A Cross-Geographical Comparative Study on Acorn Bread Heritage in the Mediterranean and the Middle East. *Foods* 2022, *11*, 3898, doi:10.3390/foods11233898.

[2] Ribet, L.; Kassis, A.; Jacquier, E.; Monnet, C.; Durand-Dubief, M.; Bosco, N. The Nutritional Contribution and Relationship with Health of Bread Consumption: A Narrative Review. *Critical Reviews in Food Science and Nutrition* 0, 1–28, doi:10.1080/10408398.2024.2428593.

[3] Amoah, I.; Cairncross, C.; Osei, E.O.; Yeboah, J.A.; Cobbinah, J.C.; Rush, E. Bioactive Properties of Bread Formulated with Plant-Based Functional Ingredients Before Consumption and Possible Links with Health Outcomes After Consumption- A Review. *Plant Foods Hum Nutr* 2022, *77*, 329–339, doi:10.1007/s11130-022-00993-0.

The authors want to acknowledge Fundação la Caixa and FCT for the financial support through the project “Plantas aromáticas do Alentejo, probióticos e farinha de bolota no desenvolvimento de pão funcional” (PD23-0025). FCT is

also acknowledged for its financial support through the Strategic Research Centre Project UIDB/00102/2020 and UIDB/05183/2020

Poster 57

Environmental persistence of *Listeria monocytogenes* originating from ruminant sources and beef (RTC and RTE) products.

M. P. Teixeira^{1,2}, P. Bernardo^{1,2}, M. H. Fernandes^{1,2}, M. J. Fernandes^{1,2}, L. Orge³, M. J. Fraqueza^{1,2}

¹CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal

²AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences

³INIAV, Instituto Nacional de Investigação Agrária e Veterinária, 2784-505 Oeiras, Portugal;

Email: mjoaofraqueza@fmv.ulisboa.pt

Listeria monocytogenes is a major foodborne pathogen responsible for severe human and animal listeriosis, a disease associated with high mortality. Contamination of ready-to-eat (RTE) meat products often occurs during processing, as raw materials, equipment, and workers can introduce the organism into the production environment. Once present, *L. monocytogenes* may persist due to its psychrotrophic growth capacity and ability to form biofilms that are more resistant to sanitizing agents. This study assessed the biofilm-forming capacity of *L. monocytogenes* isolated from ruminant clinical cases, bovine carcasses, and ready-to-cook (RTC) and RTE beef products, aiming to evaluate their potential to persist in food-processing environments. In total, 94 *L. monocytogenes* strains were characterized. Species confirmation, serogroup identification, clonal complex (CC) determination, and virulence gene profiling were performed using PCR-based methods. Biofilm formation was evaluated using the crystal violet microtiter plate assay at 37 °C for 48 h and at 10 °C and 4 °C for 10 and 20 days. Adherence capacity was classified according to established optical-density thresholds. At 37 °C, most isolates formed biofilms, with the majority classified as moderate producers. However, at lower temperatures and longer incubation periods, a reduction in the number of biofilm-forming isolates was observed, indicating that temperature and time strongly influence biofilm production. Serogroup also played a significant role: serogroup IIa strains exhibited the highest biofilm-forming capacity, outperforming all other serogroups. Serogroup IIc isolates formed significantly more biofilm than IIb and IVb, while IIb strains produced more biofilm than IVb. These results align with previous studies showing that IIa strains are frequently recovered from food-processing environments and appear better adapted to these conditions. Within serogroup IIb, clonal complex CC224 showed significantly weaker biofilm formation compared with other IIb complexes. Despite this, CC224 has been detected in food-production environments, farm settings, and clinical cases, and is associated with virulence factors such as LIPI-3. These findings demonstrate that biofilm formation in *L. monocytogenes* is strongly influenced by temperature, time, and bacterial lineage, with serogroup IIa and certain clonal complexes showing enhanced persistence traits. Identifying persistent or hypervirulent strains is therefore essential for developing targeted control strategies.

This research was supported by national funds through FCT - Fundação para a Ciência e a Tecnologia, under the projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS). The PhD scholarship of the first author was also funded by the UIDB/00276/2020.

The use of *Thymus citriodorus* and *Salvia elegans* as alternatives to nitrite in bacon

P. Bernardo^{1,2}, M. J. Fernandes^{1,2}, M. H. Fernandes^{1,2}, M. P. Teixeira^{1,2}, B. Özkan^{1,2}, L. Patarata^{2,3} and M. J. Fraqueza^{1,2*}

¹CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

²AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences

³CECAV - Animal and Veterinary Research Center, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Email: mjoaofraqueza@fmv.ulisboa.pt

Nitrites ensure safety, stability, and characteristic cured-meat colour. Their link to N-nitrosamine formation has raised concerns. Following the 2015 classification as carcinogenic and the new EU restrictions, identifying alternatives to nitrites in cured meats is a current challenge. This study evaluated the effects of reduced ingoing nitrite and the use of *Thymus citriodorus* or *Salvia elegans* infusions (as a source of nitrate), with or without a *Staphylococcus equorum* starter [to reduce nitrate], on bacon. Seven formulations were produced across three independent days: F1 (without NaNO₂), F2 (150 mg NaNO₂/kg), F3 (80 mg NaNO₂/kg), F4 (thyme infusion), F5 (thyme + starter), F6 (sage infusion), and F7 (sage + starter). Samples were collected throughout processing for analysis. Consumers focus groups were conducted to evaluate sensory attributes. Final products tested negative for *Listeria monocytogenes* and *Salmonella* spp. *Enterobacteriaceae* counts were reduced ($P = 0.02$) by the heat treatment. A formulation*time interaction ($P < 0.01$) was observed for coagulase-negative staphylococci, with the expected higher counts in starter-containing formulations during early stages, although final products did not differ ($< 3.26 \log \text{ cfu/g}$). Formulation had no effect on NaCl content, A_w , pH, or L^* ($P > 0.05$). Colour parameters a^* and b^* showed formulation*time interactions ($P < 0.01$). Nitrite-free bacon exhibited substantial a^* declines during processing, while nitrite-containing bacon maintained a stable redness (around 13), consistent with nitrosohemochrome formation. Infusion-based products were less red, as confirmed by focus group assessments. Residual nitrite differed significantly between treatments ($P < 0.01$): F2 and F3 contained 23.75 and 10.15 mg NaNO₂/kg, respectively, while infusion-containing formulations below 0.41 mg/kg. Residual nitrate was low ($< 4.67 \text{ mg NaNO}_3/\text{kg}$) across all samples. TBARS values were influenced by formulation*time interaction ($P < 0.001$). The control (F1) presented the highest lipid oxidation (1.44 mg MDA/kg), whereas all other treatments showed markedly lower oxidation ($< 0.37 \text{ mg MDA/kg}$), demonstrating the antioxidant roles of nitrite and plant phenolics. Reducing nitrite from 150 to 80 mg/kg did not compromise safety or quality. Bacon produced with plant infusions had significantly less residual nitrite and lipid oxidation, indicating their potential as natural alternatives in cured meat systems.

This study was supported by Fundação para a Ciência e a Tecnologia (FCT, Lisbon, Portugal) through CIISA project UIDB/00276/2020 (CIISA), and LA/P/0059/2020 (AL4AnimalS) with financial support of the project AL4A-PROJ-LT1.3 - Safety and acceptability assessment of Green label cured meat products. The scholarship of the first author was funded by the Portuguese Foundation for Science and Technology (FCT)— UI/BD/152824/2022 (<https://doi.org/10.54499/UI/BD/152824/2022>).

Low numbers of lactic acid bacteria and staphylococci in *Alentejano* pork *Paio do Cachaço* do not compromise microbiological safety

Conceição, S.¹, Queiroga, MC.², Laranjo, M.²

¹MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, IIFA-Instituto de Investigação e Formação Avançada, Universidade de Évora, Évora, Portugal

²MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: smpc@uevora.pt

Alentejano pig is a Portuguese autochthonous swine breed, belonging to the Iberian breed branch. *Alentejano* pork meat is considered of high-quality and therefore used in the production of several meat products (for example, *Paio do Cachaço*). All meat products must comply with specific conditions established by Portuguese law to be certified under the Protected Designation of Origin “Carne de Porco Alentejano DOP”[1]. Therefore, the aim of this work was to evaluate if there are significant differences in the microbiological characterization of a dry-fermented sausage (*Paio do Cachaço*) produced with two different raw materials (*Alentejano* pork meat and pork meat), since there are some differences in the livestock farming, such as the type of feeding. Because *Paio do Cachaço* is a long-cure fermented sausage, it is possible to understand how the curing process modulates the microbiota. For that purpose, three independent batches from each meat type were manufactured and analyzed. Two replicate samples were collected at different steps throughout the manufacturing/curing process (meat batter, half-cured sausage, and end-product). Physicochemical analyses, namely pH and a_w , and microbiological analyses (*Salmonella* spp. detection, and *Listeria monocytogenes*, enterobacteria, lactic acid bacteria (LAB), staphylococci, and moulds and yeasts counts) were performed. The presence of *Salmonella* spp. and *L. monocytogenes* was not detected in any of the analyzed samples. However, the growth of enterobacteria was inhibited, probably due to an increased number of LAB, which resulted in a decrease of the pH values along the curing process. An increase in the number of staphylococci was also observed. Significant differences ($p < 0.05$) between the two studied sausages were observed, where the regular *Paio do Cachaço* showed higher numbers of LAB, staphylococci, moulds and yeasts, as well as higher a_w values, compared to the *Alentejano Paio do Cachaço*. Despite these differences, both *Paio do Cachaço* sausages are considered microbiological safe for consumers, because the final a_w values inhibit the growth of spoilage bacteria.

This work is funded by National Funds through FCT - Fundação para a Ciência e a Tecnologia under projects: PhD Fellowship UI/BD/153510/2022 (<https://doi.org/10.54499/UI/BD/153510/2022>); MED UID/05183/2025 (<https://doi.org/10.54499/UIDB/05183/2020>); CHANGE LA/P/0121 (<https://doi.org/10.54499/LA/P/0121/2020>).

1. Charneca, R., et al., *Alentejano Pig*, in *European Local Pig Breeds - Diversity and Performance - A study of project TREASURE*, M. Čandek-Potokar and R.M. Nieto, Editors. 2019, IntechOpen: London.

Veterinary Sciences and Animal Production

Influence of grazing management practices on gastrointestinal nematode burden and clinical parameters in sheep

Maria Braz¹; Emanuel Carreira²; Flávio Silva³; Nuno Borges⁴; Sandra Travessa³; Rodrigo Albuquerque⁵; Cláudia Vicente¹; Rui Charneca¹; Alfredo Pereira¹; Elisa Bettencourt⁵; Jordana Lopes¹; Sónia Lucena⁵; Marta Laranjo⁵; Andreia Amaral¹; Ludovina Padre⁵

¹ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal

² MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

³ Departamento de Medicina Veterinária, Universidade de Évora, Portugal

⁴ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal ⁵

MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

email: maria.braz@uevora.pt

Montado is an agrosilvopastoral ecosystem, defined by the association of soil, pastures, trees and animals with the Mediterranean climate, characteristic of Alentejo. Grazing management practices vary among farmers, most adopting continuous grazing (CG) while others practice deferred grazing (DG). DG entails use of pasture plots, with grazing durations adjusted to available biomass and typically supports a higher stocking rate (SR) compared to CG. Small ruminant gastrointestinal nematode (GIN) infections threaten a cost-effective and sustainable livestock production, causing subclinical to clinical symptoms. It is generally recognised that increasing SR leads to an increase in parasitic burden. The primary objective of this study was to evaluate differences in parasitological and haematological parameters between yearling female sheep managed under CG and DG. Grazing strategy defined two groups: CG group (13 yearling ewes stocked at 1 animal unit equivalent (AUE)/ha), and DG group (37 yearling ewes stocked at a 3 (AUE)/ha). Fresh faecal samples were collected directly from the rectum, blood samples via jugular venipuncture. Haematocrit (HT) was determined with the manual microhaematocrit technique; serum total proteins (PT) were accessed using an analogic refractometer. Faecal samples were analysed using the McMaster technique, with a detection threshold of 50 eggs per gram (EPG). Following individual EPG counts, pooled samples were prepared and subjected to coproculture for morphological identification of parasitic larvae. Statistical analyses were performed using R software. Descriptive statistics of the haematological and parasitological variables (HT, PT, and EPG) was computed, including measures of central tendency and dispersion. Group comparisons were tested using the non-parametric Kruskal-Wallis test ($p < 0.05$). EPG in the CG (150.0 ± 111.8 ; 150 EPG) did not differ ($p = 0.52$) from EPG in the DG (591.9 ± 798.7 ; 150 EPG). For HT, difference was also not significant ($p = 0.43$), CG (28.5 ± 5.6 ; 31%), and DG (31.0 ± 2.6 ; 31 %). Similarly, PT values did not differ between groups ($p = 0.96$). CG: (5.0 ± 0.2 ; 5g/dL), DG: (5.0 ± 0.3 ; 5g/dL); relative abundances of GIN genera were similar across groups: *Trichostrongylus* spp. were most prevalent, followed by *Teladorsagia* spp.; *Chabertia* spp. and *Oesophagostomum* spp. exchanged ranks between groups, while *Haemonchus* spp. remained the least abundant. In conclusion, no significant differences were detected in GIN egg excretion, HT, or PT between continuous and deferred grazing systems. These results indicate that grazing management had little effect on the evaluated parameters. Larger and longer-term studies are needed to confirm these findings within the regional context.

Veterinary Sciences and Animal Production

This work was funded by the SUMO Project: Montado Sustainability (PRR-C05-i03-I-000066), investment supported by the PRR - Recovery and Resilience Plan and European Funds NextGeneration EU and by National Funds through FCT – Foundation for Science and Technology within the scope of Project UIDB/05183/2025

Ensuring safe food waste upcycling: pesticide bioaccumulation in black soldier fly larvae reared on food waste

Joana Oliveira¹, Alexandre Trindade¹, Daniel Murta^{1,2,3}, Ricardo Assunção^{1,4}

¹ Egas Moniz Center for Interdisciplinary Research (CiiEM); Egas Moniz School of Health & Science, Almada, Portugal

² Ingredient Odyssey SA – EntoGreen, Santarém, Portugal

³ Thunder Foods SA, Santarém, Portugal

⁴ Food and Nutrition Department, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

Email: joliveira@egasmoniz.edu.pt

Food waste is a global challenge with major public health implications, contributing to greenhouse gas emissions and undermining food system sustainability [1]. The larvae of the black soldier fly (*Hermetia illucens*) (BSFL) can feed on a variety of organic substrates, including food waste, and convert them into valuable resources such as biomass, oil and frass, that may be used as animal feed and soil fertiliser, through a process called bioconversion [2]. However, in the European Union, food waste is prohibited as an insect substrate partly due to concerns over chemical and microbiological contaminants [3]. Given these restrictions, assessing whether BSFL can safely process food waste without accumulating hazardous substances is crucial for determining the feasibility of this bioconversion strategy within a One Health framework. This study assessed the bioaccumulation potential of organophosphate and carbamate (OP/C) pesticides in BSFL reared on food waste at an industrial scale. To this end, a total of 1847.5 kg of pre- and post-consumer food waste from Portuguese restaurants were collected and processed into the test substrate for a BSFL bioconversion assay. The Gainesville diet, a commonly used insect feed, was used as the control substrate. The bioconversion process lasted 14 days, with samples from the substrates collected prior to inoculation with juvenile larvae (day 1), and the larvae samples were collected after being sieved from the frass on day 14. All samples were subsequently frozen at –20 °C until analysis. OP/C pesticides in both substrates and larvae were then assessed using an enzymatic colourimetry commercial kit (Gold Standard Diagnosis, Horsham, PA, USA). The results demonstrated that the larvae did not bioaccumulate OP/C pesticides ($p < 0.029$), with these compounds absent in larvae from both control and test groups. These findings comply with European Union's food and feed safety standards, supporting that BSFL bioconversion can serve as a safe and sustainable method to upcycle food waste from a One Health perspective.

References:

- [1] United Nations. (2022). *Reducing food loss and waste: Taking Action to Transform Food Systems*. United Nations. <https://www.un.org/en/observances/end-food-waste-day>
- [2] Siddiqui, S. A., Ristow, B., Rahayu, T., Putra, N. S., Yuwono, N. W., Nisa, K., Mategeko, B., Smetana, S., Saki, M., Nawaz, A., & Nagdalian, A. (2022). Black soldier fly larvae (BSFL) and their affinity for organic waste processing. *Waste Management*, 140, 1–13. <https://doi.org/10.1016/j.wasman.2021.12.044>
- [3] EFSA Scientific Committee. (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, 13(10), 4257. <https://doi.org/10.2903/j.efsa.2015.4257>

Joana Oliveira is funded by a PhD grant by FCT (grant number 2022.13540.BDANA). This work was funded by “InsectERA” (Project No. C644917393-00000032) within the WPs InBioremediation and One Health, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5—Capitalization and Business Innovation, and by CiiEM Investiga “Flywaste”, through Project 10.54499/UIDB/04585/2020, funded by FCT.

A systems approach to the tick-host immunobiology – A starting pipeline

S. Zúquete ^{1,2,3}, E. Lamy ¹, L. Padre ¹, C. Grácio ⁴

¹MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Universidade de Évora, Portugal.

²FMV – Faculdade de Medicina Veterinária, Universidade Lusófona—Centro Universitário de Lisboa, 1749-024 Lisboa, Portugal

³CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;

⁴CIMA – Centro de Investigação em Matemática e Aplicações (CIMA), Escola de Ciências e Tecnologia, Universidade de Évora, Portugal

Email: mgracio@uevora.pt

Hard ticks are among the most significant arthropod vectors impacting animal and public health, for their dual capacity for direct host damage through blood feeding and their role in transmitting an expanding array of pathogens. The interface between ticks and hosts is a dynamic battleground shaped by complex immunobiological interactions with profound consequences for hosts. Both innate and acquired immune responses in vertebrates are activated upon tick infestation, driving inflammatory and defensive pathways that aim to block ticks' feeding. However, ticks developed a diverse arsenal of countermeasures, among the most notably, the bioactive constituents of their saliva, that suppress, subvert, or modulates host immune defenses thereby facilitating prolonged feeding periods and effective pathogen transmission to the hosts. In the mid-twentieth century, molecular biology and information-communication theory emerged as transformative scientific disciplines. Despite rapid advances, initially converging in the concept of the genetic code, the integration of these fields into a predictive framework within biology remains a major challenge but, still, an essential milestone. In the realm of vector biology, where understanding the complex immunobiological interactions between ticks and their hosts is critical for next-generation preventive strategies this is of particular relevancy. A systems-level deconstruction of the tick-host interface may be closer due to the identification of hundreds of tick salivary gland molecules with immunomodulatory and pharmacological properties, that are shedding some light on how ticks may orchestrate immune evasion and on how they manipulate hosts immunity at the molecular basis. By approaching the tick-host interface as a complex biological system, it may become possible to decode molecular signals, predict immunological outcomes and design rational immunological strategies to overcome tick burden. Our network is committed to converge expertise across fields to achieve new shifts towards innovative solutions in tick control. Although, still beginning, here, we present the general strategy pipeline for this research borne within the Cost Action Dynalife.

This work is based upon work from COST Action DYNAlife (CA21169), supported by COST (European Cooperation in Science and Technology).

One Health Insights into Spillover and Recombination-Driven Evolution of HEV, PCV2 and PCV3 in Portugal

B. Santos Almeida¹

¹National Institute of Agrarian and Veterinarian Research, Quinta Do Marquês, Av. da República, 2780-157 Oeiras, Portugal

Email: bsa.almeida2002@gmail.com

Swine serve as important reservoirs for viruses of both veterinary and public health importance, underscoring the need for a surveillance approach within a One Health context. This study provides the first integrated characterization of three significant swine viruses circulating in both domestic and wild Portuguese swine populations: the zoonotic *Hepatitis E virus* (HEV) and *Porcine circoviruses types 2 and 3* (PCV2 and PCV3), economically significant in the swine industry. Using molecular, phylogenetic, phylogeographic and recombination analysis, the circulation, origins and evolutionary dynamics of these viruses were explored. A total of 280 samples from domestic pigs and wild boars across mainland Portugal were analyzed. PCV2 and PCV3 were detected in both host populations, while HEV was only assessed in the 120 wild boar samples (3.3% prevalence), with all positive animals detected in Évora municipalities bordering Spain. The sequenced HEV strain (genotype HEV-3m) clustered with human-derived Spanish and French strains, while phylogeographic analysis suggested Spain as the origin of this strain in Portugal, highlighting its zoonotic relevance and suggesting cross-border wildlife movement as a likely route of introduction into the country. For both PCV2 and PCV3, two distinct genotypes were identified, one circulating in the domestic population and the other in wild boars. Phylogeographic reconstruction suggested that these strains likely originated in China and subsequently dispersed into different European countries, revealing extensive global transmission pathways followed by regional diversification within Europe. Multiple recombination events were detected in the PCV2 strains, clearly pointing to recombination as a major driver of PCV2 evolution. No recombination signals were detected for PCV3. Overall, the findings obtained not only showcase the complex viral ecology at the domestic–wildlife interface in Europe, but also provide direct evidence of cross-border wild boar movement as a source of viral introduction in Portugal, demonstrate the spillover risk to the domestic swine population and map the global transmission routes of PCV strains. This work represents the most comprehensive characterization of HEV, PCV2 and PCV3 in Portugal to date and underscores the need for integrated One Health surveillance to monitor viral evolution and mitigate spillover threats at the wildlife–livestock–human interface.

First Report of *Megaselia scalaris* in Évora Region

Laura Cesare¹, Sara Zúquete^{1,2,3}, Teresa Rosmaninho¹, Guilhermina Pias¹, Sara Santos^{4,5}, Ludovina Padre^{1,4}, Ana Gião^{4,6}, Marta Laranjo^{1,4}, Sónia Lucena^{1,4}

¹Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal. ²CIISA Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa Avenida da Universidade Técnica 1300-477, Lisboa, Portugal. ³Faculty of Veterinary Medicine, Universidade Lusófona, Av. do Campo Grande 376, 1749-024 Lisboa, Portugal. ⁴MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Évora, Portugal. ⁵Núcleo de Alimentação e Veterinária de Reguengos de Monsaraz (NAV), Direção Geral de Alimentação e Veterinária (DGAV), Reguengos de Monsaraz, Portugal. ⁶Direção de Serviços de Alimentação e Veterinária da Região Alentejo (DSAVRA), Direção Geral de Alimentação e Veterinária (DGAV), Évora, Portugal.

Email: laura.delpinocesare@gmail.com

Introduction: *Megaselia scalaris*, commonly known as scuttle fly, is a polyphagous and synanthropic species with broad distribution and high ecological plasticity. Its presence has significant implications for public and animal health, food safety, forensic science, and beekeeping.

Objective: To report, for the first time, the presence of *M. scalaris* in Évora Region.

Materials and Methods: In October 2025, as part of an ongoing study in the south of Portugal, several samples of distinct swine muscles and arthritis liquid were inoculated in Plate Count Agar and Violet Red Bile Glucose culture media and incubated at 30°C for regular microbiological analyses. After a 72 hours incubation period, the plates were examined and several larvae were observed. The plates were maintained, allowing the observation and later identification of various developmental stages: larva, pupa, and adult. Morphological identification of the adult flies was performed, and molecular confirmation via PCR targeting the mitochondrial cytochrome oxidase subunit I (COI) gene is ongoing.

Results and Discussion: During the macroscopic examination of the Petri dishes, unusual trails on the culture media and small moving larvae were detected. Under conventional light microscopy, the larvae exhibited an elongated, translucent body approximately 6 mm in length. Morphological evaluation of adult flies led to their putative identification as *M. scalaris*, based on the morpho-anatomical features, including size and colour; the shape and dimensions of the head and thorax; wing shape, size, and venation pattern, the specimens were identified as belonging to *M. scalaris* fly. To the best of our knowledge, this is the first report of *M. scalaris* in mainland Portugal, which is noteworthy, because of its importance in food contamination, myiasis (intestinal, urogenital, ocular, vaginal, and cutaneous), and its role as a parasite and vector of pathogens affecting other arthropods, as *Apis mellifera*.

Conclusions and Future Perspectives: The accidental observation of *M. scalaris* in Évora region highlights the need for continuous monitoring of the species. Future research should assess its distribution, pathogenic potential in public health, implications for food safety, and impact on apiculture. A molecular confirmation via PCR is currently underway, and it will be the first report of molecular identification of *M. scalaris* in Portugal.

Keywords: *Megaselia scalaris*, larvae, adults, swine meat, morphology.

Effect of fish oil inclusion in diets with almond hull on lamb productive performance, carcass traits and meat quality

Liliana Cachucho^{1,2,3}, Susana P. Alves^{2,3,4}, Olinda Guerreiro^{1,5}, Diogo Henriques⁶, Kátia Paulos⁶, José Santos-Silva^{2,3,6}, M. Teresa P. Dentinho^{2,3,6}, Rui Bessa^{2,3,4}, Eliana Jerónimo^{1,5}

¹CEBAL – Alentejo Biotechnology Center for Agriculture and Agro-food/IPBeja, Beja, Portugal.

²CIISA – Center for Interdisciplinary Research in Animal Health, Lisbon, Portugal.

³AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal.

⁴Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

⁵MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Beja, Portugal.

⁶INIAV – National Institute for Agricultural and Veterinary Research, Santarém, Portugal.

Email: liliana.cachucho@cebal.pt

The partial replacement of cereals by almond hull (AH) in a 40:60 forage to concentrate diet supplemented with 5% of soybean oil (SO) increased the potentially beneficial fatty acids (FA) to human health, namely *t*11-18:1 and *c*9,*t*11-18:2, in intramuscular fat of lambs, without affecting the average daily gain (ADG) or carcass and meat quality. Marine lipids can inhibit the final steps of rumen biohydrogenation, potentiating the deposition of *t*11-18:1 and *c*9,*t*11-18:2 in tissues. This study aimed to evaluate whether the addition of fish oil (FO) to the diet with AH can intensify the rumen production of *t*11-18:1 and increase the *t*11-18:1 and *c*9,*t*11-18:2 in intramuscular fat. Thirty-two lambs, individually housed, were randomly distributed in four diets: C – 0% AH and 5% SO; CAH – 18% AH and 5% SO; CFO – 0% AH, 4% SO and 1% FO; and CAHFO – 18% AH, 4% SO and 1% FO. After a 7-day adaptation period, feed intake and body weight were monitored for 6 weeks. Following slaughter, the carcass parameters were assessed, and chemical, physical, and sensory parameters were determined for the meat. The data were analysed by the Proc Mixed of SAS. The diets did not affect: ADG, feed intake, feed conversion ratio, slaughter live weight, carcass parameters, intramuscular fat content, cooking losses, and sensory attributes ($P>0.05$), except for tenderness and flavour intensity. The CFO diet resulted in meats with higher shear force and lower tenderness ($P<0.001$). The FO increased flavour intensity ($P=0.006$). The meat colour parameters L^* , b^* , H^* and C^* ($P<0.001$) increased and a^* decreased ($P<0.001$) over 7 days at 2 °C. The FO increased L^* , b^* and H^* ($P<0.05$) in meat. In raw and cooked meat, lipid oxidation increased over the storage time, with higher levels in cooked meat from FO diets. AH and FO increased *t*11-18:1 and *c*9,*t*11-18:2 ($P<0.001$) in intramuscular fat, and FO increased n-3 polyunsaturated FA ($P=0.021$). Both AH and FO were effective in improving the nutritional value of lamb fat, without compromising productive performance and carcass quality. However, the effect of FO on meat FA composition was independent of the dietary AH levels.

This work was funded under the projects MED (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>), CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>), CIISA (<https://doi.org/10.54499/UIDB/00276/2020>), AL4Animals (<https://doi.org/10.54499/LA/P/0059/2020>) and PhD grant awarded to LC (2020.05712.BD).

Isolation of salivary glands from *Hippobosca equina* for vector potential analysis

Maria Felício¹, Sara Tudela Zúquete^{3,4}, Alexandre Leitão^{3,4}, Pedro Caetano^{1,2} and Ludovina Padre^{1, 2}

¹ MED – MED mediterranean institute for agriculture, environment and development, Universidade de Évora, Universidade de Évora Pólo da Mitra, Apartado 94, 7006-554 Évora, Portugal

² Departamento de Medicina Veterinária, Escola de Ciência e Tecnologia, Universidade de Évora

³ CIISA - Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, 1300-477 Lisboa, Portugal

⁴ AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal

⁵ Faculty of Veterinary Medicine, Universidade Lusófona, Av. do campo Grande 376, 1749-024 Lisboa

Louse flies, including *Hippobosca equina* (Linnaeus, 1758), are hematophagous insects that infest livestock and are considered potential vectors of various pathogens. Investigating their vector potential requires a detailed examination of their salivary glands, which plays an important role in pathogen transmission. The present work details a dissection and isolation technique for salivary glands, adapted from methodologies established for other *Hippoboscidae* species, such as deer keds (*Lipoptena spp.*). The method allow the abdominal region to be dissected, enabling salivary gland extraction with minimal contamination – a crucial step for molecular analysis.

From the previously described protocol, certain aspects were simplified in terms of materials. Materials like plasticine was used to secure the fly, instead of wax. For dissection, 18G needles were used instead of scalpel blades, as they allowed for more precise incisions in the abdomen due to their smaller size. Additionally, *H. equina* has wings, unlike *Lipoptena* species, which may cause difficulties in the procedure. The legs weren't removed, contrary to the method described in the original protocol.

Specimens of *Hippobosca equina* were collected from dairy cattle on São Miguel Island, Azores, and preserved in RNAlater for subsequent analysis. Dissections began with the flies being immobilised under a stereomicroscope. The abdominal cuticle was removed to expose the internal organs. The salivary glands were located and excised. Once isolated, the glands were stored for subsequent pathogen analysis to assess their role in transmitting infectious agents. The main difficulty found was dissecting the salivary glands of flies containing large larvae. Additionally, isolating the salivary glands was easier in males than in females.

This adapted technique is crucial to advancing our understanding of the biological mechanisms underlying the vector potential of *Hippobosca equina* and its role in disease ecology. This study aims to refine the process of salivary gland isolation to obtain high-quality samples with minimal contamination and a higher DNA yield for molecular analyses. This will enable the capacity of this fly to transmit pathogens to be evaluated. The results provide a foundation for future investigations into pathogen transmission by *Hippobosca equina*, contributing to a better understanding of its impact on livestock health.

This work was supported by the Bolsa João Cannas da Silva from the Associação Portuguesa de Buiatria (APB)

Water Quality: comparison of the effect of manganese in water on two groups of dairy cattle

V. Resende¹, O. Moreira², J. M. Martins¹, M. R. Lucas³, R. Branco⁴

¹MED (Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²INIAV (Instituto Nacional de Investigação Agrária), Quinta da Fonte Boa, Vale de Santarém, 2005-048 Santarém, Portugal.

³CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia), Palácio do Vimioso, Largo Marquês de Marialva, n.º 8, 7000-809 Évora, Portugal.

⁴Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749- 024 Lisboa - Portugal

Email: vania.resende@uevora.pt

The estimated increase in the global population growth rate suggests that, in the future, there will be greater demand for food, which in turn will have a direct effect on water use for agricultural purposes. Drinking water is a scarce resource in many regions of the world and plays a vital role in living organisms for the maintenance of life. On dairy farms, the use of quality water is essential to maximize milk production from animals. Manganese is naturally present in many surface and groundwater sources and in soils that can erode into these waters, and is an element that is necessary in small amounts for all living organisms. However, if concentrations are excessive, this mineral can reduce water intake and have other harmful effects on health and production.

The objective of this study was to verify the impact of water quality on the production, reproduction, and health of animals on a dairy farm in Alentejo with high manganese levels in the water.

Materials and methods: Two experimental groups were created (treated water group and untreated water group) balanced according to age, number of births, butyric acid content, and protein content. The experimental groups (n=25) were kept under the same conditions: stable, feed, ambient temperature and humidity, same number of drinking troughs, same feeding space. The factor of variation between the groups was the concentration of manganese (Mn): treated group <50 µg/L (DGAV, 2014) and untreated group >1500 µg/L. In addition to all production, reproductive, sales, and mortality data, blood samples (glucose, urea, creatinine, ALT, AST, ALP, [Mn]), urine samples (urea-N, urea, creatinine, [Mn], [Fe]), and milk samples (Mn, Fe, Ca, TMT, CCS, TB, TP, urea) were collected for 4 months. Statistical analysis was performed using SPSS.

Results: Regarding reproduction data (number of inseminations, interval between births), production levels, and health, significant differences were observed between the study groups, with the untreated group showing the worst results. However, no statistically significant differences were found for some parameters in the serum and urine analyses. The concentrations of minerals such as Mn and Fe in the blood differed between groups.

Conclusion: It was found that excess manganese in drinking water affected overall farm performance, with significant differences between groups.

Keywords: Cattle, Water, Manganese; Production and Health.

Plenary Lecture Speakers

Name	Email
Ana Alexandre	anaalex@uevora.pt
Francisco Ferreira	ff@fct.unl.pt

List of Participants

Name	Email