



# **IX PhD Students Meeting in Environment and Agriculture**

**December 11 & 12, 2024**

**Pólo da Mitra, Universidade de Évora**

## **Book of abstracts**

---



**Title:** IX PhD Students Meeting in Environment and Agriculture

**Editors:**

Marta Laranjo

Ana Alexandre

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

Copyright © 2024, all rights reserved

ISBN: 978-972-778-434-9



Dear participants,

It is our great pleasure to welcome you to the **IX PhD Students Meeting in Environment and Agriculture**, held in Évora on the 11<sup>th</sup> and 12<sup>th</sup> of December 2024. We have put together a two-day program with the aim of encouraging scientific discussion. This Meeting represents an excellent opportunity for young researchers to exchange ideas and to explore new challenges in research regarding Environmental and Agricultural Sciences.

This event is organized by MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, and IIFA – Institute for Advanced Studies and Research, University of Évora and supported by UNIMED – Mediterranean Universities Union.

The Meeting focuses on eight main areas: Agribusiness; Biology; Environment, Landscape & Sustainability; Biotechnology; Agricultural & Environmental Sciences; Food Sciences; Veterinary Sciences; and Biochemistry. The Meeting includes two invited plenary lectures and several presentations selected from the abstracts submitted by PhD students. In addition, all authors that were not selected for oral communication, will present their work as posters displayed throughout the Meeting.

The PhD Students Meeting in Environment and Agriculture intends to stimulate the interaction between PhD students, to streamline scientific discussion and highlight the ones who will become the researchers of the future.

Finally, we wish to thank the Scientific Committee as well as all the participants who have contributed to the scientific program and hope you will enjoy the Meeting and appreciate the beautiful city of Évora, an UNESCO World Heritage. You should find all detailed information in this book of abstracts, including the detailed programme, abstracts, and a list of participants.

Welcome to Évora!

The Organising Committee,  
Marta Laranjo, MED|CHANGE  
Ana Alexandre, MED|CHANGE  
Bruno Medronho, MED|CHANGE  
Cláudia Marques, IIFA  
Teresa Pinto Correia, MED|CHANGE  
Sofia Eufrazio, MED|CHANGE



## COMMITTEES

### **Organising Committee**

Marta Laranjo, Universidade de Évora - MED|CHANGE  
Ana Alexandre, 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  
Catarina Meireles, 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  
Pedro M. Anastácio, Universidade de Évora – MARE-EU  
Maria João Cabrita, Universidade de Évora – MED|CHANGE  
Marco Gomes da Silva – Universidade Nova  
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





**Organised by:**



**Supported by:**





## Programme

---

Wednesday, December 11

09:00	Registration
09:45	Opening session
	<p><b>Rui Salgado</b> Director of the Institute for Advanced Studies and Research (IIFA)</p> <p><b>Fátima Baptista</b> Director of the Mediterranean Institute for Agriculture, Environment and Development (MED)</p>

	<p>Moderator: Ana Rita Martins CEBAL, MED &amp; CHANGE</p>
10:00	<p><i>Unraveling the secrets of the grapes: GC×GC on the aromas hunt</i> <b>Daniela Fonseca</b> University of Évora, MED &amp; CHANGE</p> <p><i>Detection of Listeria monocytogenes in Beef food-chain</i> <b>Maria Pedro Teixeira</b> FMV, CIISA and AL4Animals</p> <p><i>Developing cured meat products with Thymus citriodorus and Salvia elegans extracts combined with S. equorum</i> <b>Patrícia Bernardo</b> FMV, CIISA and AL4Animals</p> <p><i>Antimicrobial resistance genes detected in pork meat products</i> <b>Sara Conceição</b> University of Évora, MED &amp; CHANGE</p>
11:00-11:45	Coffee break and poster session

11:45	<p><b>Plenary Lecture</b> <i>Laboratory Diagnostics: From Classical to Molecular and Beyond</i> <b>Miguel Fevereiro</b> INIAV</p>
12:45 – 14:45	Lunch break

	<p>Moderator: <b>André Albuquerque</b> University of Évora, MED &amp; CHANGE</p>
14:45	<p><i>Changes in the volatile profile of Citrus × limon leaves treated with ethanol and salicylic acid and infested by Trioza erytrae (Del Guercio, 1918)</i> <b>Tomás Magalhães</b> University of Algarve, MED &amp; CHANGE</p>

	<p><i>Effects of PM10 from Luanda on the viability of human A549 alveolar epithelial cells</i>  <b>Marlene Soares</b>  University of Aveiro, CESAM</p>
	<p><i>Improvement of agronomic, environmental and economic sustainability of processing tomato production system in Portugal through the application of Conservation Agriculture principles</i>  <b>Ricardo Vieira Santos</b>  University of Évora, MED &amp; CHANGE</p>
	<p><i>Developing a RfxCas13d system against ToBRFV in tomato plants</i>  <b>Joana Amaro Ribeiro</b>  University of Évora, MED &amp; CHANGE</p>
	<p><i>Potentially Toxic Elements Contamination of Soil and Compost from Urban Farms in the Porto Metropolitan Area and its Impact on Food Safety</i>  <b>Lourenço Pinto de Rezende</b>  Universidade Católica Portuguesa, CBQF</p>
16:00 – 16:45	Coffee break and poster session
16:45	<p><i>Anthelmintic resistance of parasitic gastrointestinal nematodes of Alentejo sheep and use of natural products to overcome resistance</i>  <b>Natália Faria Campbell</b>  INIAV, MED &amp; CHANGE</p>
	<p><i>Determinants for a good transfer of passive immunity in dairy calves</i>  <b>Flávio G. Silva</b>  University of Trás-os-Montes and Alto Douro, CECAV, MED &amp; CHANGE, CISAS</p>
	<p><i>Impact of production season on the fatty acid composition of fat from lambs produced in Portugal - Emphasis on trans fatty acids and conjugated linoleic acids</i>  <b>Andreia Silva</b>  CEBAL, CIISA, AL4AnimalS</p>
17:30	Ending of the first day of the IX PhD Students Meeting in Environment and Agriculture

**Thursday, December 12**

	<p>Moderator: Paola Hernández University of Évora, MED &amp; CHANGE</p>
9:30	<p><i>Unlocking the potential of lignin for hair care: from extraction to sustainable conditioners</i> <b>Catarina Fernandes</b> University of Coimbra, CERES, MED &amp; CHANGE</p>
	<p><i>Rethinking road verges to promote Mediterranean wildflowers: give biodiversity a chance</i> <b>Mariana Pucarinho Fernandes</b> University of Évora, cE3c and MED &amp; CHANGE</p>
	<p><i>Sustainable Management of Natural Remnant Habitats in the Montado: Advancing Conservation Through Practical Tools</i> <b>Erika Almeida</b> University of Évora, MED &amp; CHANGE</p>
	<p><i>Indicators to assess social sustainability: similarities and differences between three wine regions (Verdes, Douro and Alentejo)</i> <b>José Massuça</b> University of Trás-os-Montes and Alto Douro, CETRAD</p>
	<p><i>The role of five Mediterranean tree species in enhancing climate resilience in Mediterranean cities</i> <b>Pedro Matias</b> University of Algarve, CIMA, ISE, MED &amp; CHANGE</p>
10:45-11:30	Coffee break and poster session
11:30	<p><b>Plenary Lecture</b> <i>Technological Disruption, Globalization, and Sustainable Development</i> <b>Carlos Zorrinho</b> University of Évora</p>
12:30-14:30	Lunch break
	<p>Moderator: <b>Patrick Materatski</b> University of Évora, MED &amp; CHANGE</p>
14:30	<p><i>Using the dead to infer about the living: Amphibian roadkill spatiotemporal dynamics suggest local populations' reduction</i> <b>Tiago Pinto</b> University of Évora, MED &amp; CHANGE</p>
	<p><i>Different inter-row management practices on a vineyard: implications on plant physiology</i> <b>Vanessa Silva</b> University of Évora, MED &amp; CHANGE</p>

	<p><i>Somatic embryogenesis in Portuguese grapevine (Vitis vinifera L.) cultivars – a focus on the induction phase</i></p> <p><b>Catarina Estêvão</b> University of Évora, MED &amp; CHANGE</p>
	<p><i>Nanoparticle Syntheses Using Microbial Supernatants: An Environmentally Friendly Approach to Cultural Heritage Conservation</i></p> <p><b>António Carrapiço</b> University of Évora, HERCULES &amp; IN2PAST</p>
15:30-16:15	Coffee break and poster session
16:15	<p><i>Monitoring of Alternaria spp. and Alt a 1 in Évora atmosphere</i></p> <p><b>Mariana Marques</b> University of Évora, ICT</p>
	<p><i>Assessing Perceived Value in Cooperative Membership: Insights from Organic Cocoa Producers in Sao Tome and Principe</i></p> <p><b>Ibrahim Prazeres</b> University of Évora, MED &amp; CHANGE</p>
17:45	<p>Closing Session</p> <p>Best Poster Prize</p> <p>Solange de Oliveira Prize (MED)</p>

**Note: Posters will be displayed throughout the whole Meeting.**

## BEST POSTER AWARDS



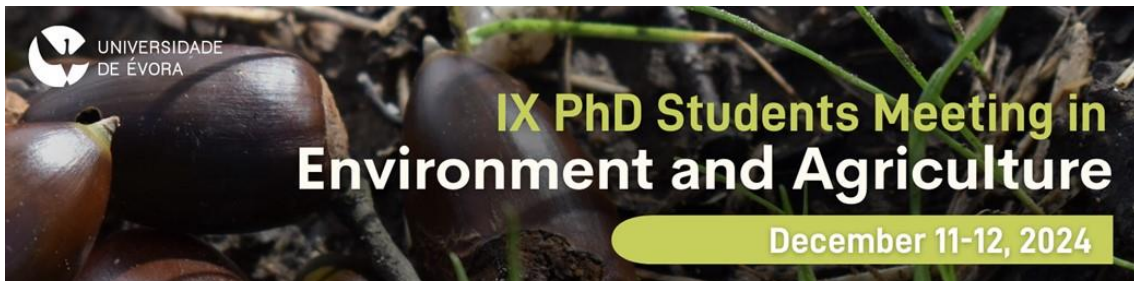
### Mónica Marques

was awarded with the **Best Poster Prize** (1<sup>st</sup> place) for the Poster

*“Molecular and phylogenetic relationships between Colletotrichum species and related fungi based on partial DNA gene analysis”*

On behalf of the Organising Committee,

Fátima Baptista  
Director of MED



### Carla Janeiro

was awarded with the **Best Poster Prize** (2<sup>nd</sup> place) for the Poster

*“Biodiversity Market: Case Study for the Montado Ecosystem”*

On behalf of the Organising Committee,

Fátima Baptista  
Director of MED





## Filipa Santos

was awarded with the **Best Poster Prize** (3<sup>rd</sup> place) for the Poster

*"Olive anthracnose in Alentejo: is there a single species to blame?"*

On behalf of the Organising Committee,

**Fátima Baptista**  
Director of MED







## Plenary Lectures

## Laboratory Diagnosis: From classical to molecular and beyond

Miguel Fevereiro

National Institute of Agricultural and Veterinary Research – INIAV

Email: [miguelfevereiro@yahoo.com](mailto:miguelfevereiro@yahoo.com)

Centuries ago, epidemics significantly impacted ancient civilizations like the Egyptians, Greeks, and Romans. The Middle Ages saw frequent outbreaks of diseases such as plague, tuberculosis, and smallpox, with medieval people attributing these events to supernatural causes. It was not until the 16th century that physicians began to propose more scientific explanations, such as Hieronymus Fracastorius' germ theory. In the 17th, Francesco Redi demonstrated that the presence of maggots in putrefying meat does not result from spontaneous generation but from eggs laid on the meat by flies. In the 18th century, discoveries like Leeuwenhoek's microscope, Jenner's smallpox vaccine, and Semmelweis's hand-washing practice advanced our understanding of diseases.

The 19th century brought crucial breakthroughs. The dominant "miasma theory" that diseases resulted from "bad air" was questioned by John Snow in 1855, who hypothesised that cholera was waterborne or foodborne. The foundation of Bacteriology begins when Louis Pasteur in 1857 proved that microorganisms were responsible for the fermentation of fluids and Robert Koch discovered *Mycobacterium tuberculosis*, and *Vibrio cholerae*. Martinus Beijerinck conceptually conceived virology, when in 1898 he reports that the infectious agent of the tobacco mosaic was present only in cells that were dividing and multiplying. In the same year, Friedrich Loeffler and Paul Frosch, isolated the first animal virus, the foot-and-mouth disease virus.

In the early 20th century, key developments included the beginning of cell culture, the discovery of yellow fever virus, bacteriophages, and influenza virus. In 1928, Alexander Fleming discovered penicillin, ushering in the era of antibiotics.

A significant milestone came in 1953 with the discovery of DNA's structure ("The secret of Life") by Watson and Crick. In the 1970s, the recombinant DNA revolution began and the discovery of reverse transcriptase, an enzyme crucial for retroviruses, laid the foundation for retrovirology and cancer biology. The development of a method for determining base sequences in DNA by Frederick Sanger, Paul Berg, and Walter Gilbert (1975), along with technologies such as PCR, developed by Kary Mullis in 1983, revolutionized the field of molecular biology and boosted molecular diagnosis.

The Human Genome Project, completed in 2003, marked a milestone, providing insight into human genetic sequences. The new era of "Big Data", the advancements in bioinformatics, Next Generation Sequencing technologies, and the rise of artificial intelligence further transformed biosciences, giving rise to fields like genomics, proteomics, microbiomics and many more "OMICS". These innovations continue to shape the future of Biosciences.

## **Technological Disruption, Globalization, and Sustainable Development**

Carlos Zorrinho

Center for Advanced Studies in Management and Economics (CEFAGE), Department of Management, Universidade de Évora, Ap. 94, 7002-554 Évora, Portugal.

Email: [czorrinho@uevora.pt](mailto:czorrinho@uevora.pt)

In an era defined by rapid technological advancements, globalization, and pressing sustainability challenges, this presentation examines the interplay between digital and energy disruptions as drivers of transformative change. Drawing upon decades of experience in academia and public policy, the discussion highlights the potential of adopting a human-centred approach to these dynamics. The European Union's dual focus on digitalization and decarbonization is presented as a case study in soft-power leadership. Through initiatives like the Green Deal and Digital Decade, the EU seeks to align its legislative priorities with Sustainable Development Goals (SDGs), addressing bureaucratic hurdles and funding disparities in the process. The presentation explores how the strategic alignment of data, purpose, and values can act as game-changers for societal progress. It emphasizes ethical globalization, digital literacy, and innovative governance as critical tools to combat challenges like disinformation, sustainability issues, and inequitable energy systems. Moreover, the integration of peacebuilding, humanitarian aid, and sustainability through the Triple Nexus approach is discussed as a promising and achievable pathway. The presentation also envisions a redefined multilateralism to address the evolving global landscape in the post-2030 SDG era. Advocating for purpose-driven innovation, co-creation, and collective action, the presentation underscores the importance of mitigating risks and fears to pave the way for a future marked by freedom, reduced inequality, and enhanced human development.





## Unraveling the secrets of the grapes: GC×GC on the aromas hunt

D. Fonseca<sup>1</sup>, N. Martins<sup>2</sup>, R. Garcia<sup>3</sup>, M. J. Cabrita<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: [daniela.fonseca@uevora.pt](mailto:daniela.fonseca@uevora.pt)

Grapes, essential for wine production, contain many volatile compounds responsible for the unique aromas and flavors that make each wine special. These compounds, known as varietal volatiles, include aldehydes, terpenes and C13-norisoprenoids, and vary according to variety, terroir and viticultural practices. However, identifying these compounds is challenging due to their low concentration and the complexity of the grape matrix, requiring advanced analysis techniques. The “hunt for aromas”, is a meticulous process facilitated by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS). This technique offers an efficient and rigorous separation of the volatile compounds in grapes, allowing for a comprehensive analysis of the aromatic profile. In the first dimension, the compounds are separated based on polarity, while in the second dimension, the separation is based on volatility. A key component of this process is flow modulation which improves the separation process by concentrating the sample and ensuring that even coeluting compounds are efficiently separated in the second dimension.

The application of GC×GC-ToFMS in grape analysis not only enhances the detection of compounds at low concentrations but also allows for the precise differentiation of compounds with similar chemical structures. In addition, the technique helps to understand how environmental factors, such as soil and climate, influence the aromatic profile of grapes. Thus, GC×GC aroma hunting unlocks the secrets hidden in grapes, providing a clearer view of the volatile varietal components that contribute to the unique aromas of each wine. In addition, this information can be used to improve the quality of wines, helping in the selection of varieties and consequently leading to the production of high-quality wines.

The objective is to introduce comprehensive two-dimensional gas chromatography, an advanced and powerful analytical technique that enables the analysis of a wide and diverse range of matrices, including oils, flavors, food, petrochemicals, forensics and environmental samples.

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>.

**Detection of *Listeria monocytogenes* in Beef food-chain**M. P. Teixeira<sup>1,2</sup>, P. Bernardo<sup>1,2</sup>, L. Orge<sup>3</sup>, M. J. Fraqueza<sup>1,2</sup>

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

<sup>2</sup>AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences

<sup>3</sup>Laboratory of Pathology - National Institute for Agricultural and Veterinary Research, I.P. (INIAV), UEISPSA, Avenida da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

Email: [mjoaofraqueza@fmv.ulisboa.pt](mailto:mjoaofraqueza@fmv.ulisboa.pt)

*Listeria monocytogenes* is a ubiquitous microorganism and the causative agent of listeriosis in humans and animals. Listeriosis is considered one of the most severe foodborne diseases due to the high fatality registered. According to the Listeriosis Annual Epidemiological Report (European Centre for Disease Prevention and Control 2023), there has been an increase in human listeriosis cases. The most common transmission route is ingesting contaminated foods, particularly ready-to-eat foods, such as meat and dairy products. To limit the introduction of *L. monocytogenes* in the food chain, controlling the pathogen at the slaughterhouse level is important. *Listeria monocytogenes* in beef food-chain is less studied than other pathogens such as *E. coli* and *Salmonella*. The objective of the work was then to study the presence of *Listeria monocytogenes* in the bovine meat food chain. The ISO standard 17604:2015 was utilized for sampling bovine carcasses, the method applied was nondestructive. The carcasses were sampled at different slaughter days at the end of the line and in carcasses in the refrigeration chamber. The sample was done on the hind leg, brisket, shoulder and neck with a total sampled area of 1000 cm<sup>2</sup>. Ready-to-cook (RTC) and ready-to-eat (RTE) beef-based products were also sampled on different days and supermarkets in the metropolitan Lisbon area. The detection and isolation of *Listeria monocytogenes* was performed according to ISO 11290-1:2017. The presumptive positive colonies were selected and collected for further identification utilizing PCR protocol adapted from Ryu *et al.* (2013). In total, 145 carcasses were sampled: 60 (41.4%) from carcasses in the refrigeration chamber and 85 (59.6%) from the slaughterhouse line. From these samples, only one carcass was positive for *Listeria monocytogenes*, leading to an incidence of 0.8%. The RTC had a higher % incidence of *Listeria monocytogenes* of 12%, with two of the 17 sampled products being positive for *L. monocytogenes*. The highest incidence was on the RTE beef products, 25%, due to the detection of the pathogen in one of the four analyzed products. These results agree with the literature, with the higher incidence of RTE products. However, more samples need to be analyzed to validate this incidence rate.

This work was financially supported by the Portuguese Foundation for Science and Technology (FCT) under projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS) with the financial support of the project Safemeatproducts PDR2020-1.0.1-FEADER-031359. The scholarship of the first author was funded by UIDB/00276/2020



**Developing cured meat products with *Thymus citriodoros* and *Salvia elegans* extracts combined with *S. equorum***

P. Bernardo<sup>1,2</sup>, M.J. Fernandes<sup>1,2</sup>, M.H. Fernandes<sup>1,2</sup>, M.P. Teixeira<sup>1,2</sup>, L. Patarata<sup>2,3</sup>, M.J. Fraqueza<sup>1,2</sup>

<sup>1</sup>CIISA, Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal;

<sup>2</sup> AL4AnimalS, Associate Laboratory for Animal and Veterinary Sciences;

<sup>3</sup>CECAV—Animal and Veterinary Research Center, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal.

Email: [mjoaofraqueza@fmv.ulisboa.pt](mailto:mjoaofraqueza@fmv.ulisboa.pt)

This study aimed to evaluate the effects of replacing nitrate with *Thymus citriodoros* or *Salvia elegans*, and a *Staphylococcus equorum* starter culture on the microbiota, quality and sensory acceptance of cured meat sausages (CMS).

Eight CMS formulas were produced: C1- without nitrate or starter; C2- with starter without nitrate; F1- 150 mg KNO<sub>3</sub>/kg; F2- 150 mg KNO<sub>3</sub>/kg with starter; F3- Sage10.6%; F4- Sage10.6% with starter; F5- Thyme10.6%; F6- Thyme10.6% with the starter. Samples were collected before filling, final product and after 60 days of 5°C storage. The colour (L\*a\*b\*) was measured with a Konica Minolta CR-400/410. Residual nitrate and nitrite were determined. Microbial analysis was performed according to ISO Standards: Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS), and *Enterobacteriaceae*. Total DNA was extracted by using the E.Z.N.A.<sup>®</sup> Food DNA Kit. Sequencing was done with Illumina PE150. Sensory analysis was carried out on CMS final product. A consumer test was performed with 111 volunteers, which included a hedonic evaluation, willingness to consume and purchase, and a Check-All-That-Apply (CATA) test. The starter produced positive effects on CMS colour. Excluding CMS with Sage, all inoculated samples exhibited higher a\* values. Staphylococci counts in CMS with starter were always above 7 Log cfu/g, while in non-inoculated were lower (4.9 to 6.3 Log cfu/g). Taxonomic characterization revealed significant levels of starter microorganisms remaining in the final product. The counts of LAB progressively increased over time, reaching values ranging from 7.5 to 8.2 Log cfu/g after 60 days, and were higher in samples without added SCN starter. The growth of SCN and LAB corresponded to a decrease in *Enterobacteriaceae* and *Pseudomonas* spp.. Multifactorial analysis of CATA test shows that thyme and *S. equorum* sausages had sensory attributes similar to those with nitrate. All formulations scored above the middle of the hedonic scale. The use of a CNS starter modulates the sausage microbiota and has a protective affect against *Enterobacteriaceae* and *Pseudomonas* throughout time. CMS with *S. equorum* also presented the characteristic cured red colour, noticed by consumers. Combining *T. citriodoros* or *S. elegans* with *S. equorum* demonstrates potential as an effective strategy for replacing nitrites.

The scholarship of the first author was funded by the Portuguese Foundation for Science and Technology (FCT)—UI/BD/152824/2022. This research was funded by FCT, under projects 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.

## Antimicrobial resistance genes detected in pork meat products

S. Conceição<sup>1</sup>, M.C. Queiroga<sup>2</sup>, M. Laranjo<sup>2</sup>

<sup>1</sup>MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, IIFA-Institute for Advanced Studies and Research, University of Évora, Polo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>Department of Veterinary Medicine, School of Sciences and Technology, University of Évora, Polo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [smpc@uevora.pt](mailto:smpc@uevora.pt)

In recent decades, the transmission of antimicrobial resistance genes (AMR) from food-producing animals to the final consumer (farm-to-fork transmission) has been investigated. The presence of these genes is linked to the use of antimicrobials in animal production. The misuse of antimicrobials contributes to an increase in antimicrobial-resistant bacterial populations, resulting in serious problems dealing with infection complications both in humans and animals. Considering the fact that pig is a huge reservoir for AMR, analysis of a fermented meat product was performed to understand how this product can be a source of AMR genes. Analyses were carried out throughout the manufacturing process at two distinct steps, meat batter (MB) and half-cured sausage (HCS), with two replicates per sample using two different approaches. In the culture-dependent approach, selective culture media with antimicrobials in their composition were used: CHROMID VRE (for the detection of vancomycin-resistant *Enterococcus*), CHROMID ESBL (to detect ESBL-producing *Enterobacteriaceae*) and CHROMID MRSA (for the detection of methicillin-resistant *Staphylococcus aureus*). For the culture-independent approach, next-generation sequencing (NGS) was used (minION MK1B, Nanopore Oxford Technologies), and the results were analyzed on the EPI2ME platform (Metricor). The preliminary results showed no resistant bacteria isolated with the culture-dependent approach. In the metagenomic analyses, four AMR genes were detected in two samples (1 MB and 1 HCS): *tetC*, *yojL*, *opmH*, *emrE*, which confer resistance to tetracycline, the peptide antibiotic microcin J25, triclosan and aminoglycoside, respectively. Apart from the *tetC* gene, which has a plasmid origin, all the other AMR genes found come from either *Pseudomonas* spp. or *Klebsiella* spp., and have a chromosomal origin. The different results obtained with the two approaches is expected, since NGS detects all the genes present in a sample, regardless of the viability of the microorganisms, while the culture-dependent approach only enables the isolation and identification of bacteria resistant to the antimicrobials present in the culture media used. In short, these two approaches are complementary, if we are to gain a better understanding of the presence and potential transmission of resistance genes in foods of animal origin.

This work was funded by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/05183/2020. S. Conceição acknowledges a PhD fellowship from FCT under the reference UI/BD/153510/2022.

## Exploring Meagre migration through fatty acids profile of heart phospholipids

André Jorge<sup>1,2</sup>, Bernardo Quintella<sup>3,4</sup>, Marco Gomes da Silva<sup>2</sup> and M. João Lança<sup>5</sup>

<sup>1</sup>MED (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

<sup>2</sup>LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

<sup>3</sup>MARE - 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

<sup>4</sup>Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal

<sup>5</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Zootécnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: [af.jorge@campus.fct.unl.pt](mailto:af.jorge@campus.fct.unl.pt)

The meagre (*Argyrosomus regius*) is a sciaenid fish species with high commercial value (9.04 €/Kg)<sup>1</sup> and is highly appreciated by consumers due to its versatility when cooked. As a result, it is actively targeted by both commercial and recreational fisheries in Portugal, especially in the Tagus region.

Over the past decade, the Tagus estuary and surrounding areas have accounted for 60% to 70% of the country's total meagre landings, with annual catches averaging around 132 tonnes<sup>2</sup>. In 2023, annual meagre catches reached 264 tonnes<sup>1</sup>.

Despite its economic importance, there is limited knowledge about the species' finer-scale distribution, movement patterns, population dynamics in the wild, and feeding grounds in marine areas.

As part of the MIGRACORV project (<https://migracorv.pt/>), this study aims to evaluate whether the fatty acid (FA) signatures of the heart can be used to identify potential differences between the feeding areas of adult and juvenile meagres at sea, as feeding grounds and food availability significantly impact the proximate composition of the fish. This methodology is possible because phospholipid FAs are genetically controlled and can serve as natural markers<sup>3</sup>. Using various sample preparation methods and chromatographic techniques, we analyzed FA profiles in different phospholipid classes. The results indicate differences in the proportion of phospholipid classes between juveniles and adults, although the overall FA profiles in phospholipids remain similar.

#### Acknowledgements:

André Jorge would like to thank Fundação para a Ciência e Tecnologia (FCT) for a PhD scholarship UI/BD/153381/2022. This work was funded by National Funds through FCT - Foundation for Science and Technology under the Project PTDC/BIA-BMA/030517/2017 and the Portuguese Science Foundation through the strategy plan for MED (Mediterranean Institute for Agriculture, Environment and Development), via project UIDB/05183/2020, for MARE (Marine and Environmental Sciences Centre), via project UIDB/04292/2020, UID/50006/2020, and under the project LA/P/0069/2020 granted to the Associate Laboratory ARNET; and for LAQV-REQUIMTE, via project UIDB/50006/2020 and UIDP/50006/2020. This research was also anchored by the RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology.

#### References:

1. Instituto Nacional de Estatística - **Estatísticas da Pesca: 2023**. Lisboa: INE, 2024. Available online (accessed 6<sup>th</sup> August 2024) <[url:https://www.ine.pt/xurl/pub/439542305](https://www.ine.pt/xurl/pub/439542305)>. ISSN 0377-225-X. ISBN 978-989-25-0679-1
2. Mota, A. (2018) - Pescarias de Corvina-legítima na região do Estuário do Tejo: caracterização das componentes lúdica e comercial. Dissertação de Mestrado, Faculdade de Ciências, Lisboa, Universidade de Lisboa.
3. Lança, M.J.; Machado, M.; Mateus, C.S.; Lourenço, M.; Ferreira, A.F.; Quintella B.R. and P.R. Almeida (2014) – Investigating population structure of sea lamprey (*Petromyzon marinus*, L.) in Western Iberian Peninsula using morphological characters and heart fatty acids signature analysis. *PLoS ONE* 9(9)

## **Evaluation of the potential use of alternative flours to traditional flours in bakery and processed food production in São Tomé and Príncipe**

A.P. Quintas<sup>1</sup>, N. Martins<sup>2</sup>, M.J. Cabrita<sup>3</sup>, M.P. Duarte<sup>4</sup>, R. Garcia<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

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

Email: [anisiopatricio23@gmail.com](mailto:anisiopatricio23@gmail.com) ; [raquelg@uevora.pt](mailto:raquelg@uevora.pt)

The recent global crises have exposed the fragility of the agri-food system in its fundamental demand of satisfying the humanity's food needs and food security.

According to the Food and Agriculture Organization of the United Nations (FAO), wheat consumption has been increasing by around 2 to 5% per year. At the same time, population growth and wheat imports in São Tomé e Príncipe Island have increased. Since the world wheat production is expected to decrease in the next years, probably it is not sufficient to meet consumption needs. It is therefore crucial and urgent to have alternative flours in order to reduce the dependence on wheat flour. In order to decrease wheat flour imports in São Tomé and Príncipe (STP) and attempting to take advantage of local agricultural production, some food industries are producing flours from taro (locally known as “matabala”), cassava, sweet potato, breadfruit, banana bread and banana prata. Indeed, these locally produced flours are being included in the baking industry and other processed foods, such as cakes and cookies.

However, the knowledge of the physicochemical and nutritional properties of these flours is relatively scarce. Thus, it is crucial a deeply evaluation of the physical, chemical, sensory and microbiological characteristics of these flours, as well as the food products produced from them. Therefore, the aim of this work is the study of the physical, chemical and microbiological characteristics of flours produced in São Tomé and Príncipe. Moreover, on the other hand, food products will be made with these flours, namely bread and other processed foods, and their sensory analysis will be carried out aiming to assess their consumer acceptance.

In summary, the production of these flours using local agricultural production will enable the design of new and differentiated products with potential nutritional boost in the diets of children and young people and moreover could also improve the economic sustainability of São Tomé e Príncipe's producers.

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).

## **Chemometric investigation of the volatile content of young varietal wines from Alentejo**

S. Gastão -Muchecha<sup>1</sup>, N. Martins<sup>2</sup>, R. Garcia<sup>3</sup>, M.J. Cabrita<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Laboratório de Enologia, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: [sousa.gastao@uevora.pt](mailto:sousa.gastao@uevora.pt)

The volatile fraction of wines is one of the most important quality factors, defining their organoleptical character and wine typicity. The flavour of young wines results from a series of different biochemical and technological processes. Formation of volatile compounds begins in the grape, while during juice production, fermentation, maturation, ageing and storage continues to change. The amount and type of compounds that influence wine flavour therefore depend on many factors including the origin of the grapes, grape varieties and ripeness, soil and climate, yeast used during fermentation and a variety of other winemaking practices. For the present study, HS-SPME-GC/TOFMS methodology was used to identify and quantify the volatile content of varietal wine produced in University of Évora winery from *Aragonez*, *Castelão*, *Merlot*, *Syrah*, and *Trincadeira* grapes, produced in the years 2021 and 2022. The methodology used allows the identification of a wide range of volatile and semi-volatile compounds. The results of the analysis of the wines under study showed a variability between the volatile profiles of wines from different grape varieties. In conclusion, exhaustive studies of the volatile composition of wines are crucial to maintaining their typicity and intrinsic aromatic characteristics.

### Acknowledges and funding

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 -Muchecha)

## **A Comprehensive Review of Aromatic Plants and Essential Oils. From Extraction Techniques to Food Applications**

Margarida Oliveira<sup>1</sup>, Isabela Vera<sup>1</sup>, Solange Magalhães<sup>2</sup>, Hugo Duarte<sup>2</sup>, Carla Varela<sup>2</sup>, Carlos Cavaleiro<sup>2</sup>, Marta Laranjo<sup>1</sup>, Bruno Medronho<sup>1,3</sup>, Maria Graça Rasteiro<sup>2</sup>, Luís Alves<sup>2\*</sup>

<sup>1</sup>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, email: [miguidaoliveira@gmail.com](mailto:miguidaoliveira@gmail.com); [ivanjos@ualg.pt](mailto:ivanjos@ualg.pt); [mlaranjo@uevora.pt](mailto:mlaranjo@uevora.pt); [bfmedronho@ualg.pt](mailto:bfmedronho@ualg.pt)

<sup>2</sup>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, email: [solangemagalhaes@eq.uc.pt](mailto:solangemagalhaes@eq.uc.pt); [hmduarte@ualg.pt](mailto:hmduarte@ualg.pt); [carla.varela@uc.pt](mailto:carla.varela@uc.pt); [cavaleir@ff.uc.pt](mailto:cavaleir@ff.uc.pt); [mgr@eq.uc.pt](mailto:mgr@eq.uc.pt); [luisalves@ci.uc.pt](mailto:luisalves@ci.uc.pt)

<sup>3</sup>FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

\*Correspondence: [luisalves@ci.uc.pt](mailto:luisalves@ci.uc.pt)

Aromatic plants and essential oils have been esteemed for their diverse properties and applications across various industries, particularly in the realm of food applications. This comprehensive review delves into the intricate world of aromatic plants and essential oils, exploring their extraction techniques, unique physicochemical properties, and diverse applications in food areas settings. Beginning with an overview of the background and significance of these natural substances, the review delves into the properties of aromatic plants and essential oils, elucidating their chemical compositions and therapeutic effects. Various extraction technologies and characterization methods employed to extract and analyze these botanical essences are discussed, providing insights into the advancements in the field. Furthermore, encapsulation methods are examined for their role in enhancing stability and bioavailability in food applications. The review culminates in a thorough exploration of the myriad food applications of aromatic plants and essential oils, highlighting their roles in flavor enhancement, preservation, and functional food development. Through this comprehensive synthesis of literature, this review aims to provide a holistic understanding of essential oils and aromatic plants, bridging the gap between extraction methodologies and culinary innovation.

### **Acknowledgements**

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.

## Impact of Geographical Origin on the Quality and Composition of *Thymus mastichina* Essential Oil

Solange Magalhães<sup>1</sup>, Margarida Oliveira<sup>2</sup>, Isabela Vera<sup>2</sup>, Hugo Duarte<sup>1</sup>, Carla Varela<sup>1</sup>, Carlos Cavaleiro<sup>1</sup>, Marta Laranjo<sup>2</sup>, Bruno Medronho<sup>2,3</sup>, Maria Graça Rasteiro<sup>1</sup>, Luís Alves<sup>1\*</sup>

<sup>1</sup>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, email: [solangemagalhaes@eq.uc.pt](mailto:solangemagalhaes@eq.uc.pt); [hmduarte@ualg.pt](mailto:hmduarte@ualg.pt); [carla.varela@uc.pt](mailto:carla.varela@uc.pt); [cavaleir@ff.uc.pt](mailto:cavaleir@ff.uc.pt); [mgr@eq.uc.pt](mailto:mgr@eq.uc.pt); [luisalves@ci.uc.pt](mailto:luisalves@ci.uc.pt)

<sup>2</sup>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, email: [miguidaoliveira@gmail.com](mailto:miguidaoliveira@gmail.com); [ivanjos@ualg.pt](mailto:ivanjos@ualg.pt); [mlaranjo@uevora.pt](mailto:mlaranjo@uevora.pt); [bfmedronho@ualg.pt](mailto:bfmedronho@ualg.pt)

<sup>3</sup>FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

\*Correspondence: [luisalves@ci.uc.pt](mailto:luisalves@ci.uc.pt)

This study investigates the impact of geographical origin on the physicochemical properties of essential oil extracted from *Thymus mastichina* (Thyme), focusing on samples from Estremoz, Mértola, and Alcoutim in Portugal. Widely used in cosmetic, pharmaceutical, and food industries due to its therapeutic properties, *Thymus mastichina* essential oil's chemical profile is influenced by environmental factors, though the extent of this influence remains underexplored. To address this knowledge gap, samples from these regions were analyzed to assess the variability of bioactive compounds, including phenols, terpenes, and antioxidants. The samples experienced detailed physicochemical analyses, including gas chromatography-mass spectrometry (GC-MS), to identify and quantify key components of the essential oil. Preliminary results indicate significant variations in chemical composition among the regions, suggesting that factors such as local climate and soil type play a direct role in shaping the properties of *Thymus mastichina* essential oil. This study provides a deeper understanding of how geographical origin influences the quality and therapeutic potential of essential oils, offering valuable insights for the development of region-specific products with tailored properties.

### Acknowledgements

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.



**Influence of edible coatings on anthocyanin content during raspberry storage**Sofia Trindade<sup>1,2</sup>, Maria Inês Rouxinol<sup>2</sup>, Ana Cristina Agulheiro-Santos<sup>3</sup><sup>1</sup>Aluno de Doutoramento em Ciências Agrárias e Ambientais<sup>2</sup>MED-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, 7006-554 Évora, Portugal<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE (Instituto para as Alterações Globais e Sustentabilidade), Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, PortugalEmail: [d50890@alunos.uevora.pt](mailto:d50890@alunos.uevora.pt)

Anthocyanins are plant pigments that belong to the flavonoid group (a class of polyphenolic secondary metabolites found in plants). They are responsible for colors blue, purple, and red that can be found in plants, vegetables and fruits, so are enhance of the visual appeal of fruits. Anthocyanins can also play a crucial role in human health, being highlighted their antioxidant, anti-inflammatory, and anticancer properties. Nowadays, their importance in food marketability is unquestioned, with studies focused on understanding how precise genotype, fertilization techniques, storage and other factors can influence their concentration. Raspberries possess various anthocyanins, primarily cyanidin and pelargonidin glycosides, which contribute significantly to the fruits' antioxidant capacity and color. However, they are highly perishable fruits, which means a fast-ripening period and senescence, hindering storage and marketing. Edible coatings and similar techniques can be used to preserve the quality of these fruits during storage. The anthocyanins in raspberries undergo significant changes during storage, depending on the storage time and temperature. Therefore, the objective of this study was to evaluate how the anthocyanin content evolved throughout the storage of raspberries of the 'Clarita' variety with edible coating. The edible coating was made with *Opuntia ficus-indica* mucilage, extracted from cladodes produced by "PepeAromas"© in Alentejo region. The fruits were coated and stored at 2 °C, 90 % R.H. using two different concentrations of the edible coating, to understand how the concentration of the edible coating solution could affect the results. The trial was carried out over nine days, with samples analyzed on day zero, and every three days. The samples were analyzed by High Pressure Liquid Chromatography with Diode Array Detector (HPLC-DAD) according to Ivanovi et al.,( 2016). It was possible to identify four anthocyanins: cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-sophoroside. Cyanidin-3-sophoroside had the highest concentration (>516 mg/mL), and cyanidin-3-rutinoside the lowest (<11 mg/mL). As a final observation, we can say that there was an increase in anthocyanin content in the control group, and a decrease in the fruits with the edible coating. The concentration of the edible coating also influences the results, with a higher concentration resulting in a bigger decrease of anthocyanins.



## Harnessing Bacterial Cellulose: an innovative food Emulsifier from the Mother of Vinegar

Hugo Duarte<sup>1\*</sup>, Pedro Silva<sup>2</sup>, João Brás<sup>3</sup>, Marta Laranjo<sup>4</sup>, Luís Alves<sup>1</sup>, Célia Quintas<sup>2,3</sup>, Bruno Medronho<sup>3,5</sup>, Anabela Romano<sup>3</sup>

<sup>1</sup>University of Coimbra, CERES, Department of Chemical Engineering, 3030 790 Coimbra, Portugal

<sup>2</sup>University of the Algarve, Engenharia Alimentar, Campus da Penha, 8005-139 Faro, Portugal

<sup>3</sup>MED—Mediterranean Institute for Agriculture, Environment and Development, CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>4</sup>MED (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.

<sup>5</sup>Surface and Colloid Engineering, FSCN Research Center, Mid Sweden University, SE-851 70 Sundsvall, Sweden

\*correspondence: [uc49007@uc.pt](mailto:uc49007@uc.pt)

Bacterial cellulose (BC) has increasingly been used as a sustainable alternative material for applications in different fields. It can be obtained through the fermentation of vegetable matter, generally led by acetic or lactic acid bacteria and is formed during the vinegar production at the air-liquid interface, as a membrane known as the “Mother of vinegar”. Due to its inherent high molecular weight (Mw) and lack of solubility, BC needs to be depolymerized into fractions of lower Mws where each Mw population might be suitable for a specific application. Therefore, it is here proposed as well, the development of an innovative and integrated strategy of *ex situ* modifications to precisely achieve populations of BC with well-defined Mw. The approach will consider the joined action from suitable microorganisms (enzymatic), ultrasounds (mechanical) and green solvents (chemical). Moreover, differently charged BC derivatives will be researched, expanding the functionalization of the polymer and consequently its range of applications. Food emulsions are, in general, oil-in-water or water-in-oil, however multiple emulsions can be utilized. A suitable emulsifier is needed for an efficient emulsion formation, which usually can include proteins or low molecular weight surfactants. Here we intend to use the obtained BC derivatives as innovative emulsion stabilizers ideal to prepare spreadable oils and creams, and replacements for the commonly used emulsifiers in bread as mono- and diglycerides of fatty acids (E471). The function of such glycerides is mainly to improve the dough’s texture and quality, creating a stable emulsion of water and oil in the dough. However, these are usually obtained from sunflower, palm, or soybean oils which increasing demand expands deforestation, building up pressure on ecosystems. Given this, BC can be a more sustainable and alternative food additive as it requires minimal processing and can be obtained as a sub-product/residue from the vinegar production or even produced from agroforestry residues. Adding to this, the obtained BC derivatives will be tested as capsules for the delivery of essential oils.

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.

## **AlBread – Aromatic plants from Alentejo, probiotics and acorn flower for the development of functional bread**

Margarida Oliveira<sup>1</sup>, Isabela dos Anjos<sup>1</sup>, Hugo Duarte<sup>2</sup>, Solange Magalhães<sup>2</sup>, Célia Cabral<sup>3</sup>, Carla Varela<sup>2,3</sup>, Carlos Cavaleiro<sup>2</sup>, Maria da Graça Rasteiro<sup>2</sup>, Sandra Gonçalves<sup>1</sup>, Anabela Romano<sup>1</sup>, Bruno Medronho<sup>1,4</sup>, Marta Laranjo<sup>1</sup>, Miguel Elias<sup>1</sup>, Maria Eduarda Potes<sup>1</sup>, Ana Agulheiro-Santos<sup>1</sup> and Luís Alves<sup>2,\*</sup>

<sup>1</sup>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.

<sup>2</sup>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.

<sup>3</sup>University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Clinic Academic Center of Coimbra (CACC), Faculty of Medicine, Azinhaga Santa Comba, 3000-548, Coimbra, Portugal.

<sup>4</sup>FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

\*Correspondence: [luislaves@ci.uc.pt](mailto:luislaves@ci.uc.pt)

Bread is one of the most popular and ancient foods in the world, with a strong presence in the daily lives of populations due to easy access to this food. 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 and probiotic microorganisms is seen as essential, because of their good biological activities and beneficial impact on human health.

On the other hand, the use of gluten-free flowers 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 these endogenous resources from the Alentejo region, essential oils extracted from aromatic and medicinal plants and acorn, 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 maintaining of Montado, from where is obtained the acorn and the aromatic and medicinal plants.

### **Acknowledgements**

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.





**Changes in the volatile profile of *Citrus xlimon* leaves treated with ethanol and salicylic acid and infested by *Trioza erytrae* (Del Guercio, 1918)**

T. Magalhães<sup>1,2</sup>, D. Ruano<sup>2</sup>, A. I. Rodrigues<sup>2</sup>, N. Rodrigues<sup>2</sup>, A. Duarte<sup>1</sup>, N. T. Marques<sup>1</sup>, J. A. Pereira<sup>2</sup>

<sup>1</sup>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.

<sup>2</sup>CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.

Email: [ttmagalhaes@ualg.pt](mailto:ttmagalhaes@ualg.pt)

The psyllid *Trioza erytrae* is a vector of the devastating citrus disease Huanglongbing (HLB), caused by the bacterium *Candidatus Liberibacter* spp.. There is no viable cure for HLB, and its management is based on vector control and inoculum elimination. In 2014, the psyllid *T. erytrae* was identified in the Iberian Peninsula, which highlights the urgent need to develop novel vector control strategies. Previous studies have demonstrated that plant volatiles play an important role in plant defense and signaling mechanisms and that salicylic acid (SA) and ethanol (ET) are effective in stimulating plant defenses. The objective of this study was to assess the potential of SA and ET as defense-inducing agents in lemon plants (*Citrus xlimon*) against this psyllid. The impact of SA and ET on the profile of volatiles emitted by lemon leaves was evaluated, both in control and infested plants. The experimental design comprised six treatments, each with five plants: 1) control (sprayed with water), 2) ET sprayed, 3) SA sprayed, 4) water sprayed plus infested, 5) ET sprayed plus infested, 6) SA sprayed plus infested. Leaf volatiles were quantified 25 days following the spray application. The infestation with adult *T. erytrae* psyllids was conducted 24h after the spray application. The total number of *T. erytrae* nymphs developed in infested samples was counted 23 days after infestation, and leaves were used immediately for leaf volatile evaluation. Leaf volatiles were adsorbed via headspace solid-phase microextraction (HS-SPME) and analysed through gas chromatography-mass spectrometry (GC-MS). The number of nymphs per plant was higher in sprayed plants with 310 in ET sprayed plants and 314 in SA sprayed plants, in comparison to the control plants that exhibited only 201 nymphs per plant. The greatest number of differentially abundant volatiles (DAV) was observed in plants sprayed with SA and subsequently infested, with 82 DAV, followed by plants sprayed with ET and subsequently infested, with 33 DAV. The remaining treatments showed 13, 15 and 16 DAV, for ET, SA and infested treatments, respectively. Sprays of ET and SA impacted the profile of leaf volatiles, despite having no effect on the plant's defence against *T. erytrae*.

This work was funded by “European Union Horizon 2020”, through the research project Grant agreement ID: 817526 (Pre-HLB “Preventing HLB epidemics for ensuring citrus survival in Europe.”). T. M. acknowledges FCT 2020.07798.BD (PhD grant) (<https://doi.org/10.54499/2020.07798.BD>).

## **Effects of PM<sub>10</sub> from Luanda on the viability of human A549 alveolar epithelial cells**

M. Soares<sup>1</sup>, C. Alves<sup>1</sup>, H. Oliveira<sup>2</sup>

<sup>1</sup>Department of Environment and Planning, Centre for Environmental and Marine Studies, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup>Department of Biology, Centre for Environmental and Marine Studies, University of Aveiro, 3810-193 Aveiro, Portugal

Email: [marlene98@ua.pt](mailto:marlene98@ua.pt)

Atmospheric particulate matter lower than 10 µm (PM<sub>10</sub>) is a major environmental concern, due to its ability to penetrate into the respiratory system, with the finer fractions capable of reaching the alveoli. The genotoxic, mutagenic, and carcinogenic properties of PM<sub>10</sub> represent significant risk factors leading to serious health outcomes, such as heart disease, stroke, COPD (chronic obstructive pulmonary disease), and lung cancer. PM<sub>10</sub> induces or enhances the generation of reactive oxygen species (ROS), leading to DNA damage and cell death. According to satellite data, African cities rank among the most polluted in the world, yet studies linking air quality to health-related outcomes are still missing. This study aimed to investigate the impact of organic extracts from PM<sub>10</sub> collected in Luanda, Angola, on the viability of A549 cells (human adenocarcinoma alveolar epithelial cell line) over 48- and 72-hours of exposure. Daily PM<sub>10</sub> samples were collected in a central location of this African megacity with a high-volume sampler for 6 months (July to November 2023). Representative filters of each month were selected to perform toxicity assays. To assess cell viability, A549 cells were exposed to increasing concentrations of the PM<sub>10</sub> extracts for 48- and 72-hours followed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. For the highest concentrations, the results showed that exposure to PM<sub>10</sub> extracts significantly reduced A549 cell viability, with the effects persisting over the longest exposure time. A 72-hour exposure resulted in a higher percentage of cell death compared to 48-hour exposure, although all extracts caused a statistically significant reduction in cell viability at both time points. These findings highlight the need for air quality monitoring and intervention strategies, aiming to mitigate the adverse health impacts associated with PM<sub>10</sub> pollution in Luanda and similar urban settings.

An acknowledgment is given to the Portuguese Foundation for Science and Technology (FCT) for funding the PhD grant 2020.04826.BD. This work was supported by the project APAM - "Air Pollution in an African Megacity: Source Apportionment and Health Implications" (2022.04240.PTDC), financially supported by national funds (OE), through FCT/MCTES (DOI: 10.54499/2022.04240.PTDC). The financial support to CESAM by FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), through national funds, is also acknowledged.

## **Improvement of agronomic, environmental and economic sustainability of processing tomato production system in Portugal through the application of Conservation Agriculture principles**

R. V. Santos<sup>1</sup>, G. Basch<sup>1,2</sup>, A. Rato<sup>1,2</sup>, M.R. Martins<sup>3,4</sup>

<sup>1</sup>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

<sup>2</sup>Departamento de Fitotecnia, Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>Laboratório HERCULES & IN2PAST, Universidade de Évora, 7000-809 Évora, Portugal

<sup>4</sup>Departamento de Ciências Médicas e da Saúde, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora, 7000-809 Évora, Portugal

Email: [rjvs@uevora.pt](mailto:rjvs@uevora.pt)

The current processing tomato production system in Portugal is based on monoculture, intensive soil tillage and fallow between tomato crops. These practices create sustainability concerns related to pest management, soil degradation and nutrient leaching. Conservation Agriculture (CA) based on principles: 1) reduced soil disturbance, 2) permanent soil cover with plants or residues and, 3) diversified crop rotation, could originate solutions opportunities. A 2-year field trial (2021/2022 and 2023/2024) was conducted comparing the current system (Conventional) against 2 CA systems based on: i) strip-till and cover crop (TomCC); and ii) strip-till, cover crop, and tomato-sunflower/maize rotation (Rotation). The main goal was to improve the agronomic, environmental and economic sustainability of the system.

The total nitrogen retention in cover crop biomass in TomCC was 91 and 127 kg ha<sup>-1</sup> in 2021/2022 and 2023/2024 respectively, and in Rotation reached 123 kg ha<sup>-1</sup> in 2021/2022 and 2023/2024, respectively, both significantly higher than in spontaneous vegetation of the Conventional with 5 and 35 kg ha<sup>-1</sup> in 2021/2022 and 2023/2024 respectively. The results clearly indicate the potential of cover crops to mitigate nitrogen leaching. The strip-till used for tomato plantation resulted in a satisfactory crop establishment in both CA systems, despite a significantly higher seedling mortality rate in Rotation in 2021/2022 with 2,50% and in TomCC in 2023/2024 with 20,67%, against Conventional with 0,33% in 2021/2022 and 4,00% in 2023/2024. The lower plant density did not compromise the marketable tomato fruit yield in both years, which in TomCC was 91,663 and 17,024 t ha<sup>-1</sup> in 2021/2022 and 2023/2024, respectively, did not differ from Conventional with 75,428 and 19,216 t ha<sup>-1</sup> in 2021/2022 and 2023/2024, respectively, but was significantly higher in Rotation reaching 109,845 and 32,206 t ha<sup>-1</sup> in 2021/2022 and 2023/2024, respectively. Although an increase in costs linked to cover crop and strip-till practices (+193,26 € ha<sup>-1</sup> in 2021/2022), both CA systems presented a higher profit than Conventional, due to higher tomato fruit yield.

The CA practices of cover cropping, strip-tillage and specially crop rotation demonstrated potential to improve the agronomic, environmental and economic sustainability of the Portuguese processing tomato production system.

This work was funded by Syngenta Crop Protection under the project TomAC - Conservation Agriculture approaches in tomato growing, and by Fundação para a Ciência e Tecnologia through the grant number 2021.07037.BD (<https://doi.org/10.54499/2021.07037.BD>). The authors acknowledge to the support of Aposolo, Sogepoc and Ag-Innov, and to research centres MED (<https://doi.org/10.54499/UIDB/05183/2020>) and Change (<https://doi.org/10.54499/LA/P/0121/2020>).

**Developing a CRISPR/RfxCas13d system against ToBRFV in tomato plants**

J. A. Ribeiro<sup>1</sup>, S. D' Adamo<sup>2</sup>, M. D. Campos<sup>3</sup>, A. Albuquerque<sup>1</sup>, M. Patanita<sup>1</sup>, F. Santos<sup>1</sup>, T. Monteiro<sup>1</sup>, M. Basaloco<sup>1</sup>, C. M. R. Varanda<sup>1,4</sup>, P. Materatski<sup>1</sup>, M. R. Félix<sup>3</sup>

<sup>1</sup>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.

<sup>2</sup>Bioprocess Engineering, AlgaePARC, Wageningen University and Research, PO Box 16, 6700 AA Wageningen, The Netherlands.

<sup>3</sup>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.

<sup>4</sup>CERNAS – 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.

Email: [joanaar@uevora.pt](mailto:joanaar@uevora.pt)

*Tomato brown rugose fruit virus* (ToBRFV) was first identified in Jordan in 2015 and since then it has spread globally, affecting a great number of tomato-growing countries and causing severe constraints in tomato cultivation such as the decrease of fruit quality and yield. Very recently this virus was renamed by the International Committee on Virus Taxonomy as *Tobamovirus fructirugosum*. Although it belongs to the *Tobamovirus* genus, it can overcome the known tobamovirus genetic resistance conferred by the Tm-1, Tm-2 and Tm-2<sup>2</sup> R genes, highlighting the need for long-term sustainable control measures relying on the development of new molecular technologies. One of the most promising tools that has been explored in recent years is based on an adaptative immune system against viruses found in prokaryotes, the Clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR/Cas). The first CRISPR/Cas systems studied were very useful for DNA targeting, but more recently identified types can specifically cleave single-stranded RNA (ssRNA) in eukaryotic cells, which was a major breakthrough, especially since most plant viruses have RNA genomes. The RfxCas13d variant has shown structural and functional advantages over the other variants, making it the best choice for generating resistance against RNA viruses. This work aimed to design and develop a plasmid containing the CRISPR/RfxCas13d system within a pk7WG2 vector, for expression first in *Nicotiana benthamiana* and then in tomato plants. Additionally, several plasmids were constructed with different RNA guides to specifically target the ToBRFV, for further testing and efficiency assessment in blocking the infection. RfxCas13d expression levels and ToBRFV interference efficacy will be measured to evaluate system performance. This work contributes to the development of virus-resistant plants through CRISPR/Cas systems and has potential applications in molecular diagnostics.

This work is funded by Portuguese National Funds through Foundation for Science and Technology (FCT) under the PhD scholarships 2022.13638.BD, attributed to Joana Ribeiro, and under the project UIDB/05183/2020 (MED)



exploratory project “CRISPR/Cas13 a new technology against plant pathogens: the case of Tomato brown rugose fruit virus”).

Oral Communication 9

## **Potentially Toxic Elements Contamination of soil and compost from Urban Farms in the Porto Metropolitan Area and its Impact on Food Safety**

Lourenço Pinto de Rezende, Joana Bastos Barbosa, Paula Teixeira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

Email: [pinto.rezende88@gmail.com](mailto:pinto.rezende88@gmail.com)

The correlation between urban agriculture, food security, and mental well-being is well documented. However, the safety of consuming products from urban agriculture remains a subject of debate. From the latter half of the 19th century, the Porto Metropolitan Area (PMA) became one of the most significant industrial centres in the Iberian Peninsula, resulting in a lasting environmental impact on the region. Today, the PMA is home to over 1.75 million residents, and although much of the industry has shifted to the outskirts, the environmental legacy of past industrial activity persists. This study assessed the contamination of potentially toxic elements (PTE) in soil and compost samples from 12 urban farms within the PMA. These results were analysed according to national and international regulations concerning permissible levels of these elements in agricultural soils.

Widespread contamination with zinc, lead, copper and chromium was observed in soil samples. Zinc levels exceeded the Italian legislation (IL) threshold in five farms, while lead concentrations surpassed both the Swedish guidelines (SG) and IL in nine and five farms, respectively. Soil samples from four farms contained copper concentrations above the FAO/EU regulations, likely due to excessive use of Bordeaux mixture. Additionally, three farms exhibited substantial chromium contamination, with two exceeding FAO guidelines. In at least one of these farms, chromium contamination may be linked to historical tanning activity in the area. Given the potential role of fertilisation in metal and metalloid contamination, organic fertilisers, primarily compost, used in these farms were also analysed. Extremely high zinc concentrations, surpassing the FAO guidelines, were observed in five samples, with one exceeding the European legislative limit. While lead concentrations remained within acceptable limits, copper levels in three fertilisers exceeded both European and FAO standards. Although chromium concentrations in the fertilisers were lower than those observed in soils, the presence of this element in high concentrations is concerning.

The long-term sustainability of urban farming requires safe environments to ensure safe products. In this study, substantial metal contamination was observed in urban farms within the PMA, highlighting the need for increased monitoring of both the environment and agricultural practices in these settings.

Financial support for the author Lourenço Pinto de Rezende was provided by the doctoral fellowship 2022.13871.BD

## **Environmental factors-induced oxidative stress alters osmolyte and secondary metabolites production in the aromatic plant *Lavandula viridis***

Inês Mansinhos, Sandra Gonçalves, Anabela Romano

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

E-mail: [ifmansinhos@ualg.pt](mailto:ifmansinhos@ualg.pt)

One of the biggest threats to plants is their ability to withstand unfavorable climatic circumstances, particularly in light of climate change. *In vitro* simulation of stressful environmental conditions is an appropriate method to study how plants react to those factors given that it is carried out under a controlled environment and is free of pathogens, which allows plant material production on a large scale and without jeopardizing the species conservation. The main goal of this work was to assess how different abiotic stressors affect some biochemical traits and secondary metabolites production on *Lavandula viridis* L'Hér, an aromatic species endemic to the Iberian Peninsula. *In vitro*-produced cultures of *L. viridis* were separately exposed to salinity, temperature, or UV-B radiation conditions. After each treatment, photosynthetic pigments, oxidative stress markers [ $H_2O_2$ , malondialdehyde (MDA)], soluble sugars, phenolic profile, and associated antioxidant activity were assessed. In addition, the effect of these abiotic factors on the essential oil profile of micropropagated plants of *L. viridis* was also evaluated. Cultures and plants grown in non-stressful conditions were used as control. It was observed that temperature induced the highest oxidative stress, evidenced by the contents of  $H_2O_2$  and MDA. Heat stimulated the biggest level of lipid peroxidation, with a direct negative consequence on total chlorophylls and carotenoids. On the other hand, phenolic compounds and antioxidant activity, as well as the osmolytes soluble sugars, were confirmed to have an important function in counteracting oxidative stress. Temperature was shown to be an excellent stimulus to the production of rosmarinic acid, the most important phenolic compound of *L. viridis*. Concerning the essential oil profile, the three environmental factors tested induced the production of the most abundant volatile components of this plant (1,8-cineole and camphor). These findings shed new light on how secondary metabolites and soluble sugars respond to unfavorable environmental conditions in *L. viridis* and demonstrate that *in vitro* culture is a viable option for producing volatile and phenolic compounds with biological properties.

This research was financed by National Funds through FCT-Foundation for Science and Technology under the Projects UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>) and LA/P/0121/2020 (<https://doi.org/10.54499/LA/P/0121/2020>). Inês Mansinhos (<https://doi.org/10.54499/SFRH/BD/145243/2019>) and Sandra Gonçalves (CEECINST/00052/2021) acknowledge the financial support from FCT.

## Geochemical dynamics and risk assessment of potentially toxic metals in irrigated agricultural soils of the Mediterranean region

Custódio, Mariana<sup>1,2</sup>, Catarino, Adriana<sup>1,2,3</sup>, Tomaz, Alexandra<sup>1,3,4</sup>, Dôres, José<sup>1</sup>, Patanita, Manuel<sup>1,4</sup>, Palma, Patrícia<sup>1,2,3,4</sup>

<sup>1</sup>Escola Superior Agrária, Instituto Politécnico de Beja, Beja, Portugal

<sup>2</sup>Center for Sci-Tech Research in Earth system and Energy (CREATE), Instituto Politécnico de Beja, Beja, Portugal

<sup>3</sup>Instituto de Ciências da Terra (ICT), Universidade de Évora, Évora, Portugal

<sup>4</sup>GeoBioTec: Universidade Nova de Lisboa, Campus da Caparica, Caparica, Portugal

E-mail of the presenting author: [mariana.custodio@uevora.pt](mailto:mariana.custodio@uevora.pt)

The European Commission report “Caring for soil is caring for life” reveals that 60-70% of European soils are unhealthy due to current management practices. Human activities have led to the accumulation of potentially toxic metals (PTMs) and other pollutants in the soil. This accumulation can disrupt soil function, degrade crop quality, and ultimately poses risks to human health through the food chain. So, the objectives of this study were: (i) to assess the level of PTMs contamination in agricultural soils from the Alentejo region (South of Portugal); and (ii) to determine soil pollution risks using several indices based on chemical and biological parameters.

The study was conducted in 10 agricultural soils (S1-S10), from the Brinche-Enxoé hydroagricultural sub-system (Alqueva system; Alentejo region), over 5 campaigns between 2018 and 2020. Samples occurred at the beginning (T1, T3), and end (T2, T4, T5) of the irrigation campaigns. Soil agronomic parameters, such as electrical conductivity, pH, and soil organic matter content were determined. The PTMs content was evaluated by inductively coupled plasma mass spectrometry (ICPMS). The degree of contamination with PTMs was assessed using the following pollution indices: Enrichment Factor (EF), Potential Ecological Risk Index (PERI), and Geoaccumulation Index (I<sub>geo</sub>).

The soils presented slightly alkaline pH (7.7-8.6), low percentage of organic matter (0.42-2.92%), and low electrical conductivity values (124.1-867.0  $\mu\text{S}/\text{cm}$ ). Regarding the PTMs quantification, the values of Cr, As, and Ni have surpassed the Portuguese limits for the good quality of agricultural soils in several campaigns (Cr: 64.41-76.11; As: 11.31-23.02, and Ni: 37.14-58.90 mg/kg). The EF values were always lower than 1.5, which points out that PTMs have a natural source, and the *I<sub>geo</sub>* results, from -9.61 to -0.42, supported this conclusion. The PERI values, ranging from 7.13 to 41.06, indicated that PTM levels posed a low ecological risk to the ecosystem. Although concentrations of certain PTMs exceed recommended limits for high-quality agricultural soils, most of these levels are naturally occurring, as the soils are part of the geologically rich Ossa-Morena Zone (OMZ). Moreover, the ecological risk index analysis revealed that PTM concentrations did not pose a significant risk to the agroecosystem.

The study is co-financed by the European Union by the European Regional Development Fund, included in COMPETE 2020 (Competitiveness and Internationalization of the Operational Program) through the project Institute of Earth Sciences (ICT; UIDB/04683/2020) with the reference POCI-01-0145-FEDER-007690, of the GeoBioTec project (UIDB/04035/2020) (funded by FCT – Foundation for Science and Technology), and by the European Agricultural Fund for Rural Development through the FitoFarmGest Operational Group (PDR2020-101-030926).

## Evaluating Co-Digestion of Olive Mill Wastewater and Olive Leaves for Biogas Production

Sofia Zantedeschi<sup>1</sup>, Francesca Valenti<sup>1</sup>, Mirko Maraldi<sup>1</sup>, Gonzalo Augustin Martinez<sup>2</sup>, Matilde Tura<sup>1,3</sup>, Enrico Valli<sup>1,3</sup>, Tullia Gallina Toschi<sup>1,3</sup>

<sup>1</sup>Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna, Viale Fanin, 40-50 and Piazza Goidanich, 60, 40127 Bologna and 47521 Cesena, Italy

<sup>2</sup>Department of Civil, Chemical, Environmental and Materials Engineering (DICASM), Alma Mater Studiorum - Università di Bologna, Via Terracini 28, 40131 Bologna, Italy

<sup>3</sup>Interdepartmental Centre for Industrial Agrofood Research, Alma Mater Studiorum - Università di Bologna, Cesena, Italy

Email: [sofia.zantedeschi2@unibo.it](mailto:sofia.zantedeschi2@unibo.it)

The olive oil industry, deeply rooted in Mediterranean culture and expanding globally, generates large quantities of by-products, including olive leaves, branches, liquid effluents, and solid residues. Efficiently managing these by-products could enable innovative applications, create secondary resources, and provide on-site energy solutions while reducing landfill dependency and environmental impact. In particular, olive leaves, separated during the preliminary cleaning at the mill, are typically dispersed onto nearby fields—an approach that entails handling costs and leaves out their possible valorization. This research investigates whether olive mill wastewater (OMWW) can be processed with olive leaves in a co-digestion setup to support biogas production with enhanced methane quality, offering a sustainable waste management approach within the olive oil sector.

Using biochemical methane potential (BMP) testing, the study evaluates how adding a small proportion of olive leaves influences the methane concentration and overall quality of biogas produced from OMWW. By comparing mono-digestion of OMWW with a combined OMWW and olive leaf digestion process, the research explores whether this by-product combination could yield a more methane-rich biogas.

Preliminary results suggest that co-digesting OMWW with a small addition of olive leaves may increase methane content, improving biogas quality by achieving a higher methane-to-carbon dioxide ratio, without reducing the overall biogas volume. Future research will aim to determine the highest effective proportion of olive leaves suitable for co-digestion, optimizing both total biogas production and methane levels. This strategy demonstrates an effective approach to utilizing olive oil production residues, simultaneously addressing environmental concerns and supporting energy resource generation. Adopting circular economy principles, the study points toward a more resource-efficient and environmentally responsible model for managing agricultural by-products in industrial processes.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

## Dynamic Analysis and Risk Assessment of Pesticides in the Lage Hydroagricultural Reservoir

Adriana Catarino <sup>a,b,c,d</sup>, Júnia Alves Ferreira <sup>a,b,c</sup>, Manuel Garcia Vara <sup>e</sup>, Mariana Custódio <sup>a,c</sup>,  
Clarisse Mourinha <sup>a,c</sup>, Pedro Anastácio <sup>d</sup>, Miren Lopez de Alda <sup>e</sup>, Patrícia Palma <sup>a,b,c,f</sup>

<sup>a</sup>Escola Superior Agrária, Instituto Politécnico de Beja, R. Pedro Soares S/N, 7800-295 Beja, Portugal

<sup>b</sup>Instituto de Ciências da Terra (ICT), Universidade de Évora, 7000-671, Évora, Portugal

<sup>c</sup>Center for Sci-Tech Research in Earth system and Energy (CREATE), Pole of the Polytechnic Institute of Beja, 7800-259 Beja, Portugal

<sup>d</sup>Marine and Environmental Sciences Centre (MARE), Universidade de Évora, 7000-671 Évora, Portugal

<sup>e</sup>Water, Environmental and Food Chemistry Unit (ENFOCHEM), Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 080834 Barcelona, Spain

<sup>f</sup>GeoBioTec, NOVA School of Science and Technology, Campus da Caparica, 2829-516 Caparica, Portugal

Email: [adriana.catarino@ipbeja.pt](mailto:adriana.catarino@ipbeja.pt)

Pesticides are increasingly used to boost farm productivity and protect crops from specific pests; however, their presence can disrupt terrestrial and aquatic ecosystems, making regular monitoring essential. This study aimed to evaluate pesticide concentrations and assess their aquatic risk in the Lage Reservoir, part of the Alqueva Hydroagricultural System in southern Portugal. The monitoring campaign was performed in two points (L and LS) of the reservoir, at four different times (Ap/M, Jl, Sp, and Dc), during two years (2020 and 2021). Fifty-one pesticides, belonging to the classes of acidics, anilides, carbamates, chloroacetanilides, neonicotinoids, organophosphates, phenylureas, quinolones, sulfonylurea and triazines, were quantified for LC/MS-MS. The environmental risk assessment was calculated based on the risk quotient (RQ) methodology. The results revealed that the highest amounts of total pesticides were observed in the dry period (May 2020) with 727.5 and 940.5 ng L<sup>-1</sup> in L and LS, respectively. Notably, MCPA, metolachlor, terbuthylazine, and terbutryn were detected in all samples. During the study, several pesticides no longer authorized in Portugal (including alachlor, atrazine, CFP, cyanazine, diazinon, diuron, irganol, isoproturon, propanil, simazine, and terbutryn) were detected, some of which posed a high environmental risk (CFP: RQ= 4.49; diazinon: RQ=6.35; irganol: RQ=13.76; terbutryn: RQ=1.92). A similar trend is observed with other pesticides still in use, which also show high RQ values, including azinphos methyl oxon (RQ = 119.00), diflufenican (RQ = 9.39), imidacloprid (RQ = 7.48), terbuthylazine (RQ = 1.93), and thiacloprid (RQ = 2.34). Analysis of the results indicated that several pesticides were present at concentrations that could pose an environmental risk to aquatic ecosystems, most of which are already banned from commercialization in Portugal.

### Acknowledgements

The work is co-funded by National funds through FCT – Fundação para a Ciência e Tecnologia, I.P. (projects UIDB/04683/2020 and UIDP/04683/2020), the European Agricultural Fund for Rural Development through the FitoFarmGest Operational Group (PDR2020-101-030926). This work is part of Adriana Catarino's PhD with scholarship 2023.04004.BD (<https://doi.org/10.54499/2023.04004.BD>).

## Hydrologic, erosive and phytotoxic responses of biochar and mulch: comparing soil-mixed and surface-applied effects in agricultural soils

Canedo, J.N.G.V.<sup>1</sup>, Coelho, L.<sup>1</sup>, Castro, L.<sup>2</sup>, Lameira, B.<sup>1</sup>, Stoutjesdijk, E.<sup>3</sup>, Keizer, L.<sup>3</sup>, Bles, M.<sup>3</sup>, Verheijen, F.<sup>4</sup>, Prats, S.<sup>1,5</sup>

<sup>1</sup>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.

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

<sup>3</sup>Wageningen University and Research. Droevendaalsesteeg, 4, 6708 PB Wageningen, Netherlands.

<sup>4</sup>Universidade de Aveiro, Campus Universitário de Santiago, Av da Universidade de Aveiro, 3810-193 Aveiro, Portugal.

<sup>5</sup>Misión Biológica de Galicia – National Spanish Research Council (MBG-CSIC), 36143 Salcedo, Pontevedra, Spain.

Email: [joao.canedo@uevora.pt](mailto:joao.canedo@uevora.pt)

The intensification of agriculture frequently contributes to soil degradation, increasing erosion and driving desertification. Organic mulching is a nature-based solution that reduces surface runoff, soil erosion and increases soil organic matter. Biochar, the product produced through the pyrolysis of biomass, can also have a positive impact in the recovery of degraded soils. The main objective of this study was to assess the effect of biochar and straw mulch on soil hydrology and erosion under different application approaches, such as soil-mixed (2.82% of biochar concentration) and surface-applied (10Mgha<sup>-1</sup> of biochar with 2Mgha<sup>-1</sup> of mulch on top), by performing rainfall simulations in two sandy loam soils under integrated management, one from a vineyard and one from an olive orchard, both from the southern Portugal Alentejo region. Nine repetitions of a 30 minutes simulated rainfall event with 85.6mmh<sup>-1</sup> intensity were applied to each treatment. We also tested how the different treatments affect germination/plant length and physical properties of the leaching water, through germinations tests and pH/EC measurements, respectively.

The bare soil treatment had an average runoff of 17mm. The biochar mix treatment reduced the runoff amount to an average figure of 8mm. The layered treatments also showed lower runoff volumes of 7, 2 and 5mm, respectively for the biochar, mulch and M+B layers. On the other side, leaching and soil water storage in the bare soil was the lowest, with only 9 and 10.8mm, respectively. Leaching was increased by 1.3, 1.6, 1.9 and 2 times, and soil water storage by 1.08, 1.34, 1.06 and 1.05 times, respectively for the biochar mix, biochar, mulch and M+B layers. Interrill erosion was the highest in the bare soil and it was reduced significantly by 55% in the biochar mix and by 59, 81 and 77% in the biochar, mulch and M+B layers, respectively. Plant length was increased significantly in all treatments with biochar (from 29 to 45% in the root and 15 to 39% in the shoot), in comparison with the bare soil. These same treatments also significantly increased soil EC, from 23 to 36%, which is directly correlated to soil fertility.

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

## Evaluation of Phenolic Content, Antioxidant Potential and Enzyme Activities in *Vitis vinifera* (var. 'Aragonês') Extracts as Indicators of Grape Quality

G. Carneiro Murta<sup>1</sup>, E.M.M Vaquinhas<sup>2</sup>, A.E Rato<sup>3</sup>, M.R Martins<sup>4</sup>, M.J Cabrita<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>Departamento de Química e Bioquímica, Escola de Ciências e Tecnologia, Universidade de Évora, Rua Romão Ramalho, 59, 7002-671 Évora, Portugal

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

<sup>4</sup>Departamento 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

Email: [gabriela.murta@fa.uevora.pt](mailto:gabriela.murta@fa.uevora.pt)

The wine industry is essential to the economy of Alentejo, one of the largest wine-producing regions of Portugal. During the maturation process, grapes naturally produce important compounds, including polyphenols and other secondary metabolites, which contribute to the production of high-quality wine. To enhance wine production quality, various vineyard treatments were applied, including foliar protectants and biostimulants such as kaolin, silicon, and *Opuntia ficus-indica* extract, to reduce oxidative stress in plants throughout the maturation cycle. This study aims to evaluate the phenolic content and antioxidant potential of grape skin extracts from the *Vitis vinifera* 'Aragonês' grape variety, as well as to monitor the enzymes activity associated with oxidative stress. The characterization of phenolic content and antioxidant activity of grapes harvested was performed at two stages of ripeness: mid-version and harvest time. Additionally, the activity of key enzymes, involved in the oxidative stress response and used as grape quality indicator, such as Superoxide Dismutase (SOD), Catalase (CAT), and Polyphenol Oxidase (PPO), was evaluated at the end of maturation. The results showed a significant increase in phenolic content and antioxidant capacity throughout maturation, particularly in the treatments with "2% kaolin" and "2% silicon." In addition, a reduction in the enzymatic activities of SOD, CAT, and PPO was observed, especially in the combined treatment of "2% kaolin + 2% silicon," indicating reduced plant exposure to oxidative stress. These findings suggest that the tested treatments effectively enhance stress resistance, contributing to the production of high-quality grapes, essential for wine production. Future studies are planned to further assess the efficacy of these and other treatments, with repeated applications throughout maturation and in different grape varieties. This is expected to provide a more detailed understanding of the processes involved, promoting the sustainability and quality of wine production in Alentejo, a region of great importance to the Portuguese economy.

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.



## **Why soil health? Stakeholders' motivations, priorities and values behind soil regeneration in the context of Montado**

Greta Winkler<sup>1</sup>, Maria Teresa Pinto Correia<sup>2</sup>

<sup>1</sup>Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, via L Borsari, 46, 44121 Ferrara, Italy.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: [greta.winkler@unife.it](mailto:greta.winkler@unife.it)

The concept of soil health reflects its understanding as a living ecosystem and its ongoing capacity to promote the pertinent functions of the land in which it is embedded. Yet soils are (mostly) privately owned assets, capable of delivering ecosystem services in the form of both private and public goods, but lacking a property rights definition that ensures a public goods interest. Stakeholders directly involved in soil management, such as farmers and landowners affect the capacity of soil to be, remain or return in a healthy status. The determination of soil's assigned value by individuals or communities remains under-explored in the literature. Improving the understanding of actors' beliefs and perceptions of soils in their contexts could help to develop new instruments of soil governance taking the value system into account.

Objective of this study is to explore a value-based understanding of soil health in the context of Montado, Alentejo, Portugal. In particular, to explore what values motivate farmers to enhance soil health, to what extent they are aware of benefits of soil health to their farming activity and durability, and what is needed to increase motivation and adoption of sustainable soil management practices. Data collection will be twofold: firstly, 15 -20 interviews with open and close ended questions will be conducted with key stakeholders participating to soil regeneration projects in the Montados of Alentejo. Target group are stakeholders already committed to soil regeneration practices. Secondly, and based on the answers, a survey will be designed and distributed to larger farmers' networks, to investigate understanding and non-adoption of soil restoration practices and assigned values.

Exploring understanding and valuation of soil health can help shedding light on priorities and motivations of farmers, as well as to inform current governance and incentive systems for more coherent and long-term action at territorial level.



## **Enhancing Buckwheat Breeding and Agronomic Performance Through Genomic Selection and Climate Adaptation Strategies**

Getalew Ayizengaw Chana, Emanuele De Paoli, Elisa Marraccini

University of Udine. Department of Agricultural, Food, Environmental and Animal Science (DI4A) via delle Scienze, 206, 33100 Udine, Italy.

E-mail: [chana.getalewayizengaw@spes.uniud.it](mailto:chana.getalewayizengaw@spes.uniud.it)

This PhD project tackles the significant challenge of enhancing breeding strategies and improving the agronomic performance of common buckwheat (*Fagopyrum esculentum* Moench), an essential crop for sustainable agriculture and a valuable plant protein source with minimal input requirements, particularly in the face of climate change. Buckwheat's reproductive system prevents the generation of inbred lines and the exploitation of heterosis. Still, this crop can be addressed with a modern approach to speed up breeding through genomic selection (GS). GS consists of building phenotype prediction models based on phenotypic and genotypic data from training populations, allowing for reducing the burden of phenotyping multiple generations. In this project, different approaches will be implemented to facilitate the application of GS to buckwheat breeding, including (i) exploitation of the genetic diversity in the available germplasm and (2) innovative technologies, such as hyperspectral imaging, for better predictive models. These are expected to significantly streamline the breeding process for the identification of varieties that are superior in drought tolerance, yield, and nutritional value. The second part of the project includes comprehensive variety adaptation trials aimed at adjusting sowing times to better equip the crop for climate-related challenges like spring frosts and summer heat waves, also given the increasing interest for this crop in low-input cropping systems. Therefore, this study will assess multiple varieties across a range of scenarios. And comprehensive evaluation of growth patterns, yield, and quality attributes will be conducted to identify varieties that offer consistent performance, resilience, and reliable options for different sowing seasons. Altogether, these methodologies aim to provide new tools and information for buckwheat cultivation, a major crop of increasing interest. By integrating cutting-edge selection techniques with agronomic trials, we will strive to produce resilient, high-yielding varieties, contributing to efforts toward sustainable food production and security.

This work is funded by the National Recovery and Resilience Plan (PNRR) with project code DOTT39PNRR118\_DI4A\_AGR\_BUDG1.2\_CHA.

## Testing a mass-capture trap prototype for the olive fly (*Bactrocera oleae*)

Dias, Ivo L. <sup>1\*</sup>; Rei, Fernando T. <sup>2</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, University of Évora, Évora, Portugal

Email: [d54251@alunos.uevora.pt](mailto:d54251@alunos.uevora.pt)

The EP 3 772 277 B1 prototype was developed as a mass-capture trap specifically targeted at the olive fruit fly (*Bactrocera oleae*), a significant pest in the olive sector. The trap aims to capture the pest selectively, minimizing the capture of other arthropods, especially beneficial ones. To achieve this, it combines a food attractant (ammonium dihydrogen phosphate ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>)), specific pheromones, and a chromotropic trap. The initial design included an electrocution system, which was replaced by an adhesive screen to eliminate fire hazards in the field. Additionally, a fan distributes the pheromone and food attractant, controlled by a Raspberry Pi system with a thermometer, powered by a battery and solar panel.

The trials were conducted in an intensive organic olive grove at Herdade do Esporão, Reguengos de Monsaraz, with a planting spacing of 5 x 7 metres. Three test zones (21 x 133 m) were established, each comprising seven rows. The first zone used six mass-capture traps positioned between the planting rows; the second served as a buffer zone; and the third employed bottles containing the same food attractant, placed alternately between trees.

To assess the pest infestation level, fruits were collected from the three zones, and viable and non-viable stings were analyzed, as well as the fruit maturation level where differences only appear in the maturation of the olives in the bottles zone, and in the infested fruits of the buffer zone which can be derived from the inexistence of attractants in the zone. Results indicated that the prototype captured fewer arthropods compared to the conventional trap (1329 to 5598), which was less selective capturing a broader range of arthropods, including non-target species. There were significant differences in the capture of *Bactrocera oleae*.

Further adjustments to the prototype are necessary to improve its efficacy in capturing the target pest, as well as repositioning the traps to the planting row area.

## Pollen Viability Analysis by Flow Cytometry and Epifluorescence Microscopy

A. Galveias<sup>1</sup>, C. Inês<sup>2</sup>, A.R. Carlos<sup>3</sup>, A.R. Costa<sup>1</sup>, C.M. Antunes<sup>1</sup>, L. Alho<sup>4</sup>, A. Cordeiro<sup>2</sup>, I. Velada<sup>4\*</sup>

<sup>1</sup>Instituto de Ciências da Terra (ICT), Instituto de Investigação e Formação Avançada (IIFA), Departamento de Ciências Médicas e da Saúde, Universidade de Évora, Rua Romão Ramalho, 59, 7000-671 Évora, Portugal;

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

<sup>3</sup>Centre for Ecology, Evolution and Environmental Changes (CE3C) & 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;

<sup>4</sup>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](mailto:ivelada@uevora.pt)

The male gametophyte (pollen) is essential for fertilization and, consequently, in fruit set, meaning that factors impacting pollen quality can significantly affect crop productivity with serious economic consequences. Pollen is highly sensitive to heat stress, therefore, in a context of climate changes where an increase in heatwave episodes is expected to occur, much attention has been given to understand the effect of high-temperature stress on pollen fitness across various plant species. In *Olea europaea* L., studies have shown a marked decline in pollen viability when subjected to a combination of high temperatures and humidity. However, many Portuguese olive varieties have not been included in such analyses, despite the importance of these varieties in the production of olives and olive oil in our country. Since olive is predominantly wind-pollinated and often self-incompatible, its pollen is likely to encounter diverse environmental challenges during its dispersal across long distances. In this study, we aim to employ a fluorescent stain-based method (dichlorodihydrofluorescein diacetate, H<sub>2</sub>DCFDA) combined with flow cytometry and epifluorescence microscopy to quickly assess cell viability in a large population of pollen from different olive varieties. As H<sub>2</sub>DCFDA also serves as an indicator of endogenous reactive oxygen species (ROS) levels, this technique will also help us evaluate how pollen from distinct olive varieties responds to heat stress. Our goal is to identify the varieties with the highest percentage of pollen viability, which could be used as pollinators in both new and traditional cultivation areas to increase productivity. Additionally, our findings may be valuable for plant breeders aiming to develop new olive varieties with improved heat tolerance in terms of pollen performance.

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UIDB/05183/2020. We acknowledge to MED (<https://doi.org/10.54499/UIDB/05183/2020>) and CHANGE (<https://doi.org/10.54499/UIDP/05183/2020>) and CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>).

## Molecular and phylogenetic relationships between *Colletotrichum* species and related fungi based on partial DNA gene analysis

F. Santos<sup>1</sup>, M. D. Campos<sup>2</sup>, M. T. Carvalho<sup>3</sup>, A. Albuquerque<sup>1</sup>, M. Patanita<sup>1</sup>, J. A. Ribeiro<sup>1</sup>, T. Monteiro<sup>1</sup>, M. Basaloco<sup>1</sup>, M. R. Félix<sup>2</sup>

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> INIAV - Instituto Nacional de Investigação Agrária e Veterinária, I. P. Estrada de Gil Vaz, Ap. 6, 7351-901 Elvas, Portugal.

E-mail: [fs@uevora.pt](mailto:fs@uevora.pt)

Olive is an important cultivated perennial crop in Portugal, contributing significantly to both the economy and the country's reputation for high quality olive oil. Over the last decade, Portugal has become a leading producer in Europe, especially due to the Alentejo region. This area not only produces large quantities of olive oils, but also offers certified products, such as Protected Designation of Origin (PDO). Native varieties, particularly 'Galega vulgar', are prized for their low acidity, oxidative stability and unique flavour profile, making them ideal for High quality olive oil and blends to improve lower quality varieties. A major challenge in olive production is olive anthracnose, a disease that causes yield losses and affects the quality of olives and oil, being 'Galega vulgar' a highly susceptible variety. This disease is associated with different *Colletotrichum* species, with infection levels influenced by olive varieties, climate and pathogen virulence. As certain *Colletotrichum* species are region-specific, disease management strategies must be tailored to the diversity of *Colletotrichum* species present in each area. In this sense, a detailed molecular and phylogenetic characterisation of these species is required. In this context, with the aim of carrying out a phylogenetic study of the most relevant species throughout Alentejo region, olives showing anthracnose symptoms were sampled and 32 *Colletotrichum* isolated cultures were obtained. Genomic DNA was extracted followed by conventional PCR amplification. To detect genetic variability between isolates, different primer pairs (ACT 512-ACT783; Bt2a-Bt2b; CHS79-CHS35; CYLH3F-CYLH3R; GDF-GDR; ITS5-ITS4) that amplified partial gene sequences of Actin (Act), Tubulin (Tub), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Chitin Synthase 1 (CHS-1), Histone 3 (HIS3) were used. Amplicons were Sanger sequenced and analysed using bioinformatic tools and phylogenetic analysis were performed. Our results revealed that all isolates belonged to the *Colletotrichum acutatum* complex, which includes the species *C. godetiae*, *C. fioriniae*, *C. acutatum* and *C. nymphaeae*, with *C. nymphaeae* being the predominant species (> 98%). The predominance of *C. nymphaeae* suggests a greater susceptibility of natives varieties to this species and raises questions about the mechanisms of interaction between the pathogen and the olive variety. Knowledge of pathogen diversity and host interactions are fundamental for the development and implementation of new strategies for disease control. Pathogenicity tests and transcriptomic studies to identify the genes involved in the plant's response mechanisms to infection will be the next step to increase the knowledge on the interaction *Colletotrichum* spp. x *Olea europaea* L. and for the establishment improvement strategies.

This work is funded by Portuguese National Funds through Foundation for Science and Technology (FCT) under the PhD scholarship 2022.13638.BD, attributed to Joana A. Ribeiro, under the project UIDB/05183/2020, and under the project "INOVCIRCOLIVE – Inovação e Circularidade no Setor Oleícola" PRR-C05-i03-I-000208.

**The potential of insect frass as an organic fertiliser: Insights from the literature**A. Fonseca<sup>1</sup>, P. Quinteiro<sup>1</sup>, A. C. Dias<sup>1</sup>

<sup>1</sup>Centre for Environmental and Marine Studies (CESAM), Department of Environment and Planning, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

Email: [anaritafonseca@ua.pt](mailto:anaritafonseca@ua.pt)

Entotechnologies are applications and innovations inspired by insects' ecological roles that are being promoted as circularity tools and sustainable solutions for food security, waste management, and biotechnology. One by-product of the insect's bioconversion process gaining interest for agricultural application is frass, composed of insect's excrements, exuviae, and leftover feedstock. Frass contains key nutrient elements like nitrogen (N), phosphorus (P), and potassium (K) that are essential for plant growth and development. However, frass's nutrient composition and effectiveness as a fertiliser can vary depending on insect species, diet, and rearing conditions, which may impact its viability and consistency.

A literature search was conducted using Scopus and Web of Science to evaluate the nutrient profile of insects' raw frass (not subjected to further composting after harvest). The search focused on specific keyword combinations: "insect AND frass," "insect AND organic fertiliser," and "insect AND compost." Only studies reporting N, P, and K content and identifying the feedstock provided to insects were included. Additionally, only species with more than three data occurrences for the NPK content were considered.

A total of 27 records met these criteria, considering two insect species: *Tenebrio molitor* (yellow mealworm) (n=8) and *Hermetia illucens* (black soldier fly, BSF) (n=19), with 38 total data occurrences (12 for yellow mealworm and 26 for BSF). In yellow mealworm studies, the insects were mostly grown on wheat bran-based feedstocks (often mixed with fruits or vegetables), and NPK frass values ranged from 2.5-7.8%, 0.98-3.3%, and 1.2-3.0%, respectively (on a dry basis). In contrast, BSF frass originated from a broader range of feedstocks, including waste materials (e.g. manure) and by-products from industrial processes (e.g. brewery spent grains), resulting in wider ranges of nutritional values, 0.6-7.9%, 0.16-5.0% and 0.23-6.7% for NPK, respectively (on a dry basis).

The variability of ranges within the same species could affect the use of frass as an organic fertiliser, especially when addressing specific crop nutrient requirements. However, it also highlights the potential to tailor frass-based fertilisers for different agricultural needs, providing flexibility for crops with varying demands for N, P, and K, and potentially reducing reliance on synthetic alternatives and unsustainable conventional products.

This study is funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation "InsectERA" (Project no. 20 with the application C644917393-00000032). The authors would like to acknowledge the Portuguese Foundation for Science and Technology (FCT)/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020 + LA/P/0094/2020), through national funds. Thanks also to PRR for the research grant granted to Ana Rita Fonseca (BI/UI64/11138/2023). Paula Quinteiro and Ana Cláudia Dias also acknowledge the research contracts 2023/06946/CEECIND and CEECIND/02174/2017 (doi: 10.54499/CEECIND/02174/2017/CP1459/CT0019), respectively.

## Effects of *Alternaria* disease on olive cultivars: Preliminary insights of pathogen prevalence across cultivars, organs and growth stages

T. Monteiro<sup>1</sup>, M. R. Félix<sup>2</sup>, J. A. Ribeiro<sup>1</sup>, F. Santos<sup>1</sup>, A. Albuquerque<sup>1</sup>, M. Patanita<sup>1</sup>, C. Campos<sup>1</sup>, H. Cardoso<sup>3</sup>, M. Basaloco<sup>1</sup>, M. D. Campos<sup>2</sup>

<sup>1</sup>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.

<sup>2</sup>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.

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: [tomas.monteiro@uevora.pt](mailto:tomas.monteiro@uevora.pt)

Olive groves have been planted in Portugal for centuries, being exceptionally adapted to the country's climate due to their low water requirements and high tolerance for hot and dry summers. Moreover, olives hold profound cultural significance, serving as an enduring symbol of Portugal's heritage, landscape and culinary traditions. The recent increase in intensive and super-intensive olive groves associated with climate change and the introduction of new olive cultivars has paved the way for the emergence of new diseases affecting olive trees. Among these, *Alternaria alternata*, previously considered an endophytic fungus, has now emerged as a notable pathogen responsible for Alternaria disease in olive trees. This disease manifests through symptoms such as leaf spots, fruit rot and premature fruit drop, causing substantial yield losses. Despite active research, there remains a gap in knowledge regarding this disease, emphasizing the need for deeper investigation into its epidemiology, diagnosis, and monitoring. The present study explored the colonization patterns of *A. alternata* in various olive tree organs across different cultivars. Samples of olive fruits, small branches, and leaves were collected from the 'Arbosana', 'Oliana', and 'Coriana' cultivars at three different time points of the tree growth cycle to assess the pathogen's prevalence. Detection and quantification of *Alternaria spp.* were performed by real-time PCR, using a TaqMan Minor Groove Binder (MGB) assay targeting the internal transcribed spacer (ITS) region specific to *Alternaria* species. This study allowed to determine whether *A. alternata* colonization is consistent throughout the olive tree cycle, to identify the specific organs in which the pathogen is prevalent, and to understand if the susceptibility varies among the different olive cultivars. Preliminary results indicate that the pathogen is present in most plant organs across all three time points, with the highest quantities found in branches, followed by leaves, and lastly, in olives. These findings will contribute to a better understanding of Alternaria disease dynamics in olive trees, contributing to future management strategies for disease control in olive cultivation.

This work is funded by Portuguese National Funds through Foundation for Science and Technology (FCT) under the PhD scholarship 2022.13638.BD, attributed to Joana A. Ribeiro; under the project UIDB/05183/2020; and under the project "INOVCIRCOLIVE - Inovação e Circularidade no Setor Oleícola" PRR-C05-i03-I-000208.

## Assessing Soil Macrofauna in Alentejo's agriculture: The Impact of Farming Practices and Soil Treatments

Maaïke Bles<sup>1</sup>, Joao Nunes<sup>1</sup>, João Canedo<sup>2</sup>, Bernardo Lameira<sup>2</sup>, Luísa Coelho<sup>2</sup>, Sérgio Prats<sup>2</sup>.

<sup>1</sup>Soil Physics and Land Management Group, Wageningen University and Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands.

<sup>2</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Núcleo da Mitra, Évora 7006-554, Portugal

<sup>3</sup>Misión Biológica de Galicia – Consejo Superior de Investigaciones Científicas (MBG-CSIC), Salcedo, Pontevedra, España.

Email: [licoelho@uevora.pt](mailto:licoelho@uevora.pt)

The Alentejo region has a thriving agricultural economy, particularly known for its olive orchards and vineyards, which plays an important role in aiding both the local economy and the region's traditional way of life. The Alqueva dam considerably increased agricultural production by providing irrigation, which led to more intensive olive groves and vineyards. While intensification has increased yields, it also poses significant environmental issues. Soil erosion, reduced soil fertility, and a decline in biodiversity within olive groves are among the most significant. These concerns underscore the critical necessity for adopting sustainable farming methods and novel agricultural technology that can strike a balance between heightened productivity and sustainability. To attain this balance, it is crucial to deepen the understanding of soil macrofauna dynamics and how various management practices affect olive grove sustainability. Monitoring soil macrofauna diversity to preserve or improve soil health will be critical to guaranteeing the long-term viability of olive cultivation in the Alentejo. This entails monitoring a variety of management practices, including organic and integrated farming systems, as well as soil treatments, like mulch and biochar and microenvironments, as tree row, rut and interrow.

In this work, we hypothesize that different olive groves management practices, like organic or integrated, and treatments, including mulch and biochar, can significantly affect the soil mesofauna biodiversity in two olive groves and two vineyards, each counting nine experimental plots. The soil macrofauna occurrences and diversity were assessed for different: i) management practices (organic or integrated), ii) treatments (mulch or combination of mulch and biochar) and iii) orchard microclimate (tree, rut and interrow). Three composite soil samples were collected in each site and macrofauna morphotypes identified. The diversity was calculated using the Shannon diversity index with the morphotype occurrences per site as input. Preliminary results indicated that only the mulch and biochar treatment positively impacted mesofauna diversity in the less biodiverse orchards, and this effect was confined to the tree microenvironment. Additionally, the influence of the treatments (untreated, mulch, and mulch & biochar) and the microenvironments (tree, row, interrow) was more pronounced in soils with lower biodiversity.

**Keywords:** management practices, treatment, soil mesofauna diversity, Shannon diversity index, morphotype.

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



## **Characterising double cropping in Friuli Venezia Giulia (Italy) using the Land Parcel Identification System**

A. Bruno<sup>1</sup>, P. Martin<sup>2</sup>, E. Marraccini<sup>1</sup>

<sup>1</sup>DI4A, Università di Udine, Udine, IT

<sup>2</sup>UMR SADAPT, Université Paris-Saclay, INRAE, AgroParisTech, UMR SAD-APT, 91120, Palaiseau, FR

Email: [bruno.antonio@spes.uniud.it](mailto:bruno.antonio@spes.uniud.it)

Double cropping (DC) indicates growing and harvesting two crops with different growing seasons in the same field in the same year. This agronomic technique, spread particularly in low-land tropical and subtropical areas, is a form of crop intensification. Where climate and soil condition are suitable, DC is a way to make more economically resilient the farm, a technique to better control diseases and weeds, and increase the general productivity without use more land. Despite its interest, in Mediterranean regions few DC systems have been investigated, e.g. in fodder or biomass production but few studies dealt with their identification and accounting at territorial level. We propose a method to account for DC using as case study the Friuli Venezia Giulia region and as data source Land Parcel Identification System (LPIS) data from 2019 to 2023. We firstly reclassified crops names to be consistent among the databases. Then, we cleaned the database by deleting polygons of less than 1000m<sup>2</sup>. Considered as DC multiple records on a same parcel we identified possible DC sequences, excluding from the analysis parcels with crop cycles overlapped or not feasible (e.g. wheat and barley or corn in winter), or where crop type was not explicit (e.g. arable crop instead of the crop name). The obtained data have been validated in a sample of five local farms. LPIS database seems a promising source of data for territorial studies on crop sequences, including DC. Information about farming practice will be collected through on-farm surveys. We made it up considering the factors affecting farm management and in particular DC (water availability, yield of previous crop). Our preliminary results show that soybean, corn and grapevine cover more than half of regional surface. Soybean, corn and sorghum are abundantly the main spring crop in DC (69% of DC area); soft wheat and barley cover more than two thirds (73% of DC area). Future work will integrate the place and the frequency of DC in regional crop rotations with data on crop management, along with the drivers of their adoption.

This study was funded by SICaNSE project, funded by Friuli Venezia Giulia region.



## Metagenomics analysis reveals fungal community differences between almond orchards

A. Faustino<sup>1,2,3</sup>, M.M. Oliveira<sup>4</sup>, M.R. Félix<sup>5</sup>, L. Marum<sup>1,2</sup>

<sup>1</sup>Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.

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

<sup>3</sup>MED – 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.

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

<sup>5</sup>MED & 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](mailto:ana.faustino@cebal.pt)

Portugal's almond culture has grown in the last years as a result of the establishment of new orchards in the Alentejo region. In the new orchards, phytosanitary problems associated with fungal pathogens have been emerging, as the primary cause of diseases in the almond trees, likely resulting in lower productivity and significant financial losses. In extreme situations, these diseases may account for tree mortality. To identify microorganisms, culture-based methods have been used, to characterize morphological and structural traits of pure fungal isolates. However, this methodology has several disadvantages. Furthermore, certain fungi exhibit very specific growth requirements and need specific conditions for development. Additionally, identification based on morphological characteristics can be subjective and dependent on the technician's experience. The development of new technologies based on sequencing such as metagenomics allowed the identification of the complete genetic repertoire of all microorganisms within a specific environment. In this work, we identified and characterized the fungal communities of 2 almond orchards in the Alentejo regions (Mora and Ferreira do Alentejo). Samples of the trunk from 5 symptomatic and 5 asymptomatic trees were selected from each orchard and analyzed by amplicon sequencing-based metagenomic approach. Briefly, DNA was isolated from each tree and used for PCR (polymerase chain reaction) amplification of the internal transcribed spacer (ITS) region of nuclear rDNA. The PCR products were purified by Agencourt AMPure XP beads, dissolved in the elution buffer, labeled for library construction, and analyzed using an Agilent 2100 Bioanalyzer. Differences in the number of species and relative abundance were observed between symptomatic and asymptomatic trees as well as between orchards. We observed more unique features (OTUs) in the orchard of Mora (281 and 334 OTUs from asymptomatic and symptomatic trees, respectively), when compared with the orchard of Ferreira do Alentejo (265 and 229 from asymptomatic and symptomatic trees, respectively). To establish sustainable management techniques for almond production, it is crucial to comprehend the fungal diversity associated with almond trees, and metagenomics can play a key role in this process by revealing the real diversity of fungi in an orchard.

This work was performed 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. Authors also acknowledge FCT for funding the R&D Units UIDB/05183/2020 (10.54499/UIDB/05183/2020, 10.54499/UIDP/05183/2020) to Mediterranean Institute for Agriculture, Environment and Development (MED) and UIDB/04551/2020 to GREEN-IT Bioresources for Sustainability, as well as LA/P/0121/2020 (10.54499/LA/P/0121/2020) to Global Change and Sustainability Institute (CHANGE), and still the Contrato – Programa de L. Marum (CEECINST/00131/2018), and the Ph.D. grant of A. Faustino (UI/BD/153511/2022).

## Optimizing irrigation in avocado (*Persea americana* Mill.) orchards in southern Portugal: the impact of oxygenated water and subsurface irrigation.

A.R. Trindade<sup>1,2</sup>, J. Sardo<sup>3</sup>, A. Arozarena<sup>4</sup>, L. Coelho<sup>5</sup>, P. Rocha<sup>6</sup>, J. Almeida<sup>7</sup>, A. Machado<sup>3</sup>, J. Bento<sup>3</sup> & A. Duarte<sup>1,2</sup>

<sup>1</sup>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.

<sup>2</sup>Faculty of Sciences and Technology, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

<sup>3</sup>JB I Group, Estrada Senhora da Saúde, Cx. Postal 297ª, 8005-148, Faro, Portugal.

<sup>4</sup>University of Lorraine, ENSAIA, 2 Av. de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy, France.

<sup>5</sup>MED (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.

<sup>6</sup>Deep Root Irrigation - Irrigation Technologies, LLC - Escanaba, Michigan EUA - Burlington, Iowa EUA.

<sup>7</sup>Moleaer, C/Escudo Português, 7. San Isidro de Níjar, Níjar 04117, Almería, Espanha.

Email: [artrindade@ualg.pt](mailto:artrindade@ualg.pt)

Avocado (*Persea americana* Mill.) farming has gained significant popularity globally, and it's projected to become the most traded subtropical fruit. While native to subtropical climates, avocado trees can thrive in drier regions like the Mediterranean Basin, making it an important source of fresh, safe fruit for European consumers. However, this region has consistently faced challenges related to unpredictable rainfall patterns. Investing in advanced water-efficient technologies is essential to ensure sustainable farming and water savings.

Nanobubble irrigation, a relatively new technique, has shown promising applications in agriculture. By infusing irrigation water with oxygen-rich nanobubbles, it enhances oxygen availability near plant roots, which is especially beneficial for crops sensitive to waterlogged conditions, like avocados. While subsurface irrigation is not a new concept, recent innovations like deep root irrigation (DRI) emphasize deep-rooted plants, ensuring water penetrates deeply into the soil. This minimizes surface evaporation and promotes drought-resilient plants. Both techniques promote a healthier microbial community and enhance soil fertility, while effectively controlling root rot diseases caused by *Phytophthora cinnamomi* Rands, a pathogenic fungus that thrives in high humidity conditions and threatens avocado production.

A field trial is underway in an avocado farm (JB I Group in the Algarve, Portugal) to compare the two advanced irrigation technologies versus the conventional method: T1, fresh water with drip irrigation; T2, nanobubble-enriched water with drip irrigation; T3, fresh water with DRI; and T4, nanobubble-enriched water with DRI. Monthly evaluations are conducted on fruit diameter growth, plant nutritional status and microbiological activity.

With the trial still ongoing, the results show that the T1 is producing the largest average fruit size at 57,76 mm, while treatment T4 yields the smallest, averaging 50,96 mm. There are no significant differences between T2 and T3, suggesting no impact on fruit size. Furthermore, none of the trees exhibited any signs of water stress.

Nanobubble irrigation and DRI seem effective in promoting soil moisture retention while creating a resilient growing environment. Preliminary results indicate that these technologies help maintain fruit size while conserving water, making them valuable tools for sustainable farming.

This trial is being carried out with the resources of the companies involved (JB I, Moleaer and DRI) and has the support of the projects PRR Agro+Eficiente (PRR-C05-i03-I-000010).

## Mapping and unlocking the valorization potential of olive oil by-products for a circular bioeconomy: Insights from the Meknès-El Hajeb region, Morocco

H. Dhaidhi<sup>1,2,3</sup>, E.A. Ajal<sup>4</sup>, E. Dornier<sup>3</sup>, S. Ennahli<sup>1</sup>, H. Abdelmoumen<sup>2</sup>, A. Carrasco-Pancorbo<sup>5</sup>, A. Bajoub<sup>1,5\*</sup>

<sup>1</sup>Laboratory of Food and Food By-Products Chemistry and Processing Technology, National School of Agriculture, km 10, Haj Kedour Road, B.P. S/40, Meknès, Morocco.

<sup>2</sup>Faculty of Sciences Rabat, Mohammed V University, Rabat, BP 1014 RP, Morocco.

<sup>3</sup>QualiSud, Montpellier University, Avignon University, CIRAD, Institut Agro, IRD, La Réunion, University, Montpellier, France.

<sup>4</sup>UPR of Pharmacognosy, Faculty of Medicine and Pharmacy of Rabat, BP 6203, Morocco.

<sup>5</sup>Department of Analytical Chemistry, Faculty of Science, University of Granada, Ave. Fuentenueva s/n, 18071, Granada, Spain.

Email: [hamza.dhaidhi@gmail.com](mailto:hamza.dhaidhi@gmail.com); [e.ajal@um5r.ac.ma](mailto:e.ajal@um5r.ac.ma); [manuel.dornier@institut-agro.fr](mailto:manuel.dornier@institut-agro.fr); [ennahlisaid@gmail.com](mailto:ennahlisaid@gmail.com); [hanabdel10@gmail.com](mailto:hanabdel10@gmail.com); [alegriac@ugr.es](mailto:alegriac@ugr.es); [bajoub.aadil@gmail.com](mailto:bajoub.aadil@gmail.com)

\*Corresponding author: A. Bajoub, [bajoub.aadil@gmail.com](mailto:bajoub.aadil@gmail.com)

The Mediterranean region, where more than 87% of the world's olive oil is produced, is currently facing significant environmental problems with the disposal of by-products, particularly olive pomace and olive mill wastewater (OMWW). In Morocco, where olive oil production is a strategic agri-food sector, the disposal and management of these by-products is a critical issue, especially as the sector grows. The Meknès-El Hajeb region, one of the most important olive growing areas in the country, exemplifies this challenge.

This study, conducted in the Meknès-El Hajeb region over four olive harvest seasons (2020-2024), initially aimed to quantify and map the by-products of 158 olive oil mills (with two-phase, three-phase and traditional extraction systems) using field surveys and GPS data. The results showed that approximately 159.25 thousand tons of olive pomace and 50.70 thousand m<sup>3</sup> of OMWW are produced annually, posing a significant threat to the environment. In addition, a total of 452 samples (277 pomace and 175 OMWW) were collected and analyzed. The results showed significant differences in terms of moisture, organic matter and nutrient content between samples according to the extraction system used. Pomace from the two-phase system had the highest moisture content, while the traditional system had lower values. The nitrogen content was slightly lower in the two-phase pomace (0.95–1.09%) than in the traditional pomace (up to 1.5%), while the phosphorus content remained the same in all samples and the potassium content was between 1.87% and 2.00%. The pH of the pomace was slightly acidic (4.35–5.17). The OMWW showed high pollution indicators, with chemical oxygen demand ranging from 150.59 to 210.56 g/L and biological oxygen demand from 21.14 to 25.19 g/L, indicating significant environmental risks. High salinity (6.15–12.81 mS/cm) and acidic pH (4.28–4.83) were also observed. The polyphenol content was highest in the pomace from the two-phase extraction (up to 3.26 g/kg dry weight), while the OMWW from the three-phase extraction and traditional systems had 2.68–3.41 g/L.

The findings of this research highlight promising potential for transforming olive oil by-products within a circular bioeconomy promoting sustainable resource use and recovery of various valuable compounds.

## Susceptibility of different olive fruit cultivars to *Bactrocera olea* oviposition along ripening

Miguel D. Ferro<sup>1,2,\*</sup>, Tânia Nobre<sup>3</sup>, M. João Cabrita<sup>4</sup>, Maria F. Duarte<sup>1,2</sup>

<sup>1</sup>Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal;

<sup>2</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal;

<sup>3</sup>MED—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.

<sup>4</sup>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

[\\*miguel.ferro@cebal.pt](mailto:miguel.ferro@cebal.pt)

Susceptibility to *Bactrocera olea* infestation is known to be cultivar specific, which directly influences the effect upon the final olive fruit production. Within the present study, seven olive cultivars were evaluated in terms of their morphological descriptors and phenolic profile, namely: ‘Galega Vulgar’, ‘Cobrançosa’, ‘Carrasquenha’, ‘Redondil’, ‘Azeiteira’, ‘Arbequina’ and ‘Picual’, along seven ripening stages (from T1 to T7). ‘Redondil’ presented the highest oviposition index from half-way maturation onwards, with a total of 44 oviposition holes in 100 olive fruits at the last sampling point (T7), followed by ‘Galega vulgar’ and ‘Picual’, with a total of 14 and 11 holes in 100 olives, respectively. Regarding fruit caliber, fruit pulp and fruit pulp to stone ratio, ‘Redondil’ was also the cultivar showing highest average values along ripening. Despite the relatively high concentration of phenolic compounds shown by ‘Redondil’ olive fruits, other factors showed a stronger correlation to the olive fruit fly oviposition, such as fruit caliber parameter, which showed to be a decisive factor regarding oviposition, with olive fruit fly revealing a greater preference for ‘Redondil’ compared to all other cultivars. ‘Galega vulgar’ showed also higher oviposition index, likely due to its early ripening characteristics.

This work was supported by the “Fundação para a Ciência e Tecnologia” (FCT—Portugal), through the research project PTDC/ASP-PLA/30650/2017. The authors further acknowledge FCT for the PhD grant to Miguel Duarte Ferro (SFRH/BD/140083/2018), for the Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and the Project LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

## Phytotoxic Activity of Sesquiterpene Lactones-Enriched Fractions from *Cynara cardunculus* L. Leaves on Pre-Emergent and Post-Emergent Weed Species and Putative Mode of Action

D. Rosa<sup>1,2,3</sup>, C. Rial<sup>2</sup>, T. Brás<sup>1,3</sup>, R.M. Varela<sup>2</sup>, F. A. Macías<sup>2</sup>, MF. Duarte<sup>1,3</sup>

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

<sup>2</sup>Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz, Spain.

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal

Email: [daniela.rosa@cebal.pt](mailto:daniela.rosa@cebal.pt)

Sesquiterpene lactones (SLs) are compounds that are highly produced in *Cynara cardunculus* leaves ( $\approx 95\text{g/kg}$  dry weight), known for their phytotoxic activity.<sup>1–3</sup> This study aims to assess SL-enriched fractions' (cynaropicrin, aguerin B, and grosheimin) phytotoxic potentials and putative modes of action, compared to an initial extract, using two approaches: first, against a panel of nine weed species in pre-emergence, and then on *Portulaca oleracea* L.'s post-emergency stage. The SL-enriched fractions demonstrated greater phytotoxic activity when compared with the *C. cardunculus* leaf initial extract. The SL-enriched fractions had higher activity at root growth inhibition over the panel tested, doubling the activity in five of them at 800 ppm. Regarding the post-emergence bioassay, the SL-enriched fractions had a higher influence on the plants' growth inhibition (67% at 800 ppm). The SL-effects on the plants' metabolisms were evidenced. The total chlorophyll content was reduced by 65% at 800 ppm. Oxidative stress induction was observed because of the enhancement in MDA levels at 800 ppm compared to control (52%) and the decrease in SOD-specific activity from 4.20 U/mg protein (400 ppm) to 1.74 U/mg protein (800 ppm). The phytotoxic properties of the SL-enriched fractions suggested a potential application in the development of a bioherbicide formulation, requiring further investigation.

This research was funded by Regional Development European Foundation and Alentejo Region Operational Program (ALENTEJO 2020) with the financial support of "MedCynaraBioTec—Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop Project (ALT20-03-0145-FEDER-039495), Fundação para a Ciência e a Tecnologia (FCT) for financing the PhD grant to Daniela Rosa (SFRH/BD/143845/2019). This work was also funded through FCT under the Project UIDB/05183/2020 to Mediterranean Institute for Agriculture Environment and Development (MED) <https://doi.org/10.54499/UIDB/05183/2020> (accessed on 17 July 2024), Project LA/P/0121/2020 to CHANGE—Global Change and Sustainability Institute <https://doi.org/10.54499/LA/P/0121/2020> (accessed on 17 July 2024), and by the Agencia Estatal de Investigación—Ministerio de Ciencia e Innovación del Gobierno de España under the project PID2020-115747RB-I00/AEI/10.13039/501100011033.

### References

- (1) Ramos, P. A. B.; Guerra, A. R.; Guerreiro, O.; Freire, C. S. R.; Silva, A. M. S.; Duarte, M. F.; Silvestre, A. J. D. Lipophilic Extracts of *Cynara Cardunculus* L. Var. *Altilis* (DC): A Source of Valuable Bioactive Terpenic Compounds. *Journal of Agricultural and Food Chemistry* 2013, 61 (35), 8420–8429. <https://doi.org/10.1021/jf402253a>.
- (2) Brás, T.; Paulino, A. F. C.; Neves, L. A.; Crespo, J. G.; Duarte, M. F. Ultrasound Assisted Extraction of Cynaropicrin from *Cynara Cardunculus* Leaves: Optimization Using the Response Surface Methodology and the Effect of Pulse Mode. *Industrial Crops and Products* 2020, 150 (March), 112395. <https://doi.org/10.1016/j.indcrop.2020.112395>.
- (3) Scavo, A.; Rial, C.; Molinillo, J. M. G.; Varela, R. M.; Mauromicale, G.; Macias, F. A. The Extraction Procedure Improves the Allelopathic Activity of Cardoon (*Cynara Cardunculus* Var. *Altilis*) Leaf Allelochemicals. *Industrial Crops & Products* 2019, 128 (August 2018), 479–487. <https://doi.org/10.1016/j.indcrop.2018.11.053>.

## The interplay between salinity stress and mycorrhizal fungi inoculation on Olive tree physiological responses

Rodrigo Eugénio<sup>1</sup>, Lénia Rodrigues<sup>2\*</sup>, Renato Coelho<sup>3</sup>, Isabel Brito<sup>3</sup>, Rafaela Santos<sup>1</sup>, Hélia Cardoso<sup>3</sup>, Catarina Campos<sup>1\*</sup>

<sup>1</sup>School of Science and Technology, Colégio Luís António Verney, Rua Romão Ramalho, 59, 7000-671 Évora, Portugal.

<sup>2</sup>MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE — Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>3</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, School of Science and Technology, Department of Biology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Corresponding authors: Lénia Rodrigues, [liar@uevora.pt](mailto:liar@uevora.pt); Catarina Campos, [mccampos@uevora.pt](mailto:mccampos@uevora.pt)

Olive tree (*Olea europaea* L.) is a widely cultivated species and a crucial economic crop for the Mediterranean, where drought and soil salinization are prevalent. This species exhibits moderate response to salinity stress but it has been shown that salt tolerance in olive appears to be cultivar dependent. The traditional Portuguese olive cv. 'Galega vulgar' remains the most representative across national olive orchards. However, there are only a few studies about its tolerance to salinity stress. This study aimed to analyze the response of cv. 'Galega vulgar' plants to salinity stress, concomitantly assessing the effect of arbuscular mycorrhizal fungi (AMF) colonization in the salinity stress response.

Plants of *O. europaea* cv. 'Galega Vulgar', previously inoculated or not with AMF, were watered with a NaCl solution (150mM) or with water and maintained for 50 days under greenhouse conditions. Plant responses were evaluated by measuring physiological parameters, including relative water content, transpiration rate, specific leaf area, chlorophyll content and stomatal conductance. The plants' mycorrhizal colonization rate was evaluated at the end of the experiment.

Some physiological patterns differed between non-inoculated and inoculated plants in response to salt stress. Stomatal conductance was higher in non-inoculated plants subjected to salt stress than in control ones. On the opposite, this parameter was higher in mycorrhizal plants not subjected to salt stress than in salt-stressed ones. Specific leaf area showed similar trend to stomatal conductance. Relative water content was not affected by salt or mycorrhizal colonization, and chlorophyll levels also remained relatively stable during the experiment.

Being olive tree a moderately salt-tolerant species, it is plausible that the increase in stomatal conductance under higher salinity in control plants may be the result of plant adaptation to salt, due to improved ion compartmentalization and osmotic adjustment. On the contrary, in mycorrhizal plants, it seems that the fungus triggers a stronger stress response to salinity, leading to a decrease in stomatal conductance.

Further studies are needed to better determine the effect of salt stress in this cultivar, particularly on the translocation of ions from roots to leaves.

### Acknowledgement

This work was financially supported by national funds through FCT (Foundation for Science and Technology) under the Project UIDB/05183/2020. The authors would like to thank the company Microplant Lda (Évora, Portugal) for kindly providing the olive plants, and to IRTA (Barcelona, Spain) for providing the AMF inocula.



## Can the *Quercus suber* L. genetic origin influence flathead oak borer (*Coroebus undatus* Fabricius)? Study proposal

J. Horta Marques<sup>1,2</sup>, F. Costa e Silva<sup>1</sup>

<sup>1</sup>CEF (Centro de Estudos Florestais) e Laboratório Associado Terra, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal.

<sup>2</sup>MED (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.

Email: [joao.horta.marques@uevora.pt](mailto:joao.horta.marques@uevora.pt)

The flathead oak borer (*Coroebus undatus* Fabricius) is a biological enemy of the cork oak (*Quercus suber* L.) that affects the cork quality, reducing its value. Associated with other harmful factors for the cork oak, such as stripping wounds, summer water scarcity, excessive stripping and soil mobilization, it increases its propensity, as well as other stress factors such as the incidence of cork ants (*Crematogaster scutellaris*), devaluing it even more.

Given the incidence of this plague, since the time of Professor Joaquim Vieira Natividade (1899-1968), many studies have been carried out within the scope of its biology, impact, combat measures, interactions with cultural operations, namely, since 1940. Due to the fact that around half of cork comes from Portugal, its devaluation has a direct impact on GDP. However, to date the influence of cork oak genetics on the incidence of flathead oak borer is unknown.

In antithesis to the initial objectives of genetic improvement (increased production, mainly quantitative), lately plant breeding has also focused on promoting resistance or tolerance to stress factors (diseases, drought and pests).

Given the interest of this issue and the fact that a cork oak provenance test was carried out, installed between 1997 and 1999, in Monte Fava (Baixo Alentejo), this work proposes the study of the effect of the genetic provenance of *Quercus suber* L. in the flathead oak borer's incidence. Since this trial will be cork harvested in 2025 it provides a unique opportunity to evaluate the differences of susceptibility in 35 provenances to the flathead oak borer.

In this genetic trial, seven countries with at least one cork oak provenance are represented: Portugal, Spain, France, Italy, Algeria, Tunisia and Morocco. In total, 35 provenances are installed. The results will allow us to evaluate the susceptibilities of each genetic origin to flathead oak borer. This knowledge is essential for afforestation planning and forest reproductive material selection. However, it's important to note that these first results will be obtained from a first cork harvest (virgin cork), which must then be regarded as a first indication of different genetic origin susceptibilities and imply the need for a continuous study.

### Dedication

To Hachemi Merouani's (1962-2016) memory, for the contribution he made to cork oak studies, especially in seed conservation.

## Evaluation of the potential of Plants in power generation for low power devices

Milton G. M. De Gussule<sup>1</sup>, Masud Rana Rashed<sup>2</sup>, Mouhaydine Tlemçani<sup>2</sup>

<sup>1</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, IIFA (Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>2</sup>ICT - Instituto de Ciências da Terra, Universidade de Évora, Colégio Luis António Verney, Rua Romão Ramalho, 59, 7002-554 Évora

Email: [d39583@alunos.uevora.pt](mailto:d39583@alunos.uevora.pt)

The extraction of electrical energy from living plants appears as a promising source of renewable bioenergy, with the potential to provide energy continuously, overcoming the intermittency of solar and wind sources [1]. This electrical potential is generated by the potential of the electrodes, which depends on the concentration of ions around the electrodes in the plant [2], originated by the metabolic processes of photosynthesis and respiration [3]. When incorporating metallic electrodes into plants, an electrochemical process occurs that induces the flow of electrons, which are captured by the electrodes and converted into electricity through oxidation-reduction reactions [4]. Thus, energy extraction from plants represents an emerging technology, offering a sustainable alternative to power low-consumption devices, such as sensor nodes, connected by autonomous Wireless Sensor Networks (WSNs) [5]. Among living plants exploited for energy harvesting, *Aloe vera* has been shown to generate the highest magnitudes of voltage and current [8][9]. The species used is an important factor, as different concentrations of electrolytes in each species influence the flow of electrons between different groups of plants [6]. The research evaluates the use of *Aloe vera* as a source of sustainable electricity for low-power devices, incorporating copper and zinc electrodes into the leaves, to explore greater potential for generating electrical current. Series and parallel experimental setups indicate that although individual power per sheet is limited, the combination of multiple sheets can achieve power levels around watts [7]. This method represents a sustainable alternative for powering monitoring sensors in wireless sensor networks, especially useful in areas such as smart agriculture and forest fire prevention. Thus, harvesting energy from plants offers an environmentally friendly and continuous solution, overcoming the intermittency of solar and wind energy to support low-power IoT devices in the field.

### References

- [1] Howe Cheng, T., Rahman, M. A., Kim, S. H., Jo, Y., & Park, J. (2018). A review on energy harvesting potential from living plants: Future energy resource. *International Journal of Renewable Energy Research*, 8(4). Recuperado de <https://www.researchgate.net/publication/330224818>
- [2] Hao, Z., Li, W., & Hao, X. (2021). Variations of electric potential in the xylem of tree trunks associated with water content rhythms. *Journal of Experimental Botany*, 72(4), 1321–1335. <https://doi.org/10.1093/jxb/eraa492>
- [3] Tcherkez, G., & Atkin, O. K. (2021). Unravelling mechanisms and impacts of day respiration in plant leaves: An introduction to a virtual issue. *New Phytologist*, 230(1), 5–10.
- [4] Choo, Y. Y., & Dayou, J. (2014). Increasing the energy output from living-plants fuel cells with natural photosynthesis. In *Proceedings of the International Conference on Environmental and Biological Sciences*.
- [5] Gupta, V., Tripathi, S., & De, S. (2020). Green sensing and communication: A step towards sustainable IoT systems. *Journal of the Indian Institute of Science*, 100(2), 383–398
- [6] Taam, A., Habaebi, M., & Islam, M. (2024). Living plant energy harvesting for WSN & IoT devices. In *Proceedings of ICOM*, 107-111. <https://doi.org/10.1109/ICOM61675.2024.10652478>.
- [7] Krinker, M., & Goykadosh, A. (2010). Renewable and sustainable energy replacement sources. In *Applications, and Technology Conference, Long Island: Institute of Electrical and Electronics Engineers*.
- [8] Chong, P. L., Singh, A. K., & Kok, S. L. (2019). Potential application of *Aloe vera*-derived plant-based cell in powering wireless device for remote sensor activation. *PLOS ONE*, 14(12), e0227153. <https://doi.org/10.1371/journal.pone.0227153>.
- [9] Sounder, J., & Deepa, N. (2022). Green electricity – *Aloe vera* plant – A review. *International Journal of Current Research*, 10(8)







## **Anthelmintic resistance of parasitic gastrointestinal nematodes of Alentejo sheep and use of natural products to overcome resistance**

Natalia Faria Campbell<sup>1,2</sup>, Helga Cardoso Waap<sup>3</sup>, Helder Carola Cortes<sup>2</sup>

<sup>1</sup>INIAV (Instituto Nacional de Investigação Agrária e Veterinária), Oeiras, Portugal

<sup>2</sup>MED (Mediterranean Institute for Agriculture, Environment and Development), Évora, Portugal

<sup>3</sup>Dutch National Institute for Public Health and the Environment, The Netherlands

Email: [natalia.campbell@iniav.pt](mailto:natalia.campbell@iniav.pt)

Commercial sheep in Portugal are important mainly for their production of meat and milk (principally for cheese) and certain breeds for wool. Additionally, grazing by sheep in arid or rocky areas, unsuitable for agricultural cultivation, reduces scrub, lowering potential forest-fire hazards. Sheep are commonly parasitised by a number of species of gastrointestinal helminths. Extensive parasitisation has a significant impact on sheep well-being, reproductive capacity and profitability of flock management. Estimated cost in loss of productivity by Portuguese sheep resulting from helminthiasis is >3.5 million euros, and cost of anthelmintic treatments >1.5 million euros, yearly (Charlier *et al.*, 2020). Moreover, anthelmintic resistance (AR) in sheep continues to be an expanding worldwide problem, especially over the past decade. Surveys for AR in sheep have been conducted in several European countries. However, to date, an AR survey has only been conducted in the Lisbon and Tagus Valley areas of Portugal (Antunes *et al.*, 2022). Our project is to expand AR surveys to encompass additional regions of Portugal. We have already surveyed a number of commercial flocks in the Alentejo Region for AR involving the commercial anthelmintics, albendazole and thiabendazole, based on an egg hatch test (EHT). Effective Concentrations (EC) at 10, 25, 50, 75 and 90% levels of egg hatch inhibition by anthelmintics were determined using probit analysis of duplicate EHT assays. AR was assigned according to EC<sub>50</sub> values equal to or exceeding values already established for resistance of respective anthelmintics. Thus far, we identified AR occurs extensively amongst flocks throughout Alentejo to both anthelmintics. We next examined the potential of combinations of redox-active natural products with these antihelmintics to reduce AR resistance. We found anthelmintic efficacy was synergised with some combinations where 90% inhibition was achieved using almost 10x less of the natural product or anthelmintic when tested, alone.

### **References**

Antunes *et al. Pathogens* (2022) 11, 1457.

Charlier *et al. Preventive Veterinary Medicine* (2020) 182, 105103.

## Determinants for a good transfer of passive immunity in dairy calves

F. G. Silva<sup>1,2,3</sup>, C. Conceição<sup>2</sup>, M. Laranjo<sup>2</sup>, P. Infante<sup>4</sup>, L. E. Hernández-Castellano<sup>5</sup>, J. Ramalho<sup>2</sup>, M. González-Cabrera<sup>5</sup>, E. Lamy<sup>2</sup>, J. O. L. Cerqueira<sup>1,3</sup>, A. Pereira<sup>2</sup> and S. R. Silva<sup>1</sup>

<sup>1</sup>Veterinary and Animal Research Centre & AI4Animals, University of Trás-os-Montes e Alto Douro, Department of Animal Science, Quinta de Prados, 5000-801 Vila Real, Portugal.

<sup>2</sup>MED-Mediterranean Institute for Agriculture, Environment and Development, and CHANGE—Global Change and Sustainability Institute, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>3</sup>Center for Research and Development in Agrifood Systems and Sustainability, Polytechnic Institute of Viana do Castelo - Agrarian School of Ponte de Lima, Rua D. Mendo Afonso, 147 Refóios do Lima.

<sup>4</sup>CIMA - Centro de Investigação em Matemática e Aplicações, Colégio Luís António Verney, Rua Romão Ramalho, 59, 7000-671 Évora

<sup>5</sup>IUSA-ONEHEALTH 4, Animal Production and Biotechnology, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Campus Montaña Cardones, s/n, 35413 Arucas, Spain

Email: [fsilva@uevora.pt](mailto:fsilva@uevora.pt)

Due to the physiological characteristics of the cow's placenta, calves are born hypogammaglobulinemic, making the transfer of passive immunity (TPI) via colostrum critical for their survival and welfare. There are several factors that affect TPI, which are related to parturition (e.g. dystocia), the calf (e.g. body weight - BW) and colostrum management (e.g. quantity and quality of colostrum). Pre-weaning mortality and morbidity rates are still unacceptably high in the dairy farms, therefore additional efforts should be made to improve the TPI process. We aimed to understand if some factors related with the calf, birth and with colostrum management are associated with a good transfer of passive immunity (serum IgG concentration  $\geq 18$  g/L; GTPI). Sixty-eight calves from three dairy farms were included, and information was collected on the calving event (i.e. difficulty and duration), the calf (i.e. breed, sex and BW) and colostrum management (i.e. time between birth and first and second feeding of colostrum, volume and method of colostrum feeding and whether heat treatment (HT) of colostrum was used). For each calf, a sample of colostrum was collected from the feeding apparatus for laboratory analysis. Chemical composition (i.e. dry matter, protein, fat and pH), immunoglobulin concentration (i.e. IgG, IgA and IgM), somatic cell count (SCC) and microbial counts of colostrum (i.e. total plate, total coliforms, enterobacteria, staphylococci and lactic acid bacteria counts) were analyzed. A blood sample was taken from each calf between 24 and 72 hours after birth to analyze hematocrit and serum IgG concentration. Data was analyzed with a logistic regression and the odds ratio calculated from the coefficients. Forty out of the sixty-eight calves (58.8%) had a GTPI. The factors significantly associated with the odds of having a GTPI were the BW and the hematocrit of the calf; the volume and method used for colostrum feeding, and the HT of colostrum; and the dry matter, protein, pH, IgG, SCC, and all microbial counts of colostrum. This study shows that the TPI is a complex process involving many factors and that a comprehensive strategy involving the peripartum and neonatal periods is required to achieve a GTPI.

This work is funded by National Funds through FCT—Foundation for Science and Technology under the Projects CECAV UIDB/00772/2020 (<https://doi.org/10.54499/UIDB/00772/2020>), MED UIDB/05183/2020 (<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>), CISAS UIDP/05937/2020 (<https://doi.org/10.54499/UIDP/05937/2020>; <https://doi.org/10.54499/UIDB/05937/2020>) and F.G.S. PhD grant UI/BD/150834/2021 (<https://doi.org/10.54499/UI/BD/150834/2021>).

## Impact of production season on the fatty acid composition of fat from lambs produced in Portugal - Emphasis on *trans* fatty acids and conjugated linoleic acids

A. Silva<sup>1,2,3</sup>, O. Guerreiro<sup>1,4</sup>, S. Alves<sup>2,3,5</sup>, J. Santos-Silva<sup>2,6</sup>, R. Bessa<sup>2,3,5</sup> and E. Jerónimo<sup>1,4</sup>

<sup>1</sup>CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo) / IPBeja (Instituto Politécnico de Beja, 7801-908 Beja, Portugal

<sup>2</sup>CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>3</sup>AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>4</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal

<sup>5</sup>Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>6</sup>INIAV – Instituto Nacional de Investigação Agrária e Veterinária, Polo de Investigação de Santarém, Quinta da Fonte Boa, 2005-424 Vale de Santarém, Portugal

Email: [andrea.silva@cebal.pt](mailto:andrea.silva@cebal.pt) / [eliana.jeronimo@cebal.pt](mailto:eliana.jeronimo@cebal.pt)

The fatty acid (FA) composition of ruminant fat depends on its complex lipid metabolism, which is influenced by several factors, including diet. Higher levels of beneficial FAs in ruminant fat, like vaccenic (18:1-*t*11) and rumenic (18:2-*c*9,*t*11) acids, are associated with use of high-forage diets. Conversely, cereal-rich concentrate diets, such as those often used in intensive ruminant finishing, promote an increase of 18:1-*t*10 in fat, a *trans*-FA associated with detrimental health effects. Thus, the variable availability of natural resources for feeding ruminants throughout the year can affect the FA composition of lamb fat. This study aimed to characterize the FA composition of fat from lambs produced in Portugal in two production seasons marked by differing pasture availability. A total of 402 samples of kidney knob channel fat from lambs, produced in different regions of Portugal, was collected at abattoirs, specifically 213 samples during the autumn/early winter (A/W, absence of pasture) and 189 during late winter/spring seasons (W/Sp, abundance of pasture). The FA methyl esters were analysed through GC-FID. Production season affected the fat content of straight-chain saturated FA, branched-chain FA, and polyunsaturated FA, revealing higher concentrations in the W/Sp season (59.4; 1.60 and 4.56 g/100g total FA (TFA), respectively) compared to A/W season (53.2; 1.41 and 4.17 g/100g TFA, respectively); while monounsaturated FA were higher in A/W season (39.9 vs. 33.9 g/100g TFA). Vaccenic acid, was the main *trans*-FA in 82% of the samples obtained during W/Sp season, ranging from 0.60 – 7.3 g/100g TFA. In these samples, the 18:1-*t*10 varied between 0.11 – 9.0 g/100g TFA. Conversely, in samples from A/W season, 18:1-*t*10 was the main *trans*-FA in 41% samples, ranging from 0.12 – 11.1 g/100g TFA, while in the remaining samples the main *trans*-FA was 18:1-*t*11, which varied between 0.22 and 7.3 g/100g TFA. Rumenic acid presented higher levels in W/Sp season (0.89 vs. 0.62 g/100g TFA). These results showed that fat from lambs produced in Portugal during late winter and spring, a period characterized by higher availability of pasture, has a more favourable nutritional value due to a higher concentration of FA considered beneficial to health.

This work was financially supported by the project “Val+Alentejo – Valorisation of small ruminant products from Alentejo” funded by Program Alentejo 2020 (ALT20-03-0246-FEDER-000049) through the European Regional Development Fund (ERDF), and the Portuguese Foundation for Science and Technology (FCT), under the projects UIDB/05183/2020 and UIDP/05183/2020 to MED (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/UIDP/05183/2020>), UIDB/00276/2020 to CIISA (<https://doi.org/10.54499/UIDB/00276/2020>), LA/P/0121/2020 to CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>), LA/P/0059/2020 to AL4AnimalS (<https://doi.org/10.54499/LA/P/0059/2020>) and the PhD studentship of A. Silva (2022.12759.BD) - FCT.

## Epidemiological Characterization of Ocular Squamous Cell Carcinoma in cattle from Azores archipelago (Portugal)

Beatriz Bilhastre <sup>1,2</sup>, Mário Silveira <sup>3</sup>, João Fagundes da Silva <sup>3</sup>, João Vidal <sup>7</sup>, Helena Vala <sup>4,5</sup>, Sandra Branco <sup>2,6</sup>, Carlos Pinto <sup>8</sup>

1 Egas Moniz School of Health and Science, University Campus, Quinta da Granja, 2829-511 Monte da Caparica, Almada [b.bilhastre@gmail.com](mailto:b.bilhastre@gmail.com)

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

3 UNICOL – Agricultural Cooperative, CRL. Rua Dr. Aníbal Bettencourt, nº281, Vinha Brava, Conceição, 9700-236 Angra do Heroísmo, Terceira, Azores, Portugal [joao.fagundes@unicol.pt](mailto:joao.fagundes@unicol.pt) [mariosilveira@hotmail.com](mailto:mariosilveira@hotmail.com)

4 CERNAS, Escola Superior Agrária de Viseu, Instituto Politécnico de Viseu, Campus Politécnico, 3504-510 Viseu, Portugal [hvala@esav.ipv.pt](mailto:hvala@esav.ipv.pt)

5 Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

6 Department of Veterinary Medicine. School of Science and Technology, University of Évora. Laboratory of Pathological Anatomy of the EU Veterinary Hospital. Colégio da Mitra, Herdade da Mitra – Valverde 7000-083 Évora. [smbb@uevora.pt](mailto:smbb@uevora.pt)

7 Associação Agrícola de São Miguel – Cooperativa União Agrícola. C.R.L, Recinto da Feira – Campo de Santana 9600-096 – Vila de Rabo de Peixe – Ribeira Grande, São Miguel, Portugal. [jmrvidal@hotmail.com](mailto:jmrvidal@hotmail.com)

8 Faculty of Agricultural and Environmental Sciences—University of the Azores, Rua Capitão João d'Ávila—Pico da Urze, 9700-042 Angra do Heroísmo, Portugal; [carlos.a.pinto@uac.pt](mailto:carlos.a.pinto@uac.pt)

Ocular Squamous Cell Carcinoma (OSCC) is a primary malignant neoplasm originating from keratinocytes, mainly affecting the third eyelid. Considered the most common ocular neoplasm in cattle, it causes a high economic impact due to carcass rejections in slaughterhouses, treatment costs and decreased production. Elderly *Bos Taurus* cattle without periocular pigmentation exposed continuously to ultraviolet (UV) solar radiation and present in higher altitudes are the most affected. The incidence is high in the cattle population in Azores islands, so the aim is to determine the factors underlying the genesis of these neoplasms. The methodology consisted of preparing an epidemiological survey to be completed by veterinarians who practice livestock species clinic in the Azores. From August 2023 to the present, seventy-six surveys regarding observed clinical cases of bovine OSCC have been completed. The variables analyzed and recorded included reproductive status, breed, altitude, age of the animal, type of reproduction on the farm, location of the lesion, periocular pigmentation and occurrence of suspected cases of pithomycotoxicosis. Of the total number of surveys, fifty-four belonged to Terceira Island, twenty-one belonged to São Miguel Island and one to Faial Island from the Azores archipelago. The animals were all female, with the Holstein-Friesian breed predominating (100% of the results) and most cases were found between the age 5 and 9 years, located in medium altitude areas (200 to 400 m). The results obtained indicate that the greater the longevity, the greater the frequency of cases. In accordance with what is described in the scientific literature, all the cases recorded correspond to animals that are grazed all year round, without stables, with permanent exposure to UV radiation from sunlight, with the third eyelid being the most frequently affected ocular structure ( $\approx 67\%$ ). Of the suspected cases of OSCC, 48% of cattle were observed with ocular pigmentation. There was a high prevalence of

suspected cases of pithomycotoxicosis on farms where OSCC was diagnosed (50%), with most of these cases ( $\approx 72\%$ ) using zinc supplementation as a preventive measure. The aim of the study is to continue evaluating the etiological factors underlying this pathology, in addition to exposure to UV radiation, such as pithomycotoxicosis or papillomavirus infection as well as the molecular biology studies of this tumors.

**Acknowledgements:**

This work is financed by National Funds through FCT – Foundation for Science and Technology within the scope of the projects CERNAS UIDB/00681/2020 and CITAB UIDB/04033/2020 ([https //doi.org/10.54499/UIDB/04033/2020](https://doi.org/10.54499/UIDB/04033/2020)). This work is financed by National Funds through FCT – Foundation for Science and Technology within the scope of the project UIDB/05183/2020-

## **Rising Popularity of Sushi in Portugal: Assessing Risks and Promoting Safe Consumption**

I. Mira<sup>1</sup>, M. Laranjo<sup>2</sup>, R. Assunção<sup>3</sup>

<sup>1</sup>MED-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, 7006-554 Évora, Portugal.

<sup>2</sup>MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, IIFA-Instituto de Investigação e Formação Avançada, & Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, 2829-511 Caparica, Almada, Portugal

Email: [d50831@alunos.uevora.pt](mailto:d50831@alunos.uevora.pt)

Portugal, a country with a long tradition of cooked fish consumption, is witnessing an increase in the popularity of sushi. This shift presents a unique opportunity to explore potential public health risks associated with increased raw fish intake, specifically exposure to histamine (a foodborne biogenic amine) and zoonotic diseases (like anisakiasis).

This study will use a questionnaire, informed by existing research in Spain and Portugal, to investigate raw fish consumption habits among the Portuguese population. The survey will cover several questions targeting sociodemographic characteristics, health status, and raw fish consumption patterns (quantity, type, and source). Distribution will occur via email to universities, high schools, and sushi restaurants, alongside social media, and word-of-mouth promotion, with a 6-month data collection period to ensure a robust sample size.

The anticipated results will undergo a risk-benefit analysis to identify potential public health concerns associated with raw fish consumption in Portugal. This will involve exploring correlations between demographics, health status, consumption patterns, and awareness of associated risks.

The current study aims to provide crucial information on raw fish consumption habits in Portugal, particularly regarding sushi. The findings will be helpful in developing preventive measures and recommendations for both consumers and sushi establishments. This knowledge is essential for protecting public health in a changing gastronomic scenario in Portugal.



## Study of the intestinal microbiome of healthy and atopic dogs

T. Lagoa<sup>1,3</sup>, S. Conceição<sup>1</sup>, M. Laranjo<sup>1,2</sup>, L. Martins<sup>1,2</sup>, C. Queiroga<sup>1,2</sup>

<sup>1</sup>MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, IIFA-Institute for Advanced Studies and Research, University of Évora, Polo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>Department of Veterinary Medicine, School of Sciences and Technology, University of Évora, Polo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>Escola Superior de Biociências de Elvas, Instituto Politécnico de Portalegre, 7350-092 Elvas, Portugal

Email: [d50889@alunos.uevora.pt](mailto:d50889@alunos.uevora.pt)

The lifestyle choices, such as diet, living habits and mental stress have profound implications for health and disease. In modern urban environments, both humans and dogs are exposed to chemicals and high levels of indoor allergens. Smaller family sizes and reduced early-life exposure to microorganisms contribute to the growing prevalence of atopic dermatitis (AD). This study argues that the development of atopic dermatitis (AD), a significant public health issue, is linked not only to the skin microbiome, but also to the gut flora, or microbiota. The term microbiota refers to the collection of microorganisms in an ecosystem and represents the largest source of non-self-antigens in the body. The resident microbiota plays a crucial role in preserving the structure and functionality of the gut and is a key factor in supporting host immunity, protecting against invading gut pathogens, and supplying nutritional benefits. Disruptions or imbalances in the microbiota, known as dysbiosis, can impact intestinal barrier function and immune responses, resulting in serious health issues in both the gastrointestinal tract and other body systems. The objective of this study is to investigate the differences in faecal microbiota from canine patients with AD and healthy control individuals, using metagenomic methodologies. We aim to show that gut alterations can contribute to the aetiology of skin pathology and to encourage discussion around the excessive use of antibiotic treatments in these animals. The administration of systemic antibiotics disrupts gut flora and impairs skin disorders, through the connection between the gut and skin – known as the gut-skin axis. This study seeks to raise awareness about antibiotic use, as well as investigating the presence of antimicrobial resistance namely antimicrobial resistance genes relevant to public health and to veterinary medicine. We propose microbiome modulation as an approach to prevent the use of antimicrobials through food, probiotics, prebiotics and faecal transplants to manipulate the intestinal microbiota. We hope that microbiome replacement strategies will shape future clinical treatments for AD.

This work was funded by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/05183/2020.

## **Influence of Temperature and Humidity on Sample Preservation prior to the Molecular Identification of Major Gastrointestinal Strongylids (GIS) in Small Ruminants**

Margarida Fitas<sup>1,2</sup>, Ana Teresa Belo<sup>3</sup>, Inês Delgado<sup>2,4,5</sup>, Sónia Lucena<sup>1</sup>, Ludovina Padre<sup>1</sup>, Alexandre Leitão<sup>2,4</sup>, Sara Zúquete<sup>1,2,4,5</sup>

<sup>1</sup>MED - Mediterranean Institute for Agriculture, Environment, and Development, University of Évora, Apartado 94, 7006-554 Évora, Portugal.

<sup>2</sup>CIISA – Interdisciplinary Centre for Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477, Lisbon, Portugal

<sup>3</sup>National Institute of Veterinary Research, I.P. (INIAV, I.P.), Quinta da Fonte Boa, Vale de Santarém, 2005-048 Santarém, Portugal.

<sup>4</sup>Associated Laboratory for Animal and Veterinary Science (AL4Animals)

<sup>5</sup>Faculty of Veterinary Medicine, Universidade Lusófona – Centro Universitário de Lisboa, 1749-024, Lisbon, Portugal.

Email: [l42978@alunos.uevora.pt](mailto:l42978@alunos.uevora.pt)

The exponential development of resistance to anthelmintics is increasingly threatening the small ruminant production sector. Current diagnostic methods for gastrointestinal strongylid infections depend on the collection of fresh faeces, maintained at a consistent refrigeration temperature of 4°C, with rapid transport and laboratory processing to prevent egg hatching. Implementing molecular diagnostic techniques offers a solution to these strict requirements, simplifying both the diagnostic process and associated logistics. To assess the influence of temperature and humidity on coprological samples prior to genomic DNA extraction, this study sought to develop a simple and rapid method using faecal samples from sheep that had been dewormed for over three months. For this experiment, half of the samples were kept at room temperature and the other half refrigerated at 4°C for 24 hours in order to investigate the influence of temperature prior to DNA genomic extraction. Samples were then pressed onto filter paper (3 mm in thickness), and, to study the impact of the natural humidity of samples on DNA extraction, half of each previous groups was placed in a oven at 25°C for 16 hours (dry samples) prior to extraction while the remaining groups were, immediately, extracted (fresh samples). The DNeasy Blood & Tissue kit (Qiagen) was used for genomic DNA extraction, successfully extracting genomic DNA from all tested samples. It was possible to correlate the extracted quantities with the level of humidity present in the samples. Amplification of a segment of the rRNA ITS1 nematode gene (Internal Transcriber Spacer) was only achieved in samples that underwent the drying process in the oven, regardless of temperature.

Although still in the early stages, the results presented here indicate that the storage temperature of the samples is not decisive for identifying gastrointestinal strongylids, but rather the humidity level is critical. The methodology is quick to execute and highly adaptable, allowing it to be performed in the field, thus facilitating the transport, storage, and routine laboratory processing and analysis of samples. Consequently, we will continue to optimise the technique under study and investigate why only oven-dried samples achieved ITS1 gene amplification.

This work was funded by SUMO: Sustainability of Montado PRR-C05-i03-I-000066; FCT – Foundation for Science and Technology, I.P. (Portugal) through projects UIDB/00276/2020; LA/P/0059/2020 - AL4Animals.

**Preliminary Assessment of the Productive Impact of *Theileria buffeli* / *orientalis* Infection in Dairy Cattle on São Miguel Island, Azores**

Maria Felício<sup>1,2</sup>, Sara Tudela Zúquete<sup>1,3,4,5</sup>, Elisa Bettencourt<sup>1</sup>, Alexandre Leitão<sup>3,4</sup> and Ludovina Padre<sup>1</sup>

<sup>1</sup>MED – MED mediterranean institute for agriculture, environment and development, Universidade de Évora, Universidade de Évora Pólo da Mitra, Apartado 94, 7006-554 Évora, Portugal;

<sup>2</sup>Cooperativa Juventude Agrícola, CRL., Centro de Bovinicultura – Arribanas- Arrifes 9500-372 Ponta Delgada – S. Miguel – Açores

<sup>3</sup>CIISA - Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, 1300-477 Lisboa, Portugal;

<sup>4</sup>AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal,

<sup>5</sup>Faculty of Veterinary Medicine, Universidade Lusófona, Av. do campo Grande 376, 1749-024 Lisboa;

The Azores archipelago is one of Portugal's most prominent regions for cattle production. Previously, our group found a prevalence of 45% for asymptomatic *Theileria buffeli/orientalis* infections across ten dairy farms on São Miguel Island. This study aimed to monitor productivity on a selected farm to evaluate potential subclinical impacts of infection.

On this farm, all animals in the milking facility—including lactating and pre-calving cows, but excluding calves, heifers, and dry cows—underwent PCR screening for *Theileria buffeli/orientalis*. Of the 74 samples, seven were confirmed positive, resulting in a 9.5% infection rate. This prevalence is lower than in our previous study, though some previously surveyed farms also showed low infection rates with only one positive case.

Over the six months following sampling, the negative group showed a higher average milk yield per cow (31.726 kg) compared to the positive group (29.574 kg). The negative group also exhibited higher protein content (3.447) than the positive group (3.362), while the positive group showed slightly higher butterfat content (4.086 vs. 4.032). Statistically, no significant association was found between these productivity measures and infection status, which is expected given that only 7 of the 74 animals tested positive.

Follow-up studies on this farm may offer further insights into the impact of *Theileria buffeli/orientalis* infections on dairy production in endemic areas. With this study ongoing, extended research across more farms and over longer periods is essential to fully capture subclinical effects, enabling a deeper understanding of the parasite's influence on productivity and animal health. By monitoring these subclinical impacts, this study contributes to advancing evidence-based management practices, supporting more informed strategies for herd health and optimizing dairy production in affected areas.







## Unlocking the potential of lignin for hair care: from extraction to sustainable conditioners

C. Fernandes<sup>1,2</sup>, B. Medronho<sup>2,3</sup>, Luís Alves<sup>1</sup>, M. G. Rasteiro<sup>1</sup>, C. Varela<sup>1,4,5</sup>

<sup>1</sup>University of Coimbra, CERES, Department of Chemical Engineering, Coimbra, Portugal.

<sup>2</sup>MED & CHANGE, Universidade do Algarve, Faro, Portugal.

<sup>3</sup>FSCN, Surface and Colloid Engineering, Mid Sweden University, Sundsvall, Sweden.

<sup>4</sup>University of Coimbra, iCBR, Clinic Academic Center of Coimbra, Coimbra, Portugal.

<sup>5</sup>University of Coimbra, CIBB, Coimbra, Portugal.

Email: [csfernandes@uc.pt](mailto:csfernandes@uc.pt)

The use of cosmetic products is a highly popular practice. However, the increasing use of these products has raised concerns about their environmental impact. For example, some compounds commonly found in hair care formulations, such as cationic conditioning agents, are reported to be toxic to aquatic organisms<sup>1</sup>. The rinsing of hair care products results in their entry into wastewater systems, and, eventually, their discharge into rivers and oceans. These problems, and the increasing preference for natural products with low environmental impact, have motivated the research on renewable feedstocks for the development of cosmetic formulations<sup>2</sup>. Biopolymers, such as lignin, are excellent candidates to be used in bio-based formulations. Lignin is a natural polyphenol that can be seen as a multi-functional cosmetic ingredient for hair care, offering antibacterial, antioxidant, and sun-protective activities<sup>2</sup>. Lignin's hydrophobic nature can help to restore the hydrophobic barrier characteristic of healthy hair and its functional groups allow chemical modifications, which can enhance the interactions with hair. In this work, lignin was extracted from acacia wood and then chemically modified to prepare cationic derivatives that could act as hair conditioning agents. The effect of the cationization conditions on the degree of substitution and surface charge of the prepared polymer were evaluated. The ability of these new lignin-based conditioning agents to efficiently repair damaged hair was assessed by evaluating their deposition into model surfaces of the hair. Lignin derivatives showed lower ecotoxicity to aquatic organisms of different trophic levels compared to a conventional commercial polymer (polyquaternium-11). These lignin-based conditioning agents were then incorporated into preliminary formulations and their stability and sensorial properties evaluated.

### References:

[1] Bujak, T., Niziol-Lukaszewska, Z., Ziemiańska, A. 2020. Amphiphilic cationic polymers as effective substances improving the safety of use of body wash gels. *Int. J. Biol. Macromol.*, 147: 973–979.

[2] Fernandes, C., Medronho, B., Alves, L., Rasteiro, M.G. 2023. On Hair Care Physicochemistry: From Structure and Degradation to Novel Biobased Conditioning Agents. *Polymers*, 15, 608.

### Acknowledgments:

This work was financially supported by the Portuguese Foundation for Science and Technology, FCT, via the projects 2022.06810.PTDC, UIDB/05183/2020, and UIDB/00102/2020, and the researcher grants CEECIND/01014/2018 and 2021.00399.CEECIND. C. Fernandes also acknowledges FCT for the PhD grant (2021.05991.BD).

## **Rethinking road verges to promote Mediterranean wildflowers: give biodiversity a chance**

M.P. Fernandes<sup>1,2</sup>, P. Matono<sup>3</sup>, G. Gomes<sup>4</sup>, C. Pinto-Cruz<sup>3</sup>, E. Almeida<sup>1</sup>, A.D.F. Belo<sup>3</sup>

<sup>1</sup>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, 7002-554 Évora, Portugal

<sup>2</sup>cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

<sup>3</sup>MED - 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

<sup>4</sup>Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

Email: [mpfernandes@uevora.pt](mailto:mpfernandes@uevora.pt)

Urban areas and their network of linear infrastructures are growing worldwide, homogenising landscapes and causing habitat fragmentation and biodiversity losses. Still, anthropogenic features can help offset the biodiversity crisis if used to promote semi-natural habitats. Road verges, if adequately managed, can sustain well-established plant assemblages, establishing novel habitats and acting as corridors between semi-natural landscapes. We have developed and applied a native wildflower seed mix to rehabilitate vegetation patches in Mediterranean road verges. This study aims to assess the dynamics of the seed mix over four years and its effect on the road verge plant community.

We applied the seed mix on three national roads in southern Portugal in two phases: a trial phase in 2018, when we sowed 7 plots, and an extended trial in 2020 with 21 plots. We also established control plots. No watering or soil fertilisation was applied. We monitored all plots in spring during four years through floristic surveys to assess species frequency and abundance. The seed mix dynamic was consistent, with similar composition and diversity levels in both trials. The sown species persisted over time, with a seed mix persistence index higher than 0.5. Nonetheless, the seed mix community changed over time: the first and second years were dominated by annual species, gradually displaced by biennial/perennial species during the third and fourth years. Flowering success was high (about 75%), contributing to the seed mix sustainability. The seed mix application benefited the existing plant community, significantly increasing species richness and diversity in the sown plots.

The tested seed mix promoted spatial diversity and the temporal heterogeneity of extant vegetation, and it was compatible with the mowing regime of road verges (mowing in early to mid-spring); therefore, it seems suitable for use on road verges or similar linear infrastructures with a Mediterranean climate. Applying native wildflower seed mixes on road verges helps offset these infrastructures' adverse effects, increasing plant diversity and providing heterogeneity of plant communities.

This work was funded by the EU Life Programme "LIFE LINES Linear Infrastructure Networks with Ecological Solutions" (LIFE14 NAT/ PT/001081) and by National Funds through FCT under the PhD grant 2020.06339.BD.



## **Sustainable Management of Natural Remnant Habitats in the Montado: Advancing Conservation Through Practical Tools**

E. Almeida<sup>1</sup>, P. Matono<sup>2</sup>, C. Pinto-Cruz<sup>2</sup>

<sup>1</sup>MED – 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.

<sup>2</sup>MED – 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](mailto:erikaroldao@gmail.com) | [d49668@alunos.uevora.pt](mailto:d49668@alunos.uevora.pt)

The Montado, a traditional agro-silvo-pastoral system, represents a High Nature Value (HNV) landscape, supporting high levels of biodiversity. Within this landscape, Natural Remnant Habitats (NRH) are small, heterogeneous patches of native vegetation that play a crucial role in maintaining ecosystem stability and biodiversity conservation. These "pockets" of biodiversity offer unique ecological niches and contribute to the provision of essential ecosystem services. However, land-use changes and intensification practices threaten the integrity of these habitats, risking biodiversity loss.

This study aims to develop a robust classification system to evaluate the conservation status of NRH within Montado landscapes. The following NRH typologies were considered: forest and shrubs patches, rocky outcrops, temporary and permanent ponds, and riparian gallery. Field data collection encompassed vegetation strata cover, plant species surveys, and additional metrics such as shadow and rock cover percentages. The fieldwork was conducted across 36 sites within 22 different estates in the Montado of Central Alentejo. A total of 230 Natural Remnant Habitats (NRHs) were sampled during the spring months from 2021 to 2023. Data analysis involved the grouping of NRH based on floristic composition and biophysical attributes, identifying high-quality reference groups, and calculating non-correlated indicators that effectively distinguish these reference groups. These indicators were then included in a scoring and classification framework to assess conservation status levels for each NRH.

The results highlighted specific indicators of conservation status for each NRH typology, integrating both biophysical parameters and characteristic species. This indicator selection enables precise assessments of NRH quality, particularly relevant to ecological management practices. A preliminary assessment based on expert judgment revealed limited concordance with the current results, aligning only for habitats at the extremes of the conservation gradient (i.e., bad and excellent). This emphasizes the importance of objective indicators to complement expert evaluations. Ultimately, this study delivers a practical tool for conservation evaluation in NRH, suitable for results-based payment schemes that support sustainable management practices.

This work is funded by National Funds through FCT under the Ph.D. scholarship 2020.07109.BD. and Project UIDB/05183/2020.

## **Indicators to assess social sustainability: similarities and differences between three wine regions (Verdes, Douro and Alentejo)**

J. Massuca<sup>1</sup>, A. Marta-Costa<sup>2</sup>, M. R. Lucas<sup>3</sup>, A. Baptista<sup>2</sup>

<sup>1</sup>CETRAD (Centro de Estudos Transdisciplinares para o Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-801 Vila Real, Portugal.

<sup>2</sup>CETRAD (Centro de Estudos Transdisciplinares para o Desenvolvimento), Departamento de Economia, Sociologia e Gestão, Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-801 Vila Real, Portugal.

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

Email: [josemassuca@utad.pt](mailto:josemassuca@utad.pt)

Studies on the sustainability of agribusinesses often overlook the social dimension of sustainability. Among the three traditional pillars of sustainability, the social dimension is clearly the least studied, and the classification and estimation of the indicators used to measure it present some weaknesses. Furthermore, existing sustainability assessment models have some drawbacks, such as political and territorial management biases, lack of comparability, and most importantly, they do not give ownership to the stakeholders. Additionally, many social indicators used in sustainability assessment models, currently applied across the agribusiness ecosystem, focus on the internal dynamics of companies without considering the comprehensive global perspective of the agribusiness sector and the region they refer to. Therefore, it is of major importance that assessment models are adapted to the overviewed context, especially in the case of complex winegrowing systems that are inherently linked to surrounding environments. Naturally, the region will dictate important priorities regarding the aspects deemed worthy of assessment within the realm of social sustainability. That is the major reason why it is crucial to gather diverse local stakeholder input during the development of a model intended for replication.

The aim of this study is to identify and weigh the most appropriate indicators for evaluating social sustainability, supporting this identification on the genuine concerns of stakeholders. Through a systematic literature review, forty social pillar indicators related to the social pillar were compiled, ensuring they align with the unique social characteristics and specificities of various regions and contexts.

To refine the selection and weighting process, a participatory and collaborative methodology was implemented using an adapted Real-Time Delphi approach. This method incorporates functional innovations to calculate consensus from responses collected during online collaborative sessions with stakeholders. Unlike the traditional Delphi method, which often narrows participants' choices to a single consensus that may not reflect their initial preferences, the adapted approach expands the range of options after each response round. This adjustment fosters a convergence toward approximate consensus while preserving and valuing diverse perspectives rather than prematurely discarding divergent viewpoints.

A total of twenty-six stakeholders participated in this process, representing three distinct geographical regions within the Vine and Wine agribusiness. The workshops included a diverse cross-section of the agribusiness sector, including small producers, winery managers, field staff, grape and wine specialists, representatives of local organizations involved in viticulture and winemaking, oenologists, professionals from the wine tourism sector, and members of civil and governmental institutions.

Results indicate a higher degree of agreement than disparity regarding the selection of indicators and their importance in the model. Notably, the "Fair Salary" indicator emerged as

the most positively significant across all regions, while the "Level of Computer Use" consistently ranked as having the greatest negative importance.

This study contributes to the establishment of a foundational framework for assessing perceptions of social sustainability. The framework is grounded in consensus-built indicators and validated through a participatory, collaborative methodology, offering a robust tool for evaluating social dimensions in diverse contexts.

This research is supported by the R&D project 'Vine & Wine PT—Driving sustainable growth through smart innovation', with the financial support of the PRR (Recovery and Resilience Plan) and EU Next Generation funds, under the auspices of the 'Agenda for mobilising reindustrialisation', and by national funds through the FCT (Portuguese Foundation for Science and Technology) under the projects UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>) and UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>); <https://doi.org/10.54499/LA/P/0121/2020>).

## The role of five Mediterranean tree species in enhancing climate resilience in Mediterranean cities

P. Matias<sup>1,2,4</sup>, J. Teigão<sup>3</sup>, M. Moreira da Silva<sup>2,3</sup>, A. Duarte<sup>1,4</sup>

<sup>1</sup>MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal;

<sup>2</sup>Centre for Marine and Environmental Research (CIMA), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal;

<sup>3</sup>Institute of Engineering, University of Algarve, Campus da Penha, 8005-139 Faro, Portugal;

<sup>4</sup>Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Email: [pmmatias@ualg.pt](mailto:pmmatias@ualg.pt)

Mediterranean cities are particularly vulnerable to climate change, which is accelerated by greenhouse gases like CO<sub>2</sub> in the atmosphere. Rising temperatures in these cities lead to more frequent heat waves and water scarcity. Incorporating nature into urban areas can help mitigate these impacts by enhancing cities resilience. Urban trees sequester and store carbon, reducing CO<sub>2</sub> in the atmosphere, produce oxygen, and remove pollutants from the air. Additionally, shading and transpiration lower local temperatures. Tree canopies also intercept rainwater, helping to manage runoff during precipitation.

To quantify their ecosystem services in an urban environment, we studied all trees from five species in the city of Faro, Portugal: *Ceratonia siliqua* (100 trees, 0.5 – 12.7 m tall), *Olea europaea* (369 trees, 1 – 15 m tall), *Pinus pinea* (40 trees, 3 – 15.4 m tall), *Quercus suber* (30 trees, 1.6 – 13 m tall), and *Quercus ilex* (18 trees, 2 – 12.5 m tall). Using the i-Tree Eco model, we found that the species demonstrated varying average CO<sub>2</sub> sequestration and O<sub>2</sub> production rates: *P. pinea* > (*O. europaea*  $\cong$  *Q. ilex*) > (*C. siliqua*  $\cong$  *Q. suber*). However, *C. siliqua*, *Q. ilex* and *Q. suber* were the most efficient in CO<sub>2</sub> sequestration per unit of leaf area. The average carbon stored per tree ranged from 87 kg in *C. siliqua* to 700 kg in *P. pinea*. *P. pinea* removed the most atmospheric pollutants on average, while *C. siliqua* and *Q. suber* removed the least, including CO, O<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub>, PM10 (particles with diameters  $\leq$  10  $\mu$ m), and PM2.5 (particles with diameters  $\leq$  2.5  $\mu$ m). Transpiration and rainwater interception were directly dependent on leaf area, with *P. pinea* showing the highest values and *C. siliqua* and *Q. suber* the lowest. The studied species play a role in enhancing urban climate resilience, highlighting their importance in the design and integration of green spaces within Mediterranean cities.

## **The impact of soil management on soil hydraulic properties in Olive orchards of Southern Portugal**

Ruben Heutink<sup>1</sup>, Loes van Schaik<sup>1</sup>, João Canedo<sup>2</sup>, Bernardo Lameira<sup>2</sup>, Luísa Coelho<sup>2</sup>, Sérgio Prats<sup>2,3</sup>

<sup>1</sup>Soil Physics and Land Management Group, Wageningen University and Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands.

<sup>2</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Núcleo da Mitra, Évora 7006-554, Portugal

<sup>3</sup>Misión Biológica de Galicia – Consejo Superior de Investigaciones Científicas (MBG-CSIC), Salcedo, Pontevedra, España.

Email: [ruben.heutink@wur.nl](mailto:ruben.heutink@wur.nl)

Olive groves in the Iberian Mediterranean landscapes have been intensified to guarantee their profitability, by means of higher planting density and higher management inputs (irrigation, fertigation, phytosanitary treatments). This trend has negative environmental implications (i.e. increased soil compaction, lowering soil organic matter) which can lead to reduced soil infiltration capacity and increased risk of soil erosion and soil degradation. In this research, the effect of some three soil treatments (Untreated control, Mulch of olive leaves and mulching+biochar) and microenvironments affecting differently the soil surface; the tree (area beneath the tree space), the rut (area affected by the wheels passage) and the vegetation strip (area between the two areas of rut) will be measured. Two orchards (part of SOLVO) located in the vicinity of Évora, an integrated intensive olive orchard and an extensive, organic rainfed olive orchard will be assessed.

The methodology consisted in determining some soil hydraulic properties, such as the soil infiltration rate, the soil moisture, the soil water repellency and the resistance of soil to the penetration in untreated, mulch and mulch+biochar plots and under tree, rut and vegetation microenvironments.

Preliminary results showed that the effect of the microenvironment was stronger than the effect of the treatments. The application of mulching in combination with biochar, followed by the mulch treatment, will result in the highest infiltration capacities. This treatment also provides the lowest resistance of the soil to penetration and thus provides a higher conductivity and soil structure will be improved when using biochar and mulching.

Keywords: Olive groves, infiltration capacity, tree, rut, vegetation strip, SOLVO, hydraulic properties.

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

## Urban atmospheric pollution affects platanus tree pollen allergenicity

Teixeira R.V.<sup>1</sup>, Galveias A.<sup>1</sup>, Lara B.<sup>2,3</sup>, Costa M. J.<sup>1,4,5</sup>, Rojo J.<sup>7</sup>, Bortoli, D.<sup>1,4,5</sup>, Antunes C. M.<sup>1,6</sup>,  
Pérez-Badia R.<sup>2</sup>, Costa A.R.<sup>1,6</sup>

<sup>1</sup>Institute of Earth Sciences, Évora, Portugal, Institute of Research and Advanced Training; University Évora.

<sup>2</sup>Institute of Environmental Science. University of Castilla-La Mancha., Toledo, Spain.

<sup>3</sup>Department of Chemical and Environmental Engineering. Polytechnic University of Cartagena, Cartagena, Spain.

<sup>4</sup>Earth remote Sensing Laboratory (EaRSLab), Évora, Portugal.

<sup>5</sup>Department of Physical, School of Sciences and Technology, University of Évora, Portugal.

<sup>6</sup>Department of Medical and Health Sciences, School of Health and Human Development, University of Évora.

<sup>7</sup>Department of Pharmacology, Pharmacognosy and Botany. Complutense University, Madrid, Spain.

Email: [d61919@alunos.uevora.pt](mailto:d61919@alunos.uevora.pt)

Air pollution aggravates asthma and respiratory allergies. Road traffic is one of the major sources of air pollutants in Iberian Cities. Aero-disseminated pollen contacts with air pollutants generated by automobile traffic, potentially changing its protein and/or allergenic profile.

The main goal was to evaluate the allergenicity of *Platanus x hispanica* (platanus) pollen from Lisbon, Toledo and Madrid in relation to the local air pollutant levels.

Pollen was harvested in 2019 in Lisbon, Toledo and in four locations within Madrid, close to Air Quality Monitoring Stations that provide air pollutant levels. Pla a 1 was quantified by ELISA. Immunoblot using pooled sera from allergic individuals was used to obtain IgE-recognition patterns. Correlation analysis between Pla a 1 and air pollutant levels were evaluated.

Pollen collected from different locations have shown different IgE-recognition profiles. Pla a 1 content varied in the range 1.2-2.2 µg/mg protein being higher in Madrid (*Retiro*) and lower in Madrid (Pz.Elíptica). Allergen was negatively correlated with pollutant levels of NO, NO<sub>2</sub> and NO<sub>x</sub> and positively with O<sub>3</sub> and PM<sub>2.5</sub>.

These results highlight an association between air pollution and platanus tree pollen allergenic content, being O<sub>3</sub> and PM<sub>2.5</sub> exposure associated with higher allergenicity.

This work was co-funded by the European Union through the European Regional Development Fund, framed in COMPETE 2020 Operational Program Competitiveness and Internationalization, by National Funds by the Portuguese Foundation for Science and Technology (FCT) through the projects PTDC/ATP-EAM/0817/2014 and UIDB/04683/2020 - ICT (Institute of Earth Sciences) and by the Ministry of Science, Innovation and Universities of the Spanish Government through the project SICAAP-CPI RTI2018-096392-B-C22 and by the University of Castilla-La Mancha (UCLM) through the UCLM Plan Propio. Costa, AR acknowledges Fundación Carolina for a 3-month research grant in the UCLM- Campus of Toledo.

## **The bio-social mapping of antimicrobial stewardship as a tool to sustainable production: the case of pig farming in Portugal**

M. Reis<sup>1,2,3</sup>, M. Cardo<sup>1,2</sup>, M. Truninger<sup>3</sup>

<sup>1</sup>Centre for Interdisciplinary Research in Animal Health, Lisbon, Portugal (CIISA)

<sup>2</sup>Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal (FMVUL)

<sup>3</sup>Institute of Social Sciences, University of Lisbon, Lisbon, Portugal (ICSUL)

Email: [mreisvet@gmail.com](mailto:mreisvet@gmail.com)

In recent years, antimicrobials have been and continue to be an important tool for controlling infectious diseases in people and animals. However, the widespread use and misuse of antimicrobials contributes to the emergence of antimicrobial resistance (AMR), which is a normal evolutionary process of microorganisms that is accelerated by the selective pressure exerted by exposure to antimicrobials. AMR already represents a serious public health problem, as there is already scientific evidence that bacteria present in human, animal and environmental ecosystems can exchange antimicrobial-resistant bacteria or resistance determinants. AMR already represents a serious social and economic burden, as it places a significant issue on healthcare systems and puts global financial pressure on economies, for example the “tragedy of the commons”. So, it is essential to understand the dynamics of AMR in different sectors to mitigate its impact on human health and rapidly control its spread.

This project aims to study how antimicrobial stewardship is performed in pig production in Portugal, between the years 2023 and 2024, using a multidisciplinary approach, taking into consideration that in terms of sustainable production, the environmental and socio-economic pillars must be considered to find solutions to the problem.

To this aim, we will conduct a study of antimicrobial stewardship on Portuguese pig farms and the significance of antimicrobial resistance events. Therefore, establishing a relationship between data from the veterinary electronic drug prescription system from pig farming and data from the national antimicrobial resistance surveillance program, as it is necessary to integrate data on antimicrobial use and resistance and to study the national overview. The contribution of farmers and veterinary practice with farm animals is also a factor in the selection and spread of antimicrobial resistance and must be recognized. Thus, another important perspective to integrate into the study of antimicrobial stewardship in livestock will be the socio-economic-demographic and organizational farm context and its drivers such as farmers knowledge, attitudes, practices, perceptions, and beliefs.

## Biodiversity Market: Case Study for the Montado Ecosystem

Carla Janeiro<sup>1,2,3</sup>, Cristina Branquinho<sup>2</sup>, Carla Pinto-Cruz<sup>1</sup>, Victor Barros<sup>3</sup>

<sup>1</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Instituto para as Alterações Globais e Sustentabilidade, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>Ce3C – Centro de Ecologia, Evolução e Alterações Ambientais & CHANGE – Instituto para as Alterações Globais e Sustentabilidade, FCUL, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

<sup>3</sup>ADAVANCE/CSG - Centro de Investigação Avançada em Gestão, ISEG, Universidade de Lisboa, Rua do Quelhas, nº 6, 1200-781 Lisboa, Portugal

E-mail: [carla.janeiro@uevora.pt](mailto:carla.janeiro@uevora.pt), [janeiro.c@gmail.com](mailto:janeiro.c@gmail.com)

In the current context of biodiversity and climate crises, finding innovative and effective solutions is essential. Market-based solutions, such as biodiversity credits, are emerging as promising methods to reverse biodiversity loss by mobilizing private financing. In mainland Portugal, the “Montado” is the largest forest system, covering extensive areas of the Iberian Peninsula and representing a High Nature Value agroecosystem.

This study aims to contribute to the design of a biodiversity credit market, as this tool could effectively enhance ecosystem services, particularly biodiversity, while providing income for “Montado” landowners. The research includes an analysis of the evolution of this concept, characterization of functioning biodiversity markets worldwide, and the design of a mechanism proposal specific to the “Montado” ecosystem. A financial framework that considers the risks involved in the proposed mechanism will also be developed.

The expected outcomes include a detailed assessment of how the biodiversity market could contribute to reversing biodiversity loss and increasing rural landowners' incomes by promoting sustainable, results-oriented environmental management practices in the “Montado”. If successful, this mechanism could benefit approximately 6 million hectares in the Iberian Peninsula, providing an innovative and significant complement to the traditional model of public financing.

**Keywords:** biodiversity credits, ecosystem services, sustainability







## Using the dead to infer about the living: Amphibian roadkill spatiotemporal dynamics suggest local populations' reduction

Tiago Pinto<sup>1,2</sup>, Neftalí Sillero<sup>3</sup>, António Mira<sup>1,2</sup>, Sara M. Santos<sup>1,2</sup>

<sup>1</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, University of Évora, Mitra, 7006-554 - Évora, Portugal

<sup>2</sup>Conservation Biology Lab (UBC), University of Évora, Mitra, 7002-554 - Évora, Portugal

<sup>3</sup>Research Centre in Geo-Spatial Sciences (CICGE), Faculty of Science, University of Porto, Alameda do Monte da Virgem, 4430-146 – Vila Nova de Gaia, Portugal

Email: [tiagop@uevora.pt](mailto:tiagop@uevora.pt)

Roads represent one of the main sources of wildlife mortality, population decline, and isolation, especially for low-vagility animal groups. It is still not clearly understood how wildlife populations respond to these negative effects over space and time. Most studies on wildlife road mortality do not consider the spatial and temporal components simultaneously, or the imperfect roadkill detection, both of which could lead to inaccurate assumptions and unreliable mitigation actions. In this study, we applied a multi-season occupancy model to a 14-year amphibian mortality dataset collected along 120 km of roads, combined with freely available landscape and remote sensing metrics, to identify the spatiotemporal patterns of amphibian roadkill in a Mediterranean landscape in Southern Portugal. Our models showed an explicit general decrease in amphibian roadkill. The Iberian painted frog (*Discoglossus galganoi*) experienced roadkill declines over time of ~70%, while the spiny common toad (*Bufo spinosus*) and the fire salamander (*Salamandra salamandra*) had a loss of nearly 50%, and the Southern marbled newt (*Triturus pygmaeus*) had 40%. Despite the decreasing trend in roadkill, spatial patterns seem to be rather stable from year to year. Multi-season occupancy models, when combined with relevant landscape and remote sensing predictors, as well as long-term monitoring data, can describe dynamic changes in roadkill over space and time. These patterns are valuable tools for understanding roadkill patterns and drivers in Mediterranean landscapes, enabling the differentiation of road sections with varying roadkill over time. Ultimately, this information may contribute to the development of effective conservation measures.

T.P. is financed by Fundação para a Ciência e Tecnologia (FCT) with a doctoral Grant 2020.04581.BD. N.S. and S.M.S. are supported by a CEEC2017 (CEECIND/02213/2017) and CEEC2023 (CEECIND/07242/2023) contracts from FCT. This research was supported by project LIFE LINES - LIFE14 NAT/PT/001081 financed by the European Commission.

## Different inter-row management practices on a vineyard: implications on plant physiology

V. Silva<sup>1</sup>, M. Vaz<sup>3</sup>, R. Coelho<sup>3</sup>, I. Brito<sup>2</sup>, A. Alexandre<sup>2</sup>

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

<sup>2</sup>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, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>CREATE (Center for Sci-Tech Research in Earth System and Energy) and 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, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal  
Email: d38740@alunos.uevora.pt

The importance of soil health to guarantee the quality of the final product led several wine growing regions worldwide to adopt soil sustainable management practices, such as introducing cover crops in the inter-row. This conservation practice is used to improve weed control, soil and soil water conservation, and vine nutrition, growth, and productivity. The soil microbiome interacts with the aboveground plant tissues and it is able to regulate and stimulate plant responses. The microbial composition, i.e. diversity and abundance, is influenced by soil management practices that impact agroecosystem in different ways, and the relationships between soil water availability to the grapevine, water loss through evapotranspiration, grapevine photosynthesis, and yield and grape composition may be affected [1]. Photosynthetic pigments allow plants to absorb energy from light, so foliar chlorophyll and fluorescence content are key factors impacting the performance of plant photosynthesis [2]. The analysis of chlorophyll fluorescence parameters is considered an important approach to evaluating the health or integrity of the photosynthetic apparatus within a leaf. In the present study, two soil management practices were analysed: absence and presence of cover crop in inter-row with *Vitis vinifera* L. cv. Alicante Bouschet and Arinto. Our objective was to evaluate the physiological parameters, such as chlorophyll content and fluorescence, at different growing seasons of the vineyard: flowering and veraison, during the three sampling years (2022 to 2024) in order to compare the productivity and photosynthetic performance of the grapevine subjected to different soil management treatments. In addition, the physiological parameters and performance index were compared with the content in major macronutrients in the petiole, namely nitrogen(N) and magnesium(Mg). The results showed that the presence of cover crop increased the chlorophyll and fluorescence during the veraison season at year 1 and year 3 and during the flowering season of year 3 in Arinto inter-rows. In Alicante Bouschet, no differences between treatments were observed. The physiological parameters showed a significance correlation with the macronutrients in the petiole. Additional research on this topic is ongoing to better understand the effects of the cover crops in the vineyards and grape quality.

### Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the Project UIDB/05183/2020 (MED). V. Silva acknowledges a PhD fellowship from FCT (UI/BD/153513/2022). The authors are grateful to Herdade de Coelhoiros for providing all the necessary means for the *in vivo* experiments.

### References

- [1] Medrano, H., Escalona, J.M., Cifre, J., Bota, J. & Flexas, J., 2003. A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: Effects of water availability from leaf photosynthesis to grape yield and quality. *Funct. Plant Biol.* 30, 607-619
- [2] Taiz, L. & Zeiger, E., 2006. *Plant physiology*. Sinauer Associates Inc., Massachusetts, USA

### Frass derived from the bioconversion of vegetable waste by black soldier fly larvae: Effect on tomato productivity and soil multifunctionality

Catarina Malheiro<sup>1</sup>, Marija Prodana<sup>1</sup>, Diogo N. Cardoso<sup>1</sup>, Sara Peixoto<sup>1</sup>, Pedro Monteiro<sup>1</sup>, Ana Rita R. Silva<sup>1</sup>, Fábio Campos<sup>1</sup>, Pedro Sarmiento<sup>2</sup>, Glória Pinto<sup>1</sup>, Isabel Henriques<sup>3</sup>, Carla Patinha<sup>4</sup>, Daniel Murta<sup>5,6</sup>, Rui G. Morgado<sup>1</sup> and Susana Loureiro<sup>1</sup>

<sup>1</sup>CESAM & Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; <sup>2</sup>Escola Profissional de Agricultura e Desenvolvimento Rural de Vagos, Estrada Florestal – Gafanha da Boa-Hora, 3840-254 Vagos, Portugal; <sup>3</sup>University of Coimbra, CEF- Centro de Ecologia Funcional & Department of Life Sciences, Faculty of Sciences and Technology, Portugal; <sup>4</sup>GeoBioTec, Department of Geoscience, University of Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal; <sup>5</sup>Ingredient Odyssey – EntoGreen, Zona Industrial de Santarém, 2005-079 Santarém, Portugal. <sup>6</sup>CiiEM – Centro de Investigação Interdisciplinar Egas Moniz, Campus Universitário, Caparica, Portugal. Email: [catarinalemosmalheiro@ua.pt](mailto:catarinalemosmalheiro@ua.pt)

In recent decades, the Green Revolution dramatically boosted agricultural yields, crucial for feeding a growing population. However, this intensification has caused significant environmental degradation and increased organic waste production, threatening ecosystem services. A new agricultural revolution is urgently needed, one that emphasizes sustainability through efficiency and circularity. Technological innovations focus on the valorisation of agroindustrial waste into innovative products, such as insect frass, aimed to reduce fertilizer dependence and maintain ecosystem integrity. Our study used an ecosystem services approach to evaluate the effectiveness and environmental impact of frass derived from the bioconversion of vegetable waste by *Hermetia illucens* larvae in a greenhouse production system. A randomized block experiment was established: 1) control, 2) mineral & manure, 3) mineral & frass, and 4) frass. The integrated assessment included the evaluation of functional indicators that were grouped into ecosystem functions and valued as ecosystem services. These indicators included the tomato yield and the evaluation of the morphology and physiology of tomato plants, the abundance, composition and functionality of the soil microbial community, the abundance of soil mesofauna, the feeding activity of soil organisms *in situ*, and the evaluation of the soil retention function. The soil's physical and chemical properties from the different treatments were also assessed. This work showed that frass improved some soil properties and increased tomato yield, without damaging the plant's physiological status. Changes in the structure and functionality of the soil microbial community were observed at the end of the experiment. Higher consumption of the upper section of the bait-lamina sticks could be explained by lower biological abundance at lower depths. The application of the solarization method and the greenhouse environmental conditions could explain the lower mesofauna abundance. Our results also suggest a low risk of eutrophication. Our results indicate that the soil multifunctionality improved with the different fertilization treatments, which might positively influence agricultural production and environmental sustainability. Our multidisciplinary approach showed that frass produced by *H. illucens* larvae can be considered an appropriate and environmentally safe fertilization management option for tomato production in poor agricultural production systems, improving the sustainability of terrestrial and aquatic ecosystems.

This study was supported by the projects ECOCENE – Ecological Effects of Nanopesticides to Soil Ecosystems (PTDC/CTA-AMB/32471/2017), ENTOSAFE (PTDC/CTA-AMB/0730/2021), supported by FCT – Fundação para a Ciência e Tecnologia, within the PT2020 Partnership Agreement and Compete2020 co-funded by the FEDER – Fundo Europeu de Desenvolvimento Regional, and by the PRR – Recovery and Resilience Plan and by the NextGenerationEU funds at University of Aveiro, through the scope of the Agenda for Business Innovation “InsectERA” (Project no. 20 with the application C644917393-00000032). Thanks are due to Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES), Portugal, for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds. FCT also supported this work through a grant to C. Malheiro (PD/BD/135577/2018) and R. G. Morgado (SFRH/BPD/123384/2016). The authors would like to thank the company Ingredient Odyssey, S.A. and Daniel Murta for providing the Black Soldier Fly larvae frass.

## Levels of metals with obesogenic potential in *Mytilus galloprovincialis* along the Portuguese coast

S Coelho<sup>1,2,3</sup>, LP Rato<sup>4</sup>, C Patinha<sup>4</sup>, S Galante-Oliveira<sup>3</sup>, C Barroso<sup>3</sup>, R Pastorinho<sup>2,5</sup>, AC Sousa<sup>1,2</sup>

<sup>1</sup>Department of Biology, University of Évora, Évora, Portugal;

<sup>2</sup>Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal;

<sup>3</sup>Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Aveiro, Portugal;

<sup>4</sup>Department of Geosciences, University of Aveiro, Aveiro, Portugal;

<sup>5</sup>Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal

Email: [susana.coelho@uevora.pt](mailto:susana.coelho@uevora.pt)

*Mytilus galloprovincialis*, a filter feeder bivalve, is highly consumed as part of the Mediterranean diet. Thus, it is important to assess the levels of contaminants to guarantee food safety. In this study, levels of the toxic metals Cadmium, Chromium, and Lead, as well as the metalloid Arsenic, were evaluated in mussels' tissues. Mussels were collected by hand at 44 locations along the Portuguese coast. In the lab, mussels' soft tissues were removed, homogenized, freeze-dried, and reduced to powder, and on average 0.25g of mussels' tissue were digested using nitric acid prior to inductively-coupled plasma mass spectrometry (ICP-MS) analysis. Quantification was done with random duplicates and the percentage recovery of the reference material (ERMCE278K) was 102, 88, 78 and 99 for Arsenic, Cadmium, Chromium, and Lead respectively. The contamination pattern along the coast varied greatly between metals and stations, with the highest levels of Arsenic being recorded at the Deep Sea Fishing Port located inside Aveiro harbour and the lowest at Guincho beach, Lisbon (8.40 and 1.52mg.kg<sup>-1</sup> Wet Weight WW, respectively). Cadmium highest concentrations were registered in Cape Sardão (Alentejo coast) and the lowest in Lagos fishing port, Algarve (1.20 and 0.05 mg.kg<sup>-1</sup> WW, respectively). Chromium highest levels were registered in Figueira da Foz fishing port and the lowest at S. Jacinto beach, Aveiro (0.68 and 0.11 mg.kg<sup>-1</sup> WW, respectively). Lead highest concentrations were registered in Portinho da Arrábida beach and the lowest in Figueira da Foz marina (1.27 and 0.05 mg.kg<sup>-1</sup> WW, respectively). When analysing the risk for consumers under the European Union values for Lead and Cadmium intake for bivalves (1.5 and 1.0 mg.kg<sup>-1</sup> WW, respectively), none of the stations sampled were above this value, except for Cadmium in Cape Sardão. The obtained results reveal relatively low metal concentrations, being in their overwhelming majority under the values for ingestion in bivalves determined for Pb and Cd by Commission Regulation 2023/915 on maximum levels for certain contaminants in food.

This work was developed within the scope of the CHRC project UIDP/04923/2020. S. Coelho acknowledges FCT for the PhD fellowship 2021.08027.BD.

## Identification of parasitism-related genes in the specialized esophageal gland cells from the plant-parasitic nematode, *Bursaphelenchus xylophilus*

Madalena Mendonça<sup>1</sup>, Cláudia S. L. Vicente<sup>1</sup>, Margarida Espada<sup>1</sup>

<sup>1</sup>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.

Email: [maria.silva@uevora.pt](mailto:maria.silva@uevora.pt)

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a migratory plant-parasitic nematode responsible for the pine wilt disease (PWD), causing economic and ecological losses in the forestry industry. Current climate change predictions emphasize an increase of PWD on conifers worldwide. The absence of effective measures to control PWN and the present restrictions on the use of chemical pesticides require the discovery of sustainable control solutions. Therefore, to develop novel control strategies is essential to comprehend the molecular mechanisms by which the PWN parasitize the host. The interactions between the nematode and the host are mediated by parasitism proteins most of which are produced in the esophageal gland cells (GC), considered specialized cells crucial in parasitism. A GC transcriptomic library was generated and provided a new set of candidate genes specific to PWN, which can be useful to explore for nematode target resistance. Therefore, this work aimed to identify putative candidates of parasitism-related genes by *in silico* analysis and to validate the spatial expression of the transcripts in the nematode tissues, using *in situ* hybridization. In the top 212 most abundant transcripts in the GCs, a set of 74 candidates were tested. Out of 74, 30 transcripts were specifically localized in the nematode GC and, from those, one third are novel genes with no sequence similarity to other genes and unknown protein domain (pioneer genes). Interestingly, most of these 30 transcripts are species and genus specific, which includes aspartic and cysteine peptidases, and saposin B-type domains. Characterizing the PWN parasitism proteins could offer new potential targets regarding the control of this quarantine pathogen.

This work is funded by National Funds through FCT - Foundation for Science and Technology under the Project NemaWAARS - 10.54499/PTDC/ASP-PLA/1108/2021 and individual funding for M. Mendonça (2024.00901.BD), M. Espada (10.54499/CEECIND/00066/2018/CP1560/CT0003) and C.S.L. Vicente (10.54499/CEECIND/00040/2018/CP1560/CT0001).

## Mycorrhizal inoculation alters the expression of DNA methylation genes in wheat subjected to manganese stress

Francisca Lázaro<sup>1</sup>, Lénia Rodrigues<sup>2</sup>, Amaia Nogales<sup>3</sup>, Hélia Cardoso<sup>4</sup>, Maria Doroteia Campos<sup>5</sup>,  
Catarina Campos<sup>2\*</sup>

<sup>1</sup>Master Student in Biochemistry, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal.

<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, IIFA (Instituto de Investigação e Formação Avançada), University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>3</sup>Institut de Recerca i Tecnologia Agroalimentàries –IRTA, Ctra. De Cabrils, Km 2, 08348 Cabrils, Barcelona, Spain.

<sup>4</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, School of Science and Technology, Department of Biology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>5</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

\*Corresponding author Catarina Campos: [mccampos@uevora.pt](mailto:mccampos@uevora.pt)

DNA methylation is an essential epigenetic mechanism that regulates gene expression in plants and occurs throughout an organism's lifetime in response to developmental cues and external stressors. This process involves the addition of a methyl group to the 5' carbon of the cytosine ring, catalyzed by DNA methyltransferases, which often leads to the transcriptional silencing of the methylated genes. In plants, DNA methylation is established primarily through the RNA-directed DNA methylation (RdDM) pathway, where the DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) catalyzes *de novo* DNA methylation across all sequence contexts (CG, CHG, and CHH, where H represents A, C, or T). DNA methylation patterns are maintained following replication by METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 2 and 3 (CMT2 and CMT3), which function in the CG, CHH, and CHG contexts, respectively. Environmental stresses, such as heavy metal exposure, are known to induce changes in DNA methylation. However, DNA methylation can also be influenced by interactions with microorganisms. Arbuscular mycorrhizal fungi (AMF) are soil-dwelling fungi that form symbiotic relationships with most terrestrial plants, often enhancing plant performance under various environmental stresses, including heavy metal stress. Manganese (Mn) is a micronutrient essential for normal plant growth and development, but when in excess, it can have detrimental effects on plants. This study aimed to investigate the effect of inoculation with the AMF *Rhizoglyphus irregularis* on wheat (*Triticum aestivum* L. var. Ardila) under manganese toxicity, focusing on global DNA methylation in leaves and the expression of genes involved in the DNA methylation process. The results showed that Mn toxicity increased the expression of methyltransferase genes, particularly in AMF-colonized plants. However, global DNA methylation, as determined by the MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric), did not show significant differences between plants exposed or not to Mn toxicity. It is plausible that an interplay of methyltransferases and demethylases, which promote active demethylation of DNA, may have prevented a detectable increase in global DNA methylation. Nonetheless, targeted methylation of specific genes in response to manganese and AMF factors cannot be ruled out and must be included in future studies.

### Acknowledgments

This work was financially supported by national funds through FCT (Foundation for Science and Technology) under the Project UIDB/05183/2020. This work was supported by the MED exploratory project - Epigenomic selection of wheat-AMF symbiotic relation under manganese stress.



## Carob tree: One Green Solution to support a Sustainable Agriculture

M. Marques<sup>1</sup>, R. Pires<sup>1</sup>, L. Rodrigues<sup>1</sup>, C. Campos<sup>1</sup>, C. Estêvão<sup>1</sup>, A. Peixe<sup>2</sup>, H. Cardoso<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (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.

<sup>3</sup>MED (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](mailto:d63881@alunos.uevora.pt)

The carob tree (*Ceratonia siliqua* L.), native to the Mediterranean region, is recognized for its notable drought tolerance and ability to colonize nutrient-poor soils, as well as its natural resistance to pests, and diseases. Beyond its ecological benefits, the carob tree has gained attention as a valuable crop in various sectors due to its high nutritional and medicinal value. Production of diverse secondary metabolites, like tannins, phenolic acids, and flavonoids, with strong antimicrobial, insecticidal, and fungicidal properties, makes this species highly interesting for the medicinal and pharmaceutical industries. These properties also make these metabolites potentially valuable for the production of phytosanitary products. The PhD project here presented aims to explore such applications by establishing an advanced *in vitro* platform based on Portuguese traditional varieties for high-value metabolite production, positioning carob as a “green cell factory” for scalable metabolite production through valorization of Portuguese carob genotypes. This research is part of the project “ALCAROB – Applicability of carob tree on sustainability of Alentejo region”, funded through “la Caixa” Foundation’s Promove Program, in partnership with the Foundation for Science and Technology (FCT) (Projetos I&D Mobilizadores 2024).

To achieve the PhD project aims, initial optimization trials will be focused on the establishment of *in vitro* liquid cultures following different strategies (e.g. cells, somatic embryos, adventitious roots). Parameters like culture media composition, growth regulators, temperature, photoperiod and light intensity/quality will be considered for optimization. Preliminary results allowed the establishment of efficient conditions for callogenesis for the vars. ‘Cardeira’, ‘Lamy’, and ‘Cavi’. Additionally, a liquid culture medium has been successfully optimized for cell cultures of the var. ‘Cardeira’.

Employing high throughput metabolomics techniques will enable the characterization of metabolome profiling of the different *in vitro* systems and identification/quantification of key bioactive compounds. Further testes comprising bacteria and fungi resistance will be done to verify the bactericide and fungicide properties.

Overall, this research enhances the scientific understanding of carob’s metabolite profile while providing practical applications across industries. Developing carob as a green cell factory offers innovative, environmentally friendly solutions for agriculture and material sciences, contributing to both economic and ecological sustainability.

**Acknowledgments:** 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.

## Genomic Insights into a Desert-Adapted *Sinorhizobium* Strain from the Tunisian Sahara

R. Ben Gaied<sup>1,2</sup>, I. Sbissi<sup>1</sup>, S. Kaur<sup>3</sup>, G. C. diCenzo<sup>3</sup>, M. Tarhouni<sup>1</sup>, C. Brígido<sup>4</sup>

<sup>1</sup>Laboratory of Pastoral Ecosystems and Promotion of Spontaneous Plants and Associated Micro-Organisms, Institute of Arid Regions, University of Gabes, Tunisia

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>3</sup>Department of Biology, Queen's University, 116 Barrie Street, Kingston, Ontario K7L 3N6, Canada

<sup>4</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Portugal

Email: [ccb@uevora.pt](mailto:ccb@uevora.pt)

Nitrogen fixation by rhizobia in symbiosis with legumes is essential for nitrogen cycling and sustainable agriculture and plays a critical role in the survival of native leguminous plants in the arid and Saharan environments of Tunisia. The *Sinorhizobium meliloti* strain IRAMC:0087, initially isolated from the root nodules of the Saharan shrub *Calobota saharae* in southern Tunisia, can establish nodulation with *Vachellia tortilis subsp. raddiana*, a tree well-suited to extreme desert climates, and colonize roots of *Trifolium subterraneum* endophytically. Phenotypic analyses of IRAMC:0087 demonstrate its resilience to high salinity, drought, and elevated temperatures. To further explore the genetic foundation of its adaptations, we sequenced the strain's complete genome (7.3 Mb), which comprises five replicons: a main chromosome (3,650,495 bp), a chromid pSymB (1,674,059 bp), and another large plasmid, plasmid Accessory A (1,247,198 bp), and two smaller plasmids, pSymA (597,953 bp) and plasmid Accessory B (197,378 bp), with an overall GC content of 61.94%. In total, we identified 6,558 protein-coding genes, 56 tRNAs, and 9 rRNAs, along with a prophage region of 53.3 kb showing homology with *Sinorhizobium* phage phiLM21. Gene clusters associated with rhizosphere interactions, production of secondary metabolites, plant growth promotion, and symbiosis were found within the genome. Notably, one additional plasmid encodes stress tolerance-related genes, including those for trehalose and osmoprotectant synthesis, likely enhancing the strain's capacity to withstand extreme environmental conditions. The ability of IRAMC:0087 to engage in both endophytic and symbiotic interactions with desert-adapted hosts highlights its unique adaptations. Comparative genomic studies with other rhizobial strains may uncover new genetic elements involved in symbiosis under harsh climates.

R. B.G. acknowledges the PhD mobility program supported by the University of Gabés (FSG) and the Ministry of Higher Education and Scientific Research of Tunisia, C. B. acknowledges a contract from the Individual Call to Scientific Employment Stimulus 2018 (CEEC-IND/00093/2018) from FCT. The authors thank to the Tunisian-South African project AFRITRUF and to NSERC for supporting Rhizobium research in the GCD laboratory.

## ***In Vitro* Propagation, Acclimatization, and transplantation into the field of *Cynara cardunculus* L. ecotypes**

A. Paulino<sup>1,2,3</sup>, C. Rodrigues<sup>1,4</sup>, A. Faustino<sup>1,3,5</sup>, A. Peixe<sup>3,5</sup>, O.S. Paulo<sup>2</sup>, M.F. Duarte<sup>1,3</sup> and L. Marum<sup>1,3</sup>

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

<sup>2</sup>Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

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

<sup>4</sup>Escola de Ciências e Tecnologia, Universidade de Évora, 7002-554, Évora, Portugal;

<sup>5</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Évora, Portugal

Email: [ana.paulino@cebal.pt](mailto:ana.paulino@cebal.pt)

*Cynara cardunculus* represents an endogenous resource, with its floral structures used in cheese production. Contemporary understanding highlights the multifunctional capabilities of this plant, extending beyond cheesemaking to include its role as a source of bioactive ingredients relevant to the food and pharmaceutical sectors, as well as its usefulness as biomass for various industrial applications. In this context, cultivating economically significant plant species, specifically through the propagation of high-value plants with the desired chemical and biochemical profiles, is paramount for the economic valorisation of cardoon, thus ensuring product differentiation and sustainability. Micropropagation is a tissue culture technique that may swiftly and effectively produce high-quality plants. The challenges of contamination, low multiplication rate, and rooting are some of the obstacles to artichoke (*Cynara cardunculus* L. var. *scolymus*) micropropagation [1,2]. Continuing the work developed by Marum et al. [3], the present study addresses two conditions that used the MS medium and the other used WPM medium both supplemented with IBA and BAP for optimal growth and development in vitro propagation of the Portuguese wild cardoon genotype. Following a period of growth, the in vitro plantlets were ready for the acclimatization phase, which involves adapting them to the conditions of the external environment. After acclimatization, the cardoon plantlets were prepared to be transplanted into the field, where their number of leaves, follicular area, and quantification of cynaropicrin content were monitored. Investigations are underway to integrate this novel understanding into forthcoming cardoon breeding initiatives.

### References

[1] Fortunato et al., Sci. Hort. 106: 472-483, 2005.

[2] Bedini et al., Cen. Eur. Jour. of Bio. 7: 680-689, 2012.

[3] Marum et al., Book of Abstract, XI Inter. Sym. on Artichoke, Cardoon, and their wild relatives 3, 2023

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), Ph.D. grant to A. Paulino (10.54499/SFRH/BD/145383/2019 (<https://doi.org/10.54499/SFRH/BD/145383/2019>)), and A. Faustino (UI/BD/153511/2022), Project UIDB/05183/2020 to MED, and Project LA/P/0121/2020 to CHANGE.







## **Somatic embryogenesis in Portuguese grapevine (*Vitis vinifera* L.) cultivars – a focus on the induction phase**

Catarina Estêvão<sup>1</sup>, Mónica Marques<sup>1</sup>, Rita Pires<sup>1</sup>, Catarina Campos<sup>1</sup>, Lénia Rodrigues<sup>1</sup>, José Luís Silva<sup>2</sup>, Amândio Rodrigues<sup>2</sup>, Ana Carina Neto<sup>2</sup>, Rui Flores<sup>2</sup>, Augusto Peixe<sup>3</sup>, Maria João Cabrita<sup>3</sup>, Hélia Cardoso<sup>4\*</sup>

<sup>1</sup>MED—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

<sup>2</sup>Esporão S.A., Herdade do Esporão, Apartado 31, 7200-999 Reguengos de Monsaraz, Portugal

<sup>3</sup>MED—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

<sup>4</sup>MED—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](mailto:hcardoso@uevora.pt)

Grapevines (*Vitis vinifera*) are essential to the wine industry and regional economic stability but are increasingly challenged by climate change, and the emergence of new pests and diseases. Developing new resilient grapevine cultivars requires advanced biotechnological strategies. The use of genetic engineering tools, based on cisgenesis, and the use of the new genomic techniques (CRISPR/Cas) require the support of an efficient *in vitro* regeneration system, being somatic embryogenesis (SE) the most used. Typically, SE protocols begin with the induction phase, where plant tissue used as initial explant is stimulated to produce embryogenic callus through the influence of plant growth regulators. Establishing appropriate conditions to induce SE is a key factor in achieving successful plant regeneration via SE.

This study evaluated SE induction across eight Portuguese grapevine cultivars, with high interest in the Alentejo region, including the disease-resistant cv. 'Defensor'. Three growth regulator combinations were tested in a solid Nitsch & Nitsch (1969) basal medium: Medium A (1.33  $\mu$ M BAP, 5.42  $\mu$ M 2,4-D), Medium B (1.33  $\mu$ M BAP, 2.72  $\mu$ M 2,4-D), and Medium C (8.87  $\mu$ M BAP, 4.52  $\mu$ M 2,4-D). Carpels and stamens, aseptically excised from immature flower buds, were used as initial explant.

Results indicate that Medium C achieved the highest callogenesis rates in most cultivars, independent of the explant type used, demonstrating that lower concentration of BAP, even combined with higher concentration of 2,4-D, has limited efficacy in promoting callus formation. The cv. 'Trincadeira' exhibited significantly higher callogenesis rates across all media compared to the other cultivars, while 'Touriga Franca' proved to be the most recalcitrant, showing the lowest callogenesis rates. Furthermore, carpels generally yielded higher callogenesis rates than stamens across all media and cultivars, implying that explant type strongly influences SE outcomes.

In addition to the effect of genotype, evidenced by the high variability in SE efficiency across cultivars, the results obtained enabled the selection of carpels as the most suitable explant type, as well as the most effective combination of growth regulators (Medium C) to further proceed with optimizing the next phase of SE protocol (expression phase) for the eight genotypes.

The work is financed by the project BioGrapeSustain. Vine&Wine – SubProject Proposal – Project 81. Agenda Mobilizadora: Driving Sustainable Growth Through Smart Innovation (Reference C644866286-00000011) and by National Funds through FCT under the Project UI/BD/153509/2022 and Project UIDB/05183/2020.

## **Nanoparticle Syntheses Using Microbial Supernatants: An Environmentally Friendly Approach to Cultural Heritage Conservation**

A. Carrapiço<sup>1,2,3,\*</sup>, M. R. Martins<sup>1,2,4</sup>, P. Barrulas<sup>1,2,5</sup>, E. Carreiro<sup>6</sup>, A. T. Caldeira<sup>1,2,5</sup>, J. Mirão<sup>1,2,7</sup>, L. Dias<sup>1,2,3</sup>

<sup>1</sup>HERCULES Laboratory, University of Évora, Portugal

<sup>2</sup>IN2PAST - Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Portugal

<sup>3</sup>Institute for Research and Advanced Training (IIFA), University of Évora, Portugal

<sup>4</sup>Department of Medical Sciences and Health, ESDH, University of Évora, Portugal

<sup>5</sup>Department of Chemistry and Biochemistry, ECT, University of Évora; Portugal

<sup>6</sup>LAQV-REQUIMTE, IIFA, University of Évora, Portugal

<sup>7</sup>Department of Geosciences, ECT, University of Évora, Portugal

Email: [antonio.carrapico@fa.uevora.pt](mailto:antonio.carrapico@fa.uevora.pt)

Biocontamination poses a significant threat to the conservation of Cultural Heritage (CH) materials leading to its deterioration. Although conventional conservation techniques are widely used, they often rely on chemical treatments that can harm materials and carry environmental risks. This study presents an innovative, eco-friendly alternative by synthesizing Metal Nanoparticles (MNPs) using microbial culture supernatants, offering a sustainable pathway for CH preservation. The biosynthetic approach aligns with broader sustainability goals, minimizing the use of toxic chemicals and reducing energy demands typically associated with nanoparticle production. In this study, we characterize MNPs synthesized through microbial supernatants, using UV-Vis, SEM-EDX, XRD, DLS, and ICP-MS to analyze how particle properties vary with reaction media and physical and chemical parameters such as pH and precursor concentration. Antimicrobial testing revealed effective inhibition of microorganisms isolated from CH materials, with metal ions playing a primary role in microbial suppression. These findings emphasize the importance of nanoparticle shape, surface area, and diffusion rate, particularly for applications in porous materials. Future work will focus on testing MNP performance on stone mock-ups and assessing potential ecological toxicity, contributing to greener conservation methods that align with environmental priorities.

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.



**MEC working-conditions integral analysis for bioelectrochemical H<sub>2</sub> production**A. Baía<sup>1</sup>, A. I. Arroyo-Escoto<sup>2,3,\*</sup>, N. Ramos<sup>1</sup>, A. Lopes<sup>1</sup>, M. C. Fernandes<sup>2,3</sup>, A. Fernandes<sup>1</sup><sup>1</sup>FibEnTech-UBI, Department of Chemistry, Universidade da Beira Interior, 6201-001 Covilhã, Portugal<sup>2</sup>Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/ Polytechnical Institute of Beja (IPBeja), Apartado 6158, 7801-908 Beja, Portugal<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal.\* Corresponding author: [alonso.escoto@cebal.pt](mailto:alonso.escoto@cebal.pt)

Winery-wastewater (WW) is normally stated as one of the most relevant problems for this industry due to the different stages endured, and the organic and chemical load carried during the different steps of its production. Normally the technologies destined for its treatment are based on the application of high amounts of energy with no return or applicability beyond the water reintegration to the environment. The purpose of this work is to develop an adapt microbial consortium to degrade the compounds found in the WW and test its capacities as a source of protons for H<sub>2</sub> production through electrochemical means. To assess these capabilities, the system was grown in a Microbial Electrochemical Cell to develop its electroactive biofilm under a constant voltage difference application. The degradative capacities of the system were followed by High Pressure Liquid Chromatography (HPLC) analysis of the compounds, the constantly increasing oxidative state reaching of the Open Circuit Potential (OCP), the Optical Density of the cells developing in the medium (OD600), along with the Electrochemical Impedance Spectroscopy and Cyclic Voltametry as a way of conjunctly understand the feasibility and improvement of the technique compared to the non-recovery winery-ww treatments. The experiment is carried out using a controlled medium for the development of the bacteria to establish the conditions of the treatment to be implemented on the real effluent. The degradative conditions improvement tested for this work will settle the basis for the needs of H<sub>2</sub> production.

**Acknowledgments:** Fundação para a Ciência e a Tecnologia for: projects Wine4H2 (2022.02566.PTDC), FibEnTech-UBI (UIDB/00195/2020), MED (UIDB/05183/2020), and CHANGE (LA/P/0121/2020); PhD grants 2022.11077.BD (A. Baía), and UI/BD/153579/2022 (A.I. Arroyo-Escoto); and contract CEECINST/00016/2021/CP2828/CT0006 (A. Fernandes).

## Exploring Antimicrobial Activity of Pristine Environment Bacterial Extracts Against Biodeteriogenic Microorganisms

P. Gatinho<sup>1,2,3</sup>, C. Salvador<sup>2</sup>, A. Z. Miller<sup>3,2</sup>, A. T. Caldeira<sup>2,4,5</sup>

<sup>1</sup> Institute for Advanced Studies and Research, IIFA, University of Évora, Évora, Portugal

<sup>2</sup> HERCULES Laboratory & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Évora, Portugal

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

<sup>4</sup> Department of Chemistry and Biochemistry, School of Sciences and Technology & City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science University of Évora, Évora, Portugal

<sup>5</sup> City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal.

patricia.gatinho@uevora.pt

Biodeterioration caused by biodeteriogenic microorganisms is a significant challenge for the preservation of Cultural Heritage. These microorganisms can harm important artworks, monuments, and historical sites, leading to their deterioration over time. Traditional methods to combat this issue often rely on biocides, which are chemical agents that kill or inhibit these harmful organisms. However, many biocides are highly toxic and can have negative effects on the environment and living organisms, making it essential to find safer alternatives.

One promising solution is to use bioactive metabolites produced by bacteria found in pristine environments. These unique bacteria have evolved to survive in extreme conditions, and they produce antimicrobial compounds that help them fend off other microorganisms. By tapping into these natural compounds, we can create biocontrol agents that are less harmful to the environment and effective against the microorganisms that threaten Cultural Heritage.

The bioactive metabolites from these bacteria can inhibit the growth of competing microorganisms, thus protecting valuable cultural sites. This method could lead to the development of green-safe biocides agents that are both effective and environmentally friendly. By investigating the potential of these natural products, we can make important advances in bioremediation.

Exploring bioactive metabolites from bacteria to combat biodeterioration is a promising direction for future efforts. This method offers a safer and more sustainable approach to preserving Cultural Heritage while also benefiting other sectors, showcasing the potential of nature in solving modern challenges.

This work was funded by FCT, FCT—Foundation for Science and Technology, within the scope of the projects MICROCENO (DOI 10.54499/PTDC/CTA AMB/0608/2020), ART3mis (DOI 10.54499/2022.07303.PTDC), and R&D Units HERCULES Lab (UIDB/04449/2020), IN2PAST - Associate Lab (DOI 10.54499/LA/P/0132/2020), as well as the individual support to P. Gatinho (2024.00316.BD) and C. Salvador (DOI 10.54499/DL57/2016/CP1372/CT0019).

## Ecotoxicity assessment of hydrophobically-modified and non-modified cationic cellulose extracted from Acacia wood

Solange Magalhães<sup>1,\*</sup>, Cátia Venâncio<sup>2</sup>, Isabel Lopes<sup>2</sup>, Bruno Medronho<sup>3,4</sup>, Luís Alves<sup>1</sup>, Maria da Graça Rasteiro<sup>1</sup>

<sup>1</sup>University of Coimbra, CERES, Department of Chemical Engineering, 3030 790 Coimbra, Portugal

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

<sup>3</sup>MED–Mediterranean Institute for Agriculture, Environment and Development, CHANGE–Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal

<sup>4</sup>Surface and Colloid Engineering, FSCN Research Centre, Mid Sweden University, SE-851 70 Sundsvall, Sweden

\*Correspondence: [solangemagalhaes@eq.uc.pt](mailto:solangemagalhaes@eq.uc.pt)

The use of cellulose derivatives for the flocculation of microplastics presents a promising and sustainable strategy to help minimize their environmental impact. However, the development of these derivatives requires careful fine-tuning to ensure they do not themselves become an environmental burden. This study aimed to characterize the physicochemical properties of these polymers and assess their ecotoxicity in four freshwater trophic level representative species: *Raphidocelis subcapitata* (biomass and growth rate), *Daphnia magna* (mortality), *H. viridissima* (mortality) and *Danio rerio* (mortality, malformation, and heartbeat rate). Different concentrations of cellulose derivatives were tested (i.e., 0.007%, 0.01%, 0.015%, 0.023%, 0.034%, and 0.051% (w/w)). Whenever possible the median lethal and sublethal concentrations (LC50 and EC50) were determined. All derivatives were found to be very toxic for *H. viridissima* (100% mortality at all tested concentrations) and *R. subcapitata*. In *R. subcapitata*, all polymers induced high aggregation and sedimentation of the microalgae cells. Considering the results based on *D. magna* and *D. rerio*, the hydrophobically-modified cellulose polymer was found to be one of the least toxic polymers for the former and most toxic for the later species. All cationic-modified polymers induced toxicity to *D. magna* in the same order of magnitude (0.022% < LC50 < and 0.024%), whilst for *D. rerio* the toxicity increased with the cationic degree of substitution. It is hypothesized that low degrees of substitution limits complete polymer solubilization in aqueous media, causing interference with biological membranes, whilst high DS leads to strong interaction with the cell wall, potentially causing higher toxicity.

Based on the responses observed in the four species representing different trophic levels, it is important to emphasize that the development of eco-friendly methodologies to address plastic pollution must be carefully evaluated prior to marketing; otherwise, these methods themselves may become a cause for concern within ecosystems.

### Acknowledgements

This work was financially supported by the Portuguese Foundation for Science and Technology, FCT, via the Strategic Research Centre Projects from CERES (doi:10.54499/UIDB/00102/2020 and doi:10.54499/UIDP/00102/2020), MED (doi:10.54499/UIDB/05183/2020 and doi:10.54499/UIDP/05183/2020) and CHANGE (doi:10.54499/LA/P/0121/2020) and the research grants 2020.07638.BD, CEECIND/01014/2018/CP1540/CT0002 (DOI: 10.54499/CEECIND/01014/2018/CP1540/CT0002) and 2021.00399.CEECIND (DOI: 10.54499/2021.00399.CEECIND/CP1656/CT0025).

## LBG-Based Hydrogels: Innovative Solutions for Sustainable Agricultural Practices

Brás J<sup>1\*</sup>, Boutoub O.<sup>1</sup>, Mendoza A<sup>2</sup>, Duarte H<sup>1</sup>, Medronho B<sup>1,3</sup>, Antunes M<sup>4</sup>, Aliaño-González MJ<sup>1,5</sup>, Guerra R<sup>6,7</sup>, Gonçalves S<sup>1</sup>, Costa A<sup>6</sup>, Romano A<sup>1</sup>

<sup>1</sup>MED—Mediterranean Institute for Agriculture, Environment and Development, CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>2</sup>Laboratory of Nanotechnology, Biological Systems and Industrial Applications, Polytechnic University of Pachuca, Zempoala, Hidalgo, Mexico

<sup>3</sup>FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

<sup>4</sup>Departamento de Ciências Biológicas e Bioengenharia, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>5</sup>Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, 11510 Cadiz, Spain

<sup>6</sup>Center for Electronics, Optoelectronics and Telecommunications (CEOT), Faculdade de Ciências e Tecnologia, Universidade do Algarve, Faro 8005-139, Portugal

<sup>7</sup>Departamento de Física, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

\*E-mail: [jbras@ualg.pt](mailto:jbras@ualg.pt)

As resources diminish and populations increase, the need for eco-friendly solutions become a worldwide priority. Agroforestry is rising to the challenge, offering a variety of natural compounds that could replace unsustainable fossil-based products and protect ecosystems from further degradation. One promising agroforestry product is derived from the carob tree (*Ceratonia siliqua*), prevalent in the Mediterranean region, including the Algarve, with Portugal as one of the world's main producers. The commercial value of carob lies primarily in its seed endosperm, used to produce carob bean gum, commonly known as locust bean gum (LBG). LBG is widely accessible and serves as a natural additive (E410) with applications in the food industry as a thickener and stabilizer, as well as in the pharmaceutical and biomedical industries as an excipient in medications. The applications of LBG could be expanded further through the development of novel charged derivatives. To achieve this, an initial hull extraction using eco-friendlier solvents was performed, followed by chemical modification of LBG into two anionic (sulfate and carboxylate) and one cationic (trimethylammonium) derivatives. The novel derivatives, alongside with unmodified LBG, were tested for their gelation ability. These hydrogels might find potential applications in agriculture, particularly in enhancing water retention. Tests included LBG dissolution, crosslinking with citric acid and water uptake, swelling and stability assays. The resulting hydrogels were further characterized by Fourier transform infrared spectroscopy and rheometry. This project aims to develop innovative solutions to address the growing problem of drought and water scarcity, which increasingly affects not only Portugal but also the global landscape, while simultaneously supporting local economies.

This work was funded by National Funds through FCT – Foundation for Science and Technology under the Project “BIG opportunities in a small SEED: valorization of carob by-products into novel hydrogels for sustainable agriculture” (2022.07519.PTDC). FCT is further acknowledged for 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>) and the research grant CEECIND/01014/2018/CP1540/CT0002 (DOI: 10.54499/CEECIND/01014/2018/CP1540/CT0002). National Council for Science and Technology (CONACYT-Mexico) for A.I. Canales-Mendoza scholarship (813864).

## Biosynthesized zinc oxide nanoparticles added to *in vitro* multiplication of *Agave applanata*

Canales-Mendoza, Ana Itzel<sup>1</sup>, Mercado-Flores, Yuridia<sup>2</sup>, Villanueva-Ibáñez, Maricela<sup>1</sup>

<sup>1</sup>Laboratorio de Nanotecnología, sistemas Biológicos y Aplicaciones industriales, Universidad Politécnica de Pachuca, Zempoala C.P. 43830 Hidalgo, México

<sup>2</sup>Aprovechamiento Integral de Recursos Bióticos, Universidad Politécnica de Pachuca, Zempoala C.P. 43830 Hidalgo, México

E-mail: [villanueva@upp.edu.mx](mailto:villanueva@upp.edu.mx)

The application of nanoparticles (NPs) in plant biotechnology has been successfully reported in various processes, including plant regeneration, callus induction, and the removal of microbial contaminants. Due to their size and structure, NPs can function as novel chemical elicitors in plants, acting as signalling agents that positively influence a range of morphological, physiological, and biochemical processes within plant metabolism. Micropropagation has enabled the successful rescue of species such as *Agave applanata*, an endemic plant of Mexico that is difficult to reproduce in its natural habitat. In addition, *A. applanata* and *Agave salmiana* are the only species known to serve as high-protein hosts for chinicuil larvae, which are of interest in entomophagy. The plant also yields valuable products and by-products, such as distillates and fructans.

The objective of this study was to multiply *A. applanata* by incorporating biosynthesized NPs into the culture medium. NPs were obtained using a cell-free filtrate of *Mucor fragilis* as a reducing agent and characterized for their size and structure. The multiplication culture media were supplemented with the growth regulators benzylaminopurine (BAP) and 2,4-dichlorophenoxyacetic acid (2,4-D), with NPs added as a fixed factor. A 3<sup>2</sup> factorial experimental design was applied, with five replicates per treatment, and seedlings previously obtained through germination were used. The synthesized NPs had an average size of 80 nm, a hemispherical morphology, and a hexagonal Wurtzite structure, remaining stable in colloidal solution. Results showed that not all NP concentrations allowed the sprouts formation. However, the most successful treatment was the combination of 0.025 mg/L BAP, 1 mg/L 2,4-D, and 50 mg/L NPs, which resulted in an average of 18 ± 3 shoots. These sprouts were generated within 30 to 45 days, a shorter time frame compared to that reported for other *Agave* species.

We thank National Council for Science and Technology (CONACYT-Mexico) for A.I. Canales-Mendoza scholarship (813864), and CITNOVA Hidalgo, México.

**An alternative methodology for citrus tree micrografting**Matos I.<sup>1</sup>, Duarte A.<sup>2</sup>, Cabrita L.<sup>3</sup>, Marques N.<sup>2</sup><sup>1</sup>Faculdade de Ciências e Tecnologia, Universidade do Algarve, 8005-139 Faro, Portugal.<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development and Change - Global Change and Sustainability Institute, Faculty of Science and Technology, University of Algarve, 8005-139 Faro, Portugal.<sup>3</sup>CCDR Algarve – Agricultura e Pescas, 8005-511 Faro, Portugal.Email: [nmarques@ualg.pt](mailto:nmarques@ualg.pt)

In vitro micrografting is a technique that has been developed to obtain virus-free and viroid-free plants. The application of this biotechnological technique enables the development of plants displaying enhanced phytosanitary quality, the conservation of commercially valuable species and varieties, and the advancement of their clonal multiplication. In vitro micrografting is conducted in aseptic conditions and involves the grafting of stem apices from diseased plants onto young rootstock stems cultivated in vitro. The micrografting technique is of particular importance in the management of viral infections, including the Citrus tristeza virus (CTV), among others that affect citrus trees. The objective of this study was to clean 12 citrus species from the DRAPALG collection, which demonstrated high commercial potential and resistance to drought conditions, as part of the AGRO+EFICIENTE project. The stem apices of the various citrus species were grafted onto the Carrizo citrange rootstock. As outlined by Navarro et al. (1976), the micrografting technique yields a low success rate (14%) with regard to the union between the stem apex and the rootstock. An alternative micrografting technique was developed in this study, comprising the grafting of three stem apices into the rootstock stem. The procedure resulted in an enhanced success rate of union between the rootstock and the grafted variety, reaching 42%, and promoted the regeneration of micrografted citrus plants.

The authors acknowledge the financial support provided by PRR-C05-i03- I-000010-“Valorização de Recursos Genéticos Tradicionais”, PRR-C05-i03-I-000010-LA4.1 “Conservação e fertilidade do solo”, PRR-C05-i03-I-000010-LA4.3 “Gestão dos recursos hídricos”, PRR-C05-i03-I000010-LA4.4 “Variedades adaptadas às alterações climáticas” and PRR-C05-i03-I-000010-LA4.7 “Promoção de ações de divulgação”.







### Monitoring of *Alternaria spp* and Alt a 1 in Evora atmosphere

M. Marques<sup>1</sup>, R.M., Rodriguez-Arias<sup>2</sup>, A. Galveias<sup>1</sup>, A. R. Costa<sup>1,5</sup>, R. P. Badia<sup>2</sup>, P. Palma<sup>1,3</sup>, M.J. Costa<sup>1,4</sup>, C.M. Antunes<sup>1,5,6</sup>

<sup>1</sup>ICT, Institute of Earth Sciences, Polo de Évora, Rua Romão Ramalho, 59, 7000-671, Évora, Portugal

<sup>2</sup> Institute of Environmental Sciences (Botany), University of Castilla-la-Macha, Toledo, Spain.

<sup>3</sup>Department of Technologies and Applied Sciences, Agrarian School, Polytechnic Institute of Beja, Rua Pedro Soares, 7800-295 Beja, Portugal

<sup>4</sup>Department of Physics, School of Science and Technology, University of Évora, Rua Romão Ramalho, 59, 7000-671, Évora, Portugal

<sup>5</sup>Department of Health and Medical Sciences, School of Health and Human Development, University of Évora, Rua Romão Ramalho, 59, 7000-671, Évora, Portugal.

<sup>6</sup>Centro Académico Clínico do Alentejo, C-TRAIL, Évora, Portugal

Email: [mrmarques@uevora.pt](mailto:mrmarques@uevora.pt)

Fungal allergies affect 3% to 40% of the population and vary by region, country, sex, and age. *Alternaria spp* is one of the most allergenic fungi, primarily through its glycoprotein Alt a 1. Monitoring *Alternaria* is essential for public health to manage the risks of exposure to airborne spores, which can exacerbate allergies, asthma, and fungal infections. This study focused on *Alternaria spp* and Alt a 1 level in the region of Évora, Portugal, from February to October 2023 using a Digitel pump sampler. Alt a 1 level using ELISA after extraction with ammonium bicarbonate buffer. Airborne fungal spore concentration (EN16868:2019 guidelines). *Alternaria* spore levels varied from 0.055 to 2.255 spores/m<sup>3</sup> and Alt a 1 concentration ranged from 0.016 to 3.471 pg/m<sup>3</sup>, displaying seasonality and peaking together in spring and late summer/autumn, posing higher risk of exposure. A positive correlation between *Alternaria* spore concentration and Alt a 1 levels was observed, suggesting that fungal spores are the main carrier of the allergen. These results elucidate the period of higher risk for *Alternaria* exposure and potential health impacts. In conclusion, monitoring airborne *Alternaria* spores and Alt a 1 levels contributes to raise awareness and is vital to develop preventive actions to reduce allergic diseases, asthma flare-ups, and fungal infections associated with *Alternaria* exposure.

M.M. thanks the FCT. Portugal. and the ICT– University of Évora for the Ph.D. Grant UI/BD/150935/2021. This work was financed by national funds through FCT, in the scope of the ICT projects with the references UIDB/04683/2020 and UIDP/04683/2020.

## From Biofilms to Preservation: Addressing Microbial-Induced Stone Discoloration at Batalha Monastery

I. Silva<sup>1</sup>, C. Salvador<sup>1</sup>, A. Z. Miller<sup>2,1</sup>, A. Candeias<sup>1,3,4</sup>, A.T. Caldeira<sup>1,3,4(\*)</sup>

<sup>1</sup>HERCULES Laboratory & 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.

<sup>2</sup>Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain

<sup>3</sup>Chemistry and Biochemistry Department, School of Sciences and Technology, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

<sup>4</sup>City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal.

(\*) [atc@uevora.pt](mailto:atc@uevora.pt)

Throughout history, stone has been a preferred material for the construction of iconic structures due to its aesthetic appeal and intrinsic properties. However, like other construction materials, stone is subject to continuous deterioration by microorganisms, making its preservation critical for the protection of cultural heritage.

Batalha Monastery (Portugal), a UNESCO World Heritage Site, exhibits notable surface alterations in the stone architectural elements of both the Founder's Chapel and the church, including extensive pink discoloration affecting the walls and columns. The use of both culture-dependent and -independent methods for identifying the colonizing microbiota indicated that the main microorganisms forming the observed biofilms were the bacterial genera *Halalkalicoccus*, *Bacillus*, *Gordonia*, *Serratia*, and *Methylobacterium*.

In this study, one of the bacterial isolates identified in the stone, from the genus *Gordonia*, was selected to carry out biocolonization tests on limestone, aiming to mimic the discoloration process observed in the monument. Two stone mock-ups were prepared, one kept in darkness and the other exposed to sunlight, to compare the bacterial behavior under different light conditions. Colorimetry and RAMAN spectroscopy techniques were employed, allowing for the in-situ characterization of color changes and pigments produced by the isolate. Furthermore, cleaning tests were also carried out on the mock-ups, in an attempt to remove the carotenes produced. The results from the aforementioned techniques revealed that the bacteria used in the tests, induced significant colorimetric changes in the stone. These changes were more pronounced when the stone was exposed to sunlight compared to those observed under dark conditions. In addition, the results of the cleaning tests demonstrated that the concentrations of the cleaning solution used are highly effective in removing pigments and can safely be suitable for heritage use.

In order to prevent the deterioration of this cultural asset and ensure its preservation, we consider this study to be a critical step towards formulating a precise and effective conservation and intervention strategy for the Batalha Monastery.

**Acknowledgements:** This work is funded by national funds through FCT—Foundation for Science and Technology, I.P.—under the projects UI/BD/153582/2022 (I. Silva PhD Grant), C. Salvador (DOI: 10.54499/DL57/2016/CP1372/CT0019), UIDB/04449/2020 (HERCULESLab), IN2PAST (DOI:10.54499/LA/P/0132/2020), ART3mis (DOI:10.54499/2022.07303.PTDC), MICROCENO (DOI: 10.54499/PTDC/CTA-AMB/0608/2020) and Old Goa Revelations (DOI: 10.54499/2022.10305.PTDC). 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 Doctor Antónia Tinturé, Direção-Geral do Património Cultural for allowing them to perform this study.

## Salivary Omics in xerostomia treatment with pilocarpine: a proteomic and metabolomic investigation

L. Carreira<sup>1</sup>, J. Zamora<sup>2</sup>, J. Nunes<sup>2</sup>, J. Coromina<sup>2</sup>, C. Cordeiro<sup>3</sup>, E. Lamy<sup>4</sup>

<sup>1</sup>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;

<sup>2</sup>Dry mouth institute, Teknon Medical Center, 08022 Spain; <sup>3</sup>FT-IRC and Structural Mass Spectrometry Laboratory, Faculty of Science, University of Lisbon, Campo Grande 1749-016, Lisbon, Portugal;

Email: [lrec@uevora.pt](mailto:lrec@uevora.pt)

Xerostomia is a medical condition that can be caused by autoimmune diseases, radiotherapy, multidrug use, or unknown factors. It affects oral functions such as speaking, swallowing, and food perception due to changes in saliva, which normally lubricates and protects the mouth. This condition alters the biochemical properties of saliva, impacting its role in food oral perception and overall oral health. Pilocarpine, a common treatment, increases saliva production; however, its impact on the biochemical properties of saliva remains less understood.

This study investigates the effects of pilocarpine on the salivary proteome and metabolome and xerostomia symptoms. Saliva samples were collected from patients at various stages of pilocarpine treatment (0, 3, 6, 9, and 12 months). To achieve our objective, techniques such as SDS-PAGE and two dimensional-electrophoresis for proteomics and FTICRMS for metabolomics were used.

Focusing on symptomatology, patients with severe symptoms presented higher albumin levels, while those with milder symptoms exhibited lower levels of cystatins, amylase, although having higher flow rate (sialometry) prior to starting treatment. After three months of pilocarpine treatment, a decrease in the polymeric immunoglobulin receptor was observed. Further analysis through 2DE protein profiling revealed, after more than 6 months of treatment, reductions in amylase, albumin, acidic proline-rich proteins, and carbonic anhydrase VI, the latter being crucial for maintaining pH balance in the oral cavity. The observed reductions in immunoglobulin and albumin levels indicate a lowered infection and inflammation, respectively, which aligns with the typical oral health challenges associated with xerostomia.

Metabolomic analysis revealed fluctuations in metabolite levels throughout the treatment period, suggesting dynamic changes in metabolism, reflected in saliva. These fluctuations, changes in salivary metabolome after 3 and 9 months pilocarpine treatment indicate mechanisms of re-balancing of oral metabolic pathways. Identifying these metabolites will provide deeper insights into how pilocarpine alters saliva and its effects on xerostomia.

In conclusion, pilocarpine not only increases salivary flow but also alters the biochemical composition of saliva, what may have important consequences for oral health, food oral perception and consequent nutrition. More details about proteomics and metabolomics results will be presented and discussed.

This work was funded by National Funds through FCT - Foundation for Science and Technology which granted a PhD acknowledge fellowship to L. Carreira under the reference SFRH/BD/06273/2021 and supported the Project UIDB/05183/2020.

## From Skin to Script: Molecular and Microbial Characterization of Historical Parchments

M. J. Penetra<sup>1</sup>, A. Candeias<sup>1,2,3</sup>, C. Miguel<sup>1,3</sup>, A. T. Caldeira<sup>1,2,3</sup> (\*)

<sup>1</sup>HERCULES Laboratory, Évora University, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal; [mariajpenetra@gmail.com](mailto:mariajpenetra@gmail.com); [cpm@uevora.pt](mailto:cpm@uevora.pt); [atc@uevora.pt](mailto:atc@uevora.pt);

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

<sup>3</sup>City University of Macau Chair in Sustainable Heritage, University of Évora, Casa Cordovil, 7000-651 Évora, Portugal

\*Email: [atc@uevora.pt](mailto:atc@uevora.pt)

Parchment, a historic writing medium crafted from animal skins—primarily those of sheep, calves, and goats—has played a crucial role in safeguarding human knowledge and cultural heritage across centuries. However, pinpointing the specific animal origin of these parchments poses a challenge, as traditional methods, including molecular DNA analysis, often fall short due to the intensive processing treatments and varying preservation conditions.

This study conducts an extensive analytical examination of a collection of historical parchments. A key objective is to identify molecular markers that can help differentiate parchments based on their animal origin. For this, we analyzed samples, focusing on mitochondrial DNA and employing metabarcoding of the 16S region to profile associated microbial communities. Additionally, non-invasive methods like Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) and Raman Spectroscopy were used to investigate the molecular fingerprint unique to parchment, offering a comprehensive approach to their characterization.

By correlating microbiome composition with substrate type and molecular fingerprint, this study opens new possibilities for the analysis of historical artefacts, supporting improved conservation strategies and enhancing our understanding of ancient production techniques.

This work is funded by national funds through FCT – Foundation for Science and Technology, under the project 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/04449/2020) and City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural.

## Catalase as a Key Regulator of Adventitious Root Formation in Olive (*Olea europaea* L.)

C. Morais<sup>1</sup>, L. Rodrigues<sup>2</sup>, C. Campos<sup>2</sup>, A. Peixe<sup>3</sup>, H. Cardoso<sup>4</sup>

<sup>1</sup>School of Science and Technology, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>2</sup>MED (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.

<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & 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.

<sup>4</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

Olive tree (*Olea europaea* L.) is mostly propagated by semi-hardwood cuttings, where indole-3-butyric acid (IBA) is applied as an auxin to initiate adventitious root (AR) formation. The process of adventitious rooting, which drives AR development, is influenced by internal and external factors, including the wounding and the immersion in the auxin IBA solution. Although some cultivars exhibit high rooting rates, others demonstrate recalcitrant behavior, such as some traditional Portuguese cultivars.

During AR development, reactive oxygen species (ROS) act as signaling molecules that influence cellular differentiation. However, an excess of ROS could lead to oxidative damage and impairing root initiation. Catalase (CAT), an essential oxidative enzyme, can play a key role in the process of adventitious rooting by modulating the plant's response to oxidative stress. To assess the involvement of CAT in AR formation, an *in vitro* assay with olive microcuttings of cv. 'Galega vulgar' was established using IBA as adventitious rooting inducer. The basal region the microcuttings and the leaves were further collected from IBA treated and non-treated microcuttings at 0h, 6h, 24h, and 72h after *in vitro* inoculation. *OeCAT* gene expression was assessed by RT-qPCR, and analysis of CAT expression and activity was performed through Western blot and spectrophotometry, respectively. ROS levels were quantified employing the fluorogenic dye 2',7'-dichlorofluorescein diacetate (DCFDA).

The results achieved for *OeCAT* gene expression demonstrate its involvement in AR formation. Expression levels differed at the basal region of the microcuttings, being significantly higher in non-IBA treated microcuttings relative to IBA-treated, for all time points. An inverse relationship between ROS levels and CAT enzyme activity was observed in leaf and basal region of IBA-treated microcuttings - a significant increase in CAT activity at 24h and 72h coincided with a decrease in ROS levels.

To our knowledge, this study was the first to relate the activity of CAT to the adventitious rooting process in olive trees. Here, a significant variation in the activity of this enzyme was observed amongst IBA non-treated and IBA-treated microcuttings, suggesting a regulation of ROS levels by the enzyme CAT in response to rooting stimulation.

This research was funded by National Funds through FCT—Foundation for Science and Technology under the Projects UID/AGR/00115/2019 and UIDB/05183/2020, and by FEDER and National Funds through the ALENTEJO 2020 (Regional Operational Program of the Alentejo), Operação ALT20-03-0145-FEDER-000014—“Valorização das Variedades de Oliveira Portuguesas (OLEAVALOR)”. Authors would thank Virginia Sobral by the help on the establishment of the *in vitro* trials.

## NOx Sensing – Smart solutions for the control of ammonia in the aquatic environments

S. Rodrigues Gaspar<sup>1</sup>, M. Gabriela Almeida<sup>1,2,3</sup>, M. Ascensão Reis<sup>1</sup>, P. Pousão-Ferreira<sup>4</sup>

<sup>1</sup>UCIBIO/i4HB– Applied Molecular Biosciences Unit, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal

<sup>2</sup>Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Monte da Caparica, Almada, Portugal

<sup>3</sup>NS2 – Nitrogen Sensing Solutions, Leiria, Portugal

<sup>4</sup>IPMA – Instituto Português da Atmosfera IPMA, Av. 5 de Outubro, Olhão, Portugal

Email: sb.gaspar@campus.fct.unl.pt

Aquaculture is the world's fastest-growing agriculture sector, with Recirculating Aquaculture Systems (RAS) playing a crucial role in fish production. Despite the benefits of RAS, including improved safety and reduced carbon footprint, the need for advanced technology to monitor water quality remains critical and a hot topic. Specifically, the absence of real-time sensors for quantifying onsite the highly toxic nitrogen compounds - ammonia, nitrite, and nitrate. These compounds establish significant risks as they can accumulate in closed systems and compromise entire aquaculture stocks with the loss of entire production. Among these, ammonia is the most harmful N-compound with its impact varying depending on the conditions (pH, temperature, and concentration). To address this issue, we are developing two electrochemical biosensors using distinct approaches: a) biosensor based on whole *Nitrosomonas europaea* cells, and b) biosensor using commercial Glutamate dehydrogenase (GLDH), for ammonia quantification. Initial efforts have focused on cultivating *N. europaea*, a biofilter-associated microorganism that performs ammonia oxidation, under optimal conditions. Currently we have explored the use of GLDH, for detecting ammonia. Our prototype biosensors involve modifying disposable strips, according to the approach (a or b), coupled with a handheld reader to provide immediate results regarding the ammonia levels.

Preliminary laboratory tests of the whole cell-based ammonia biosensor demonstrate the need for refinement in the cell culture process to enhance both biomass yield and enzymatic activity post-harvest. For the biosensor modified with the commercial enzyme, the results indicate effective quantification within a linear range of 0.56 and 4.5  $\mu\text{M}$ , although optimization is needed to reduce the interferences caused by the enzyme's high working potential.

Future work will focus on increasing the yield of *N. europaea* biomass and gaining deeper insights into its protein mechanisms, including protein purification. Simultaneously, efforts to refine the ammonia biosensor will prioritize improving its linear concentration range and sensitivity by testing alternative matrices and electrode substrates to lower the working potential.

This work was funded by National Funds through Fundação para a Ciência e a Tecnologia, under the Project "NOx Sensing – Smart solutions for the control of nitrogen-based nutrients in the aquatic environments" with reference 2023.01176.BDANA.

## Application of molecular techniques to detect and quantify Poaceae pollen in outdoor environment

Raquel R. Ramalhosa<sup>1</sup>, Ana Galveias<sup>2</sup>, Alexandra Marchã Penha<sup>2,3</sup>, Ana Rodrigues Costa<sup>2,3,4</sup> & Célia M. Antunes<sup>2,3,4</sup>

<sup>1</sup>School of Science and Technology, University of Évora, Évora, Portugal

<sup>2</sup>Institute of Earth Sciences, University of Évora, Évora, Portugal

<sup>3</sup>Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal

<sup>4</sup>Centro Académico Clínico do Alentejo – C-TRAIL, Évora, Portugal.

\*Corresponding author: [m56818@alunos.uevora.pt](mailto:m56818@alunos.uevora.pt)

Poaceae pollen is a major aeroallergen globally and a leading cause of pollen allergies in developed countries. This study aimed at quantifying Poaceae pollen in Évora atmosphere using molecular methods. Poaceae pollen was collected using a Hirst-type volumetric spore trap and a two-stage (PM<sub>>10</sub>-XL; PM<sub>10</sub>-M) high-volume-ChemVol<sup>®</sup> cascade impactor in Évora, 2017-2021. The main pollen season was determined using logistic methods, Pollen Season Duration (PSD) and Seasonal Pollen Index (SPIn) were calculated (Hirst methodology). DNA was extracted from the ChemVol samples and quantified using real-time PCR. Pearson's correlations were used to study the relationship between pollen and Poaceae DNA concentrations. Pollen was detected mainly in April and May from 2017-2021. The SPIn ranged yearly from 1,800-10,000 pollen/m<sup>3</sup>, peaking in 2021 (9,272 pollen/m<sup>3</sup>). The PSD ranged 46-80 days, being longest in 2017 (79 days) and 2018 (68 days). The peak concentration ranged yearly between 176-748 pollen/m<sup>3</sup>. Poaceae DNA concentrations were higher in XL (34.3–866.2 ng/m<sup>3</sup>) compared to M fraction (0.05–50 ng/m<sup>3</sup>). Pollen and DNA concentrations were highly correlated in XL ( $p < 0.001$ ), but not in M fraction. Molecular approaches are crucial for detecting and quantifying Poaceae pollen, aiding in preventive measures to reduce allergy risks.

### References

1. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. *Allergy*. 2007;62:976-990.
2. Hirst, J.M., 1952. An automatic volumetric spore trap. *Ann. Appl. Biol.* 39, 257e265.
3. Demokritou, P., Kavouras, I.G., Ferguson, S.T., Koutrakis, P., 2002. Development of a high volume cascade impactor for toxicological and chemical characterization studies. *Aerosol Sci. Technol.* 36, 925e933.









## **Assessing Perceived Value in Cooperative Membership: Insights from Organic Cocoa Producers in Sao Tome and Principe**

I. Prazeres<sup>1</sup>, M. Raquel Lucas.<sup>2</sup> A. Marta-Costa<sup>3</sup>, P. D. Henriques<sup>2</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gestão, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>CETRAD (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.

Email: [ibrahim.prazeres@uevora.pt](mailto:ibrahim.prazeres@uevora.pt)

The literature suggests that Sweeney and Soutar's (2001) original consumer perceived value scale, known as PERVAL, can be effectively applied to studies worldwide to explore various dimensions of perceived value. This scale, which consists of four dimensions—functional, emotional, monetary, and social values—across 19 items, is widely utilized in scientific research. However, shorter, non-redundant versions of the PERVAL scale have demonstrated comparable dimensional properties and predictive validity, making them suitable for diverse applications. This study evaluates the applicability of an 8-item short form of PERVAL in the context of organic cocoa producers in São Tomé and Príncipe, focusing on how the dimensions of perceived value influence producers' perceptions of cooperative membership. Data were collected through face-to-face questionnaires administered from June to December 2021 to a convenience sample of 810 organic cocoa producers, drawn from a population of 3,274 organic producers. The survey captured perceptions of membership in the two major cooperatives—CECAB or CECAC11—or other smaller residual organizations. These cooperatives serve as crucial intermediaries between farmers and the national and international chocolate industry, providing access to resources, information, and markets. The questionnaire responses offer insights into how organic cocoa producers perceive the value of cooperative membership across various dimensions. The analysis employed structural equation modelling (SEM) using partial least squares (PLS-SEM) and was conducted with Smart-PLS 4 software (version 4.0.9.1). SEM constructs a theoretical model based on latent variables, with relationships represented by regression or path coefficients between observed and/or latent variables. The findings indicate that, among the four dimensions of perceived value, emotional and social values are most highly perceived and are associated with a greater likelihood of adopting a multi-crop system. In contrast, the functional value of cooperative membership is less emphasized and more strongly linked to mono-crop cocoa production. The discussion highlights the implications of these findings for research and practical applications. For producers and sector governance, particularly cooperative strategies, these insights can guide efforts to enhance governance models and better align them with the needs of organic cocoa producers in São Tomé and Príncipe.

This research is supported by national funds through the FCT (Portuguese Foundation for Science and Technology) under projects UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>), and UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/LA/P/0121/2020>). Funding was additionally provided by FCT under the research contract PRT/BD/152273/2021 to Ibrahim Prazeres.

## Comparative Analysis of Circular Economy Practices in the Agri-Food Sector: A Study of Brazil and Italy

Gabriela Daiana Christ<sup>1</sup>, Crislaine Colla<sup>2</sup>, Adele Finco<sup>3</sup>, Pery Francisco Assis Shikida<sup>4</sup>

<sup>1</sup>PhD candidate in Regional Development and Agribusiness at Western Paraná State University (PGDRA/UNIOESTE/Brazil), and Visiting PhD student at Università Politecnica delle Marche (D3A/UNIVPM/Italy); ORCID: 0000-0002-5457-5884; E-mail: [gabrielachrist@gmail.com](mailto:gabrielachrist@gmail.com)

<sup>2</sup>Professor at Western Paraná State University (UNIOESTE/Brazil); ORCID: 0000-0002-5721-9412; E-mail: [crislaine.colla@unioeste.br](mailto:crislaine.colla@unioeste.br)

<sup>3</sup>Professore Ordinario at Università Politecnica delle Marche (UNIVPM/Italy); ORCID: 0000-0002-9859-901X; E-mail: [a.finco@univpm.it](mailto:a.finco@univpm.it)

<sup>4</sup>Professor at Western Paraná State University (UNIOESTE/Brazil); ORCID: 0000-0001-9621-1520; E-mail: [pery.shikida@unioeste.br](mailto:pery.shikida@unioeste.br)

The global agri-food sector is in direct need of transitioning into sustainable consumption and production patterns (Liu et al., 2021). With an increasing focus on sustainability, the circular economy (CE) has emerged as a key strategy for reducing environmental impact, particularly within the agri-food sector. CE promotes the reintegration of waste and by-products into production chains (Chiaraluca et al., 2021). For its successful implementation, managers and technicians must explore solutions to recover and valorize waste (Chiaraluca et al., 2023). This research addresses the challenge of sustainable development – across environmental, social, and economic dimension – as a mechanism for managing sustainable regional development. The study's general objective is to analyze the implementation of CE practices in the agri-food sector, focusing on the development of circularity indicators and the influence of circular economy principles on business models in regional contexts, with case studies from Paraná State, Brazil, and Marche Region, Italy. Specific objectives include a) to identify and analyze circular economy practices implemented in the agri-food sector around the world; b) to develop and estimate an indicator for measuring circularity in the agri-food sector from a regional perspective: the six Intermediate Geographic Regions (RGInt) of Paraná, Brazil, and the five major regions of Italy; and c) to analyze how the principles of the circular economy influence and reshape the architecture of existing business models in the agri-food sector in RGInt Cascavel, Paraná State, Brazil and Marche Region, Italy. This study will employ a mixed-methods approach, integrating a literature review with both quantitative and qualitative analyses, and holds significant social, scientific, and economic relevance. The expected outcomes are both theoretical and practical. On the theoretical side, it will offer a comprehensive review of circular business models, examining their core principles while incorporating practices, case studies, and theoretical frameworks for the agri-food sector from a regional perspective. On the practical side, the research will provide actionable guidelines for stakeholders, along with policy recommendations to facilitate the transition to a circular agri-food economy in both countries.

### References

- Chiaraluca, G., Bentivoglio, D., & Finco, A. (2021). Circular Economy for a Sustainable Agri-Food Supply Chain: A Review for Current Trends and Future Pathways. *Sustainability*, 13(16), 9294. <https://doi.org/10.3390/su13169294>
- Chiaraluca, G., Bentivoglio, D., & Finco, A. (2023). The circular economy model in the agri-food sector: A new strategy for the regional development. *AIMS Agriculture and Food*, 8(3), 851–872. <https://doi.org/10.3934/agrfood.2023045>
- Liu, Y., Wood, L. C., Venkatesh, V. G., Zhang, A., & Farooque, M. (2021). Barriers to sustainable food consumption and production in China: A fuzzy DEMATEL analysis from a circular economy perspective. *Sustainable Production and Consumption*, 28, 1114–1129. <https://doi.org/10.1016/j.spc.2021.07.028>

This work was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, under the Project 88881.933678/2024-01, 194/2024 – PDSE – Programa de Doutorado Sanduíche no Exterior.

**Growing in the future: redevelopment of brownfields through vertical farming**Alessandro Sebastian Amici<sup>1\*</sup>, Diego Appicciutoli<sup>1</sup>, Deborah Bentivoglio<sup>1</sup>, Adele Finco<sup>1</sup><sup>1</sup>Department of Agricultural, Food and Environmental Sciences (D3A). Università Politecnica delle Marche (UNIVPM/Italy),\*Email: [a.s.amici@staff.univpm.it](mailto:a.s.amici@staff.univpm.it)

It has been estimated that by 2050 arable land will be one-third less than that documented in 1970 (FAO, 2021). This reality predicts reduction in available land at a time when 70% more food needs to be produced to meet population growth (Bergoglio et al., 2023). Feeding a growing global population and dealing with various crises, such as resource depletion and climate change, is one of the biggest challenges for the agricultural world (Fussy et al., 2022). Moving to Italy, a survey conducted by ISTAT in 2012 revealed that about 3% of the national territory is occupied by brownfields, equivalent to about 9,000 km<sup>2</sup>, 30% of which are in urban settings (ISTAT, 2012). These abandoned spaces represent an important opportunity for urban redevelopment and regeneration. The use of these areas for off-soil agriculture could offer a concrete opportunity for the development of short supply chains, thus contributing to the reduction of emissions related to logistics and food transport, as well as improving urban sustainability. In line with the overall goal of the PNRR VITALITY project, which aims to create innovative ecosystems to make regional production systems more competitive and improve sustainability.

The proposed study aims to analyze the economic sustainability of redeveloping brownfield through the implementation of above-ground technologies aimed at creating vertical agriculture. The analysis is going to take as a case study companies practicing vertical farming in Italian territory. To date, data has been collected from four Italian companies active in the field of soilless agriculture. Various aspects required for setting up a farm were analyzed through an interview protocol. Through this study, together with the exploitation of unusable spaces, several favorable points emerged: greater production efficiency, considerable environmental sustainability, prospect of produce innovative product. Some critical factors include inability to certify products as organic, arduous pathway to identifying as a farming or processing company, high initial costs and difficult to have access to fundings. Further studies are necessary to have a complete scenario over the soilless farming reality in Italy.

This research has received funding from the project Vitality – Project Code ECS00000041, CUP I33C22001330007 - funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.5 - 'Creation and strengthening of innovation ecosystems,' construction of 'territorial leaders in R&D' – Innovation Ecosystems - Project 'Innovation, digitalization and sustainability for the diffused economy in Central Italy – VITALITY' Call for tender No. 3277 of 30/12/2021, and Concession Decree No. 0001057.23-06-2022 of Italian Ministry of University funded by the European Union – NextGenerationEU.

**References**

- [1] Fussy, A., & Papenbrock, J. (2022). An overview of soil and soilless cultivation techniques—chances, challenges and the neglected question of sustainability. *Plants*, *11*(9), 1153.
- [2] FAO. Land statistics and indicators 2000–2021 Global, regional and country trends. FAOSTAT ANALYTICAL BRIEF 71. 2021
- [3] Bertoglio, R., Corbo, C., Renga, F. M., & Matteucci, M. (2021). The digital agricultural revolution: a bibliometric analysis literature review. *IEEE Access*, *9*, 134762-134782.
- [3] ISTAT 2012. Camera dei deputati. (2024). Proposta di legge n. 3683 [https://documenti.camera.it/dati/leg18/lavori/schedela/apriTelecomando\\_wai.asp?codice=leg.18.pdl.camera.3683.18PDL0195040](https://documenti.camera.it/dati/leg18/lavori/schedela/apriTelecomando_wai.asp?codice=leg.18.pdl.camera.3683.18PDL0195040)

## Soilless Farming: A Bibliometric Study of Trends, Innovations, and Future Outlooks

Diego Appicciutoli<sup>1\*</sup>, Deborah Bentivoglio<sup>1</sup>, Adele Finco<sup>1</sup>

<sup>1</sup>Department of Agricultural, Food and Environmental Sciences (D3A) Università Politecnica delle Marche (UNIVPM/Italy)

\*E-mail: [d.appicciutoli@staff.univpm.it](mailto:d.appicciutoli@staff.univpm.it)

Faced with challenges such as resource depletion, climate change and the recent food crisis, the agricultural sector is enduring one of its darkest times (Fussy et al., 2022). Adding to these concerns is the growing of global population, which is expected to reach 9 billion by 2050, and the consequent need to produce an estimated 70% more food (Bertoglio et al., 2023). To address these pressing issues, various programs are providing funding to support the implementation of innovative approaches aimed at ensuring healthy food for everyone. The funding of our activity came from the PNRR-funded project VITALITY. The goal of this project is to establish innovation ecosystems that enhance the competitiveness of regional production systems while improving sustainability and quality of life in both urban and rural areas. Among various emerging trends, soilless farming is recognized as an optimal approach to adapt the agricultural sector to upcoming demands (Klerkx, L. et al 2022). In light of that, we aim to conduct a bibliometric analysis coupled with a literature review that examines soilless farming research landscapes, spanning from 2008 to 2023. With a comprehensive analysis of 256 publications through VOSviewer software, the study examines key themes, emerging trends and influential works in the field. The VOSviewer software tool is used to generate a bibliographic map based on co-occurrences of the keyword. The results emphasize the increasing significance of soilless cultivation and its integrated systems. The synergy between urban agriculture and soilless methods offers a promising line of research to support the development of a sustainable agricultural model. As existing case studies focus exclusively on the urban farming scenario, future research should explore the reality of businesses. In addition, consumer perceptions towards soilless vegetables remain unexplored. Coupling this with the lack of economic viability analyses, it is essential to direct scientific focus toward the practical adoption of these technologies in global vegetable production. Addressing these gaps, soilless policies should be clarified to facilitate funding access. By building on the advancements of the last decade and incorporating economic frameworks, soilless farming could become a strong contributor to the food supply chain.

This research has received funding from the project Vitality – Project Code ECS00000041, CUP I33C22001330007 - funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.5 - 'Creation and strengthening of innovation ecosystems,' construction of 'territorial leaders in R&D' – Innovation Ecosystems - Project 'Innovation, digitalization and sustainability for the diffused economy in Central Italy – VITALITY' Call for tender No. 3277 of 30/12/2021, and Concession Decree No. 0001057.23-06-2022 of Italian Ministry of University funded by the European Union – NextGenerationEU.

### References

- Fussy, A., & Papenbrock, J. (2022). An overview of soil and soilless cultivation techniques—chances, challenges and the neglected question of sustainability. *Plants*, *11*(9), 1153.
- Bertoglio, R., Corbo, C., Renga, F. M., & Matteucci, M. (2021). The digital agricultural revolution: a bibliometric analysis literature review. *Ieee Access*, *9*, 134762-134782.
- Klerkx, L., & Rose, D. (2020). Dealing with the game-changing technologies of Agriculture 4.0: How do we manage diversity and responsibility in food system transition pathways? *Global Food Security*, *24*, 100347.

## Multifunctional agriculture and innovative models of development in rural areas

M. Mogetta<sup>1\*</sup>, D. Bentivoglio<sup>1</sup>, A. Finco<sup>1</sup>

<sup>1</sup>Department of Agricultural, Food and Environmental Sciences (D3A) – Università Politecnica delle Marche (UNIVPM), Italy.

\*Email: [m.mogetta@pm.univpm.it](mailto:m.mogetta@pm.univpm.it)

The concept of multifunctional agriculture strongly links to the European agricultural model and respective rural development policies, positioning it as a competitive and sustainable model of agriculture (Cardillo *et al.*, 2023). Today, the multifunctionality of agricultural enterprises is defined as their ability to produce different kinds of secondary goods and services in addition to their primary activity, such as public goods and externalities, but also all those ancillary activities that can be, for example, processing and direct sales, up to the realization and sale of cultural, recreational, rehabilitative, social and environmental products and services (Aimone *et al.*, 2006). Given the changes in the agricultural sector and in our society, the challenges of territories, and particularly in rural areas, the multifunctionality of agriculture presents itself as an increasingly relevant factor in addressing several critical issues (such as economic and demographic decline). This research seeks to analyse the role of multifunctional agriculture and the study of innovative development models to spread the sustainability of rural areas. Particular attention is dedicated to the innovative models of governance found in rural areas that involve multifunctional agricultural activities and can represent an innovation for local development by enhancing social and territorial capital available in these areas. The first part of the research activity is dedicated to an overview of the concept of multifunctionality in agriculture through a literature review, and then a focus is dedicated to the reality of community-based cooperatives as a new model of development in rural areas, specifically in Italy. Community-based cooperatives may represent fascinating case studies; they are realities that arise from the direct involvement of citizens, and through which an attempt is made to respond to specific territorial needs through the enhancement of local resources, promoting inclusive and sustainable development models. The analysis of these new development models is based on qualitative approaches, such as semi-structured interviews. This work is intended as an initial starting point for further comparisons of the concepts and methodologies of analysis applicable to these realities.

This work was financed by the XXXIX cycle of Doctoral Programme-Università Politecnica delle Marche (UNIVPM), Department of Agricultural, Food and Environmental Sciences (D3A).

### References

- Aimone, S., Cassibba, L., Cagliero, R., Milanetto, L., & Novelli, S., (2006). Multifunzionalità dell'azienda agricola. IRES: Torino, Italy.
- Cardillo, C., Cimino, O., De Rosa, M., & Francescone, M., (2023). The Evolution of Multifunctional Agriculture in Italy. *Sustainability* 2023, 15, 11403. <https://doi.org/10.3390/su151411403>

## **Assessing stakeholder strategies and perceptions for enhancing sustainability in the Montado and Dehesa agrosilvopastoral ecosystem**

M. Advinha<sup>1</sup>, M.R. Lucas<sup>2</sup>, A. Marta-Costa<sup>3</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

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

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

Email: [miguel.fm.advinha@gmail.com](mailto:miguel.fm.advinha@gmail.com)

Characterized by its multidisciplinary functionality, the Montado (known as Dehesa in Spain) agrosilvopastoral ecosystem represents an important component of agroforestry practices in southern and central Portugal and Spain. Known for its remarkable capacity to support a diverse array of flora and fauna, while simultaneously enabling relevant economic activities, and providing essential services and products, there's a need to support the claims on the importance of this ecosystem to the economic, social, and environmental well-being of local communities. Anchored by cork and holm oak trees and its soil health, the Montado/Dehesa faces numerous challenges that contribute to its ongoing decline. This reinforces the need to preserve and enhance the value of this unique ecosystem. The present study aims to explore strategies and stakeholders' perceptions on the valorisation of the Montado/Dehesa agrosilvopastoral system. Structured into three phases, the project begins with a systematic literature review to identify dynamics, challenges and gaps within the Montado and Dehesa value chains. The second phase analyses business and government strategies for the sustainability and valorisation of Montado/Dehesa goods and services through semi-structured interviews with producers/landowners and focus groups involving other key stakeholders. In the third phase, consumer willingness-to-pay for Montado and Dehesa-sourced goods and services will be assessed through a survey targeting different types of consumers. This research can provide valuable and strategic insights for producers and decision-makers, fostering informed policy development, and the promotion of sustainable practices. The findings will not only deepen scientific understanding of the Montado/Dehesa ecosystem, but also highlight its economic value and its role supporting the well-being of dependent communities. By considering both Portuguese and Spanish contexts, this study will real any differences in ecosystem dynamics and management practices across the two territories.

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



## Mobile Slaughter Unit: Contribution to the Sustainability of the Meat Value Chain

B. Traça<sup>123</sup>, A. Dionisio<sup>2</sup>, J. Aranha<sup>1</sup> & S. Campos<sup>3</sup>

<sup>1</sup>Universidade de Trás-os-Montes e Alto Douro - Escola de Ciências Humanas e Sociais, Quinta de Prados, 5000-801 Vila Real, Portugal

<sup>2</sup>CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>CISAS (Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentabilidade), IPVC - Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e comercial de Nun'Álvares,34, 4900-367 Viana do Castelo

Email: [barbaratraca@gmail.com](mailto:barbaratraca@gmail.com)

“Mobile Slaughter Unit: Contribution to the Sustainability of the Meat Value Chain” proposes the implementation of Mobile Slaughter Units as a sustainable solution for the meat value chain, focusing on small and medium-sized producers, including those practicing organic production. The primary aim is to promote a fairer and more balanced food system that meets the growing consumer demand for ethical and sustainable practices, respecting animal welfare and supporting the development of rural areas.

By decentralising meat slaughter and processing, the mobile slaughter model facilitates shorter and more transparent supply chains, enabling direct sales to local consumers and reducing the carbon footprint associated with meat transportation and processing. In this context, the project seeks to characterise the current system of slaughterhouses, transport, and slaughter in Portugal, identifying improvement opportunities that can strengthen the local economy and highlight the role of small producers in the meat value chain.

To achieve these objectives, the study employs a mixed methodology, combining quantitative and qualitative approaches. Two surveys will be conducted, targeting producers and economic operators, to assess perceptions of transport and slaughter challenges, interest in adopting mobile slaughter, and key sustainability indicators already implemented by economic operators. Subsequently, a Geographic Information System (GIS) will be developed to analyse land use evolution and the distribution of slaughterhouses over the past 30 years, complemented by mathematical modelling to optimise transport and slaughter networks.

One of the main expected outcomes is the creation of a sustainability assessment model for the meat industry, integrating environmental, social, and economic indicators. Inspired by the BIA-Sustainability methodology, the model will be adapted to the sector, evaluating factors such as greenhouse gas emissions, water consumption, waste management, and working conditions, aligned with Sustainable Development Goals (SDGs) 8, 10, 11, 12, and 13 and the FEAST project, which aims to catalyse the transition to a healthy and fair food system in Europe.

This work was funded by National Funds through FCT, by granting a scholarship from CECA 3rd Edition of the PhD Research Scholarship Competition under the Consortium of Schools of Agricultural Sciences (CECA).

## **Sustentabilidade e Competitividade no Enoturismo: Um Estudo das Empresas Vitivinícolas do Alentejo**

S. Rosa<sup>1</sup>, A. Guedes<sup>2</sup>, J. Ventura<sup>3</sup>, R. Fragoso<sup>4</sup>

<sup>1</sup>Universidade de Trás-os-Montes e Alto Douro (UTAD), Universidade de Évora (UE)

<sup>2</sup>Universidade de Trás-os-Montes e Alto Douro (UTAD)

<sup>3</sup>Universidade de Évora (UE)

<sup>4</sup>Universidade de Évora e Centro de Estudos Avançados em Gestão e Economia da Universidade de Évora (CEFAGE)

Email: [stelagsflrosa@gmail.com](mailto:stelagsflrosa@gmail.com)

Wine tourism has been gaining prominence as an activity that not only promotes regional wine, but also values the cultural and natural heritage of wine-producing regions. The Alentejo region, one of the largest wine producers in Portugal, is characterized not only by the quality of its wines, but also by the growing recognition of its sustainable practices. The integration of sustainability practices into wine-producing companies is seen as a promising strategy to ensure the long-term competitiveness of wine tourism, but there are few empirical studies that explore this direct relationship. Thus, this study aims to fill this gap by investigating how sustainable practices implemented by wine companies in the Alentejo region affect the competitiveness of their wine tourism activity. To this end, the study has three specific objectives: (1) to identify and characterize the sustainability practices adopted by wine companies with wine tourism activity in the Alentejo; (2) to determine the motivations and challenges faced in implementing these practices; and (3) to analyze the impact of these practices on the competitiveness of the wine tourism. The methodology adopted will be a multiple case study focused on several wine companies in Alentejo that offer wine tourism experiences. Data collection will include three main approaches: documentary analysis of companies' sustainability reports, application of questionnaires to wine tourism consumers and semi-structured interviews with winery managers and owners. The analysis of sustainability reports will allow identifying the main ecological practices, such as the use of renewable energy, efficient water management and the promotion of biodiversity. The questionnaires and interviews will provide insights into consumers' perceptions of sustainability and managers' views on the challenges and opportunities created by these practices. The results of this study are expected to not only contribute to the scientific literature by filling the existing gap on the relationship between sustainability and competitiveness in wine tourism, but also offer practical recommendations for business managers and policy makers, reinforcing the position of Alentejo as a sustainable and competitive wine tourism destination.

## Knowledge and Perception of Concepts Related to Food Sustainability: Segmentation and Analysis of Consumer

E. Venancio<sup>1</sup>, M. Raquel Lucas.<sup>2</sup> A. Marta-Costa<sup>3</sup>, P. D. Henriques<sup>2</sup>

<sup>1</sup>PhD student in Agribusiness and Sustainability - Universidade de Évora, Largo dos Colegiais, Nº 2, 7004-516 Évora

<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Gestão, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

<sup>3</sup>CETRAD (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.

Email: eunice.venancio@uevora.pt

The growing challenge of food sustainability requires a better understanding of consumer knowledge and perceptions. This study addresses this issue by exploring how consumers perceive concepts related to food sustainability. The research was carried out through an online survey, applied to a random sample of 444 individuals in Portugal, in which 95% of the participants are Portuguese citizens, between June 2023 and February 2024. The survey included questions on topics such as ecological footprint, carbon footprint, environmental impact, biodiversity, local food, greenhouse gas emissions, ecosystem services, food waste, water footprint and genetically modified organisms (GMOs). Participants were asked to indicate their level of knowledge about each concept, using the options “I don't know the meaning,” “I've heard the term, but I don't know the meaning,” and “I know the meaning.” The data was analyzed using SPSS, applying cluster analysis to identify groups of consumers, classified as follows: (1) working-aged men, employed, with a high income and academic background; (2) mature women with a high income and academic background; and (3) young students, mostly unemployed. The younger group showed greater familiarity with the concepts, except for the concept of local food, which was better understood by mature women. In addition, the mature women showed a deeper understanding of the concepts compared to the men in the first group. It is important to note that the results reflect the interviewees' perceptions of their own knowledge, rather than an objective measurement of their understandings. This suggests that while younger participants feel more confident about the concepts, older ones tend to be more cautious, recognizing the grey areas inherent in these broad concepts. This study can serve as a basis for the development of educational strategies and policies that promote more sustainable consumption practices, meeting the specific needs of each demographic group and thus contributing to a more responsible and food-conscious future.

This research is supported by national funds through the FCT (Portuguese Foundation for Science and Technology) under projects UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>), and UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/LA/P/0121/2020>). Funding was additionally provided by FCT under the research contract PRT/BD/152339/2021 to Eunice Venâncio.

## Sustainable Practices in Olive Oil Production Cooperatives in Alentejo

M. Romão<sup>1</sup>, M.R. Lucas<sup>2</sup>, M. L. Tibério<sup>3</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal and CETRAD (Centro de Estudos Transdisciplinares para o Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-801 Vila Real, Portugal.

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

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

Email: [mariamromao2@hotmail.com](mailto:mariamromao2@hotmail.com)

Olive oil production in Alentejo, one of Portugal's foremost olive-growing regions, faces challenges ranging from the need to enhance competitiveness to adapting to climate change and addressing environmental pressures. Olive oil production cooperatives play a central role in this sector and must find solutions that ensure a sustainable future while maintaining the quality and productivity that have made Alentejo's olive oil renowned worldwide. This study aims to investigate the sustainability practices adopted by olive oil production cooperatives in Alentejo, focusing on the perceptions of the key stakeholders involved and on strategies that could support a successful transition to a more sustainable production model. The research will employ a mixed-method approach, combining qualitative and quantitative methodologies. Interviews with experts, as well as surveys with producers, cooperative managers, and other key stakeholders, will be conducted to understand how olive oil cooperatives are implementing sustainable practices within their operations. This includes efficient resource management, biodiversity conservation, waste reduction, and byproduct treatment. Furthermore, the study will assess the social, economic, and environmental impacts of these practices. Another focal point of the research will be the role of technological innovation in promoting sustainability. Solutions such as smart irrigation systems, remote crop monitoring, and the use of renewable energy will be analyzed to highlight the benefits these technologies offer for efficiency and reducing environmental impact. The study will also examine the regulatory framework and public policies in place, exploring how government incentives can support the shift toward sustainability within the cooperative sector. This study is expected to demonstrate that the adoption of sustainable practices is not only feasible but also essential for ensuring the economic and environmental viability of the olive oil sector in Alentejo. The conclusions will provide practical recommendations for olive oil cooperatives and policymakers, emphasizing both the challenges and opportunities in promoting a more sustainable and competitive olive oil production industry.

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

## **Access and adherence to E-Voucher Models in Agriculture: Recent findings from the FAO PROMOVE Program in Mozambique\***

Mohomede Saide<sup>1,2</sup>, Pedro D. S. Henriques<sup>1</sup>, Carlos J. Fonseca<sup>2</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Departamento de Economia da Escola de Ciências Sociais, Pólo da Mitra, AP. 94, 7002-554 Évora, Portugal.

<sup>2</sup>Universidade de Trás-os-Montes e Alto Douro (UTAD) Quinta de Prados, 5000-801, Vila Real, Portugal.

Email: [mohomede.saide@uevora.pt](mailto:mohomede.saide@uevora.pt)

Agricultural development programs are of capital importance in rural areas in Mozambique. The country is dependent on agriculture: About 70% of the population lives in rural areas and 80% of the economically active population is involved in agricultural work (STSAN, 2014). In the 2015/2016 agricultural campaign, the Food and Agriculture Organization of the United Nations (FAO), within the framework of the Millennium Development Goal 1c (MDG1c) program, funded by the European Union, launched an electronic voucher system (e-voucher) as a tool to i) improve access and use of quality agricultural inputs and ii) strengthen the agricultural input distribution chain through the involvement of various stakeholders. The objective of this study was to analyze the implementation of the PROMOVE e-Voucher, focusing on economic, environmental and social sustainability components. The methodology includes, in sequence: i) systematic literature review; ii) analysis of sociodemographic profile of target farmers (database with 35,000 observations); and iii) evaluation of barriers and facilitators to e-voucher adoption through semi-structured interviews with farmers, association/cooperative leaders, managers, stakeholders and agro-dealers. The initial findings of the research point to the following facts: the population in several target districts of the program is vulnerable to drought (practices rainfed agriculture). The sustainability of the system represents a critical challenge, considering two key aspects: climate change and pressure on natural resources. Reality demands rapid changes and innovations in soil use practices and in the rural development model itself. Overall, this study shows that determining key barriers and facilitators to adoption and maintenance in the PROMOVE Program. This study contributes to demonstrating the need to diversify system approaches in climate-risk regions.

Key-words: Voucher, E-Voucher, Agriculture, Mozambique, FAO

\*Note: This work was funded by Fundação Calouste Gulbenkian (Portugal) n.º.255182: Formação para os PALOP e Timor-Leste.

## Food Waste in Portugal: Development and Validation of a Regionally Focused Survey

R. Carvalho<sup>1</sup>, M.R. Lucas<sup>1</sup> A. Marta-Costa<sup>2</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gestão, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora, Portugal.

<sup>2</sup>CETRAD (Centre for Transdisciplinary Development Studies), University of Trás-os-Montes (UTAD), Quinta de Prados, Pole II, ECHS, Room 1.14, 5000-801 Vila Real, Portugal.

Email: [rakel.karvalho@hotmail.com](mailto:rakel.karvalho@hotmail.com)

Food waste is a complex, multifaceted issue with significant impacts on the economy, the environment, and global food security. In Portugal, this challenge has gained attention, particularly due to consumption and retail practices that contribute heavily to waste generation. Although the importance of tackling food waste is widely acknowledged, there remains a substantial gap in research examining the behavioral and contextual factors that lie at its core. This study aims to outline the development and validation process of a survey designed to map food waste across Portugal, with particular focus to regional differences. The objective is to build an instrument capable of gathering representative and reliable data on the behaviors and perceptions of consumers regarding food waste, within a comprehensive national sample.

The survey will be developed in two main stages. The first phase involves planning the survey, with a clear definition of objectives, hypotheses, and the operationalization of concepts based on scientific literature. Variables such as attitudes and values, motivation, habits, perceived social norms, knowledge, and skills related to behavior and resources will be considered. The second phase includes preparing the data collection instrument, involving both the construction and pre-testing of the questionnaire. This process will assess the clarity and suitability of the questions, resulting from revisions and improvements prior to large-scale application. Validation will be conducted through pre-testing, followed by expert review, to ensure content validity and clarity of the questions. Additionally, best methodological practices will be employed to ensure the survey's internal consistency and its reliability in measuring the intend concepts and variables.

This study provides an innovative approach, as no similar study on food waste has been conducted in Portugal. Alongside providing a comprehensive national perspective, the survey contributes a specific focus on regional dynamics, making it a valuable tool for future investigations across diverse areas in the country.

This work was funded by National Funds through FCT (Portuguese Foundation for Science and Technology) under the Projects UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>); <https://doi.org/10.54499/LA/P/0121/2020>) and UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>).

**New business models sustaining the silvopastoral system of the *Montado***N.E. Fatahi<sup>1,2\*</sup>, H. Belhouchette<sup>2</sup>, T.P. Correia<sup>1</sup> and J.T. Marques<sup>1</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Portugal.

<sup>2</sup>IAMM, Montpellier, France.

\*Presenting author, Email: [d56257@alunos.uevora.pt](mailto:d56257@alunos.uevora.pt)

The *Montado*, a unique silvopastoral system in Southern Portugal, faces challenges from climate variability, economic pressures, and evolving agricultural policies, impacting its sustainability. Our research investigates alternative business models for *Montado* farms, aiming to balance economic viability with ecological resilience.

We selected three representative *Montado* farms in the Alentejo region, each chosen for its distinct characteristics in terms of intensification, farm size, and integration of silvopastoral components. These farms embody the diversity within the *Montado*, allowing us to capture varied management strategies and adaptation potential. The typology was informed by empirical data from public statistics, in-farm surveys and expert knowledge on *Montado* practices, giving a comprehensive foundation for understanding farm-level decisions and interactions with external pressures.

To refine our model, we are working collaboratively with local stakeholders, particularly *Montado* farmers, to develop relevant scenarios. Preliminary discussions held over a questionnaire enabled us to capture farmer priorities and concerns, particularly around market dependencies, subsidy structures, and climate adaptation. This input has been crucial for defining scenarios that reflect real-world challenges and practical solutions.

These scenarios will inform a bio-economic model built in GAMS (General Algebraic Modeling System), enabling us to assess potential impacts on income, labor needs, and ecological outcomes under changes and different management approaches. Although the model is still in development, this scenario-based approach highlights the complexity of decision-making within *Montado* systems and the need for adaptive strategies that align with farmer priorities and regional goals.

The ongoing model aims to serve as a decision-support tool, offering insights into sustainable practices for *Montado* farms facing a changing climate and market landscape.

**Keywords:** Economic modeling, silvopastoral system, Montado.

## Consumer Perception on the Sustainability of Geographical Indications in Portugal

L. Gomes<sup>1</sup>, M.R. Lucas<sup>1</sup> C. Costa<sup>2</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gestão, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora, Portugal.

<sup>2</sup>CETRAD (Centre for Transdisciplinary Development Studies), University of Trás-os-Montes (UTAD), Quinta de Prados, Pole II, ECHS, Room 1.14, 5000-801 Vila Real, Portugal.

Email: [lu\\_gomes22@yahoo.com.br](mailto:lu_gomes22@yahoo.com.br)

Although consumers frequently purchase GI (Geographical Indication) products, their awareness and understanding of GI labels are often limited. At the same time, they are increasingly concerned about the sustainability of food systems, seeking production methods with lower environmental and climate impacts, greater social responsibility, and a shift towards more responsible consumption in response to climate change. However, there is often a discrepancy between these attitudes and actual purchasing behaviour. This research employs a multi-method approach, combining different techniques—World Café dialogues, a quantitative survey, experiments, and a Delphi study—to understand and enhance how consumers perceive and value GIs and their willingness to pay for high-quality products that align with sustainability and healthy eating goals. The work includes the following objectives and correspondent tasks: 1. Enhance consumer understanding of GIs and encourage engagement with GI products by fostering responsible purchasing behaviours and the selection of healthy, nutritious, and sustainable foods. This will be achieved through qualitative methods, particularly World Café dialogues, which will bring consumers and GI producers together in a constructive, idea-sharing process that promotes mutual learning. 2. Assess consumer perception and willingness to pay for GI products that align with sustainability and health goals. This objective involves a national online consumer survey with discrete choice experiments that evaluate perceptions of GI products' health benefits and contributions to sustainability. 3. Analyse consumer processing of GI information and promotional messages to identify effective communication strategies for increasing consumer support for GIs. It will involve interconnected experimental studies to examine how consumers process information and promotional messages about GIs. 4. Develop a guide with strategic policy recommendations informed by the research findings, aimed at enhancing the role of GIs in promoting sustainability in line with the European Union's "Farm to Fork" strategy. It is expected that this research bridge the gap between consumer attitudes and behaviours toward GI by enhancing understanding, engagement and willingness to support GI products that align with sustainability and health goals, ultimately contributing valuable insights to support policy recommendations within the EU's "Farm to Fork" strategy.

This work was funded by National Funds through FCT (Portuguese Foundation for Science and Technology) under the Projects UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>); <https://doi.org/10.54499/LA/P/0121/2020>) and UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>).



## Sustainability of Geographical Indications in Portugal

M. Ruiz<sup>1</sup>, M.R. Lucas<sup>1</sup> C. Costa<sup>2</sup>

<sup>1</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gestão, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora, Portugal.

<sup>2</sup>CETRAD (Centre for Transdisciplinary Development Studies), University of Trás-os-Montes (UTAD), Quinta de Prados, Pole II, ECHS, Room 1.14, 5000-801 Vila Real, Portugal.

Email: [maylidis79@gmail.com](mailto:maylidis79@gmail.com)

The development of Geographical Indications (GI) and the legal frameworks of DOP (Protected Designation of Origin) and PGI (Protected Geographical Indication) are regarded as part of cultural heritage, providing a means to resist commodification while maintaining high quality standards and contributing to rural development. However, there is controversy regarding their environmental impact and overall sustainability, particularly as growing market demand encourages the intensification and specialization of production systems and the exploration of alternative technological innovations. In the literature, GIs are widely recognized for their role in economic development and are also viewed as a strategy to create value and support rural growth, specifically through the diversification and preservation of agro-biodiversity within their territorial context, and the enhancement of landscape and socio-ecological values. Nonetheless, significant sustainability challenges persist. Nonetheless, significant sustainability challenges persist. These challenges involve addressing the environmental footprint of GI production systems, ensuring their resilience to climate change, and meeting societal expectations for fair labour practices and community benefits. Society is increasingly concerned about the sustainability of food systems, which entails adopting production methods that minimize environmental and climate impacts, preserve biodiversity, and provide meaningful social and economic contributions. While GI schemes have the potential to address these concerns, they also face barriers, including political, regulatory, social, cultural, and market-related challenges, as well as the urgent need to transition to agro ecological practices and adapt to climate change. This research aims to analyse and enhance the contributions of GIs to sustainable development in Portugal by addressing specific objectives: 1) To evaluate the environmental, economic, and social dimensions of sustainability in GI production systems, particularly in relation to their compliance with the European Union's "Farm-to-Fork" strategy, agro ecological transitions, and climate change adaptation; 2) To identify and assess sustainable GI production practices and technological innovations that balance productivity with ecological integrity and socio-economic benefits; 3) To understand GI producers' perceptions of the barriers and incentives for adopting sustainable practices, with a focus on how policies and market dynamics influence these decisions; 4) To explore strategies for strengthening the role of GI schemes in promoting biodiversity, reducing greenhouse gas emissions, and fostering rural resilience. By adopting a mixed-method approach, this research seeks to clarify the pathways through which GI schemes can be leveraged as instruments of sustainable development. Ultimately, it will provide actionable insights for aligning GI systems with ecological and climate goals, while enhancing their contributions to the well-being of rural communities and the broader environment.

This work was funded by National Funds through FCT (Portuguese Foundation for Science and Technology) under the Projects UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>); <https://doi.org/10.54499/LA/P/0121/2020>) and UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>).

**Alternative Scenarios for Family Farming Systems practiced in Huambo Province: Essay with Ethnographic Linear Programming Models (PLE)**A. Kamutali<sup>1</sup>, P.D. Henriques<sup>2</sup>, M.R. Lucas<sup>3</sup>, A. Marta-Costa<sup>4</sup>

<sup>1</sup>Faculty of Agricultural Sciences, Universidade José Eduardo dos Santos, Angola and MED (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.

<sup>2</sup>MED (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.

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

<sup>4</sup>CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Portugal, Departamento de Economia, Sociologia e Gestão, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.

Email: [ninokamutali@gmail.com](mailto:ninokamutali@gmail.com)

The agrarian structure of Huambo is primarily composed of family farmers, who play a critical role in the region's agricultural landscape. However, their production capacity remains insufficient to meet local needs due to low productivity and inconsistent supply. This study aims to identify and test alternative sustainability scenarios in economic and social dimensions that account for the context, potential, and challenges faced by local households, with the goal of achieving well-being levels above the poverty line. To accomplish this, linear programming models (PLE) were developed for different household in Huambo Province, Angola. These models consider available resources, potential agricultural activities, and the interconnections between them, allowing for an analysis of the current conditions in family farming systems. The goal is to identify viable solutions that enable sustainable income levels for farming households, enhancing their resilience and capacity to overcome challenges. The construction of the model followed four key phases: i) designing a questionnaire to gather essential data on household activities, resources, and needs; ii) testing and validating the questionnaire to ensure its effectiveness and reliability; iii) selecting specific households to provide data to validate and adjust the model, ensuring its relevance and accuracy; and iv) developing the PLE model based on this data. Several alternative scenarios were tested, including the introduction of certified seeds, adjustments in fertilizer use, expansion of farming area, and the addition of livestock (goats) with two breeding cycles per year. The findings indicated that for small (area  $\leq 3$  ha), medium ( $3 < \text{area} \leq 5$  ha), and large (area  $> 5$  ha) farming units, the introduction of high-yield seed varieties and a livestock system with two goat breeding cycles per year were particularly effective. These changes not only increased productivity but also improved household incomes, allowing them to surpass the poverty line. In conclusion, this study provides valuable insights into sustainable strategies for improving the productivity and resilience of family farming in Huambo. Targeted interventions, such as adopting improved seed varieties and optimizing livestock breeding cycles, can significantly raise household incomes and support long-term rural development in the region.

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

## Family Farming and Sustainable Development in São Tomé and Príncipe – an approach via Ethnographic Linear Programming

G. Tiny<sup>1</sup>, M.R. Lucas<sup>2</sup>, P.D. Henriques<sup>3</sup>, A. Marta-Costa<sup>4</sup>

<sup>1</sup>CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Portugal, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal e MED (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.

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

<sup>3</sup>MED (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.

<sup>4</sup>CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Portugal, Departamento de Economia, Sociologia e Gestão, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.

Email: [gilkson.tiny@uevora.pt](mailto:gilkson.tiny@uevora.pt)

Achieving sustainable development goals and reducing poverty requires supporting family farmers to improve productivity, income, well-being, resource efficiency, and environmental sustainability. Family farmers face numerous challenges, including limited subsistence resources, restricted market access, rising production costs, and the impacts of climate change. In São Tomé and Príncipe (STP), family farmers play a crucial role in food production, particularly in staples like cereals, roots, tubers, and legumes, yet their production systems are characterized by low productivity and insufficient output to meet local needs. This study aims to enhance the understanding, definition, and characterization of family agricultural systems in STP to assess their challenges, potential, food security for families, income generation above the poverty line, and sustainability. Data collection was based on research with a convenience sample of 50 farmers, both men and women, from all seven districts of STP: Mé-zóchi (15), Cantagalo (11), Caué (7), Pagué (5), Água Grande (4), Lobata (4), and Lembá (4). Key variables considered included property size, labor use, production technology, input costs, monthly expenses, total revenue, and the diversity of crops and animal species. The questionnaire addressed: (1) the farmer and family demographics, (2) property characteristics, (3) farming activities (crops, livestock, and technologies), and (4) sources of household income and monthly expenses. The collected data, after cleaning and verification, provide the foundation for PLE models designed to analyse the socio-economic behaviour of households, their resource efficiency, suitability of farming practices for local soil and climate conditions, income generation above poverty thresholds, and potential for sustainable development. It is expected that the study provides essential insights into the dynamics of family farming systems in Sao Tome and Principe, highlighting their challenges and potential, and offering a foundation for developing strategies that promote food security, poverty reduction, and sustainable development within these communities.

This work was funded by National Funds through FCT (Portuguese Foundation for Science and Technology) under the Projects UIDB/05183/2020 (<https://doi.org/10.54499/UIDB/05183/2020>; <https://doi.org/10.54499/LA/P/0121/2020>) and UIDB/04011/2020 (<https://doi.org/10.54499/UIDB/04011/2020>). Funding was additionally provided by FCT under the research contract PRT/BD/154669/2022 to Gilkson Tiny.

**Cassava Value Chain Analysis: Case Study in Malanje Province (Angola)**A. Gonçalo<sup>1</sup>, P.D. Henriques<sup>2</sup>, M.R. Lucas<sup>3</sup>

<sup>1</sup>MED (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.

<sup>2</sup>MED (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 Colegais, 2, 7000-803 Évora, Portugal.

<sup>3</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Gestão, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegais, 2, 7000-803 Évora, Portugal.

Email: [rosanvunda11@hotmail.com](mailto:rosanvunda11@hotmail.com)

The value chain consists of a sequence of interrelated activities carried out by different stakeholders, aimed at adding value to products and/or services, ultimately benefiting the consumer. Value chains play a crucial role in organizational management, as they allow for analysis of both internal and external environments and enable the reformulation of cost and pricing strategies. Angola produces approximately 11 million tons of cassava annually, underscoring its economic significance. To enhance the cassava value chain, a sustainable development approach is essential, one that leverages local potential and integrates productive, commercial, and institutional activities. A comprehensive study of the cassava value chain is needed, focusing on participants involved in production, processing, transportation, distribution, and retail to understand how value is added at each stage. The primary goal of this research is to analyse the optimal operating model for the cassava value chain, ensuring profitability and sustainability for all stakeholders, while considering the impact of government policies and strategies. The research uses a mixed-method approach, combining quantitative and qualitative data collection through questionnaires and interviews with two convenience samples: one from farmers, greengrocers, truck drivers, and end consumers from six municipalities in Malanje Province; the other from GEPE technicians at MINGRIF, GEPE in Malanje, six Municipal Administrators, and local agricultural company managers and Traditional Authorities. The findings of this study provide a foundation for designing a model to address current challenges in the cassava value chain. Such a model would aim to increase profitability, sustain all activities, ensure equitable distribution of costs and risks, and improve access to innovative production techniques, training, information, credit, and agricultural insurance. This contribution can help reduce poverty and unemployment, foster rural development, and improve the quality of life in rural communities. In other words, the study offers a framework for optimizing the cassava value chain in Angola, with the potential to enhance economic returns, promote sustainable practices, and significantly improve livelihoods in rural communities.

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>)

## Plenary Lecture Speakers

Name	Email
Carlos Zorrinho	<a href="mailto:czorrinho@uevora.pt">czorrinho@uevora.pt</a>
Miguel Fevereiro	<a href="mailto:miguelfevereiro@yahoo.com">miguelfevereiro@yahoo.com</a>

## List of Participants

Name	Email
Adriana Catarino	<a href="mailto:adri.catarino@hotmail.com">adri.catarino@hotmail.com</a>
Agostinho Gonçalo	<a href="mailto:d49855@alunos.uevora.pt">d49855@alunos.uevora.pt</a>
Alessandro Sebastian Amici	<a href="mailto:a.s.amici@staff.univpm.it">a.s.amici@staff.univpm.it</a>
Alonso Escoto	<a href="mailto:alonso8623@gmail.com">alonso8623@gmail.com</a>
Ana Elisa Rato	<a href="mailto:aerato@uevora.pt">aerato@uevora.pt</a>
Ana Faustino	<a href="mailto:ana.faustino@cebal.pt">ana.faustino@cebal.pt</a>
Ana Fonseca	<a href="mailto:anaritifonseca@ua.pt">anaritifonseca@ua.pt</a>
Ana Galveias	<a href="mailto:acgjorge@uevora.pt">acgjorge@uevora.pt</a>
Ana Itsel Canales Mendoza	<a href="mailto:itselmendoza2@gmail.com">itselmendoza2@gmail.com</a>
Ana Paulino	<a href="mailto:ana.paulino@cebal.pt">ana.paulino@cebal.pt</a>
Ana Rita Martins	<a href="mailto:rita.martins@cebal.pt">rita.martins@cebal.pt</a>
Ana Rita Trindade	<a href="mailto:arctrindade28@gmail.com">arctrindade28@gmail.com</a>
Ana Traça	<a href="mailto:barbaratraca@gmail.com">barbaratraca@gmail.com</a>
André Albuquerque	<a href="mailto:andrealb@uevora.pt">andrealb@uevora.pt</a>
André Jorge	<a href="mailto:d52876@alunos.uevora.pt">d52876@alunos.uevora.pt</a>
Andreia Silva	<a href="mailto:andreia.silva@cebal.pt">andreia.silva@cebal.pt</a>
Anísio Patrício Quintas	<a href="mailto:anisiopatricio23@gmail.com">anisiopatricio23@gmail.com</a>
Antonino Kamutali	<a href="mailto:cellmaleao@gmail.com">cellmaleao@gmail.com</a>
Antonio Bruno	<a href="mailto:166051@spes.uniud.it">166051@spes.uniud.it</a>
António Carrapiço	<a href="mailto:d53109@alunos.uevora.pt">d53109@alunos.uevora.pt</a>
Aurelie Arozarena	<a href="mailto:arozarena.aurelie34@gmail.com">arozarena.aurelie34@gmail.com</a>
Beatriz Duarte Bilhastre	<a href="mailto:b.bilhastre@gmail.com">b.bilhastre@gmail.com</a>
Bruno Medronho	<a href="mailto:bfmedronho@ualg.pt">bfmedronho@ualg.pt</a>
Cândida Jerónimo	<a href="mailto:d55811@alunos.uevora.pt">d55811@alunos.uevora.pt</a>
Carla Janeiro	<a href="mailto:carla.janeiro@uevora.pt">carla.janeiro@uevora.pt</a>
Catarina Campos	<a href="mailto:mccampos@uevora.pt">mccampos@uevora.pt</a>
Catarina Estêvão	<a href="mailto:d56882@alunos.uevora.pt">d56882@alunos.uevora.pt</a>
Catarina Fernandes	<a href="mailto:csfernandes@student.uc.pt">csfernandes@student.uc.pt</a>
Catarina Malheiro	<a href="mailto:catarinalemosmalheiro@ua.pt">catarinalemosmalheiro@ua.pt</a>
Clarisse Brígido	<a href="mailto:ccb@uevora.pt">ccb@uevora.pt</a>
Daniela Filipa Firmino Rosa	<a href="mailto:dffr@uevora.pt">dffr@uevora.pt</a>
Daniela Pedro Fonseca	<a href="mailto:daniela.fonseca@uevora.pt">daniela.fonseca@uevora.pt</a>
Diego Appicciutoli	<a href="mailto:d.appicciutoli@staff.univpm.it">d.appicciutoli@staff.univpm.it</a>
Eduarda Potes	<a href="mailto:mep@uevora.pt">mep@uevora.pt</a>
Erika Roldão Lemos de Almeida	<a href="mailto:d49668@alunos.uevora.pt">d49668@alunos.uevora.pt</a>

Eunice Venâncio	<a href="mailto:eunycassia@gmail.com">eunycassia@gmail.com</a>
Fatahi Nour-Elhouda	<a href="mailto:d56257@alunos.uevora.pt">d56257@alunos.uevora.pt</a>
Filipa Santos	<a href="mailto:fs@uevora.pt">fs@uevora.pt</a>
Flávio Silva	<a href="mailto:fsilva@uevora.pt">fsilva@uevora.pt</a>
Gabriela Carneiro Murta	<a href="mailto:gabriela.murta@gmail.com">gabriela.murta@gmail.com</a>
Gabriela Daiana Christ	<a href="mailto:gabrielachrist@gmail.com">gabrielachrist@gmail.com</a>
Getalew Ayizengaw Chana	<a href="mailto:getalewayizengaw@gmail.com">getalewayizengaw@gmail.com</a>
Gilkson Tiny	<a href="mailto:gilmtiny@hotmail.com">gilmtiny@hotmail.com</a>
Greta Winkler	<a href="mailto:greta.winkler@unife.it">greta.winkler@unife.it</a>
Guerra Joaquim M. Govanhica	<a href="mailto:guerragovanhica19@gmail.com">guerragovanhica19@gmail.com</a>
Hamza Dhaidhi	<a href="mailto:hamza.dhaidhi@gmail.com">hamza.dhaidhi@gmail.com</a>
Hélia Guerra Cardoso	<a href="mailto:hcardoso@uevora.pt">hcardoso@uevora.pt</a>
Hugo Manuel Matias Duarte	<a href="mailto:hmduarte@ualg.pt">hmduarte@ualg.pt</a>
Ibrahim Prazeres	<a href="mailto:d44718@alunos.uevora.pt">d44718@alunos.uevora.pt</a>
Inês Mansinhos	<a href="mailto:ifmansinhos@ualg.pt">ifmansinhos@ualg.pt</a>
Inês Mira	<a href="mailto:d50831@alunos.uevora.pt">d50831@alunos.uevora.pt</a>
Inês Silva	<a href="mailto:d51300@alunos.uevora.pt">d51300@alunos.uevora.pt</a>
Isabel Cristina Soares Curado de Matos	<a href="mailto:icmatos@ualg.pt">icmatos@ualg.pt</a>
Isabel Velada	<a href="mailto:ivelada@uevora.pt">ivelada@uevora.pt</a>
Ivo Lourenço Dias	<a href="mailto:d54251@alunos.uevora.pt">d54251@alunos.uevora.pt</a>
Joana Amaro Ribeiro	<a href="mailto:d55735@alunos.uevora.pt">d55735@alunos.uevora.pt</a>
Joana Silva	<a href="mailto:jmsilva@uevora.pt">jmsilva@uevora.pt</a>
João Canedo	<a href="mailto:joao.canedo@uevora.pt">joao.canedo@uevora.pt</a>
João Lourenço Afonso Horta Marques	<a href="mailto:joao.horta.marques@uevora.pt">joao.horta.marques@uevora.pt</a>
João Teixeira Brás	<a href="mailto:jtbras@ualg.pt">jtbras@ualg.pt</a>
José Massuça	<a href="mailto:jose.massuca@gmail.com">jose.massuca@gmail.com</a>
Laura Carreira	<a href="mailto:d47128@alunos.uevora.pt">d47128@alunos.uevora.pt</a>
Lénia Rodrigues	<a href="mailto:lrodrigues-88@hotmail.com">lrodrigues-88@hotmail.com</a>
Liliana Cachucho	<a href="mailto:liliana.cachucho@cebal.pt">liliana.cachucho@cebal.pt</a>
Lourenço Pinto de Rezende	<a href="mailto:pinto.rezende88@gmail.com">pinto.rezende88@gmail.com</a>
Ludmila Gomes	<a href="mailto:lu_gomes22@yahoo.com.br">lu_gomes22@yahoo.com.br</a>
Luís Alves	<a href="mailto:luisalves@ci.uc.pt">luisalves@ci.uc.pt</a>
Luísa Coelho	<a href="mailto:lcoelho6@gmail.com">lcoelho6@gmail.com</a>
Madalena Mendonça	<a href="mailto:maria.silva@uevora.pt">maria.silva@uevora.pt</a>
Margarida Fitas	<a href="mailto:l42978@alunos.uevora.pt">l42978@alunos.uevora.pt</a>
Margarida Oliveira	<a href="mailto:miguídaoliveira@gmail.com">miguídaoliveira@gmail.com</a>
Maria Doroteia Campos	<a href="mailto:mdcc@uevora.pt">mdcc@uevora.pt</a>
Maria Felício	<a href="mailto:d56858@alunos.uevora.pt">d56858@alunos.uevora.pt</a>
Maria Gafanhoto	<a href="mailto:m.inesgafanhoto@gmail.com">m.inesgafanhoto@gmail.com</a>
Maria João Caldinhas	<a href="mailto:d56880@alunos.uevora.pt">d56880@alunos.uevora.pt</a>
Maria João Maia Penetra	<a href="mailto:d53086@alunos.uevora.pt">d53086@alunos.uevora.pt</a>
Maria Pedro Ribeiro Teixeira	<a href="mailto:mariaribeiro.3995@gmail.com">mariaribeiro.3995@gmail.com</a>
Maria Pôla Romão	<a href="mailto:d57519@alunos.uevora.pt">d57519@alunos.uevora.pt</a>
Maria Rebelo Braz	<a href="mailto:d61690@alunos.uevora.pt">d61690@alunos.uevora.pt</a>
Mariana Isabel dos Santos Custódio	<a href="mailto:d61882@alunos.uevora.pt">d61882@alunos.uevora.pt</a>
Mariana Marques	<a href="mailto:d49785@alunos.uevora.pt">d49785@alunos.uevora.pt</a>
Mariana Patanita	<a href="mailto:d46100@alunos.uevora.pt">d46100@alunos.uevora.pt</a>
Mariana Pucarinho Fernandes	<a href="mailto:mpfernandes@uevora.pt">mpfernandes@uevora.pt</a>
Mariana Reis	<a href="mailto:mariana.reis@uevora.pt">mariana.reis@uevora.pt</a>
Marlene Soares	<a href="mailto:marlene98@ua.pt">marlene98@ua.pt</a>

---

Mattia Mogetta [m.mogetta@pm.univpm.it](mailto:m.mogetta@pm.univpm.it)  
Maylidis Valdés Ruiz [maylidis79@gmail.com](mailto:maylidis79@gmail.com)  
Miguel Advinha [miguel.fm.advinha@gmail.com](mailto:miguel.fm.advinha@gmail.com)  
Miguel Ferro [d43463@alunos.uevora.pt](mailto:d43463@alunos.uevora.pt)  
Milton Gussule [d39583@alunos.uevora.pt](mailto:d39583@alunos.uevora.pt)  
Mohomede Saide [d49842@alunos.uevora.pt](mailto:d49842@alunos.uevora.pt)  
Mónica Marques [monicasgmarqueswork@gmail.com](mailto:monicasgmarqueswork@gmail.com)  
Natália Campbell [d53935@alunos.uevora.pt](mailto:d53935@alunos.uevora.pt)  
Natália Marques [nmarques@ualg.pt](mailto:nmarques@ualg.pt)  
Paola Hernández [paolaher@uevora.pt](mailto:paolaher@uevora.pt)  
Patrícia Bernardo [patriciarbernardo@gmail.com](mailto:patriciarbernardo@gmail.com)  
Patricia Coelho Lage [d55833@alunos.uevora.pt](mailto:d55833@alunos.uevora.pt)  
Patrícia Gatinho [patricia.gatinho.s@gmail.com](mailto:patricia.gatinho.s@gmail.com)  
Patrick Materatski [pmateratski@uevora.pt](mailto:pmateratski@uevora.pt)  
Pedro Matias [pmmatias@ualg.pt](mailto:pmmatias@ualg.pt)  
Raquel Carvalho [d56786@alunos.uevora.pt](mailto:d56786@alunos.uevora.pt)  
Ricardo Vieira Santos [d51301@alunos.uevora.pt](mailto:d51301@alunos.uevora.pt)  
Rita Teixeira [rvteixeira99@gmail.com](mailto:rvteixeira99@gmail.com)  
Ruben Heutink [ex64354@alunos.uevora.pt](mailto:ex64354@alunos.uevora.pt)  
Sara Conceição [d44723@alunos.uevora.pt](mailto:d44723@alunos.uevora.pt)  
Sara Rodrigues Gaspar [sb.gaspar@campus.fct.unl.pt](mailto:sb.gaspar@campus.fct.unl.pt)  
Sofia Trindade [d50890@alunos.uevora.pt](mailto:d50890@alunos.uevora.pt)  
Sofia Zantedeschi [sofia.zantedeschi2@unibo.it](mailto:sofia.zantedeschi2@unibo.it)  
Solange Magalhães [solangemagalhaes@eq.uc.pt](mailto:solangemagalhaes@eq.uc.pt)  
Sónia Lucena [slucena@uevora.pt](mailto:slucena@uevora.pt)  
Sousa Gastão Muchecha [sousa.gastao@uevora.pt](mailto:sousa.gastao@uevora.pt)  
Stela G. S. Fé Lorenzo da Rosa [d57494@alunos.uevora.pt](mailto:d57494@alunos.uevora.pt)  
Susana Coelho [d51244@alunos.uevora.pt](mailto:d51244@alunos.uevora.pt)  
Susana Lucas [d55710@alunos.uevora.pt](mailto:d55710@alunos.uevora.pt)  
Tânia Lagôa [d50889@alunos.uevora.pt](mailto:d50889@alunos.uevora.pt)  
Tiago Martins [tiagolcmartins@gmail.com](mailto:tiagolcmartins@gmail.com)  
Tiago Pinto [tiagop@uevora.pt](mailto:tiagop@uevora.pt)  
Tomás Magalhães [ttmagalhaes@ualg.pt](mailto:ttmagalhaes@ualg.pt)  
Tomás Pina Monteiro [d60730@alunos.uevora.pt](mailto:d60730@alunos.uevora.pt)  
Vanessa Amaral [s.schnitzervanessa@gmail.com](mailto:s.schnitzervanessa@gmail.com)

---