



UNIVERSIDADE
DE ÉVORA

VII Encontro de Estudantes de Doutoramento em **Ambiente e Agricultura**
VII PhD Students Meeting in **Environment and Agriculture**

12 e 13 de dezembro | 12th and 13th December 2022

VII Encontro de Estudantes de Doutoramento em Ambiente e Agricultura

12 e 13 de dezembro 2022

VII PhD Students Meeting in Environment and Agriculture

12th and 13th December 2022

Pólo da Mitra, Universidade de Évora

Book of abstracts

Title: VII PhD Students Meeting in Environment and Agriculture

Editors:

Marta Laranjo

Ana Alexandre

Bruno Medronho

Cláudia Marques

Address:

Universidade de Évora,

Largo dos Colegiais, 2

7004-516 Évora

Published:

UE – Universidade de Évora

Copyright © 2022, all rights reserved

ISBN: 978-972-778-298-7

Dear participants,

It is our great pleasure to welcome you to the **VII Encontro de Estudantes de Doutorado em Ambiente e Agricultura (EEDAA) / VII PhD Students Meeting in Environment and Agriculture**, held in Évora on the 12th and 13th of December 2022. 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 EEDAA focuses on four main areas: Veterinary Sciences and Animal Production; Agricultural Sciences and Food Sciences; Environment, Landscape and Sustainability; and Biology and Biochemistry. The meeting includes four 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.

This meeting 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 program, 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

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

Scientific Committee

Ana Teresa Caldeira, Universidade de Évora – HERCULES|IN2PAST

Elsa Leclerc Duarte, Universidade de Évora – MED|CHANGE

Fátima Baptista, Universidade de Évora – MED|CHANGE

Luiz Gazarini, Universidade de Évora – MED|CHANGE

Maria João Cabrita, Universidade de Évora – MED|CHANGE

Raquel Lucas, Universidade de Évora – CEFAGE|CHANGE

Teresa Pinto Correia, Universidade de Évora – MED|CHANGE

Organised by:



Supported by:



Programme

Monday, December 12 2022

09:00	Registration
09:45	Opening Session
	Paulo Quaresma Vice-Rector - Universidade de Évora
	Rui Salgado Director - Instituto de Investigação e Formação Avançada (IIFA)
	Teresa Pinto Correia Director - Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento (MED)
10:00	Plenary Lecture <i>Genomics for sustainability: exploring local genetic resources in Portuguese-speaking African countries</i> Andreia Amaral Universidade de Évora, CIISA & AL4AnimalS

Veterinary Sciences and Animal Production	
	Chair: Jordana Lopes Universidade de Évora – MED & CHANGE
Communications selected from the submitted abstracts	
10:45	<i>Effect of almond hull inclusion in lamb diets on growth performance and carcass and meat quality</i> Liliana Cachucho CEBAL, CIISA
	<i>Systematic identification of genetic markers associated with carcass weight in Portuguese Preta cattle using medium density SNP-chip</i> Maria Feliciano Universidade de Évora – MED & CHANGE, Universidade Lusófona
11:15	Coffee-Break & Poster Session
11:45	<i>The Black Soldier Fly as a sustainable management of urban organic waste: a One Health Perspective</i> Joana Oliveira Instituto Universitário Egas Moniz - CiiEM

Agricultural Sciences and Food Sciences

Chair: **Nicasio Morillo**
Universidade de Évora – MED & CHANGE

Communications selected from the submitted abstracts

- 12:00** *Manufacturing cultured meat through a scalable and cost-effective bioprocess*
Hélder Tavares
Instituto Superior Técnico – iBB & i4HB, CCMAR
- Improving the sustainability of rainfed olive orchards by using zeolites and early-maturing annual legumes cover crop*
Sandra Martins
Universidade de Trás-os-Montes e Alto Douro – CITAB & Inov4Agro

12:30 – Lunch
14:30

- 14:30** **Plenary Lecture**
Water, Nature, and Carbon Balance in Food Production - Looking for a Smart and Sustainable Living
Manuela Moreira da Silva
Universidade do Algarve – CIMA & ARNET, CEiiA

Agricultural Sciences and Food Sciences (continued)

Chair: **Nicasio Morillo**
Universidade de Évora – MED & CHANGE

Communications selected from the submitted abstracts

- 15:15** *The added value of aged beef*
Sara Ricardo Rodrigues
Universidade de Évora – MED & CHANGE
- Emergence of Antibiotic Resistant Coagulase Negative Staphylococci in the Pork Meat Chain*
Maria Teixeira
Faculdade de Medicina Veterinária da Universidade de Lisboa – CIISA & AL4Animals
- 15:45** Coffee-Break & Poster Session
- 16:30** *Can Biscogniauxia mediterranea be the new causal agent of disease in almond trees?*
Ana Faustino
CEBAL-MED & CHANGE, Instituto Politécnico de Beja
- Cover crop potential to mitigate N-NO₃⁻ leaching in Portuguese processing tomato production system - 1st year highlights*

17:30	<p>Ricardo Vieira Santos Universidade de Évora – MED & CHANGE</p>
	<p><i>Host plant response to the application of nematicidal phytochemicals</i></p> <p>Pedro Barbosa Universidade de Évora – MED & CHANGE</p>
	<p><i>Green Options to Substitute Nitrate in Cured Meat Products: Thymus citriodorus and Salvia elegans</i></p> <p>Patrícia Bernardo Faculdade de Medicina Veterinária da Universidade de Lisboa – CIISA & AL4AnimalS</p>
	<p>Closing of the first day of the VII EEDAA</p>

Tuesday, December 13 2022

Environment, Landscape and Sustainability

Chair: **Sérgio Prats Alegre**
Universidade de Évora – MED & CHANGE

Communications selected from the submitted abstracts

9:30

Grey infrastructures: an opportunity to promote native plants

Mariana Pucarinho Fernandes

Universidade de Évora – MED & CHANGE, cE3c & CHANGE

Lignin extraction from agroforest residues using natural deep eutectic solvents

Catarina Fernandes

Universidade de Coimbra – CIEPQPF, MED & CHANGE

Mapping colluvial mesovoid shallow substratum habitats: a case study in karst (Arrábida Natural Park, Portugal)

Rita Pereira Eusébio

Faculdade de Ciências da Universidade de Lisboa - cE3c & CHANGE

Nature-based solutions as climate change adaptation measures in Mediterranean watersheds

Miguel Rodrigues

Faculdade de Ciências da Universidade de Lisboa - cE3c & CHANGE

Spatiotemporal analysis of marginalization drivers in Gennargentu- Mandrolisai inland areas

Pietro Todde

Università degli studi di Sassari

10:45

Coffee-Break & Poster Session

11:15

Integrated assessment of ecological and human dimensions in the functional dynamics of Atlantic mangroves: studies in the Tarrafes do Rio Cacheu Natural Park (Guinea-Bissau)

Arthur Veronez

Universidade de Coimbra - CFE

11:30

Plenary Lecture

Ecosystem Services in the Portuguese Biosphere Reserves

Helena Freitas

Universidade de Coimbra - CFE

12:15-
14:30

Lunch

14:30	<p>Plenary Lecture <i>Antimicrobial peptides as an innovative therapeutic approach in human and veterinary medicine</i> Manuela Oliveira Faculdade de Medicina Veterinária da Universidade de Lisboa – CIISA & AL4AnimalS</p>
--------------	--

Biology and Biochemistry	
	Chair: Pedro A. Salgueiro Universidade de Évora – MED & CHANGE
Communications selected from the submitted abstracts	
15:15	<p><i>Endophytic bacteria associated with spontaneous legumes in arid zones of Tunisia: Genetic diversity, metabolic functionalities and potential application to mitigate the impact of climate change</i> Roukaya Ben Gaied University of Gabes - Arid Lands Institute of Medenine</p> <hr/> <p><i>HSI modelling derived from GPS tracking data, shows spatial variability and significant historic suitability loss in a declining grassland bird</i> Tiago Crispim Mendes Universidade de Évora – MED & CHANGE</p>
15:45	Coffee-Break & Poster Session
16:30	<p><i>Rewilding and rewiring animal-plant interactions in road verges: measuring the provision and regulation of ecosystem services in a Green Infrastructure</i> Carmo Silva Universidade de Évora – MED & CHANGE</p> <hr/> <p><i>Natural Remnant Habitats – a key for biodiversity conservation in Montado agroecosystems</i> Erika Lemos de Almeida Universidade de Évora – MED & CHANGE</p> <hr/> <p><i>Soil fungal community structure is shaped mostly by edaphic properties and cultivated crops in the subtropical and temperate agroecosystems</i> Maria Ornelas Oliveira Universidade da Madeira – ISOPlexis, Universidade de Aveiro - CESAM</p>
17:15	Closing Session Best Poster Award Solange de Oliveira Award

Note: The posters will be displayed throughout the Meeting.

BEST POSTER AWARDS



Cristina Mendes

was awarded with the **Best Poster Prize** for the Poster

“Transcript analysis of Plastid Terminal Oxidase (PTOX) reveals a putative role in adventitious roots and somatic embryos development in Olea europaea L.”

in the area of **Biology and Biochemistry**

On behalf of the Organising Committee,

Teresa Pinto Correia
Director of MED



Fábio Teixeira

was awarded with the **Best Poster Prize** for the Poster

“Whole genome sequencing of Landim pigs from Mozambique: a contribution for the exploration of African genetic resources in Portuguese-speaking countries

in the area of **Veterinary Sciences and Animal Production**

On behalf of the Organising Committee,

Teresa Pinto Correia
Director of MED





José Massuça

was awarded with the **Best Poster Prize** for the Poster

“The purpose of the Delphi methodology on a participatory approach for the assessment of the Social Dimension of Sustainability in Agribusiness”

in the area of **Environment, Landscape and Sustainability**

On behalf of the Organising Committee,

Teresa Pinto Correia
Director of MED



Mariana Patanita

was awarded with the **Best Poster Prize** for the Poster

“An overview of grapevine defence mechanisms and associated biological pathways during fungal and oomycete infections towards a sustainable management strategy”

in the area of **Agricultural Sciences and Food Sciences**

On behalf of the Organising Committee,

Teresa Pinto Correia
Director of MED



Genomics for sustainability: exploring local genetic resources in Portuguese-speaking African countries

Andreia J. Amaral^{1,2,3}

¹Universidade de Évora, Évora, Portugal.

²Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal.

³Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

Email: andreia.fonseca@uevora.pt

Sustainability is defined by ‘the ability to sustain’. Since the last decades, the concept of sustainability was broadened into the concept of sustainable development. In 1972, the first UN conference on Human environment was held and several important documents were produced such as “The limits of growth” and “Blueprint for survival”, creating the path for the increase of awareness for the fact that limits exist for the use of natural resources. Genomics may be defined as research by means of large-scale characterization of genes and gene products into the elucidation of the way genes, RNA, proteins and metabolites interact in the functioning of cells, tissues, organs and the complete organism and its environment, both in an individual, in a population as well as between species. Thus, allowing the view that genomics focuses on the explanation of life in terms of the interactions of genes and molecules. Which consequently implies that, genomics allows to focus in the whole of an organism and its interaction with the environment therefore providing a holistic approach into biology.

The domestication of livestock species has been a process through which human populations have selected from an original gene pool of a species, ecotypes, which are the possible combinations of gene forms adapted to an environment. During the 20th century, with the development of animal breeding practices, these ecotypes originated pure lines with defined breeding goals. Thus, resulting in the loss of genetic diversity of livestock species.

The combination of sustainability and genomics is a concept of multiple dimensions with the use of complex technology in which, genomic researchers must focus into the translation of their activities into sustainable traits. The exploration of the animal genetic resources that still subsist in remote regions of Portuguese-speaking countries using genomic approaches presents an opportunity to discover alleles that provide abilities such as feed efficiency, and disease resilience, much appreciated in a context of climate change. Genomics can therefore be the tool for discovery and increase of genetic diversity promoting food security for human populations.

This work was supported by FCT (UIDB/00276/2020; LA/P/0059/2020 - AL4AnimalS; 2021.02058.CEECIND).

Water, Nature, and Carbon Balance in Food Production - Looking for a Smart and Sustainable Living

M. Moreira da Silva

Universidade do Algarve - Institute of Engineering, Campus da Penha, 8005-139 Faro, Portugal; CIMA-ARNET—Centre for Marine and Environmental Research, Campus de Gambelas, 8005-139 Faro, Portugal; CEiiA—Centre of Engineering and Development, Avenida D. Afonso Henriques 1825, 4450-017 Matosinhos, Portugal1

Email: msanti@ualg.pt

Global demographic trends, modern societies' consumption patterns and climate change are putting increasing pressure on natural water resources, threatening habitats and biodiversity, particularly in more vulnerable regions such as the Mediterranean. Worldwide, agriculture uses around 70% of the total water used in human activities. In addition, the demand for food and animal feed production tends to increase with the growth of the world population. Meanwhile, freshwater use has exceeded recharge levels, leading to the no flow on water streams. Concomitantly, the groundwater over-extraction has promoted saline intrusion phenomena in several coastal areas, posing additional constraints to agricultural irrigation, decreasing production, and lowering crop yields. To face this scenario, the agriculture sustainability in more vulnerable regions, such as the south of Portugal, where water scarcity is a common reality, involves the choice of an alternative water supply and more efficient irrigation systems, as well as crop selection. The current technological advances in the wastewater treatment plants (WWTP) often allow the use of reclaimed water as a safe water source for different purposes, such as for the irrigation of some crops. Crop irrigation with tertiary treated effluent can preserve the biological and biochemical properties of the soil and provide nutrients for plants. Nitrogen, phosphorus, and potassium, present in treated effluents, can reduce the use of synthetic fertilizers, contributing to the decrease in N_2O and CO_2 emissions. In general, agriculture accounts for 12% of the total greenhouse gas (GHG) emissions by human activities, due to diverse field practices, including irrigation and fertilization. The sustainable management of these practices is the most promising mitigation pathway to reduce GHG emissions from agricultural soils. Traditional irrigation and fertilization practices are responsible for N_2O emissions between 30% and 50% higher than fertigated crops, due to the excessive application of nitrogen in traditional practices which led to higher nitrification rates. The carbon emissions (CE) related to synthetic fertilizer include the direct and indirect GHG emissions caused by its production, transportation, and application. However, agriculture has the potential to remove atmospheric carbon and orchards can function as carbon sinks, contributing to the mitigation of GHG emissions.

Previous studies in Algarve, showed that the reuse of urban wastewater is technologically feasible and environmentally advantageous, avoiding the overexploitation of the local aquifer and preventing the eutrophication of aquatic ecosystems, contributing to the improvement of soil characteristics, and decreasing the carbon emissions in orange production. Furthermore, it was found that during the five-month experimental period, the citrus orchard sequestered 87.5% of the CO_2e emitted by WWTP in the effluent treatment, converting 72,623 kg of sequestered CO_2 into orange biomass.

ECOSYSTEM SERVICES IN THE PORTUGUESE BIOSPHERE RESERVES

Helena Freitas

Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

Email: hfreytas@uc.pt

Biosphere Reserves (BRs) are territories recognized by UNESCO's Man and Biosphere committee for their natural and cultural heritage, emphasizing their role in promoting solutions to conserve biodiversity, restore and enhance ecosystem services, and foster sustainable use and management of natural resources. On a global scale there are in total 727 Biosphere Reserves in 131 countries. These BRs cover 6% of the earth's surface and are home to 225 million people. Furthermore, BRs are identified as "laboratories for sustainable development", where society acts as key-drivers for valuing and developing the territory sustainability. We will briefly discuss how ecosystem services may play a fundamental role in the sustainability of the BRs, as well as in the mitigation and adaptation to climate change.

Antimicrobial peptides as an innovative therapeutic approach in human and veterinary medicine

M. Oliveira^{1,2}

¹CISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal

²Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS)

Email: moliveira@fmv.ulisboa.pt

The development of new approaches for the prevention and control of emerging bacterial infectious diseases constitutes one of the main priorities and challenges for current science. Considering the spread of resistant strains at a global level, new antibacterial strategies are urgent, among which the application of antimicrobial peptides (AMPs) stands out.

AMPs are important components of the innate immune system of living beings that act as endogenous antimicrobial compounds. These multifunctional molecules are produced by members of all kingdoms, including bacteria, fungi, plants, insects and vertebrates, as part of their defense strategy against colonization and invasion by pathogenic microorganisms. Besides presenting antimicrobial activity, AMPs act as immunomodulators and have antitoxic activity. Some AMPs also have the ability to prevent biofilm formation, and to eradicate pre-formed biofilms. Moreover, the development of bacterial strains resistant to these antimicrobial compounds is very rare.

For all these reasons, the development of therapeutic protocols based on AMPs for application in human and veterinary medicine is extremely promising. In addition to the compounds being currently tested, some PAMs are already commercially available for use in human medicine, including bacitracin A, polymyxin B, colistin or polymyxin E, gradamycin and daptomycin. In veterinary medicine, although the studies in this area are less frequent, reports are available regarding the advantage of applying these compounds as antimicrobial agents, growth promoters, immunomodulators and regulators of animals' intestinal microbiota. In addition, there are two AMP-based therapeutic products approved and marketed for use in veterinary medicine: the Wipe Out[®] wipes, supplemented with nisin, approved and marketed in the US for dairy cow udder asepsis at pre-milking; and Peptivet[®], an ear gel for dogs and cats, whose composition includes the antimicrobial peptide AMP2041.

In conclusion, the growing number of studies focusing on AMPs and of commercially available compounds demonstrate that they constitute one of the most promising innovative antimicrobial approaches to control the spread of antimicrobial resistant bacterial strains, and it is expected that the number of molecules to be evaluated in clinical trials will increase in the near future.

**Veterinary Sciences and
Animal Production**

Effect of almond hull inclusion in lamb diets on growth performance and carcass and meat quality

Liliana Cachucho^{1,2}, Manuel Varregoso^{3,4}, Cláudia Costa³, Kátia Paulos³, João Almeida^{2,3,5},
Susana P. Alves^{2,5,6}, José Santos-Silva^{2,3,5}, M^a Teresa P. Dentinho^{2,3,5}, Eliana Jerónimo^{1,7}

¹CEBAL – Alentejo Biotechnology Center for Agriculture and Agro-food/IPBeja, Beja, Portugal

²CIISA - Centre for Interdisciplinary Research in Animal Health Lisbon, Portugal

³INIAV – National Institute for Agricultural and Veterinary Research, Fonte Boa, Santarém, Portugal

⁴ISA - Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

⁵AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences

⁶FMV – Faculty of Veterinary Medicine, University of Lisbon, Alto da Ajuda, Lisbon, Portugal

⁷ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Beja, Portugal

Email: liliana.cachucho@cebal.pt

Almond production is growing in the Mediterranean region, which results in increased availability of its by-products, namely Almond hull (AH). AH consists of the green outer covering of the almond and represents about 50% of the total fruit fresh weight, containing high sugar content (18-30% dry matter (DM)). The objective of this work was to evaluate the effect of partial replacement of cereals in the lamb diets by increasing levels of AH on the growth performance and carcass and meat quality. Twenty-four ram lambs were individually housed and randomly assigned to the 3 diets (8 lambs per diet), with feed offered *ad libitum*. All diets included 40% dehydrated lucerne, 6% soybean oil, soybean meal 44, sunflower meal 28, and cereals (maize, barley, and wheat), which were stepwise replaced by AH, reaching 0% DM (control), 9% DM (AH9) and 18% DM (AH18) of AH in diets. The trial started after an adaptation period of 7 days, and average daily gain (ADG) and feed intake were evaluated over 6 weeks of the experiment. The carcass traits were evaluated and in meat was determined the colour, cooking loss, shear force, and sensory attributes. The partial replacement of cereal in the diets by the AH up to levels of 18% DM did not affect the ADG ($P=0.152$, 349 g/d) and the dry matter intake ($P=0.468$, 1783 g/d). However, 18% of AH in diets increased the feed conversion ratio ($P=0.004$, 6.54 (AH18) vs 5.31 (Control/AH18)). The diets did not affect carcass parameters, namely hot and cold carcass weights, dressing percentage, and chump and shoulder tissues composition. No effects were observed in meat colour parameters and cooking loss, however increasing levels of AH in diets reduced the meat shear force ($P=0.017$, 5.83, 5.55 and 5.39kg in Control, AH9 and AH18, respectively). Regarding to meat sensory attributes, only flavour acceptability was lower in AH9 diet ($P=0.013$; 6.70 (AH9) vs 6.97 (Control/AH18)). The use AH in animal feed could be a promising strategy to replace conventional raw materials and can be incorporated in lamb diets without compromising the growth performance or meat quality.

This work is funded by project SubProMais (PDR2020-101-030988, PDR2020-101-030993) and by National Funds through FCT - Foundation for Science and Technology projects UIDB/05183/2020 (MED) and UIDB/00276/2020 (CIISA), and the PhD grant awarded to LC (2020.05712.BD).

Systematic identification of genetic markers associated with carcass weight in Portuguese Preta cattle using medium density SNP-chip

M. C. Feliciano^{1,2}, A. J. Amaral^{3,4}, E. Bettencourt¹, F. Ferreira⁵, L.T. Gama^{3,4}

¹ MED. University of Évora, Polo da Mitra, 7002-554 Évora, Portugal;

² University Lusófona, Campo grande 376, 1749-024 Lisboa, Portugal

³ CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine. University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal;

⁴ AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal.

⁵ ACBRP, E.N. 10, 2139-909 Samora Correia, Portugal;

Email: mcarmo.vet@gmail.com

Preta is a Portuguese local cattle breed raised in the Ribatejo and Alentejo regions. With the aim of increasing the accuracy of the breed management plan that has been in place for nearly 20 years, genotyping using the *GGP Bovine 100K SNP array* has been performed since 2020. A total of 317 animals with available information for carcass weight were selected for this study. Data on carcass weight per day of age were first analysed with an Animal Model, using phenotypic records from 4194 animals, collected since 2002, and the full relationship matrix (n= 48,728). The estimated breeding values were deregressed for genome-wide association analyses. Genotype data has filtered using PLINK. Markers with minor allele frequency 10^{-6}, missing genotypes >5%, not in Hardy-Weinberg equilibrium 0.001 and located in sexual chromosomes were removed. The association between SNPs and carcass weight was performed using PLINK, which performs an asymptotic version of Student's t test to compare two means. After quality filtering, 83,689 SNPs remained. We have identified markers located in BTA6 and in BTA26 which displayed significant association ($5E-5$) with carcass weight gain. These overlapped *LCORL* and *RNLS* genes, respectively. The identified markers also overlap previously described QTLs for body height and carcass weight, which suggests that these novel variants are relevant for the future improvement of this trait in Preta cattle breed.

Support disclosure: Financial support by: PDR2020; CIISA (UIDB/00276/2020) and AL4AnimalS (LA/P/0059/2020).

The Black Soldier Fly as a sustainable management of urban organic waste: a One Health Perspective

J. Oliveira¹, A. Trindade^{1,2}, D. Murta^{1,3}, R. Assunção^{1,4}

¹Centro de Investigação Interdisciplinar Egas Moniz (CiEM), Instituto Universitário Egas Moniz (IUEM), Egas Moniz-Cooperativa de Ensino Superior, CRL, Portugal

²Centro Interdisciplinar de Investigação em Sanidade Animal (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

³Ingredient Odyssey SA (EntoGreen), Santarém, Portugal

⁴CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Portugal

Email: joliveira.joana@gmail.com

Climate change currently represents a major issue of concern. Greenhouse gas emissions, and water and soil pollution contribute to the environmental changes that contribute to the climate change, and the agri-food system constitutes a great responsible for this. Land used by the agriculture, and food/feed production are important contributors for the environmental impact of food systems. The inadequate management of the food waste is likewise a cause of environmental pollution and contributes to the spread of associated pathogens. *Hermetia illucens* (Black Soldier Fly - BSF) larvae have been indicated as organisms that could convert urban organic waste (UOW) into new products, such as animal feed and soil fertilizers, in a process known as bioconversion [1]. Hence, it has been presented as a sustainable solution for promoting circular economy, once it uses the concepts of reusing wastes and reintroducing them into the agri-food chain as new valuable products, conferring economic and environmental benefits. The One Health approach defines that different sectors should work together to optimize human, animal, and environmental health. In that sense, it is imperative to properly understand if the process of bioconversion is safe for human, animal, and environmental health. The present project aims to improve the safety of the bioconversion process of UOWs by BSF larvae and its recovery into new products. To achieve this goal, there are four main specific objectives: i) to produce genome-edited BSF larvae, to trace (physically and genetically) the BSF used in each production process, bioconversion or not; ii) to assess the microbiological and chemical risk of UOWs; iii) to assess the microbiological and chemical risk of larval biomass and fertilizer; and, iv) to establish risk mitigation strategies of larvae grown on UOW through the application of processing methods and its transformation into new insect-derived products. This study will lead to a better knowledge of the safety of insects reared in UOWs as a sustainable source of protein and nutrients for animals and soils, safeguarding human, animal, and environmental health, in a One Health perspective.

This study is funded by Fundação para a Ciência e a Tecnologia, grant number 2022.13540.BDANA

REFERENCES:

L. Gasco, I. Biancarosa, and N. S. Liland, "From waste to feed: A review of recent knowledge on insects as producers of protein and fat for animal feeds," *Curr Opin Green Sustain Chem*, vol. 23, pp. 67–79, Jun. 2020, doi: 10.1016/j.cogsc.2020.03.003.

Backfat transcriptome of castrated and intact Alentejano pigs fed commercial and fibre-rich experimental diets

N. Garrido¹, A. Albuquerque^{1,2}, J.M. Martins^{1,2}, R. Charneca^{1,2}, F. Costa¹, C. Marmelo¹,
A. Ramos^{1,3}, and L. Martin^{1,3}

¹ECO-PIG Consortium, Z.I. Catraia, Ap. 50, 3441 S. Comba Dão (eco-pig@outlook.com)

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

³Escola Superior Agrária de Coimbra, Bencanta, 3045-601 Coimbra, Portugal

Email: Nicolas.osa@uevora.pt

This work, included in the framework of the ECO-PIG Project, aims to reveal the differences in the backfat transcriptome of 30 male Alentejano pigs (AL) raised outdoors and divided equally into surgically castrated (**C**), intact (**I**) and intact pigs consuming an experimental diet (**IE**). These animals were fed *ad libitum* with commercial diets from 40 to 130 kg body weight. From 130 to 160 kg (slaughter weight) **C** and **I** were fed with a commercial diet, while **IE** ate an isoproteic and isoenergetic experimental diet with the addition of locally produced ingredients and agro-industrial by-products. These were chosen to provide enough insoluble dietary fibre and beet pulp to reduce boar taint in intact pigs as mentioned in the bibliography. Backfat samples were collected in the slaughterhouse and stored at -80 °C. RNA was extracted from these samples using Purelink RNA mini-kit and the RNA-seq technique was performed with the Illumina protocol. The results were organized in three comparisons (**I** vs. **C**, **IE** vs. **C** and **IE** vs. **I**). Major differences between groups were found in **I** vs. **C** comparison, with a total of 2726 differently expressed genes (DEGs), 1170 upregulated in **I** and 1556 upregulated in **C**. The fewest differences were found in **IE** vs. **I** group with only 28 DEGs, 16 upregulated in **IE** and 12 upregulated in **I**. In **IE** vs. **C** comparison, 1639 DEGs were found, 724 upregulated in **IE** and 915 upregulated in **C**. Overall, genes overexpressed in **I** and **IE** are related to muscle development, and collagen metabolism, while the ones overexpressed in **C** are involved in lipid and fatty acid metabolism. These results are in line with those obtained by the functional analyses, in which the intact animals (**I** and **IE**) had differences when compared to **C** in general development pathways, lipid metabolism, fatty acid biosynthesis and catabolic processes. Meanwhile, there were not many significant pathways identified between the two intact groups (**I** vs. **IE**). From these results, it can be stated that the main differences in the backfat transcriptome are due to the surgical removal of the testis, and that the experimental diet has a marginal effect.

This work was funded by “Fundo Europeu de Desenvolvimento Regional” (FEDER) of the European Union through the program of operational competitiveness and internalization under the project POCI-01-0247-FEDER-072226 and FCT - Foundation for Science and Technology under the Project UIDB/05183/2020.

Comparison of blood cell counts in *Alentejano* vs commercial (LWxLR) pigs

Beatrice Tadeu-Querido^{1,2}, Luísa Mateus^{1,2}, Fábio Teixeira^{1,2}, Graça Ferreira Dias^{1,2}, Maria Salomé Gonçalves, Pamela Valente^{1,2}, Elisa Bettencourt³, Luís Telo da Gama^{1,2}, Carlos Bettencourt⁴, Andreia J. Amaral^{1,2,5}

¹Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinarian Medicine, University of Lisbon, Lisbon, Portugal; ²Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal, ³Mediterranean Institute for Agriculture, Environment and Development (MED), Évora, Portugal. ⁴Direcção Geral de Agricultura e Pescas do Alentejo (DRAPAL), Évora, Portugal. ⁵University of Évora, Évora, Portugal.

Email: andreaamaral@uevora.pt

Hemograms in pigs vary according to sex, breed, growth rate, diet, stage of gestation, management practices or season. Blood cell counts may be used to investigate adaptation of pig breeds to different environments and these studies are still lacking in many local pig breeds including *Alentejano*, a well-adapted breed to the acorn fields of Southern Portugal.

Alentejano sows born in May 2021 and F1 sows (Landrace x Large White) born in July 2021 were raised in extensive system for a study aiming to investigate the effect of epigenetic regulation on litter size. Sows were not of the same age, but at similar physiological development since breeds have different growth rates and different age at sexual maturity. Sows were submitted to estrous synchronization in May 2022. Peripheral blood was collected in EDTA tubes and hemograms were performed using a CELL-DYN 3700 System Analyzer which performs simultaneous impedance measurements.

Obtained results of hemograms displayed values within normal range for the species. However, when compared with *Alentejano*, the F1 sows had higher number of erythrocytes and platelets ($P \leq 0.05$) and lower levels of MCV, MCH and of MCHC ($P \leq 0.05$). These differences may be attributed to physiological differences between breeds, but can also be due to the adaptation of F1 sows to the extensive system. Future perspectives should include the comparison of F1 sows raised in intensive system in order to discriminate between the effect of management practices and breed.

Authors have no conflict of interest to declare. Authors acknowledge the support GEroNIMO reference 101000236 funded by the H2020 program. AJA was funded by FCT 2021.02058.CEECIND.

Continuous Wave High Level Laser Therapy promotes deep wound healing in dogs and cats

F. Cabecinhas¹, C. Queiroga²

¹ IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Ap. 94, 7002-554 Évora, Portugal.

² MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) e Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Ap. 94, 7002-554 Évora, Portugal.

E-mail: d53947@alunos.uevora.pt

Photobiomodulation is a type of therapy that is known to stimulate healing of tissues through signaling of cell chromophores. Thus, it can decrease inflammation, promote angiogenesis and neovascularization, induce fibroblast proliferation, collagen synthesis, and stimulate granulation. Most of studies developed in this field used pulsed wave Low Level Laser Therapy (LLLT-PW) as phototherapy over damaged skin, particularly superficial wounds, with or without photosensitizers use. This means that low light energy reaches the tissues making its effect over deeper wounds uncertain. Instead, High Level Laser Therapy (HLLT) is known to reach higher amounts of energy in a shorter period. In addition to saving time, more powerful beams of light reach deeper cells, as the depth of penetration increases with increased power; however, the thermal effect produced by their light beams suggests that it can be deleterious to live tissues. The aim of this study was to report HLLT effectiveness over a group of animals with traumatic, infected deep skin wounds associated with standard medical wound management, through debridement, irrigation, and dressing. A specific HLLT continuous wave (HLLT-CW) protocol was applied before covering the wound, daily over the first five days, and then every-other-day until epithelization phase was established. Wound healing was assessed using an adapted Bates-Jensen Wound Assessment Tool (BWAT). BWAT scores significantly decreased over time, meaning that wounds drastically improved, particularly during the first days of treatment. Our results highly suggest that HLLT-CW was effective stimulating wound healing on these patients, promoting granulation tissue formation and deep wound filling at an early stage, with no reported adverse effects. These findings agree with the studies denoting that laser light alters cellular DNA and RNA synthesis, modulates the inflammatory response, promotes extracellular matrix production, and modulates prostaglandin production, leading to improvements on wound microcirculation, nutrition, and cellular metabolism. According to our results, HLLT is a safe, effective tool that stimulates the repair process, making it especially attractive for this type of wounds and patients.

Effect of the early-life feeding management on the productive performance of lambs finished with a high-cereal diet

Letícia Fialho^{1,2}, Olinda Guerreiro^{1,3}, Rui Bessa^{2,4,5}, Eliana Jerónimo^{1,3}

¹CEBAL – Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Polytechnic Institute of Beja (IPBeja), Beja, Portugal

²CIISA - Center for Interdisciplinary Research in Animal Science, Lisbon, Portugal

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

⁴Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

⁵AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal.

Email: leticia.fialho@cebal.pt

In sheep farming, several systems are applied for managing the young animals that can affect the productive performance of lamb during fattening phase, which increasingly occurs with the use of concentrate diets rich in cereals. The aim of this work was to evaluate the effect of the early-life feeding management on the productive performance of lambs finished with a high-cereal diet. Thirty-two lambs and their dams were randomly divided into four treatments: M) lambs separated from their mothers and fed with milk replacer; P) lambs and ewes remained in pasture with access only to pasture; F) lambs and ewes fed with dehydrated lucerne; and C) lambs and ewes fed with concentrate for lactating ewes. Hay was provided in C and F treatments. At weaning (about 50 days), lambs were separated from their mothers and housed in pairs (2 lambs from the same pre-weaning treatment/pen). Lambs from all pre-weaning treatments were gradually adapted to the fattening diet. After adaptation period, lambs were fed with a diet composed by concentrate (high-cereal diet) and forage for 30 days. Feed was offered *ad libitum* during the pre- and post-weaning periods. Lambs were individually weighed weekly throughout the trial, and the feed intake daily during the fattening period. The statistical analysis was performed in SAS. During pre- and post-weaning periods, C lambs had the highest average daily gain (ADG) and M lambs the lowest, while P and F lambs had intermediate ADG. Live weight at weaning was lower for M lambs (16.8 kg) than for lambs from other treatments (24.8 kg). However, at the end of the finishing period the P lambs showed the lowest slaughter weight (29.1 kg) and hot carcass weight (14.1 kg), while for other lamb groups the slaughter weight varied between 31.4-33.7 kg and hot carcass weight between 16.5-17.4 kg. Feed intake was higher in C and F lambs than in M and P lambs. The feed conversion ratio was not affected by the pre-weaning dietary management. The early-life feeding management may interfere with the adaptive capacity of the ruminal community to post-weaning diet, affecting the growth performance during fattening phase.

This work was funded by National Funds through FCT - Foundation for Science and Technology under the research grant to LF (2020.04456.BD) and the projects UIDB/05183/2020 (MED) and UIDP/CVT/00276/2020 (CIISA).

Fatty acid composition of imported lamb fat

Andreia Silva¹, Olinda Guerreiro^{1,2}, Letícia Fialho^{1,3}, Susana P. Alves^{3,4,5}, José Santos-Silva^{3,5,6}, Rui J. B. Bessa^{3,4,5}, Eliana Jerónimo^{1,2}

¹CEBAL – Alentejo Biotechnology Center for Agriculture and Agro-food /IPBeja, Beja, Portugal

²MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Beja, Portugal

³CIISA – Center for Interdisciplinary Research in Animal Science, Lisbon, Portugal

⁴FMV – Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

⁵AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, Portugal

⁶INIAV – National Institute for Agricultural and Veterinary Research, Santarém Research Center, Vale de Santarém, Santarém, Portugal

Email: andreia.silva@cebal.pt | eliana.jeronimo@cebal.pt

Lamb meat is an important source of different fatty acids (FA). Ruminant fat is characterized by high saturated FA (SFA) content, low polyunsaturated FA (PUFA) levels and variable amounts of *trans* FA, attributes that are generally associated with detrimental health effects. However, there are FA, such as vaccenic (*t*11-18:1) and rumenic acid (*c*9,*t*11-18:2), that have beneficial health effects, whereas a higher level of *t*10-18:1 is related with negative health effects. A distinction of the different FA is therefore of the utmost importance for the consumers knowledge. Portugal imports lamb meat from different origins, which, depending on the production system applied, can result in meats with different FA compositions. So, the objective of the present work was to evaluate the FA composition of fat from lamb meat imported to Portugal. A total of 24 lamb samples, from United Kingdom (17), Spain (2), Greece (2) and Ireland (3), were obtained from different supermarkets located in Alentejo and Ribatejo regions, between November 2020 and August 2021. The subcutaneous fat from each sample was separated and used for FA analysis. The FA methyl esters were analysed by GC-FID. The subcutaneous fat total fatty acid (TFA) content averaged 411 g/kg of dry matter (DM), ranging between 249 and 498 g/kg DM. The FA composition, both individuals and sums, presented a higher variability between samples. Saturated FA ranged between 49.1 and 61.7 g/100g TFA. Monounsaturated FA varied between 32.9 and 44.5 g/100g TFA, whereas PUFA changed from 2.82 to 4.95 g/100g TFA. The main PUFA, linoleic acid (18:2n-6) ranged between 0.65 and 3.75 and linolenic acid (18:3n-3) between 0.34 and 1.28 g/100g TFA. Regarding *trans*-FA, the *t*11-18:1 was the predominant *trans*-FA in 75% of samples, ranging from 0.58 and 6.85 g/100g TFA, whereas the *t*10-18:1 varied between 0.21 and 7.10 g/100g TFA. Rumenic acid content varied between 0.037 and 1.55 g/100g TFA. Subcutaneous fat from imported lamb meat showed great variability in the FA content, with a reduced proportion of samples with higher levels *t*10-18:1 and several samples with considerable contents of healthy FA (*t*11-18:1, *c*9,*t*11-18:2 and PUFA).

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 (MED) and UIDP/CVT/00276/2020 (CIISA), and the PhD studentship awarded to L.F. (2020.04456.BD).

Identification and antimicrobial resistance profile of bacteria isolated from the uterus of mares

S. Conceição¹, I. Bessa de Carvalho¹, H. Guimarães¹, C. Queiroga^{1,2}, M. Laranjo¹, E. Bettencourt^{1,2}

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

²Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: smpc@uevora.pt

The main cause of infertility in mares is endometritis, characterized by acute or chronic inflammation of the endometrium. One of the main causes of the occurrence of inflammation in the endometrium is the response to bacterial infection. When the infection overcomes the defense capacity of the host it leads to the development of bacterial endometritis, often caused by *Streptococcus equi subsp. zooepidemicus*, an opportunistic pathogen. The present study aimed to evaluate the presence of bacteria in the uterus of mares before insemination. For this purpose, uterine washings were performed with sterile saline solution. Samples were centrifuged at 8000 *g* for 10min at 4°C, and the pellet was streaked onto Blood Agar and MacConkey plates. The obtained isolates were identified using biochemical (VITEK 2 Compac and API, Biomerieux) and molecular identification methodologies (16S rRNA gene sequencing). Moreover, antimicrobial susceptibility tests (AST) were performed with VITEK 2 Compac, for fast growing bacteria and disc diffusion method, for fastidious bacteria. A total of 62 uterine washings were analyzed. A positive culture was obtained in 66% of the specimens, resulting in 57 isolates, with 57% of Gram-positive bacteria isolated. Regarding prevalence, the most frequently isolated genera were *Streptococcus* (33%), *Escherichia* (25%) and *Staphylococcus* (18%), while the most frequent species was *Escherichia coli*, followed by *S. equi subsp. zooepidemicus*. Most Gram-positive bacteria were sensitive to the following three antimicrobials, namely tetracycline, ceftiofur, and enrofloxacin. Regarding Gram-negative bacteria, over 90% of the isolates were sensitive to ceftiofur and gentamycin, while over 60% of the isolates were sensitive to enrofloxacin.

This work was funded by national funds and co-funded by the European Union under project “EQUI MAIS – Melhor produção equina” (ALT20-03-0246-FEDER-000055) and also by National Funds through FCT-Fundação para a Ciência e a Tecnologia under project UIDB/05183/2020. S. Conceição acknowledges a PhD fellowship from FCT (Ref. UI/BD/153510/2022).

Proteomic changes in saliva in pigs with *Escherichia coli* diarrhea

M. Rodrigues¹, M.J. López Martínez², A. Ortin Bustillo², J.J. Ceron², E. Lamy¹

¹Student from Veterinary Medicine Department, School of Science and Technology, University of Evora

² Veterinary School, Regional Campus of International Excellence Mare Nostrum, University of Murcia

³MED (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: l33453@alunos.uevora.pt

The use of saliva as a source of biomarkers for different physiological and pathological conditions is gaining interest both in humans and animals. The fact that saliva collection being non-invasive makes this fluid to be particularly useful. Diarrheas produced by the gram-negative bacteria *E. coli* are one of the main problems of pig farms, potentially leading to animal deaths and big losses for the producers. The possibility of detecting the disease as early as possible, will allow for increases in the success of the interventions. The objective of the present work is to identify potential alterations in salivary proteome of pigs with diarrhea caused by *E. coli* that can help to better understand the physiopathology of the disease and may act as potential biomarkers. Whole saliva samples were collected to 10 young pigs with diarrhea caused by *E. coli* and 10 match healthy controls, using sponges. SDS-PAGE and two-dimensional gel electrophoresis (2-DE) profiles were compared, and the proteins observed to be present at different levels were in-gel digested, with trypsin, for identification by mass spectrometry. One of the main findings is the inter-individual variability in salivary proteomes of these animals. Besides this, the main changes in salivary proteome induced by *E. coli* will be presented and discussed.

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

The relationship between calving, colostrum management and passive immune transfer

F.G. Silva^{1,2,3}, C. Conceição², J.O.L. Cerqueira^{1,3,5}, S. Pedro², I. Azevedo², J. Ramalho⁴, P. Caetano⁴, A. Pereira², L. Martins⁴, and S.R. Silva^{1,3}

¹CECAV (Veterinary and Animal Research Centre), Department of Animal Science, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal.

²MED (Mediterranean Institute for Agriculture Environment and Development), Department of Zootechnic, University of Évora, Évora, Portugal.

³Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Portugal.

⁴MED (Mediterranean Institute for Agriculture Environment and Development), Department of Veterinary Medicine, University of Évora, Évora, Portugal.

⁵Agrarian School of Viana do Castelo Polytechnic Institute, Ponte de Lima, Portugal

Email: fsilva@uevora.pt

Failure of passive immune transfer is a widespread problem in dairy farming. Recent scientific recommendations and welfare requisites indicate that passive immune transfer (PIT) should not be treated as a binary variable but as a continuous variable. In other words, the calf benefits from the best immunization possible. So, the objective of this study was to investigate the management factors associated with the PIT in dairy calves. Blood sampling was performed in 35 calves within 24 to 72 h after birth, from 2 dairy farms in Alentejo, Portugal, and total protein in serum (TPS) was measured with a refractometer. A corresponding sample of the first colostrum meal was collected and Brix % was measured with a Brix refractometer. Heart girth (HG) was measured in every calf within 72h of birth. Calving difficulty (CD), calving time (CT), colostrum volume (Vcol) and delivery time (Tcol) were recorded. Correlations and a multi-regression model were performed to analyze the influence of Brix, Tcol, Vcol and HG on TPS. The influence of CD and CT on TPS was tested in a two-way ANCOVA, using Brix as a covariate. 78.1% of calving events occurred with “No assistance” and 21.9% with “Easy assistance”. CT was not measured in 14.3%, from the remaining, 73.3% was less than 60 min and 26.7% more than 60 min. Vcol varied from 2.9 to 4 L, with a mean of 3.8 L, being administered between 50 to 455 min after birth, with a mean value of 141.6 min and a mean Brix of 25.6 % (17.7 to 34.6 %). Calves HG varied between 59.5 and 84.5 cm (mean of 76.3 cm). TPS had a mean of 6.7 and ranged from 5.2 to 8.6 g/dL. Brix was the only variable with a significant correlation with TPS. Vcol, Tcol, CD and CT did not influence the TPS in this study. For each increase in 1% of Brix, TPS is expected to increase by 0.179 g/dL ($r=0.728$; $R^2=0.53$). These results suggest that colostrum quality is the most crucial factor intervening in PIT process for calves born and managed under the conditions described in this study.

This work is funded by National Funds through FCT—the Foundation for Science and Technology—under the Project UI/BD/150834/2021 and UIDB/05183/2020.

Water quality in dairy cattle farms: impact on animal production, reproduction and health.

V. Resende¹, O. Moreira², J. Martins¹, R. Lucas³, R. Branco⁴

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Zootecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²INIAV (Instituto Nacional de Investigação Agrária), Quinta da Fonte Boa, Vale de Santarém, 2005-048 Santarém, Portugal.

³CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia), Palácio do Vimioso, Largo Marquês de Marialva, n.º 8, 7000-809 Évora, Portugal.

⁴Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749-024 Lisboa - Portugal

Email: vjgr33@gmail.com

Climate change is currently one of the great global challenges, affecting in particular the water sector, namely the lack of precipitation and consequent periods of prolonged drought. Drinking water is a scarce resource in many regions of the world. Water is essential for the life of animals, since it intervenes in various metabolic processes. An inadequate water supply could reduce the health and performance of the animals. In dairy farms, the use of quality water is essential to maximize the milk production of animals. The objective of this study is to verify (1) the importance of water quality on intensive dairy farms (2) water quality affects animal production, reproduction and health. The preliminary results (1) of the questionnaires indicate that 51% of the farms consider that only the quantity of water is the most important factor on dairy farms; 98% of the producers mention that in the context of climate change, water scarcity is very worrying; 91% of the farms use their own water (borehole); 40% of the farms do NOT perform water quality analysis; 86% of the farms do NOT monitor water consumption; 88% of the farms do NOT treat water. The main water quality problem is associated with microbiological quality, followed by the presence of iron, nitrates and manganese; 45% of the farms consider the decrease in milk quality and the appearance of mastitis as one of the main consequences of poor water quality. Preliminary results (2) of the case study show that the group of animals without treated water has a 22% reduction in kg of feed consumption and 26% reduction in water consumption; - 879 liters/lactation; -2,8L/cow/day; + 63 days of drought; + 2,4 inseminations; + 57 days calving interval; + 33% embryo mortality rate; 19% use of heat synchronization protocols. This study helps to verify the importance attributed by national dairy cattle producers to the availability and quality of water on their farms.

Whole genome sequencing of Landim pigs from Mozambique: a contribution for the exploration of African genetic resources in Portuguese-speaking countries

Fábio Teixeira^{1,2,3}, Pedro Sá^{1,2}, Dulce Santos^{1,2}, Carmen Garrine⁴, Rosa Zimba⁵, Laurinda Sousa⁴, Hermenegildo Chiaia³, Alexandre Leitão^{1,2}, José Moras Cordeiro³, Luís Telo da Gama^{1,2}, Andreia Jesus Amaral^{1,2,6}

¹ CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Alto da Ajuda, 1300-477 Lisbon, Portugal

² AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal

³ Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola

⁴ Faculty of Veterinary Medicine, University Eduardo Mondlane, Maputo, Mozambique

⁵ Escola Superior de Desenvolvimento Rural de Vilankulo, University Eduardo Mondlane, Mozambique

⁶ University of Evora, Evora, Portugal

Email: fteixeira@fmv.ulisboa.pt

Landim pigs, a population of native pigs from Mozambique, suffered a significant decrease in population size over the last decades. The remaining populations are threatened by the recent introduction of European exotic breeds. This population has never been characterized at the genome level. In this study, we aimed to provide a comprehensive genetic characterization of Landim pigs using whole-genome sequencing (WGS). We obtained genomes of Landim pigs (N=6), that were further compared with the genomes of local pigs of Angola (N=4) and with genomes from European and Asian domestic pigs and wild boars, currently in the public domain (N=74). Identification of polymorphisms was performed. Results showed suggest that Landim pigs harbour missense SNPs associated with immune system response. Our analysis further suggests that Landim pigs display a large duplication in chromosome 4 reported only in Chinese domestic pigs, overlapping the *TBX19* gene, a transcription factor involved in the regulation of developmental processes, that can activate *POMC* gene expression and repress thyroid-stimulating hormone beta promoters. This study represents the first assessment of the genetic relationship between native pig populations from Mozambique and pigs from around the world using WGS, showing that these pigs might have been selected towards disease resilience and metabolic adaptation.

Support/interest disclosure: Authors have no conflict of interest to declare. Authors acknowledge the support of Centre for Interdisciplinary Research in Animal Health - CIISA (Project UIDB/00276/2020, funded by FCT) and of Associate Laboratory for Animal and Veterinary Science (LA/P/0059/2020 - AL4AnimalS). AJA was funded by FCT 2021.02058.CEECIND.

Agricultural Sciences and Food Sciences

Manufacturing cultured meat through a scalable and cost-effective bioprocess

Hélder Tavares^{1,2,3}*, Patrícia Lopes^{1,2}, Pedro Fonte³, Ana Fernandes-Platzgummer^{1,2}

¹ Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

² Associate Laboratory i4HB - Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

³ Centre for Marine Sciences (CCMAR), University of Algarve, 8005-139 Faro, Portugal.

*Email: helderdbtavares@tecnico.ulisboa.pt

Background and motivation: In recent years, there has been an increasing interest in cellular agriculture, an emerging field that aims to overcome most issues associated with livestock meat consumption, such as its environmental impact, risk of foodborne diseases and animal slaughtering. Different approaches have been attempted to produce cultured meat, but high production costs and scalability limitations have hampered this technology. Bioprocess scale-up is crucial to ensure the product's economic viability and availability, so key factors must be optimized, such as the cell source, bioreactor configuration, culture medium, scaffolding and biomaterials to support cell proliferation and achieve the desired properties.

Experimental approach: To address this, our work focused on optimizing cell proliferation and differentiation into relevant cell lines under static conditions. Mesenchymal stromal cells (MSC) were chosen as the starting cell line due to their high proliferation potential and ability to differentiate into myocytes (the principal constituent of meat) and adipocytes (which contribute to the organoleptic properties of meat).

Results and discussion: MSC isolation from bovine umbilical cord was successfully performed using explant cultures of three different zones: Wharton's jelly, cord lining membrane and umbilical arteries. The resulting cells were characterized according to the expression of key MSC surface markers (CD105, CD90, CD29, CD44), which were found to be positive, and expanded under different 2D culture conditions for culture media optimization. Optimal cell growth was observed using 20% fetal bovine serum (FBS)-supplemented culture medium and WJ-derived MSC achieved the highest cell yield among the sources studied.

Conclusions and future prospects: Optimal bovine MSC source and culture medium were identified, which sets the basis for the development of a large-scale platform for cultured meat production. Future studies will focus on the optimization of cell proliferation and differentiation, both under static and dynamic conditions to attain the necessary cell numbers for a large-scale process. To achieve this, it will be necessary to establish a bioreactor system and develop appropriate microcarriers composed of food-grade biomaterials, which will be optimized concerning size, surface charge, morphology, and porosity, among other key properties, and assessed according to the resulting organoleptic properties of the cultured meat product.

This work was funded by FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDB/04565/2020 and UIDP/04565/2020 of the Research Unit Institute for Bioengineering and Biosciences – iBB, the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. We also would like to acknowledge Dr. Isabel Bogalho, Dr. Mariana Mendes and Dr João Carço for the collaboration concerning the donation of bovine umbilical cord units.

Improving the sustainability of rainfed olive orchards by using zeolites and early-maturing annual legumes cover crop

S. Martins ¹, E. Silva ², C. Brito ^{1,3}, L. Pinto ⁴, C. Martins-Gomes ^{1,5}, A. Gonçalves ⁴, M. Arrobas ⁶, M. Â. Rodrigues ⁶, F. M. Nunes ⁵, C. M. Correia ^{1,3}

¹CITAB - Inov4Agro - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Universidade de Trás-os-Montes e Alto Douro, 5001-801, Vila Real, Portugal

²Association BLC3—Technology and Innovation Campus, Centre Bio R&D Unit, Rua Comendador Emílio Augusto Pires, 14, Edifício SIDE UP, 5340-257 Macedo de Cavaleiros, Portugal

³Inov4Agro - Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

⁴MORE - Collaborative Laboratory Mountains of Research, Brigantia Ecopark, Bragança, Portugal

⁵CQ-VR – Food and Wine Chemistry Laboratory, Chemistry Research Centre – Vila Real, University of Trás-os-Montes e Alto Douro, 5001-801, Vila Real, Portugal

⁶CIMO – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal

Email: scpmartins@utad.pt

Olive cultivation is considered as one of the most significant agricultural activities in Mediterranean region, from a financial, social and ecological point of view. Under climate change threats, there is a growing need to adapt the conventional agronomic practices used in rainfed olive orchards by sustainable practices, in order to preserve soil health, while ensure olive tree performance and olive oil quality. During three years, the effects of leguminous cover crops (LC) and its combination with zeolites (ZL) were evaluated on soil properties, olive tree physiological performance, yield, composition and quality of olive fruits and oil, and compared with soil tillage (T). Results show that both LC and ZL strategies were able to improve photosynthetic performance and crop yield, relatively to T. However, ZL strategy was clearly more efficient in improving soil quality, through decreasing acidity, improving soil N, P and B availability, CEC and soil microbiology. Considering the effects of these practices on olive fruit and oil composition, ZL increased the oleic/linoleic ratio on fruits, and the levels of 3,4-dihydroxyphenylglycol, tyrosol, verbascoside and caffeic acid on olive oil. In short, both sustainable soil management strategies appear to be promising practices to implement in olive orchards under rainfed conditions, but the innovative strategy of combining zeolites with legumes cover crops confer advantages from a nutritional and technological point of view, while preserving soil quality. Nevertheless, studies subjected to long-term use of these practices should be experienced to ensure the sustainability of crop yield and olive oil quality.

This work was funded by Doctoral fellowship under the Doctoral Program “Agricultural Production Chains—from fork to farm” (PD/00122/2012) provided by the FCT-Portuguese Foundation for Science and Technology to S. Martins (PD/BD/135327/2017). This research was funded by the Operational Group “Novas práticas em olivais de sequeiro: estratégias de mitigação e adaptação às alterações climáticas”, funded by PT2020 and EAFRD (European Agricultural Fund for Rural Development). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CQ-VR (UIDB/00616/2020), CIMO (UIDB/00690/2020) and CITAB (UIDB/04033/2020) and also to AgriFood XXI Project, No. NORTE-01-0145-FEDER-000041, co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (North Regional Operational Program 2014–2020).

The added value of aged beef

S. Ricardo-Rodrigues^{1*}, A. C. Agulheiro-Santos^{1,2}, M. Laranjo¹, M. E. Potes^{1,3}, M. Elias^{1,2}

¹ 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.

² Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

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

Email: sirr@uevora.pt

Beef ageing does not refer to the age of the cattle, but to the amount of time that the meat has been stored and refrigerated after slaughter. Beef ageing is a common practice in the meat industry, because it improves the sensory quality of meat (increased juiciness and better aroma and flavour), and the texture is effectively improved through complex changes in muscle metabolism after slaughtering. Two types of beef ageing are considered: vacuum or wet ageing, and dry ageing. Vacuum ageing is a widely used practice in the beef industry in which meat is aged in a sealed barrier package under controlled low temperature to improve tenderness and extend the shelf-life. On the other hand, dry ageing, refers to ageing meat without packaging, and requires greater environmental control practices (strictly controlled conditions, including temperature, moisture, and air speed) to achieve a consistent product quality. For this reason, vacuum ageing is widely used in industry due to its high production yield and convenient storage and transport, whereas dry ageing is an expensive process due to high ageing shrinkage, trim loss, contamination risk, and requirement of ageing conditions and space. However, when consumers become familiar with this type of meat, they are willing to pay more for dry-aged products and may also prefer the dry-aged flavour. For the academic community it remains controversial whether the sensory quality of dry-aged beef is higher than that of wet-aged. Beef ageing is considered a premium product, with an added value, and its price is substantially higher than that of good quality fresh meat. For this reason, it is of extreme importance for meat producers, but also for consumers, that the method of obtaining ageing meat is well studied and defined to guarantee constant high quality and safety standards. The Food Technology group at the University of Évora has recently developed a study on the safety and quality of Cachena meat. In this autochthonous breed, beef ageing has been considered an opportunity to valorize some meat cuts, with lower tenderness, that are usually sold at lower prices than the so-called noble meat cuts.

Work supported by project PDR2020-1.0.1-FEADER-030803, co-funded through ERDF, COMPETE, POFC, and national funds through FCT/MCTES under project UIDB/05183/2020 (MED). S. Ricardo-Rodrigues acknowledges a PhD grant from FCT (2021.07663.BD).

Emergence of Antibiotic Resistant Coagulase Negative Staphylococci in the Pork Meat Chain

Teixeira MP^{1,2}, Fernandes MJ^{1,2}, Fernandes MH^{1,2}, Bernardo P^{1,2}, Camoez, M.³, Bouchami, O.³,
Miragaia M³, Fraqueza MJ¹

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

²AL4Animals - Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Portugal.

³Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica (ITQB-NOVA) António Xavier, 2780-157 Oeiras, Portugal

Email: mariaribeiro.3995@gmail.com

Antimicrobial resistance is considered a global problem that threatens the health of humans and animals. Its rapid emergence is endangering the efficacy of antibiotics against bacterial infections. The overuse and misuse of antibiotics in farm animals have increased selective pressure on commensal microorganisms, such as coagulase-negative staphylococcus (CNS). These microorganisms can then be a potential reservoir of antibiotic-resistance genes for pathogenic bacteria present in the food chain. Because of this, it is essential to study the prevalence of antibiotic resistance in this group. As such, this work aimed to identify CNS isolated in a Portuguese pork meat chain, characterizing their antimicrobial resistance. The experimental work was performed on 281 coagulase-negative staphylococci isolates, isolated from samples of swine, slaughterhouse environment, workers, pork cut pieces and consumers. These isolates were clustered utilizing PCR fingerprinting and identified by multiplex PCR. After identification, the antibiotic sensibility was tested (n=104) using the disc diffusion method, according to the EUCAST (2021) and CLSI (2018) standards. In addition, ETESTs (Biomérieux, France) and broth microdilution methods were also used to assess resistance. It was possible to identify 94 *S. equorum* (33.7%), 90 *S. carnosus* (32.3%), 28 *S. saprophyticus* (10%), nine *S. epidermidis* (3.2%), eight *S. warneri* (2.9%), one *S. aureus* (0.4%), one *S. xylosus* (0.4%), and one *S. capitis* (0.4%). Antibiotic resistance was assessed with high resistant rates being observed, with 91% of isolates resistant to at least one antibiotic. Susceptible isolates were only found for *S. carnosus* (5%) and *S. equorum* (27%). Multiresistance (41%) was found in all species except for *S. warneri* and *S. capitis*, most isolated from swine and final consumers sources. *S. equorum* multiresistant isolates shared a common profile and were clustered together in the dendrogram indicating possible transmission from swine to workers. Nevertheless, the transmission of CNS bacterial strains between meat and consumer families was not evident. Overall, the high presence of antibiotic resistance on CNS from the pork meat chain confirms the emergence of antibiotic resistance linked to the food chain.

Support/interest disclosure: This work was supported by national funds through FCT - Fundação para a Ciência e a Tecnologia, I. P.: Project FARM2FORK PTDC/CVT-CVT/29510/2017, Projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4Animals).

Can *Biscogniauxia mediterranea* be the new causal agent of disease in almond trees?

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

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

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

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

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

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

Email: ana.faustino@cebal.pt

Biscogniauxia mediterranea is known as the causal agent of charcoal disease in a wide range of hosts. It is a known fungal pathogen in the Mediterranean Basin associated with the decline of cork oak¹. Although the disease is more frequent in the genus *Quercus*, some reports indicated *B. mediterranea* as responsible for the appearance of canker in other species².

In the last years, new almond cultivars have been introduced in new regions of Portugal. The adaptability of these cultivars to the local edaphoclimatic conditions, as in Alentejo region, has never been studied and can affect the health and productivity of almond trees. The intensive production observed in these new orchards associated with the mechanized harvesting and irrigation systems creates favorable conditions for the emergence of new diseases.

The main goal of this work was to identify the fungal community of almond trees in an orchard in Alentejo region. Samples from symptomatic and asymptomatic trees were collected, disinfected superficially, and inoculated in PDA medium. The fungal isolates were analyzed by PCR amplification of the internal transcribed spacer (ITS) regions of nuclear rDNA followed by Sanger sequencing. After bioinformatic analysis of the generated sequences, we could detect several fungi, among which *B. mediterranea* was found as the predominant agent.

Alentejo region is known for its typical ecological ecosystem, Montado, in which the main dynamic species is *Quercus suber*. The analysis of the sequences of *B. mediterranea* from this work presented a higher similarity with the sequence described in cork oak, indicating a possible source of the inoculum. To date, only one study has identified the presence of this fungus in almond, specifically in wild almond, *Amygdalus scoparia*, where it was found in cankered tissues³. The microbiome of “Montado”, together with climatic changes, mechanization, and the introduction of new species, may have made *B. mediterranea* the new causal agent of disease in commercial almond trees responsible for the onset of symptoms that start emerging.

References:

[1] J. Henriques, M.J. Barrento, L. Bonifácio, A.A. Gomes, A. Lima, E. Sousa, Factors Affecting the Dispersion of *Biscogniauxia mediterranea* in Portuguese Cork Oak Stands, *Silva Lusitana* (2014) 22(1):83-97.

[2] I. Yangui, M.L.B. Jamaâ, M.Z. Boutiti, A.M. Vetraino, A. Vannini, C. Messaoud, Occurrence of *Biscogniauxia mediterranea* in cork oak stands in Tunisia. *Phytoparasitica* (2021) 49:131–141.

[3] M. Rostamian, M. R. Kavosi, E. Bazgir, First report of *Biscogniauxia mediterranea* causing canker on wild almond (*Amygdalus scoparia*), *Australasian Plant Dis. Notes* (2016) 11:30.

Agricultural Sciences and Food Sciences

This work was performed in the scope of Inov-Amendo-AL: Microenxertia in vitro de amendoeirias seleccionadas 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 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 to Global Change and Sustainability Institute (CHANGE), and still the Contrato – Programa of L. Marum (CEECINST/00131/2018), and the Ph.D. grant of A. Faustino (UI/BD/153511/2022).

Cover crop potential to mitigate N-NO₃⁻ leaching in Portuguese processing tomato production system – 1st year highlights

R. V. Santos*, G. Basch*, A. Rato*

*MED – Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: ricardo.vieira.santos88@gmail.com

After the harvest of processing tomato, considerable amounts of nutrients remain available in the soil. Especially nitrate nitrogen (N-NO₃⁻) is prone to be leached during the winter period. The establishment of a cover crop (CC) is a practice recommended in Conservation Agriculture (CA), trying to enhance a permanent soil cover by growing plants serving as catch crops for available nutrients in the soil, contributing to mitigate the risk of leaching.

A field trial was carried out testing two alternative production systems, comparing the conventional monoculture against the CA systems based on: i) reduced soil disturbance and CC (TomCC); and ii) reduced soil disturbance, CC, and sunflower-tomato (Rotation2-ST) and tomato-sunflower (Rotation1-TS) rotation. A CC mixture of grasses and legumes grew during the tomato-free period. This trial aims evaluating the potential of a CC to mitigate N-NO₃⁻ leaching in Portuguese processing tomato production system.

At CC termination, the average total nitrogen (TN) concentration in aboveground biomass in both Rotation systems was similar (22.9 g kg⁻¹ DW) and 26.5 % higher than in the Conventional (16.8 g kg⁻¹ DW), while TomCC (17.7 g kg⁻¹ DW) did not differ significantly from the other systems. Aboveground biomass yield of CC in TomCC, Rotation2-ST and Rotation1-TS (on average, 5.157, 5.678 and 2.870 ton DW ha⁻¹, respectively) was significantly higher ($p \leq 0.05$) than of weeds in Conventional (0.303 ton DW ha⁻¹). Regarding these yields and TN concentrations, the TN retained in CC systems TomCC, Rotation2-ST and Rotation1-TS (on average: 91, 126 and 67 kg ha⁻¹) was significantly higher ($p \leq 0.05$) than in the Conventional (5 kg ha⁻¹). TN concentration in the soil was similar in all systems (1.49 g kg⁻¹), but the N-NO₃⁻ concentration in the Conventional (3.68 mg kg⁻¹) was significantly higher ($p \leq 0.05$) than in TomCC, Rotation2-ST and Rotation1-TS (0.88, 1.81 and 0.70 mg kg⁻¹, respectively). The higher N-NO₃⁻ content in soil of the Conventional, and the higher TN retained in CC systems, reveals potential of CC to mitigate N-NO₃⁻ leaching in processing tomato production system. Further studies measuring the N-NO₃⁻ leached are needed to complement the CC role in the process.

The work is funded by Syngenta Crop Protection through the project TomAC - Conservation Agriculture Approaches in Tomato growing, and by Fundação para a Ciência e Tecnologia through the Research Grant 2021.07037.BD. The authors acknowledge the support of Universidade de Évora, APOSOLO – Associação Portuguesa de Mobilização do Conservação do Solo and Ag-Innov – Centro de Excelência Agrícola do Grupo Ortigão Costa.

Host plant response to the application of nematicidal phytochemicals

Pedro Barbosa¹, Rui Machado², Renato Coelho³, A. Cristina Figueiredo⁴, Manuel Mota^{1,5}
Cláudia S. L. Vicente¹

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

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

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

⁴Centro de Estudos do Ambiente e do Mar (CESAM Ciências), Faculdade de Ciências da Universidade de Lisboa, Biotecnologia Vegetal (CBV), DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal.

⁵Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal.

Email: pbarbosa@uevora.pt

Potato (*Solanum tuberosum*) is one of the world's most common agricultural crop for human consumption and considered an excellent source of essential nutrients and vitamins. The productivity of this crop is threatened by several plant-parasitic nematodes, among which the root-lesion nematode *Pratylenchus penetrans* is already considered to have a significant impact. Over the years, due to an increasing need of food resources and absence of knowledge, synthetic nematicides were extremely applied on crop fields. However, new policies, supported by the Sustainable Development Goals, impose serious limits to the application of such products. Volatile organic compounds naturally produced by plants have long been recognized for their nematicidal effectiveness. In previous work, the nematicidal activity of several standards of naturally occurring phytochemicals was *in vitro* assessed on *P. penetrans*. Only 4 compounds caused more than 99% nematode mortality, namely benzaldehyde, carvacrol, octanol and thymol. The aim of this work was to determine the most suitable approach to apply the selected compounds in soil in order to maintain their nematicidal effectiveness without compromising host health. Two consecutive trials were performed using potato seed plants (cv. Agria), namely: (i) to evaluate the effect of three solvent agents - 10% (v/v) dimethyl sulfoxide (DMSO), 5 mg/mL TRITON-X, and 1% (v/v) acetone - in plant growth; and (ii) to evaluate the best solvent agent spiked with 2 mg/mL of each selected compound. For both trials, a control treatment with water was considered. Pots were maintained in growth chamber conditions, routinely watered and fertilized. Plant growth related parameters were assessed at collection time. In the 1st trial, potato plants were unable to grow in the presence of 10% DMSO solution or the 5 mg/mL TRITON-X. The potato seeds could germinate only in the presence of 1% acetone. In the 2nd trial, potato seeds could germinate in the presence of the 4 compounds dissolved in 1% acetone. No statistical differences ($p > 0.05$) were recorded between the control treatment and the different compound applications in terms of growth parameters observed. Ongoing research is now accessing the nematicidal activity of each of the four compounds in the presence of the potato plant.

This work was funded by National Funds through FCT to: SFRH/BD/134201/2017 (to PB), and projects PratyOmics (PTDC/ASP-PLA/0197/2020) + UIDB/05183/2020 to MED, UIDP/50017/2020+UIDB/50017/2020 + LA/P/0094/2020 to CESAM.

Green Options to Substitute Nitrate in Cured Meat Products: *Thymus citriodorus* and *Salvia elegans*

P. Bernardo¹, M.J. Fernandes¹, M.H. Fernandes¹, M.P. Teixeira¹, J.M. Lorenzo^{2,3}, L. Patarata⁴,
M.J. Fraqueza¹

¹CIISA, Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal;

²Centro Tecnológico de la Carne de Galicia, Adva. Galicia no. 4, Parque Tecnológico de Galicia, 32900 San Cibrao das Viñas, Spain.

³Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain.

⁴CECAV—Animal and Veterinary Research Center, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal.

Email: mjoaofraqueza@fmv.ulisboa.pt

Nitrites and nitrates are precursors of N-nitrosamines, that might be involved in the risk associated with colorectal cancer. Nitrite has several technological functions, namely the formation of the color in cured meat products, being its replacement difficult. This work aims to develop a clean-label meat product, using *Thymus citriodorus* and *Salvia elegans* infusions as natural replacers of nitrate in a cured meat sausage (CMS), combined with a *S. equorum* starter. Three batches of the following formulas of CMS were produced: C1- Control without nitrate and starter; C2- Control with starter without nitrate; F1- 150 mg KNO₃/kg; F2- 150 mg KNO₃/kg with starter; F3- Sage10.6%; F4- Sage10.6% with starter; F5- Thyme10.6%; F6- Thyme10.6% with starter. Analysis was done on days 0 and 60 (final product and end of shelf-life). Microbial analysis was performed according to ISO Standards: Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS), and *Enterobacteriaceae*. A_w and pH were evaluated. The $L^*a^*b^*$ color was measured with a Konica Minolta CR-400/410 (Konica Minolta, Japan) illuminant D65. Residual nitrate and nitrite, chlorides, and TBARS were determined. Staphylococci counts in the product conditions with starter was approximately 7 Log cfu/g for both days 0 and 60. LAB counts presented an increase in the course of time, being above 7 Log cfu/g on day 60. *Enterobacteriaceae* counts were always inferior to 4 Log cfu/g, indicating that the product was satisfactory, according to Portuguese guidelines. On day 0, all products inoculated, except for Sage formula, presented higher a^* values. F2 and F6 products were significantly redder at both points of analysis. At day 0, residual nitrate levels were generally inferior to 5 mg NaNO₃/kg, except for formulations with added nitrate, without starter (F1= 77.0 mg NaNO₃/kg) and with starter (F2= 40.1 mg NaNO₃/kg). It has been challenging to achieve the typical redness of CMS with green nitrite options. CMS produced with thyme and starter presented a reddish cured color, similar to those with nitrate and starter. Results from this study show that formulation with *Thymus citriodorus* infusion might be an excellent solution to reduce synthetic additives.

This work was funded by Parceria n. 49/Iniciativa n. 77 PDR2020-1.0.1-FEADER-031359; FCT Project UIDB/00276/2020 financed by national funds through FCT - Fundação para a Ciência e a Tecnologia, I. P. and LA/P/0059/2020 - AL4AnimalS- Associate Laboratory for Animal and Science. The scholarship of the first author was funded by Fundação para a Ciência e Tecnologia (FCT)— UI/BD/152824/2022. J. M. Lorenzo and M. J. Fraqueza is a member of the Red CYTED HEALTHY MEAT network (119RT0568).

Analysis of the sustainability of rice from the Albufera Valenciana (es) by the Sustainable Rice Platform (SRP)

Adonir Both¹, Margarida Saraiva², Manuel Tibério³

¹Programa de Doutoramento em Agronegócios e Sustentabilidade, Centro de Estudos e Formação Avançada em Gestão e Economia (CEFAGE), Universidade de Évora, 7002-554 Évora, Portugal. Centro de Estudos Transdisciplinares e Desenvolvimento (CETRAD), Universidade de Trás-Os-Montes e Alto Douro, 5000-801 Vila Real, Portugal.

²Departamento de Gestão, Universidade de Évora, 7002-554 Évora, Portugal. BRU – Business Research Unit – Instituto Universitário de Lisboa, 1649-026 Lisboa, Portugal.

³Escola de Ciências Humanas e Sociais, Departamento de Economia, Sociologia e Gestão, Centro de Estudos Transdisciplinares e Desenvolvimento (CETRAD), Universidade de Trás-Os-Montes e Alto Douro, 5000-801 Vila Real, Portugal.

Email: d44601@alunos.uevora.pt, adonir.both@gmail.com

The Rice Sustainability Platform (SRP), headquartered in Thailand, is an organization driven by the United Nations Environment Program (UNEP) whose aim is to develop tools, mobilize stakeholders, towards best practices for rice production and sustainability. It is considered the world's first sustainable standard for rice. With 12 indicators and 41 requirements, the SRP seeks to identify practices that lead to sustainable rice production. This standard scores the different levels of compliance according to the requirements and establishes minimum points limits to ratify that a producer is seeking or already producing sustainable rice. “The establishment of quantifiable sustainability standards in the field of sustainability reporting is, however, in its infancy. In several thematic areas, quantitative standards are still not widely recognized” he says (SRP, 2019). The general objective of this research is to identify the indicators used and verify the level of sustainability in the production of Paella rice in the Valencian Albufera, using the Rice Sustainability Platform (SRP) at Level 2: B, Socioeconomic Area. Questionnaires with questions about the Environmental Area, Socioeconomic Area and Institutional Area were sent to rice producing companies in the Albufera Valenciana. The measurement methodology to be applied will be the same as the PRS, which contextualizes the impact and reveals a transformative potential, as well as identifying a trend of the main referenced indicators. Overall, this study highlights the use and improvement of management practices for rice sustainability in Albufera Valenciana. This study contributes to the knowledge and dissemination of specific sustainability indicators for rice production, as well as a modus operandi on how to assess and measure the sustainability levels of this production.

Keywords: Sustainable Rice Platform; Indicators; Sustainability; Rice.

An innovative tool based on CRISPR/Cas13 to protect tomato plants against ToBRFV

J. A. Ribeiro^{1*}, C. M. R. Varanda¹, P. Materatski¹, M. D. Campos¹, A. Albuquerque¹, M. Patanita¹, M. R. Félix²

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, IIFA (Institute for Advanced Studies and Research), Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²MED (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.

*Email: joanaar@uevora.pt

Tomato is considered one of the world's most important and widespread horticultural crops. The production of industrial tomato is affected by numerous diseases that play a key role in the productivity and quality of the crop. *Tomato brown rugose fruit virus* (ToBRFV) causes an emerging viral disease responsible for extreme economic losses around the world. At present, there are no resistant tomato cultivars against ToBRFV and control strategies rely only on preventive sanitary measures. Symptoms consist of yellowing of leaf veins, deformation of young leaves and necrosis; fruits show necrotic spots and rugose symptoms, which make them unmarketable. In order to find an approach to successfully manage this disease, the present research plan intends to develop ToBRFV resistant tomato plants, by developing a strategy based on Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, a natural defense system present in bacteria. To achieve this goal, the first step will be to obtain an efficient CRISPR/Cas machinery that can be used against ToBRFV in plants, using a Cas endonuclease that degrades RNA (Cas13). CRISPR/Cas13 machinery will be delivered by an autonomously replicating virus-based vector, *Olive mild mosaic virus* (OMMV), that will also be responsible for the subsequent expression of this system in the targeted plant cells. This presents an alternative to the generation of transgenic lines, which are subjected to many public concerns and regulatory barriers. Furthermore, functionality of CRISPR/Cas13 in *Nicotiana benthamiana* plants will be assessed by measuring the interference activity of the CRISPR/Cas13 machinery against ToBRFV-GFP recombinants, through visualization of the chlorophyll fluorescence under ultraviolet light and quantitative real time PCR (qPCR). Finally, functionality of CRISPR/Cas13 against ToBRFV will be tested in tomato plants, using the optimized OMMV vector. To validate the inheritance and function of the CRISPR/Cas13 machinery, progeny plants will be inoculated with ToBRFV and tested for resistance by qPCR and dot blot analysis. This study will give great advances, not only on the control of ToBRFV in tomato, but also on the control of plant viruses in other plants, being a powerful tool to protect plants against non-treatable diseases.

This work was funded by the projects GAFAPROTECT (PTDC/ASP-PLA/28263/2017) and TOMVIRPROTECT (PTDC/ASP-PLA/28266/2017), both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 and ALGARVE 2020 and through the Foundation for Science and Technology, in its national component. J. Ribeiro and M. Patanita are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships 2022.13638.BD and SFRH/BD/145321/2019, respectively. P. Materatski was supported by FCT with a contract 2021.01553.CEECIND in the frame of the Individual Call to Scientific Employment Stimulus.

An overview of grapevine defence mechanisms and associated biological pathways during fungal and oomycete infections towards a sustainable management strategy

M. Patanita^{1*}, M. D. Campos¹, A. Albuquerque¹, C. Varanda¹, P. Materatski¹, J. A. Ribeiro¹,
R. S. Ramiro², D. Pimentel², M. R. Félix³

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

²InnovPlantProtect Collaborative Laboratory, Estrada de Gil Vaz, Ap. 72, 7350-999 Elvas, Portugal.

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

*Email: mpatanita@uevora.pt

Downy mildew, powdery mildew, grey mold, black rot, and grapevine trunk diseases (GTDs) are among the major diseases caused by fungi or oomycetes that affect grapevine, with a high economic impact in all growing areas. In recent decades, extensive efforts have been made to reduce the use of agrochemicals in viticulture, so the management of these diseases has become a major challenge. Considering the urgent need to find and develop new sustainable and effective protection strategies, the study of plant defence mechanisms and associated biological pathways is crucial as an attempt to obtain more tolerant plants and to provide valuable insights for disease management. Although several resistance genes have been functionally identified in grapevine against some pathogens, little is known about the complex molecular mechanisms underlying the defence responses.

Recent advances of new omics technologies, such as RNA-seq analysis, have enabled a better understanding of the molecular mechanisms involved in grapevine-pathogen interactions, with identification of key functional genes associated with important regulatory roles, namely pathogenesis-related proteins, antimicrobial peptides, transcription factors, secondary metabolites, and other defence-related genes. Overexpression of these genes, through genetic engineering techniques, is one of the main biotechnological tools exploited to induce resistance against specific pathogens. Furthermore, gene silencing and genome editing can also be exploited effectively to control these diseases. In this study, we discuss the grapevine defence mechanisms, their regulatory components, and the associated biological pathways during different fungal and oomycete infections. We also focus on the use of functional genomics as a mean to study grapevine immunity to different pathogens, with a special focus on newly emerging molecular technologies that are enabling a deeper knowledge towards a sustainable plant breeding.

This work is funded by Portuguese National Funds through FCT/MCTES under the PhD scholarships SFRH/BD/145321/2019 and 2022.13638.BD, attributed to Mariana Patanita and Joana Ribeiro, respectively, and under the project UIDB/05183/2020 (MED exploratory project ID 04_2019). It is also supported by the projects GAFAPROTECT (ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017) and TOMVIRPROTECT (ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017), both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through the FCT, in its national component.

Application of the *Stearoyl-ACP Desaturase 2 (OeSAD2)* in olive genotyping through *High Resolution Melting*

L. Rodrigues¹, C. Campos¹, A. Peixe², H. Cardoso¹

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

²MED (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.

Email: hcardoso@uevora.pt

Olive tree (*Olea europaea L.*) is one of the most important fruit species around the world, particularly in the Mediterranean basin, given the increasing interest in olive oil due to its benefits for human health and its exceptional organoleptic properties. In Portugal, namely in the Alentejo region, olive tree has a great socioeconomic importance, and traditional cultivars are the mark of the Portuguese olive oils' typicity. Nowadays, olive tree genotyping is carried out according to molecular DNA-based techniques focused on DNA-based markers, namely Simple Sequence Repeats (SSRs), also called microsatellites. However, despite being highly reproducible and reliable, this technique is time-consuming and highly expensive. In order to overcome these disadvantages, the development of alternative strategies that could be applied to the identification of cultivars, able to detect adulterations and frauds, is very welcome. Thus, the present work describes the preliminary results of the establishment of a methodology based on real-time PCR analysis and subsequent analysis of the melting curve – High Resolution Melting (HRM). To establish the HRM methodology there were considered the traditional Portuguese cultivars ('Galega vulgar', 'Cobrançosa', 'Cordovil de Serpa', 'Carrasquenha' and 'Verdeal Alentejana'), and two foreign cultivars nowadays used in orchards plantations ('Picual' and 'Arbequina'). The DNA was extracted from leaves collected from trees located at four orchards in the Alentejo region (2 plants per orchard). Primers for *OeSAD2* were designed based on the sequence available at the olive genome database (<http://denovo.cnag.cat/olive>, Oe6 browser). For HRM analysis it was used SensiFAST HRM (Bioline) with 10 ng of DNA and 300 nM of each primer. Reactions were run in duplicate in a PikoReal® 24 (Thermo Scientific) thermocycler. Results revealed cultivar-specific melting curves allowing to perform discrimination among the cvs. 'Galega vulgar', 'Cobrançosa', 'Cordovil de Serpa', 'Verdeal Alentejana', 'Picual' and 'Arbequina'. According to the achieved results we can conclude that HRM is a promising technique for olive genotyping based on *OeSAD2* analysis.

This research was funded by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/05183/2020 and through FEDER funds under the Program Alentejo 2020 with the project GESCERTOLIVE - ALT20-03-0246-FEDER-000058.

Bacterial and fungal communities associated to almond decline

A. Albuquerque¹, M. Patanita¹, N. Garrido², C. Varanda¹, P. Materatski¹, J.A. Ribeiro¹, M.D. Campos¹, M.R. Félix³

¹MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, IIFA – Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²Research Fellow, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³MED - 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.

Email: andrealb@uevora.pt

Almond (*Prunus dulcis*) is a tree crop in great expansion in Portugal, particularly in the Alentejo region, where planting intensive orchards is growing, competing in areas previously dominated by other traditional crops. Young almond trees decline is responsible for severe losses, increasing the costs due to the replacement of the dead trees. Symptomatic trees show decline and a hull amber-colored gummosis, in addition to the internal necrosis of the trunks and vascular discoloration, similar to what is described for grapevine trunk diseases. The pathogens associated to these diseases are also known to be comparable to those seen in grapevines, which include *Diaporthe* spp., *Diplodia* spp., *Eutypa* spp., *Neofusicoccum* spp. and *Phytophthora* spp., among others. This work aims to acquire new knowledge on the microbial communities present in almond trees associated with trunk diseases. For that, three field samples from almond trees showing the described symptoms and one asymptomatic from a nursery house were macerated with liquid nitrogen. Genomic DNA was extracted and sent for next-generation sequencing using the 16S ribosomal RNA and the internal transcribed spacer region to respectively identify the bacterial and fungal communities present. Surprisingly, few of the commonly associated pathogenic agents for trunk diseases were identified in our study, with only *Bionectria solani* found in one of the field samples (<0.1%). Some other pathogenic agents identified in all samples include *Alternaria* spp., *Mycosphaerella tassiana*, *Sclerotinia sclerotiorum* and *Stemphylium majusculum*. The most abundant bacteria found in the nursery plant was *Xanthomonas* spp. (35%), responsible for causing bacterial spot in the *Prunus* genus, but it was not identified in any of the tested field plants. The second most abundant bacteria in the asymptomatic plant was *Pantoea* spp. (12%), which can be associated to the appearance of cankers in branches, but found with a relative abundance lower than 0.2% in the field samples. Despite nurseries being frequently associated with sources of inoculum, its suggested that field conditions tend to modulate the microbial communities associated with the plants. Our results also suggest that the presence of almond trunk symptoms can be triggered by different pathogenic agents besides the ones commonly described for the trunk diseases.

This work is funded by Portuguese National Funds through FCT/MCTES under the PhD scholarships SFRH/BD/145321/2019 and 2022.13638.BD, attributed to Mariana Patanita and Joana Ribeiro, respectively, and under the project UIDB/05183/2020.

Degradability of sesquiterpene lactones high expressed in *C. cardunculus* leaves

D. Rosa^{1,2,3}, T. Brás^{1,3}, C. Rial², R. Varela², B. Maçãs⁴, MF. Duarte^{1,3}, F. A. Macías²

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

²Allelopathy 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.

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

⁴INIAV - National Institute for Agrarian and Veterinarian Research, Estrada Gil Vaz, Ap. 6, 7350-901 Elvas, Portugal

Email: daniela.rosa@cebal.pt

The emerging worldwide need to find alternatives to synthetic herbicides. Considerable interest has been devoted to natural sources as potential herbicides [1]. Sesquiterpene Lactones (SL) with high expression in *C. cardunculus* leaves ($\approx 95\text{g/kg}$ dry weight) represent an interesting group studied for their broad phytotoxic activity [2]–[4]. In the context of final agronomic use, factors such as biodegradability and chemical structure, must be taken into consideration [5]. The present study aimed to evaluate the degradability of main SL present in *C. cardunculus* leaves extract (CCLE): aguerin B, cynaropicrin and grosheimin, described with higher phytotoxic activity [3]. CCLE aqueous solution was studied in terms of compounds degradability over time in water, standard soil, and sterilized soil. Experiments were made at room temperature and samples collected during 24h. SL quantification was performed using UHPLC-MS/MS [6]. SL pure compounds degradability were also studied at the same conditions.

Results demonstrated that both CCLE and pure SLs were stable in water with decrease less than $15\pm 2\%$, except for pure cynaropicrin with $25\pm 4\%$ decrease after 24h. The same behavior wasn't observed on standard soil, with a $97\pm 2\%$ and $97\pm 3\%$ reduction after 24h for CCLE (cynaropicrin and aguerin B) and $93\pm 3\%$ for pure cynaropicrin. Regarding degradability in sterilized soil, a less pronounced decrease was observed for aguerin B, and cynaropicrin (CCLE) ($41\pm 3\%$) compared to cynaropicrin (pure) ($63\pm 4\%$), after 1 h. Nevertheless, after 24h, $87\pm 12\%$ and $96\pm 4\%$ reduction was observed, respectively, for cynaropicrin and aguerin B. Regarding grosheimin (CCLE) a lower degradability was achieved ($62\pm 9\%$) after 24h.

Results obtained evidence a possible chemical degradation of SL on both soils, being more pronounced within the latest, most probably due to concomitant microbial degradation. Further studies are being conducted, to understand the mechanisms underlying the SLs degradation in soil, namely chemical characterization of SL degradation products, as well as the assessment of their phytotoxic activity.

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 also acknowledge FCT for PhD grant to D. Rosa (SFRH/BD/143845/2019) and A. Paulino (SFRH/BD/145383/2019), Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and

Development (MED), LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute, and Project PID2020-115747RB-I00 / AEI / 10.13039/501100011033 to Agencia Estatal de Investigación - Ministerio de Ciencia e Innovación

References

- [1] J. H. Westwood *et al.*, “Weed Management in 2050: Perspectives on the Future of Weed Science,” *Weed Sci.*, vol. 66, no. 3, pp. 275–285, 2018, doi: 10.1017/wsc.2017.78.
- [2] F. A. Macías, F. J. R. Mejías, and J. M. G. Molinillo, “Recent advances in allelopathy for weed control. From knowledge to applications,” *Pest Manag. Sci.*, no. October 2018, 2019, doi: 10.1002/ps.5355.
- [3] C. Rial, P. Novaes, R. M. Varela, J. M. G. Molinillo, and F. A. Macías, “Phytotoxicity of Cardoon (*Cynara cardunculus*) Allelochemicals on Standard Target Species and Weeds,” 2014, doi: 10.1021/jf501976h.
- [4] P. A. B. Ramos *et al.*, “Lipophilic extracts of *cynara cardunculus* L. var. *altilis* (DC): A source of valuable bioactive terpenic compounds,” *J. Agric. Food Chem.*, vol. 61, no. 35, pp. 8420–8429, 2013, doi: 10.1021/jf402253a.
- [5] F. A. Macías, A. Oliveros-Bastidas, D. Marín, C. Carrera, N. Chinchilla, and J. M. G. Molinillo, “Plant biocommunicators: Their phytotoxicity, degradation studies and potential use as herbicide models,” *Phytochem. Rev.*, vol. 7, no. 1, pp. 179–194, 2008, doi: 10.1007/s11101-007-9062-4.
- [6] A. Scavo, C. Rial, R. M. Varela, J. M. G. Molinillo, G. Mauromicale, and F. A. Macías, “Influence of Genotype and Harvest Time on the *Cynara cardunculus* L. Sesquiterpene Lactone Profile,” *J. Agric. Food Chem.*, vol. 67, no. 23, pp. 6487–6496, 2019, doi: 10.1021/acs.jafc.9b02313.

Energy crops: the decarbonized solution for low-quality land

M. Abreu¹, A. Reis¹, A.L. Fernando²

¹LNEG—National Laboratory of Energy and Geology, I.P., Bioenergy and Biorefineries Unit, Estrada do Paço do Lumiar, 22, 1649-038 Lisbon, Portugal.

²MEtRICs, Ciências e Tecnologia da Biomassa, Departamento de Química, NOVA School of Science and Technology | FCT NOVA, Universidade NOVA de Lisboa, *Campus* Caparica, 2829-516 Caparica, Portugal.

Email: mariana.abreu@lneg.pt

Energy is fundamental for the evolution of mankind, well-being and the economic sector. The actual energetic crisis caused by the war in Ukraine and the increase of energy demand, particularly the depletion of oil resources and over-exploitation of fossil fuels, have prompted climate change and the pressure created by the search for alternative renewable energies, including biomass for bioenergy. Energy crops are industrial species (e.g. annual and perennial crops, woody species, microalgae, among others) that can be cultivated to generate energy, biofuels and also bioproducts. Due to their renewable characteristics, energy crops value chains, in a biorefinery setting, may help reducing greenhouse gases (GHG), contributing to fight climate change and to achieve the goals preconized in the EU green deal. Energy crops are an alternative feedstock to different industrial processes. To name a few, oilseeds can be applied in the production of biodiesel, sugar and starch rich crops match the production of bioethanol, and different biomasses can be processed to different products, namely biogas, biohydrogen, syngas, electricity and heat, bio-oil, or biochar. Moreover, several types of bioproducts (bioplastics, biolubricants, among others) in simple or more complex biorefinery units may be derived from those crops. Energy crops cultivation in Low Indirect Land Use Change (ILUC) risk areas, like marginal soils (e.g. salinity soils), degraded soils (due to natural causes that allow modifications in their physical, chemical or biological nature, e.g. desertification), or contaminated soils (pollution with polymetallic agents and other toxic elements) is a promising alternative and is being considered a decarbonized solution for low-quality land. Indeed, it promotes the environmental, economic and social sustainability of the different value chains once it reduces the conflicts caused by competition for food and feed, and contributing positively to economic growth. With this study, the aim is to present the energy crops more interesting to be explored in Portugal, the diversity of products, applications and uses that can be attained from those crops, in biorefineries, substituting the current non-renewable options, the marginal soils more adequate for their cultivation, and the process methodologies that can be applied to identify those areas.

This work was funded by National Funding by FCT, Foundation for Science and Technology [Fundação para a Ciência e a Tecnologia, I. P. (FCT, I. P.)], MCTES, Portugal, through the individual PhD research grant of Mariana Abreu (2020.06236.BD). This work was supported by Biomass and Bioenergy Research Infrastructure (BBRI)-LISBOA-01-0145-FEDER-022059, funded by Operational Programme for Competitiveness and Internationalization (PORTUGAL2020), by Lisbon Portugal Regional Operational Programme (Lisboa 2020) and by North Portugal Regional Operational Programme (Norte 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). This work was also supported by the Mechanical Engineering and Resource Sustainability Center—MEtRICs, which is financed by national funds from FCT/MCTES (UIDB/04077/2020-2023 and UIDP/04077/2020-2023). This research was funded by national funding by FCT, (SFRH/BD/144346/2019) of J.R.A.P.

Establishment of a sensitive Taqman-based approach to monitor *Fusarium* spp. in tomato plants

M.D. Campos¹, C. Varanda¹, M. Patanita¹, J.A. Ribeiro¹, P. Materatski¹, A. Albuquerque¹, M.R. Félix²

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

²MED (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: mdcc@uevora.pt

Members of *Fusarium* species are among the most important phytopathogenic fungal communities. They are ubiquitous soil-borne pathogens of a wide range of horticultural and food crops and cause destructive vascular wilts, rots, and damping-off diseases. In tomato, *Fusarium* spp. is responsible for severe yield losses and for increasing the carbon footprint caused by the phytosanitary treatments available. Although *F. oxysporum* f. sp. *lycopersici*, the causal agent of tomato vascular wilt disease, is reported as a highly destructive fungi, other *Fusarium* species have been constantly evolving, and have been increasingly associated with several wilt and rot diseases. The establishment of an approach that enables an early and accurate detection of *Fusarium* spp. in tomato plants is highly interesting, leading to a better control of the diseases associated to these fungi and providing an additional tool in the screening of resistant plants.

In this context, a molecular-based tool was developed to increase the accuracy of detection and quantification of *Fusarium* spp. genomic DNA (gDNA) in tomato plants. The methodology was based on real-time PCR, through the development of a specific TaqMan MGB (Minor Groove Binder) assay, with the selective amplification of the internal transcribed spacer (ITS) region of *Fusarium* spp. This work involved i) the design of a specific TaqMan probe for *Fusarium* spp.; ii) studies on the specificity and sensitivity of the assay; iii) the applicability of the assay in tomato plants growing under field conditions. To ensure the specificity of the assay for *Fusarium*, 45 isolates associated to tomato diseases, including 29 *Fusarium* isolates were tested. Sensitivity was assessed with serial dilutions of *Fusarium* gDNA and the limit of detection was 3.05 pg. No interference of host gDNA was observed when *Fusarium* gDNA was diluted on the gDNA of tomato. Finally, to determine the reliability of the assay, the detection and quantification of *Fusarium* spp. was successfully performed in 30 potentially infected tomato plants, from an experimental field, and in 16 control plants growing under controlled conditions. The established methodology allows a reliable, sensitive, and reproducible estimation of *Fusarium* accumulation in infected tomato plants, gaining new insights for disease control.

This work was funded by the projects GAFAPROTECT (PTDC/ASP-PLA/28263/2017) and TOMVIRPROTECT (PTDC/ASP-PLA/28266/2017), both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 and ALGARVE 2020 and through the FCT in its national component. Joana Ribeiro and Mariana Patanita are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships (2022.13638.BD and SFRH/BD/145321/2019, respectively). This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.

New tools to protect olive plants against anthracnose for a sustainable disease management: I. Candidate genes for new sources of resistance

D. Inácio¹, M.R. Félix², C. Campos³, M. Patanita³, J.A. Ribeiro³, C. Varanda³, P. Materatski³, A. Albuquerque³, A. Peixe², M.D. Campos³

¹Master student in Agronomic Engineering, Universidade de Évora, Largo dos Colegiais 2, 7004-516 Évora, Portugal.

²MED (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.

³MED (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: mdcc@uevora.pt

The olive tree (*Olea europaea* L.) is one of the most economically important fruit trees in Mediterranean countries, with the phytopathogenic fungi causing relevant losses in most of the growing areas. Among these diseases, we highlight anthracnose caused by fungi of the genus *Colletotrichum*. Although there is a natural phenotypic variability of resistance/susceptibility to *Colletotrichum* spp. among olive cultivars, the symptoms of anthracnose usually occur on ripe fruits, in the high humidity conditions of autumn, and are characterized by dark lesions, with abundant production of orange masses of conidia, premature fruit drop and a consequent decrease in the oil quality. Additionally, it can also cause defoliation of trees compromising the production of the following years. Currently the best control strategy is based on application of synthetic fungicides, with negative impact on the environment and human health, and with a regulatory pressure in agriculture worldwide to limit its use.

The goal of the work here presented is to explore in olive the transcriptional changes of selected target genes, previously identified in the literature to be involved in plant-pathogen interaction, in response to *Colletotrichum* sp. infection. The cultivar selected for the studies was 'Galega vulgar', known to be extremely susceptible to this disease. Plants used in the experiments were from *in vitro* culture (to warranty their healthy status), transplanted to pots and maintained under controlled conditions. In half of the experiment, leaves of the olive plants were inoculated with a spore suspension of *Colletotrichum* sp., while in the other half of the experiment mock inoculated plants received only water and were maintained under similar conditions. Leaf samples were collected at two defined timepoints and transcriptomic studies of target genes are being performed.

This work still in course will give an important contribution for identification of key functional genes in olive susceptible responses to infection by *Colletotrichum* sp. and in the understanding of the molecular basis of compatible interactions. We emphasize the importance of this study for the identification of candidate genes to incorporate new sources of resistance of olive trees to anthracnose with the promotion of the development of sustainable management strategies.

This work was funded by the projects GAFAPROTECT (PTDC/ASP-PLA/28263/2017) and TOMVIRPROTECT (PTDC/ASP-PLA/28266/2017), both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 and ALGARVE 2020 and through the FCT in its national component. Joana Ribeiro and Mariana Patanita are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships (2022.13638.BD and SFRH/BD/145321/2019, respectively). This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.

New tools to protect olive plants against anthracnose for a sustainable disease management: II - Early disease detection by near-infrared spectroscopy.

D. Farinha¹, M.B. Mendes¹, D. Inácio², M.R. Félix³, M.D. Campos¹, A. Peixe³, H. Cardoso¹, L. Rodrigues¹, A.E. Rato³

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

²Master student in Agronomic Engineering, Universidade de Évora, Largo dos Colegiais 2, 7004-516 Évora, Portugal.

³MED (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: aerato@uevora.pt

Plant health monitoring is one of the crucial actions among crop diseases control to minimise the risk of future contaminations and optimize the plant disease management. Currently, the presence of pathogens is associated with the appearance of the first symptoms in the plants. Early disease detection is difficult and requires sensitive techniques. Near-infrared (NIR) spectroscopy is a rapid technique used to identify the chemical composition of plant tissues and more recently has been recognized as a high throughput phenotyping tool in plants. Many reports have been shown in plants the ability of vibrational spectroscopy to predict the properties and composition of a large number of samples.

The main goal of the work here presented is to explore the ability of NIR spectroscopy for a presymptomatic detection of *Colletotrichum* spp. infection in ‘Galega vulgar’. Despite being one of the most representative olive varieties in Portugal and a highly appreciated one, ‘Galega vulgar’ is extremely susceptible to *Colletotrichum* spp. infection. Plants used in this study came from *in vitro* culture, transplanted to pots and maintained under controlled conditions. In half of the experiment, leaves of the olive plants were inoculated with a spore suspension of *Colletotrichum* sp., while in the other half received only water and were maintained under similar conditions. NIR Spectroscopy as a very sensitive technique to detect changes in the chemical composition, is applied as a method to detect metabolism changes when plants are infected by microorganisms. Considering this, spectra were collected in several timepoints with a MicroNIR On Site-W in four leaves per plant in inoculated and non-inoculated plants, with the first time point spectra collected before *Colletotrichum* sp. inoculation. NIR spectroscopy combined with machine learning techniques will be used to develop predictive models to execute a supervised classification-based disease. The most accurate model will be able to separate infected and uninfected plants at early stages of disease development i.e., during the asymptomatic phase. NIR spectroscopy is here proposed as a promising technique to be used in field for early disease detection, contributing to a better crop management and a more sustainable agriculture.

This work is also funded by National: Funds through FCT under the Project UIDB/05183/2020 and by FEDER and National Funds through the Programa Operacional Regional ALENTEJO 2020 (ALT20-03-0246- FEDER- 000064)—QualFastNut—Utilização da espectroscopia NIR para a análise rápida da qualidade em frutos secos.

Impact of nutritional status and starch content of walnut trees on deficient kernel development

D.Farinha¹; M.B. Mendes¹; J.M. Barroso²; A. Peixe²; A.B. Dias³; A.E. Rato²

¹ 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.

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

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

Email: dff@uevora.pt

Walnut (*Juglans regia* L.) is one of the most consumed nut species. This species, although known for a long time in national territory, has recently shown an important increment with the establishment of new orchards from North to South of Portugal. Nowadays many health benefits are attributed to walnut consumption. Walnuts are rich in polyunsaturated fatty acids and tocopherols, being linoleic acid the most abundant fatty acids. Considering these reasons, there is a growing interest in expanding the walnut production area which makes it essential to have an in-depth knowledge about the walnut physiology. The kernel shrivel is one of the problems that depreciates the walnut quality and there is no effective control due to its unknown origin.

During the kernel development the accumulation of seed reserves depends on several factors associated with the tree physiology. The main goal of the work here presented is to evaluate nutritional status and the nonstructural carbohydrate (NSC) level in the walnut trees, to understand the origin of this physiological anomaly in kernel development.

It was found that NSC seems to be the parameter most correlated with the occurrence of kernel shrivel. In the less vigorous varieties (Lara and Howard) it was quantified a higher starch content and a lower predisposition for the occurrence of this damage. This seems to indicate that, varieties with a lower percentage of kernel shrivel are less vigorous and consequently with lower investments in vegetative growth, becoming available more starch reserves to seed development. We also hypothesize that the levels of radiation captured by plants seems to have a major importance in the occurrence of kernel shrivel being the shaded plants more vulnerable to this physiological disorder.

This work is also funded by National: Funds through FCT under the Project UIDB/05183/2020 and by FEDER and National Funds through the Programa Operacional Regional ALENTEJO 2020 (ALT20-03-0246- FEDER- 000064)—QualFastNut—Utilização da espectroscopia NIR para a análise rápida da qualidade em frutos secos.

Evaluation of the susceptibility of *Pseudomonas savastanoi* pv. *savastanoi* strains to different antimicrobial peptides (AMPs)

A.C. Correia¹, C. Varanda², P. Materatski², M. D. Campos², M. Patanita², A. Albuquerque², J. A. Ribeiro², T. Carvalho³, A.B. Dias⁴, M. R. Félix⁵

¹Master student of Master course in Agronomy, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

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

³INIAV—Instituto Nacional de Investigação Agrária e Veterinária, I. P. Estrada de Gil Vaz, Apartado 6, 7351-901 Elvas, Portugal.

⁴MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Engenharia Rural, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

⁵MED (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: carlavaranda@uevora.pt

Olive (*Olea europaea* L.) is a crop of special economic importance in Portugal and in the Mediterranean basin. Olive productions are greatly affected by the presence of the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Pss) which causes galls on branches, leading to hypertrophy and death of branches. This disease is particularly devastating in new intensive olive groves, where bacteria can easily spread from infected trees to healthy trees through wounds. Currently, there are no efficient means of controlling this disease, and its control is done through the use of large amounts of copper that inhibit the replication of the bacterium, but only with a temporary effect. In addition, the increasing resistance to these substances, together with the limitations to their use that will probably lead to their ban in the coming years by the European Union, turns essential the search for alternative ways of control for this phytopathogenic bacterium.

Antimicrobial peptides (AMPs) are small sequences of amino acids that interact directly with the membranes of target organisms. These sequences have been successfully used as an alternative to conventional antibiotics, especially for the treatment of infections in plants that have shown antibiotic resistance. AMPs have several advantages such as their broad spectrum of antimicrobial activity and the fact that they are non-toxic to plants and animals.

Although AMPs have common characteristics, their activities and targets are difficult to predict by simply analyzing their sequences. To ensure the desired activity, it is essential that AMPs are tested *in vitro* and *in vivo*. In this research plan it is intended to isolate and identify the most common Pss strains present in olive groves, in Alentejo, and to test the ability of different AMPs to control the growth of the most commonly found Pss strains.

Mariana Patanita and Joana Ribeiro are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships SFRH/BD/145321/2019 and 2022.13638.BD, respectively. Patrick Materatski is supported by FCT with a contract (2021.01553.CEECIND) in the frame of the Individual Call to Scientific Employment Stimulus.

Genome-wide investigation of pentatricopeptides in *Olea europaea* L. shows a possible involvement in the induction of adventitious root formation

C. Campos¹, L. Rodrigues¹, A. Peixe², H. Cardoso¹

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

²MED (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.

Email: hcardoso@uevora.pt

The pentatricopeptides repeat (PPR) proteins form a large family involved in post-transcriptional processing of RNA in mitochondria and chloroplasts, thereby playing important roles in organelle biogenesis and ultimately in plant development. Based on its motif structure, PPR proteins can be classified into two subfamilies, PLS and P-subfamily. Proteins from PLS-subfamily always harbour L or S variant PPR motifs and additional C-terminal domains (E, E+, and DYW) and are strongly associated with post-transcriptional alteration of the RNA sequence. P-subfamily proteins are mostly associated with passive RNA binding to non-coding sequences and have roles in intron splicing, protection of RNAs from nuclease attack, and influencing RNA folding to allow translation. Despite the importance of this subfamily proteins on organelle biogenesis and function, and consequently on photosynthesis, respiration, plant development, and environmental responses, little is known on how PPRs are involved in the process of adventitious root (AR) formation. Adventitious rooting depends on a diversity of endogenous and exogenous factors in which are included two abiotic stress factors, the wounding and the action of an exogenous auxin. Some genotypes with high interest in horticulture and forestry present a recalcitrant behavior regarding AR formation, which is not of minor importance considering the economic impact of those cultures. Some traditional Portuguese olive (*Olea europaea* L.) cultivars are characterized by this recalcitrant behavior when propagated by semi-hardwood cuttings. In this work, the expression of *OePPRs* was investigated through transcriptomic analysis during the induction phase of AR formation on the cv. ‘Galega vulgar’ (a difficult-to-root cultivar), and a phylogenetic analysis of *OePPR* was performed. It was observed that genes encoding *OePPR* have a dynamic behavior during the induction phase of AR formation, and most of the differences between control and auxin-treated microcuttings were observed at early stages of induction (6h and 24h post-induction). A time course analysis revealed three clusters of *OePPR* genes with different expression profiles during the induction phase. Phylogenetic analysis of *OePPR* proteins and domain analysis showed a clear division between P- and PLS-subfamilies. This study provides the first insights into the *OePPR* family and how it can be involved in the AR process.

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)”. The authors would like to thank Virginia Sobral for the help on the establishment of the *in vitro* rooting experiments.

Mycorrhizal inoculation affects wheat growth and gene expression under manganese stress

J. Raeder¹, A. Nogales², L. Rodrigues³, H. Cardoso³, C. Campos³

¹Escola de Ciências e Tecnologias, Universidade de Évora, Colégio Luís António Verney, Rua Romão Ramalho 59, 7000-671 Évora, Portugal.

²LEAF—Linking Landscape, Environment, Agriculture and Food Research Center. Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

³MED (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: mccampos@uevora.pt

Arbuscular mycorrhizal fungi (AMF) are soil-inhabiting fungi that form symbiotic relationships with most terrestrial plants and can enhance plants' performance under various environmental stresses, including heavy metal stress. Manganese (Mn) is an essential micronutrient for normal plant growth and development, but in excess, it can have highly detrimental effects on plants. The present work aimed to study the effect of AMF inoculation on wheat (*Triticum aestivum* L. var. Ardila), in a context of manganese toxicity, on growth and on the expression of genes related to manganese transport and oxidative stress responses. Wheat plants were grown in a sterilized soil, inoculated or not with the AMF species *Rhizoglyphus irregularis*, under greenhouse conditions. Half of the pots were supplemented with 7.5 ppm of Mn. Plants were grown during 7 weeks. Plant weight was then measured, and samples of roots and aerial parts were taken for RNA extraction and evaluation of root colonization. Real-time qPCR analysis of genes related to manganese transport and oxidative stress response on the aerial part of wheat plants was performed using SYBRGreen chemistry. Colonization rate was not altered by Mn addition. However, shoot fresh weight was higher in inoculated plants, more evident in the Mn-addition treatment. Most of the genes were also up-regulated by Mn, with some differences between non-inoculated and inoculated plants. The Mn-specific transporter *MTP8.1* increased with AMF inoculation in the Mn treatment, whereas *CAX2* decreased in colonized plants in the no-Mn treatment. For the genes related with oxidative stress response, *SOD1* increased with AMF inoculation in the no-Mn treatment, *ascorbate peroxidase* and *glutathione peroxidase* increased with Mn-addition in non-inoculated and inoculated plants, *catalase* increased with Mn-addition in non-inoculated plants, and *thioredoxin* increased with Mn addition in inoculated plants. This work shows that Mn transporters and genes related with oxidative stress greatly respond to Mn stress, and AMF inoculation seems to have specific effects on each gene. Altogether, this study can contribute to elucidating the effect of AMF on alleviating Mn toxicity, which has great relevance for a sustainable management of crops in degraded soils.

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

Identification of fungal resistant *loci* in the Portuguese *Vitis vinifera* cv. 'Defensor'

A. Yu Chen¹, R. Martins¹, L. Rodrigues², C. Campos², J. Böhm³, D. Tavares³, M. R. Félix⁴, A. Peixe⁴, H. Cardoso²

¹Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

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

³Viveiros PLANSEL Lda., Apartado 2, 7050-909 Montemor-o-Novo

⁴MED—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

Email: hcardoso@uevora.pt

Powdery and downy mildew are the two major diseases that affect *Vitis vinifera* L. cultivars, showing a high impact at economic, social and environmental level. Breeding programs are currently running at different countries aiming to develop new genotypes resistant to the pathogens *Uncinula necator* (responsible by powdery mildew disease) and *Plasmopara viticola* (responsible by downy mildew disease). In Portugal, a breeding program is running at the Viveiros Plansel Lda (Montemor-o-Novo) aiming to develop Portuguese varieties resistant to both pathogens. A first resistant hybrid, named 'Defensor' was recently registered as *V. vinifera* in frame of this breeding program. The identification and characterization of the resistant *loci* present on this cultivar is of high interest for its use as progenitor in further crosses. Here it is presented the results regarding the identification of the resistant *locus* Rpv 3.1 through the use of SSRs, and the characterization of a gene involved in plant response upon fungal infection, located at that *locus*. The gene Resveratrol O-methyltransferase (*VvROMT*), a gene involved in biosynthetic pathway of pterostilbene previously reported as involved in *P. viticola* grapevine plant defense was isolated at gDNA level. For gene expression analysis it was established an experiment using inoculum of *Plasmopora viticola* collected from plants maintained under field conditions. Samples were collected from plants inoculated and non-inoculated (control) at 24 and 72h, and 15 days post-inoculation (six biological samples). The *VvUBI* and *VvGAPDH* were used as reference genes for transcript data normalization. At transcript level it was possible to see that plants not inoculated maintained its transcript level constant over all the experiment, contrarily with inoculated plants that exhibited an increase reaching the peak 72 h post-inoculation. The present research corresponds to the first studies performed at the Portuguese *V. vinifera* cv. 'Defensor' contributing for its further use in breeding programs.

This research was funded by National Funds through the Projeto PDR2020-784-042746 "Programa de conservação e melhoramento genético da videira" and FCT - Foundation for Science and Technology under the Project UIDB/05183/2020 and the fellowship given to A. Yu Chen under the program "Verão com Ciência – MED2022".

Influence of inter-row soil management practices on the soil enzymatic activity: the study case of an Alentejo vineyard

V. Silva¹, I. Brito², A. Alexandre²

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

²MED (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: d38740@alunos.uevora.pt

Understanding the function of vine associated microbiome will facilitate our ability to enhance the health and quality of wine grape production and will also contribute to face the upcoming agricultural and viticultural challenges regarding sustainability and the climate change. It is known that vineyards harbour a specific set of microorganisms. Research has been conducted on the association between soil microbiome and wine's terroir (Burns *et al.*, 2015; Zarraonaindia *et al.*, 2015). Cover cropping is commonly associated with increased soil health and crop growth (Tosi *et al.*, 2022). The soil microbial community plays an important role in plant productivity and ecosystem functioning. Therefore, the overall goal of this research was to investigate how soil microbial activity was influenced by the inter-row soil management. Two different soil management practices were analysed: absence of cover crop in inter-row and presence of cover crop in inter-row with *Vitis vinifera* cv. Alicante Bouschet and Arinto. The activity of the enzymes arylsulfatase, phosphatase, β -glucosidase and urease was analysed on soil samples collected at different growing seasons of the vineyard: flowering and veraison. The results showed that the different soil management treatments had the most significant effects on the urease activity. Overall, the presence of cover crop increased the activity of the analysed enzymes, compared to those of bare soil, with the exception of phosphatase activity. However, more research is still needed to assess the relationships between soil management practices and the soil microbiome.

Acknowledgments

V. Silva acknowledges a PhD fellowship from FCT (UI/BD/153513/2022).

References

- Bokulich, N., Van der Lie, D., Mills, et al (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. *mBio*. DOI: 10.1128/mBio.02527-14
- Burns, K. N., Kluepfel, D. A., Strauss, S. L., Bokulich, N. A., Cantu, D., and Steenwerth, K. L. (2015). "Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by geographic features". *Soil Biol. Biochem.* 91, 232–247. doi: 10.1016/j.soilbio.2015.09.002
- Tosi, M., Drummel-Smith, J., Obregón, D. *et al.* Cover crop-driven shifts in soil microbial communities could modulate early tomato biomass via plant-soil feedbacks. *Sci Rep* 12, 9140 (2022). <https://doi.org/10.1038/s41598-022-11845-x>

Influence of weight loss on biogenic amines content of dry-cured sausages

M. Laranjo¹, C. M. Alfaia^{2,3}, S. Ricardo-Rodrigues¹, M. J. Fernandes^{2,3}, M. H. Fernandes^{2,3}, M. E. Potes¹, M. Elias¹, M. J. Fraqueza^{2,3}

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

²CIISA—Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

³AL4AnimalS – Laboratório Associado para a Ciência Animal e Veterinária, Portugal

Email: cpmateus@fmv.ulisboa.pt

Fermentation has been a means of preserving foods since ancient times. More recently, fermented foods have often been referred in the literature for the nutritional and health benefits they confer. Dry-cured sausages are fermented meat products highly appreciated for their exquisite organoleptic properties, such as their characteristic aroma and flavour. However, they might also pose some health risks, namely the production of biogenic amines, mainly due to the metabolism of enterobacteria and some genera of the lactic acid bacteria group. The aim of the present study was to assess the influence of weight loss in the biogenic amines content of a Portuguese dry-cured sausage with high calibre throughout a period of eight months storage. Three independent batches of *Paio* were manufactured in a small industry. Three replicate sausages per batch were collected at 0, 4 and 8 months of storage under vacuum at room temperature. Water activity (a_w) and pH were determined. Microbiological analyses (enterobacteria, lactic acid bacteria, staphylococci and *Listeria monocytogenes*) and biogenic amines profile (tryptamine, β -phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine) were performed according to international standards and previously established methodologies. Statistical Analysis System (SAS) program (SAS Institute Inc., Cary, NC) was used to perform ANOVA and multiple comparisons of least square means adjusted with the Tukey–Kramer method. *L. monocytogenes* was absent in all tested samples. The final product weight loss and the storage time did not influence staphylococci counts. Conversely, lactic acid bacteria decreased in dry-cured sausages with 45% weight loss and throughout storage ($p < 0.001$). A similar behaviour was observed for enterobacteria concerning 35% weight loss sausages. In addition, enterobacteria remained below the counting limit in sausages with 45% weight loss. Regarding biogenic amines, the mean contents ranged from 3.21 to 262 mg/kg fresh matter with higher amounts of cadaverine, tyramine and putrescine. Moreover, β -phenylethylamine and spermidine were significant higher ($p < 0.001$) in dry-cured sausages with 35% weight loss. Still, histamine and tyramine levels remained below the acceptable limits. Overall, dry-cured sausages can be considered safe until 8 months of storage.

This research was supported by projects PDR2020-1.0.1-FEADER-031373 and PDR2020-1.0.1-FEADER-031359, funded by national funds through Fundação para a Ciência e a Tecnologia (FCT)/MCTES and co-funded through the European Regional Development Fund (ERDF), and by projects UIDB/05183/2020 (MED) and UIDP/00276/2020 (CIISA) financed by national funds through FCT. S. R.-R. acknowledges a PhD grant from FCT (2021.07663.BD).

Introduction of exogenous AMF species alters the biological diversity and functionality of AMF communities associated with cowpea

V.C. Leal¹, C. Brígido², I.J.S. Souza¹, G.V.L. Júnior³, M. Carvalho², P.I. Fernandes-Júnior⁴, L.M.V. Martins¹

¹Programa de Pós-Graduação em Agronomia: Horticultura Irrigada, Universidade do Estado da Bahia (UNEB), Juazeiro, Bahia, Brazil

²MED – 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

³Embrapa Meio Ambiente, Jaguariúna, São Paulo, Brasil

⁴Embrapa Semiárido, Petrolina, Pernambuco, Brazil

Email: ccb@uevora.pt

The salinity in arid and semi-arid areas of the world is rapidly expanding due to climate change and anthropogenic activities. The use of inoculants containing beneficial microbes (e.g. arbuscular mycorrhizal fungi (AMF) and rhizobia) is a promising alternative to improve plant production in these regions. Here, we investigated the effect of common agricultural practices such as the use of beneficial microbes as inoculum and crop rotation on cowpea growth and on its association with soil microbes under non- and salt-stressed conditions. Plant experiments were carried out using non-sterilized soil (supplemented or not with NaCl) under greenhouse conditions. *Bradyrhizobium yuanmingense* BR 3267 strain and a commercial mixture of AMF (Endoplant Riego) were used as inoculants. In parallel, we assessed cowpea growth following succession of buffelgrass (*Cenchrus ciliaris*) with or without prior soil disturbance. Plant and symbiotic parameters, nutrient content in leaves and AMF and root nodule communities through DNA metabarcoding were evaluated. Under non-stressed conditions, inoculation with AMF and/or BR 3267 strain led to significant increase of cowpea biomass production and higher N or P content in leaves. The imposed saline condition affected the cowpea growth although without significantly affecting the symbiotic parameters. Moreover, the increase of AMF propagules available in the soil at buffelgrass sowing through the inoculation of commercial AMF was a determining factor to mitigate the effects of soil tillage and salinity on cowpea growth. The bacterial communities in the root nodules were affected by AMF communities rather by rhizobia inoculation. Benefits of commercial AMF could be explained by changes in the biological and functionality of the AMF communities associated with cowpea. This study reveals that microbial inoculation and crop rotation are effective practices for improvement of cowpea growth and on mitigating the harmful effects of salt.

This work was financed by FEDER funds through the COMPETE 2020 (POCI) and FCT - Fundação para a Ciência e a Tecnologia in the framework of the project POCI-01-0145-FEDER-016810 (PTDC/AGR-PRO/2978/2014), by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES), Universidade do Estado da Bahia (UNEB) - Brazil, and Embrapa Semiárido – Brazil, and INCT. CB acknowledges a contract from the Individual Call to Scientific Employment Stimulus 2018 (CEECIND/00093/2018) from FCT. The authors thank Embrapa Agrobiologia (Seropédica, Rio de Janeiro state, Brazil) for kindly providing the rhizobial inoculant.

Is music the new sugar? Using sweet music to enhance the sensory and hedonic attributes of food products with varying sugar levels

D. Guedes¹, M. Prada¹, E. Lamy², M. V. Garrido¹

¹Iscte – Instituto Universitário de Lisboa, CIS_Iscte, Portugal

²MED (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: dhfgs@iscte-iul.pt

Sugar consumption is among the most pressing health issues at the global scale. Yet, the high hedonic appeal and the growing availability of sweet products remain important challenges for sugar reduction initiatives. In an effort to meet consumers' nutritional needs with minimal drawbacks for sensory acceptance, food manufacturers have relied on multisensory strategies such as the addition of colorants and aromas. Based on the emerging evidence showing the multitude of multisensory determinants of taste perception, here we examined the applicability of music to improve sweetness perception and acceptance of foods with varying sugar levels.

In one within-subjects experiment ($N = 106$, 64% women), participants tasted two products (vegetables and cookies), with higher (carrots and cookies) and lower sweetness levels (cucumbers and 0% sugar cookies). All samples were presented twice, each time with a different soundtrack (in counterbalanced order). The two soundtracks were previously shown to be highly (vs. lowly) associated with sweetness, controlling for valence and arousal.

Results showed that the high "sweetness" soundtrack increased the sweetness ratings of all products compared to the low "sweetness" soundtrack. Participants also reported higher preference and more favorable intentions of future consumption when the high "sweetness" soundtrack was played. Overall, these findings suggest that while listening to music is hardly comparable to adding a spoonful of sugar, it still may have a relevant impact on our perception and enjoyment of foods. In the future, music may be a relevant ingredient for multisensory interventions targeting sugar reduction.

This research was financially supported by Project LISBOA-01-0145-FEDER-028008, co-funded by the Lisboa 2020 Program, Portugal 2020, and European Union through FEDER funds and by national funds through the Fundação para a Ciência e Tecnologia. Part of this research was funded by Fundação para a Ciência e Tecnologia with a grant awarded to the first author (SFRH/BD/145929/2019).

Low temperature boosts the production of phenolic compounds in *Lavandula viridis* L'Hér *in vitro* cultures

I. Mansinhos¹, S. Gonçalves¹, R. Rodríguez-Solana^{1,2}, A. Romano¹

¹ 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

² Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Avenida Menendez-Pidal, SN, 14004 Córdoba, Spain

Email: ifmansinhos@ualg.pt

Temperature is considered an important abiotic factor affecting several physiological and biochemical traits in plants, as well as, the production of secondary metabolites. In this study, *in vitro* cultures and micropropagated plants of *Lavandula viridis* L'Hér were subjected to different temperatures (15, 20, 25, and 30 °C), for two weeks, and the levels of photosynthetic pigments, lipid peroxidation, and osmoprotectants, as well as the phenolic profile and two intermediates of the secondary metabolism [phenylalanine ammonia lyase (PAL) activity and shikimic acid content] were determined. Overall, the highest temperature caused an increase of lipid peroxidation in both *in vitro* cultures and micropropagated plants, but the osmoprotectant response was different depending on the plant material. Phenolics' production was boosted with the decrease of the temperature in the *in vitro* cultures, while the opposite occurred in the micropropagated plants. The biosynthesis of the most abundant phenolic compound present in cultures of this species – rosmarinic acid – was considerably stimulated at 15 °C. The findings obtained provide evidence that *in vitro* culture is a good alternative to produce this valuable bioactive compound and that temperature greatly influences not only secondary metabolism but also other biochemical traits in this species.

This research was funded by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/05183/2020. Inês Mansinhos (Grant SFRH/BD/145243/2019) is funded by national funds through FCT and Raquel Rodríguez Solana is supported by a Juan de la Cierva – Incorporation contract from the Spanish Ministry of Science, Innovation, and Universities (IJC2018-036207-I).

Naturally occurring phytochemicals against root lesion nematodes

Pedro Barbosa¹, Jorge M. S. Faria^{2,1}, A. Cristina Figueiredo³, Manuel Mota^{1,4}, Cláudia S. L. Vicente^{1,2}

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

²INIAV, I.P., National Institute for Agrarian and Veterinarian Research, Quinta do Marquês, 2780-159 Oeiras, Portugal.

³Centro de Estudos do Ambiente e do Mar (CESAM Ciências), Faculdade de Ciências da Universidade de Lisboa, Biotecnologia Vegetal (CBV), DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal.

⁴Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal.

Email: pbarbosa@uevora.pt

Plants are able to synthesize more than 200.000 distinct chemical compounds, most of which come from specialized biosynthetic pathways. Secondary metabolites can be broadly defined as natural products synthesized by an organism that are not essential to support growth and life. The high research interest on these metabolites is mainly focused on their insecticidal, nematocidal or bactericidal activities. *Pratylenchus penetrans*, one of the most damaging plant parasitic nematodes, is a serious threat to many crops, being extremely difficult to manage with the common agricultural practices. Additionally, the disease symptomatology can be aggravated by the presence of soilborne pathogens such as fungi and bacteria. Pest management has been mainly performed through an intense use of conventional nematicides. However, new policies, supported by the Sustainable Development Goals (SDGs), impose serious limits to the application of these synthetic chemicals. In the present study, the nematocidal activity of 45 plant secondary metabolites (monoterpenes, phenols and resin acids) was assessed for *P. penetrans*, as a contribution to the development of sustainable pest management practices for the control of potato nematodes. Bioassays were performed following the standard direct contact methodology, by exposing the nematodes to 2 mg of compound / mL of suspension during 24 h. The root lesion nematode was remarkably resistant to the tested compounds, with the exception of three oxygen-containing molecules (benzaldehyde, carvacrol and thymol), where full mortality was achieved. These compounds showed extremely fast activity times with approx. 60 min needed to achieve 50% mortality. Additionally, seven compounds showed higher activities than the conventional nematicide Oxamyl. Further studies should be conducted in order to evaluate the effect of these compounds on the plant hosts and their impacts in other soil organisms.

This work was funded by National Funds through FCT to: SFRH/BD/134201/2017 (to PB), and projects PratyOmics (PTDC/ASP-PLA/0197/2020) + UIDB/05183/2020 to MED, UIDP/50017/2020+UIDB/50017/2020 + LA/P/0094/2020 to CESAM.

Near infrared spectroscopy to evaluate soil microbial activity in the *Montado* SystemD. Bailote¹, A.E. Rato², J. Serrano³, I. Brito⁴

¹MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Apartado 94, 7002 – 554 Évora, Portugal

²MED – 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.

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

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

Email: m48156@alunos.uevora.pt

The *Montado*, is an agro-silvo-pastoral ecosystem, explored in several extents. Its arboreal stratum is mainly composed by cork oak (*Quercus suber*) and holm oak (*Q. rotundifolia*) while the ground cover is formed by natural biodiverse pastures growing on poor soils and grazed by animals in an extensive regime. One of the major factors affecting the productivity of pastures in the *Montado* system is soil acidity. The objective of this work is to evaluate the effect of the tree canopy and dolomitic limestone application for soil acidity correction, on soil microbial activity in the *Montado* ecosystem by using the near infrared spectroscopy technique (wavelength region above the visible region, between 700 and 2500 nm). Soil samples were collected under and outside the tree canopy, in amended and unamended areas (6 replicates) in an experimental field (4ha) located in Mitra farm in February 2022. The soil samples were subjected to laboratory analyses to evaluate microbial activity parameters (dehydrogenase, β -glucosidase) and to obtain the NIR spectra of the soil samples. The NIR signature of the soil was able to discriminate the effect of canopy and dolomitic limestone application which also have different biological activity, attested by dehydrogenase and β -glucosidase activity. The activity of these enzymes was significantly higher under the canopy than outside. The results observed are directly related to the tree litter deposition and greater availability of organic matter under the canopy. Soil amendment for pH correction had no significant influence in the β -glucosidase activity but significantly impacted dehydrogenase activity increasing its values outside the tree canopy, the soil acidity correction certainly allows the activity of a wider range of microbes. NIR spectra also reflect these differences. Near infrared spectroscopy can be seen in this case as a viable alternative to conventional laboratory analyses for assessing and monitoring soil quality, proving to be a fast, non-destructive, and cost-effective technique capable of distinguishing soil microbial activity. These results allow us to perceive the effect of the canopy and soil acidity correction on soil microbial activity in the *Montado* and provide important information regarding the holistic management of this ecosystem.

This work was funded by National Funds through FCT (Foundation for Science and Technology) under the Project UIDB/05183/2020 and by the projects PDR2020-101-030693 and PDR2020-101-031244 (“Programa1.0.1-Grupos Operacionais”).

Phenology and quality assessment in two cultivars of 'Citrinos do Algarve' IGP – looking towards varietal ripening profile differences in navel orange

Rosa Pires^{1*}, M. Dulce Antunes², António Ferreira³, Márcia Rosendo Lucas⁴, Ana M. Cavaco¹

¹CEOT, Universidade do Algarve, Campus de Gambelas, 8005-189 Faro, Portugal

²MED, Universidade do Algarve, Campus de Gambelas, 8005-189 Faro, Portugal

³Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Rua Diogo Botelho, 1327, 4169-005 Porto, Portugal

⁴CACIAL CRL, Vale da Venda Apartado 128, 8001-902 Faro, Portugal

*Email: rmpires@ualg.pt

Citrus fruit is one of the world's most valuable fruit crops, and a major source of vitamin C to human diet. The intervarietal time lag harvest among the various cultivars impacts positively the citrus supply chain, providing a continuous fruit production and consumption throughout the year. A few studies on the transcriptomics, proteomics of late-ripening citrus suggest the involvement of several pathways that need further investigation regarding their effective role on the intervarietal ripening time lag. The aim of this first study focused on the comparison of the tree phenology, fruit growth, development, and ripening of two cultivars of navel orange (*Citrus sinensis* (L.) Osbeck) with different harvest optimal dates, namely, 'Newhall' (early-season) and 'Lane Late' (mid-season). The trees phenological assessment started in January 2022 and showed that both cultivars behaved similarly up to fruit setting and through the physiological June drop. From May 2022, 33 days after anthesis (DAA), five fruit per tree were harvested throughout time, from five trees of each cultivar grown in the same commercial orchard, located at Mata Lobos (Faro, Portugal), and managed by CACIAL. Despite being a non-climacteric fruit, ethylene was further measured in detached fruit as an additional parameter in the suggested hormone cross-talk regulating citrus growth and development. Growth and quality were assessed by standard methodologies [1]. For ethylene determination, 5 fruit were placed air-tight jars during 1 h at room temperature, and 1 ml of headspace gas sample was withdrawn into a gas chromatograph for measurements [2]. In both cultivars, ethylene was produced only in phase I (until June drop), decreasing afterwards to a non-detectable content. Both fruit growth and size followed similar curves in both cultivars, and it was possible to identify clearly, phases I (cell division) and II (cell expansion). Although phase III (ripening) is not yet evident on those curves, the level and pattern of various quality attributes, such as rind thickness, soluble solids content (SSC), maturity index (MI), firmness and the citrus color index (CCI) strongly suggest that ripening is delayed in 'Lane Late' in comparison with 'Newhall', the early-season cultivar.

[1] Cavaco, Ana M. *et al.* (2018) Validation of short wave near infrared calibration models for the quality and ripening of 'Newhall' orange on tree across years and orchards. *Postharvest Biology and Technology* 141: 86-97.

[2] Gago, C. *et al.* (2022) 1-Methylcyclopropene and lemongrass essential oil nanocoatings effect on the preservation of cold stored 'Rocha' pear. *Postharvest Biology and Technology* 192: 111992

The authors acknowledge CACIAL (Almancil, Portugal) for providing the access to the orchard followed in this study. This work is supported by CEOT strategic project UIDB/00631/2020 CEOT BASE and UIDP/00631/2020 CEOT PROGRAMÁTICO and Rosa Pires is funded by FCT - Fundação para a Ciência e a Tecnologia, Portugal through PhD Studentship (reference 2021.05979.BD).

Prickly Pear Edible Coating effect on Raspberries' Shelf-life. A preliminary study

Sofia Trindade¹, Maria Inês Rouxinol², Sara Martillanes³, Ana Cristina Agulheiro-Santos⁴

¹Aluno de Doutoramento em Ciências Agrárias e Ambientais, Universidade de Évora; MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE (Instituto para as Alterações Globais e Sustentabilidade), Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²Aluno de Doutoramento em Bioquímica, Universidade de Évora; MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE (Instituto para as Alterações Globais e Sustentabilidade), Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

³Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX), Pol. Ind. El Prado, C/Pamplona s/n,06800 Mérida, Espanha.

⁴MED (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, Portugal

Email: d50890@alunos.uevora.pt

Raspberries are highly perishable fruits, which means a fast-ripening period and senescence, hampering storage and marketing. Their postharvest life is determined by the susceptibility to water loss, softening, mechanical injuries, and the presence of pathogens. To preserve the quality of these fruits, techniques such as modified atmospheres, cooling, heat and osmotic treatments, and edible coatings can be used. The use of edible coatings allows the reduction of respiration and transpiration rates, firmness retention, and decay control. Prickly Pear (*Opuntia ficus-indica* (L.) Mill) cactus is a xerophyte plant, with the ability to thrive in different edaphic-climatic conditions, with high temperatures, and low water availability. This cactus produces a large quantity of cladodes, that is characterized by the existence of a hydrocolloid commonly known as mucilage, with a very complex polymeric structure. Researchers have demonstrated the ability of mucilage, an eco-friendly and renewable substance, to form edible films and coatings. The objective of this preliminary study was to evaluate the potential of *Opuntia ficus-indica* mucilage edible coating to increase raspberries shelf-life. The mucilage was extracted from cladodes of the regional "orange" variety, 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; a control sample was also stored without the application of the coating. The samples were analyzed (weight loss, SST, color $L^*a^*b^*$, texture) on day zero and after eight days. The raspberries' juice was frozen for later analyses of phenolic content and antioxidant activity. As a final observation, we can say that the mucilage possesses the ability forms a coating around the fruits. This coating contributes to maintain some characteristics, such color and texture. However, this coating doesn't prevent weight loss since the fruits coated show a higher weight loss than the control sample, as some authors have already observed. It's apparent that further research and trials are necessary on this subject.

Ripening of Maçã de Alcobaça PGI 'Rubin Fuji' from two orchards with different cultural practices: assessment of the optimal harvest date

P. Vicente¹, C. Sánchez¹, R. Guerra², A. M. Cavaco²

¹INIAV I.P./ENFVN (Instituto Nacional de Investigação Agrária e Veterinária, Estação Nacional de Fruticultura Vieira Natividade), Estrada de Leiria, 2460-059 Alcobaça, Portugal.

²CEOT (Centro de Eletrónica, Optoeletrónica e Telecomunicações), Universidade do Algarve, Campus de Gambelas, 8005-189 Faro, Portugal.

Email: patricia.silva@iniav.pt

“Maçã de Alcobaça” is a Protected Geographical Indication (PGI) for apple cultivars grown in the West (Oeste) Region of Portugal, which specific edaphoclimatic conditions result in unique fruit organoleptic features. Following an approach “farm to fork”, based on the non-destructive assessment of fruit quality through visible near infrared spectroscopy across the supply chain, the study presented herein, aimed to establish the ripening and the optimal harvest date (OHD) in two orchards of 'Rubin Fuji' with different cultural practices, located within the Alcobaça area. Sampling started 113 days after full blooming (DAFB), in the middle of August, and ended in the middle of October (170 DAFB), at the beginning of the commercial harvest season. In each sampling date, four fruit were collected from ten selected trees in an experimental orchard (EO) and in a commercial orchard (CO) managed by INIAV and Frubaça, respectively. The quality attributes (QA) of each fruit were determined by the standard procedures [1]. Whereas the fruit height/diameter ratio and the dry matter (%) tended to remain constant through time in both orchards, weight and size increased over time, although more steeply in the CO, with heavier and larger fruit. Color changed accordingly, although the decreasing pattern of ^aHue was similar in the two orchards. The same trend was observed in both orchards for soluble solids content (SSC), juice pH and the starch regression index. Firmness, the Streiff Index (SI) and the titratable acidity (TA) presented the inverse trend. Yet, the latter was consistently higher in the CO, except at harvest. Otherwise, the Thiault index (TI) increased more steeply in the EO, despite the higher levels in the CO before harvest. Overall, fruit from the two orchards attained the breaking points established in the PGI (starch regression index ≥ 7 ; SSC $\geq 15\%$), and similar levels for all the QA mentioned, except for the weight and size. When evaluated organoleptically by a semi-trained sensorial panel, the fruit from the two orchards scored as high-quality items and were equally recommended commercial although differing in sweetness (higher in the EO) and color (higher in the CO).

[1] Sánchez, C. and Leão de Sousa, M. (2021) Avaliação preliminar da qualidade dos frutos de novos clones de macieiras da cultivar 'Gala'. In book: Agrárias: Pesquisa e Inovação nas Ciências que Alimentam o Mundo VI (pp.81-91).

The authors acknowledge FRUBAÇA and INIAV/ENFVN (Alcobaça, Portugal) for providing the access to the commercial and experimental apple orchards followed in this study, Anabela Maurício for the technical assistance on the field, Ana Pina (INIAV/ENFVN) for the technical assistance in the determination of fruit quality attributes. This work is supported by CEOT strategic project UIDB/00631/2020 CEOT BASE and UIDP/00631/2020 CEOT PROGRAMÁTICO (FCT) and INIAV, I.P.

Sheep grazing and dolomitic limestone application effects on the production and quality of biodiverse pastures in the Montado ecosystem

Emanuel Carreira¹, João Serrano¹, Carlos Pinto-Gomes¹, Alexandre Pilirito², José Lopes de Castro¹, Mário de Carvalho¹ & Alfredo Pereira¹

¹MED (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.

²Aluno de Mestrado em Engenharia Zootécnica, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

e-mail: emanuel.ruben@hotmail.com

The Montado is an agro-silvo-pastoral ecosystem, characteristic of the Alentejo region. Their soils tend to be poor, as well as the natural pastures installed on them. This work aimed to evaluate the effect of different types of grazing (continuous vs. deferred) and the application of dolomitic limestone on the production and quality of pasture in the Montado. A 4ha field was divided into 4 plots, with the following treatments: plot 1- without application of dolomitic limestone and continuous grazing (7 sheep/ha) (P1UC); plot 2- without application of dolomitic limestone and deferred grazing (16 sheep/ha) (P2UD); plot 3- application of dolomitic limestone and deferred grazing (16 sheep/ha) (P3TD); plot 4- application of dolomitic limestone and continuous grazing (7 sheep/ha) (P4TC). The experimental trial took place between November 3rd and June 30th, at the University of Évora (Mitra experimental farm). In each plot, 12 points representing the plant communities of the pasture were identified. Height measurements and sampling were carried out. These measurements and collections occurred in Autumn (November 3rd), later Winter (March 12th) and Spring (May 22nd). At each of those moments, determinations of dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) were also determined. When pasture had less than 3 cm height in the P2UD and P3TD plots, sheep were removed, counting the number of grazing days and pasture rest. As for the height of the pasture, there were highly significant differences among dates (value $p=0.000^{**}$) and for the fence (value $p=0.001^{*}$), with $\alpha=0.05$. The amplitudes of the pasture heights shows that there was selectivity in all plots, being more evident in the plots of continuous grazing. The highest CP values were found in P2UD and P3TD. P3TD and P4TC led to higher DM values in autumn and winter, with no differences in spring. This results of this study shows that the application of dolomitic limestone leads to greater growth and production of dryland pastures in the Montado, and deferred grazing with greater biotic loads enhances the quality of the grass.

Soil amendment with Zeolites and Biochar influenced soil properties, photosynthetic performance and olive fruit and oil composition

S. Martins ¹, E. Silva ^{1,2}, C. Brito ^{1,3}, C. Martins-Gomes ^{1,4}, A. Gonçalves ⁵, M. Arrobas ⁶, M. Â. Rodrigues ⁶, F. M. Nunes ⁴, C. M. Correia ¹

¹CITAB - Inov4Agro - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, - Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Universidade de Trás-os-Montes e Alto Douro, 5001-801, Vila Real, Portugal

²Association BLC3—Technology and Innovation Campus, Centre Bio R&D Unit, Rua Comendador Emílio Augusto Pires, 14, Edifício SIDE UP, 5340-257 Macedo de Cavaleiros, Portugal

³Inov4Agro - Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

⁴CQ-VR – Food and Wine Chemistry Laboratory, Chemistry Research Centre – Vila Real, University of Trás-os-Montes e Alto Douro, 5001-801, Vila Real, Portugal

⁵MORE - Collaborative Laboratory Mountains of Research, Brigantia Ecopark, Bragança, Portugal

⁶CIMO – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal

Email: scpmartins@utad.pt

Olive orchards represent a key agricultural system in the Mediterranean basin. Soil degradation processes associated to unsustainable agronomic practices and climate change could severely impact the sustainability of Mediterranean rainfed olive orchards. In this context, soil amendments are important tools that can be used to enhance soil fertility for sustained environmental quality and plant performance. For two years, we evaluated, under rainfed conditions, the effects of a fertilizer compound (FC) and its combination with zeolites (ZL) and biochar (BC) amendments on soil moisture, photosynthetic activity, yield, fruit and oil composition and quality indices. Although no significant effects were observed on crop yield, the application of ZL and BC improved plant photosynthetic performance due to lower stomatal limitations. The effects on soil quality were more evident in ZL amendment, due to the reduction of acidity, and enhanced moisture, cation exchange capacity, microbial biomass carbon and enzymatic activity. Results relative to olive composition show that the polyphenolic content was strongly influenced by treatments. ZL improved fruit fatty acid composition and oil quality, while BC enhanced the concentrations of polyphenols with high nutritional value (3,4-dihydroxyphenylglycol, oleuropein and rutin). In contrast, olive oil from FC fruits showed the poorest quality. The applied soil amendments appear to be a promising sustainable strategy to implement in olive rainfed orchards, and can be an interesting complement to mineral fertilization, in order to promote soil quality, increase physiological performance and olive oil quality.

This work was funded by Doctoral fellowship under the Doctoral Program “Agricultural Production Chains—from fork to farm” (PD/00122/2012) provided by the FCT-Portuguese Foundation for Science and Technology to S. Martins (PD/BD/135327/2017). This research was funded by the Operational Group “Novas práticas em olivais de sequeiro: estratégias de mitigação e adaptação às alterações climáticas”, funded by PT2020 and EAFRD (European Agricultural Fund for Rural Development). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CQ-VR (UIDB/00616/2020), CIMO (UIDB/00690/2020) and CITAB (UIDB/04033/2020) and also to AgriFood XXI Project, No. NORTE-01-0145-FEDER-000041, co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (North Regional Operational Program 2014–2020).

Tailoring the cultivable bacterial microbiota as a source of stage-specific biofertilizers

Rocha, R.^a, Lopes, T.^a, Fidalgo, C.^b, Alves, A.^b, Cardoso, P.^b, Figueira, E.^b

^aDepartment of Biology, University of Aveiro, 3810-193, Aveiro, Portugal

^bCESAM-Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal

Email: ricardorochoa16@ua.pt

World population is increasing and a major pressure on food production is expected. The present agricultural methodologies are vulnerable to the erratic climate, increasing urbanization, industrialization, and agrochemical pollution. To achieve food security, sustainable practices must be pursued. The common bean (*Phaseolus vulgaris* L.) is one of the most produced legumes worldwide and, as a reliable source of high-quality protein, can reduce the environmental impact of meat production. However, high yields are dependent on heavy and expensive fertilization. Plant growth promoting rhizobacteria (PGPR) are emerging as a sustainable prospect to increase agricultural production, yet this interaction is not fully understood, especially the chronological variations in the microbiota. Thus, a deeper understanding on the interaction and dynamics between plants and microorganisms may boost the beneficial effects of microorganisms on plants. To reach this goal, the cultivable microbiota of the bean root was isolated and identified at distinct stages of plant development (early vegetative growth (V1), late vegetative growth (V2), flowering (F), and pod (P)) and root compartments (rhizoplane (out), endosphere (in), and nodules (nod)). Diversity and abundance of cultivable bacteria associated to root compartments differed throughout plant life cycle. Bacterial plant growth promotion and protection abilities (indole-3-acetic acid production, siderophores synthesis, and antifungal activity) were determined and associated to the plant phenology, suggesting that among the cultivable bacteria associated to the plant root several strains had an active role on the response to plant biological necessities at each development stage. Several strains stood out for their ability to display one or more plant growth promoting (PGP) traits, being excellent candidates as efficient stage-specific biostimulants to be applied in precision agriculture.

This research was funded by FCT through the financial support to the H2O value project (PTDC/NAN-MAT/30513/2017) also supported by FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership agreement and Compete 2020 (CENTRO-01-0145-FEDER-030513). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020) through national funds.

The effects of the application of *Cynara cardunculus* L. leaf extracts on the shelf life of poultry

C.H. Barbosa ^{1,2}, M.A. Andrade ^{1,3,4}, F. Vilarinho ¹, A. Sanches Silva ^{3,5,6}, A.L. Fernando ²

¹Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisbon, Portugal

² METRICS, Departamento de Química, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal;

³ University of Coimbra, Faculty of Pharmacy, Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

⁴ REQUIMTE/LAQV, Rua D. Manuel II, Apartado 55142, Oporto, Portugal

⁵ National Institute for Agrarian and Veterinary Research (INIAV), I.P., Rua dos Lagidos, Lugar da Madalena, 4485-655, Vairão, Vila do Conde, Portugal;

⁶ Center for Study in Animal Science (CECA), ICETA, University of Oporto, Oporto, Portugal

Email: cassia.barbosa@insa.min-saude.pt

Cynara cardunculus L. (Asteraceae), commonly named cardoon, is a multipurpose crop native to the Mediterranean area. Cardoon can be used for different purposes, such as an ingredient in the Mediterranean cuisine, biomass production, and its flower acts as a vegetable rennet in the production of some cheeses. It comprises the globe artichoke (var. *scolymus* (L.) Fiori), the cultivated cardoon (var. *atilis* DC.), and the wild cardoon (var. *sylvestris* (Lamk) Fiori). This plant develops in extreme weather conditions and still have excellent beneficial properties. For instance, the leaves are known for its excellent polyphenol profile, with interesting antioxidant and antimicrobial activities, and medicinal applications (high blood cholesterol levels, diabetes, and cancer). Regarding the food industry, cardoon leaves can be used to prolong the shelf-life of foods, protecting it against lipid oxidation and retarding microbiological growth. Thus, this study aims to evaluate the effectiveness of *Cynara cardunculus* L. leaves ethanolic extract on poultry meat preservation. The poultry meat was mixed with the extract (total phenols: 81.98 mg/g GAE; antioxidant activity: EC50 = 2.10 mg/mL by DPPH assay) at different concentrations, 0.5%, 1% and 2% (w/w), and stored under refrigeration (5 °C ± 2 °C) for 15 days. To evaluate the microbiological status, total mesophilic aerobic microorganisms, total psychrotrophic aerobic microorganisms, and *Enterobacteriaceae* were measured. The freshness of the poultry meat was determined by Total Volatile Basic Nitrogen (TVBN) and the lipid oxidation by Thiobarbituric Acid Reactive Substances (TBARS). The moisture, pH, acidity, and color were also determined to characterize the adequacy of the poultry meat. Results indicate that cardoon extract was able to maintain a constant pH and level of acidity that allowed to delay microbial growth. Poultry meat with extracts showed a difference of 8 log CFU/g to control samples (without extract), minimizing the release of volatile basic nitrogen at the end of the assay (day 15). However, the extracts effect on poultry meat reduced only slightly the lipid oxidation compared to the control. The color of extracts can be a constraint due to the greenish-yellow color that is seen in the meat.

Acknowledgements: Cássia H. Barbosa would like to thank for the Ph.D. scholarship from Fundação para a Ciência e Tecnologia (FCT) — 2021.08154.BD. Funding: This work was financially supported by the Mechanical Engineering and Resource Sustainability Center—METRICS, which is financed by national funds from the FCT/MCTES (UIDB/04077/2020 and UIDP/04077/2020)

The (Environmental) Scanning Electron Microscopy in cork from *Quercus suber* L. analysis

Poeiras A. P.¹, Vogel C.², Günther B.², Camilo-Alves C.¹, Surový P.³, Silva E.⁴ and Ribeiro N. A⁵

¹Mediterranean Institute for Agriculture, Environment and Development (MED), School of Sciences and Technology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal;

²Chair of Soil Resources and Land Use, Institute of Soil Science and Site Ecology, Technische Universität Dresden, Piennner Str. 8, 01737 Tharandt, Germany;

³Department of Forest Management, Faculty of Forestry and Wood Science, Czech University of Life Sciences Prague, Kamýčká 129, 165 00 Prague-Suchdol, Czech Republic;

⁴Department of Forest Sciences and Landscape Architecture, University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5001-801 Vila Real, Portugal;

⁵Department of Plant Science, Institute of Earth Science (ICT), School of Sciences and Technology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: apcp@uevora.pt

Cork cells are produced from the phellogen of cork cambium, which lives through the lifespan of the tree and is most active during two periods: spring and autumn. Cells dimensions and cell wall thickness differ according cork rings (autumn and spring). Cell size depends on seasonal growth: cells produced during the autumn growth period are smaller in size than cells produced during the spring growth period. Cell wall features have an influence on cork characteristics, such as cork structure and density, which associated with other cork characteristics are responsible for the final cork products quality. To analyze the cell wall thickness in raw, after swelling and boiling, the Environmental and Scanning Electron Microscopy (SEM and ESEM) was applied. This approach (ESEM), not so often used in cork tissue analysis, allows the dynamic process of water interaction and the thermodynamic stability of moist samples, and can preserve the hydration of samples within the analytical chamber.

The objective of this study was the analyses of cork cell walls and their behavior during swelling and boiling processes, demonstrating the importance of this technique to some specific cork samples analysis.

This study is developed in Poeiras, A.P.; Vogel, C.; Günther, B.; Camilo-Alves, C.; Surový, P.; Silva, M.E.; de Almeida Ribeiro, N. A Cork Cell Wall Approach to Swelling and Boiling with ESEM Technology. *Forests* 2022, *13*, 623. <https://doi.org/10.3390/f13040623>, which belong to the author' PhD dissertation.

The relationship between fatty acids and fish: lesser-known aspects and the need for increasingly efficient analytical techniques

André Jorge^{1,2}, Bernardo Quintella³, Marco Gomes da Silva² and M. João Lança^{1,4}

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

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

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

⁴Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: d52876@alunos.uevora.pt

When we associate the word fatty acids with fish, most people identify fish as sources of fatty acids from the omega families. This relationship is always present in the valuation of nutritional quality of fish. However, fatty acids have many other crucial functions in fish. The oldest studies related to fatty acids focused essentially on their role as sources metabolic energy in the form of ATP through β -oxidation. Many studies referred the fatty acid role for migrations, energy production during growth and egg formation, among others. Soon fatty acids quickly began to be studied from the point of view of source of essential nutrients for several physiological processes and integral components of biological membranes. The high content of highly polyunsaturated fatty acids found in fish is related to the fact that they are poikilothermic. In the presence of various temperature scenarios, fish can effectively exploit a wide chemical diversity of membrane fatty acid profiles, to defend their physical properties, such as fluidity. In the last decades, fatty acids have been used as either biomarkers because they are limited to certain *taxa* and they can be transferred conservatively from primary producers to higher trophic level or stock identifiers because the fatty acid composition of phospholipids in some body tissues have a stable genetics basis.

In the last decades multiple techniques have been used for fatty acid analysis. As a rule, before the instrumental analysis, the sample requires 2 previous steps. These comprise extraction of lipids from the matrix and derivatization. The combination of these two stages generates multiple methodologies that are not always standardized. Soon, the analysis of fatty acids tends to reduce and standardize these previous analyses while the instrumental analysis should solve problems related to similar mass spectra of the isomers and coelution between fatty acids.

Acknowledgements: André Jorge would like to thank to Fundação para a Ciência e Tecnologia (FCT) for a PhD scholarship UI/BD/153381/2022.

This work was funding 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.

Varietal comparison of six red seedless grape cultivars produced in Alentejo

A. J. Serranito¹, M. I. Rouxinol¹, S. Ricardo-Rodrigues¹, A. C. Santos^{1,2}

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

²Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: mir@uevora.pt

Consumers are becoming more aware of the importance of food quality and safety, thus characteristics like the appearance, size of bunches and berries, and the uniform color, flavor, and texture typical of each cultivar are essential to determine quality. In the last years, there has been a growing interest in seedless table grapes, leading to an increase of their production in numerous improved varieties as an attempt of producers to respond to the consumers high demand on these products. Red table grapes are rich in phenolic compounds, that provide high antioxidant capacity which is associated with countless health benefits.

In this work, six red seedless table grape varieties were studied to assess product quality attributes, including skin firmness (using a penetration test with a 2 mm diameter stainless steel cylindrical probe using a TA.HD.Plus), phenolic composition (Folin-Ciocalteu method), antioxidant capacity (radical DPPH assay), total soluble solid content (by refractrometry) and titratable acidity. The varieties chosen were 'Timco', 'Melody', 'Scarlota', 'Allison', 'Autumn Royal' and 'Crimson'. The grapes were produced and collected in their commercial ripe stage in Herdade do Vale da Rosa, Alentejo, located in Ferreira do Alentejo (38°05' 23,80" N; 8°04' 52,7 1" O). The differences in the quality parameters measured among the six varieties are crucial for their evaluation. Scarlota and Autumn Royal were that varieties that showed a higher soluble solid content, while Crimson and Timco had the lowest. Despite the higher phenolic content was found in Crimson, the higher antioxidant capacity was found in Autumn Royal. Finally, the grape skin firmness was higher in Crimson and lower in Autumn Royal. These results specifically characterizing the varieties, can provide detailed information to consumers and for helping marketing strategies, for each variety.

This work was supported by Vale da Rosa Sociedade Agrícola Lda in the project "Investigação e desenvolvimento de novas tecnologias de conservação pós-colheita de uva de mesa sem grainha e na Promoção e divulgação dos resultados de I&D", UID/AGR/00115/2013 and FCT, UIDB/05183/2020

What are seedless table grapes?

S. Ricardo-Rodrigues^{1*}, M. Laranjo¹, M. I. Rouxinol¹, A. Serranito¹, A. C. Agulheiro-Santos^{1,2}

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

²Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: sirr@uevora.pt

Grapes are one of the most commonly produced fruit crops in the world and an important fruit commodity that allows good profits to producers. According to their characteristics, grapes can be considered for different uses: wine production (varieties with higher acidity and moderate sugar content), table grapes for fresh consumption (varieties with low acidity, low sugar, and specific standards of size, color, and shape), and raisins (varieties with low acidity and high sugar). In the last years cultivation and consumption of table grapes (*Vitis vinifera* L.) has increased considerably, mainly due to seedless varieties. Consumers appreciate the absence of seeds and are willing to pay more for sweeter and more firm seedless grapes. However, for many people the term “seedless” has a negative connotation, associated with negative health effects or even genetic manipulation. Seedless grapes exist naturally or may be manipulated by plant breeders without using genetic engineering techniques. Is possible to distinguish two mechanisms of seedlessness: parthenocarpy (when the ovary can develop without fertilization of the ovum) and stenospermocarpy (embryo abortion occurs after fertilization, and partially developed seeds or traces of seed are visible). Shelf-life of seedless grapes is expected to be longer than seeded fruits, because seeds produce hormones that activate senescence. The current table grapes breeding programmes are designed to obtain varieties with good characteristics and mandatorily seedless. So, the absence of seeds, shape, colour, skin thickness, resistance against diseases and pests, ability to be transported without damage and shelf-life are the main criteria to select new varieties. However, those who think that these new seedless table grape varieties are a new development, you are mistaken! There are records of this type of grapes since the 19th century. The most widely commercialized seedless table grapes varieties in the world are Thompson seedless (first seedless variety cultivated in the world), Crimson and Autumn Royal. In recent years, the post-harvest research group at the University of Évora has developed several works, in partnership with regional enterprises, in the characterization and quality evaluation of several varieties of seedless table grapes.

This work was supported by Portuguese National Funds through FCT – *Fundação para a Ciência e a Tecnologia* under Project UIDB/05183/2020 (MED). S. Ricardo-Rodrigues acknowledges a PhD grant from FCT (2021.07663.BD).

**Environment, Landscape
and Sustainability**

Grey infrastructures: an opportunity to promote native plants

M.P. Fernandes^{1,2}, P. Matono¹, A. Nunes², F. Moreira³, A.D.F. Belo⁴

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

²Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, C2, Piso 5, 1749-016 Lisboa, Portugal

³CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

⁴MED - 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: mpfernandes@uevora.pt

The widespread of the linear infrastructures network and the intensification of land use are significant drivers of biodiversity loss, highlighting the need to safeguard semi-natural refuge areas in human-dominated landscapes. Proper management of linear infrastructures related habitats (LIRH), such as road verges or power lines rights-of-way, may represent an opportunity to increase the potential of fragmented landscapes and contribute to the conservation of natural values, particularly native flora that is disappearing from the adjacent landscape. To take advantage of this opportunity, research on ecological restoration and maintenance of LIRH is needed, so that their potential is attained and their demand as a conservation tool is met, especially in the Mediterranean.

This PhD project aims to promote Mediterranean native flora along LIRH through appropriate management and restoration measures, replicable in other Mediterranean environments. The study takes place on national roads and high-voltage power lines rights-of-way of Alentejo (southern Portugal).

As a first step, this study is assessing the potential for vegetation conservation in road verges and power lines rights-of-way in a Mediterranean semi-natural landscape and develop a methodology to determine priority areas as native plant refuge areas. In a second step, it intends to improve the ecological values in habitats related to linear infrastructures through the development, application and follow-up of management and restoration techniques.

Increasing vegetation diversity in LIRH and enlightening their role as biodiversity refuges is an urgent need and a challenge for which information available is scarce. This PhD project enables both scientific and practical outputs, actively contributing to improve biodiversity of habitats related to linear infrastructures and their management practices in a Mediterranean landscape.

This work is funded by National Funds through FCT under the PhD grant 2020.06339.BD.

Lignin extraction from agroforest residues using natural deep eutectic solvents

C. Fernandes^{1,2}, B. Medronho^{2,3}, L. Alves¹, M. G. Rasteiro¹

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

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

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

Email: csfernandes@uc.pt

The world population growth and the increase in waste generation that results from it are motivating the adoption of a circular economy approach, where residues are treated as useful resources to produce new materials of added value. In this respect, lignin, derived from lignocellulosic materials, has received increased attention as an alternative renewable raw material. Lignin is an abundant by-product from the pulp and paper industry, often treated as waste. It is the most abundant natural aromatic feedstock, and its efficient extraction could expand the utilization of biomass and reduce the dependence on fossil fuels. However, the complex molecular structure of lignin makes its isolation from the other biomass components non-trivial. Among the different extraction methods available, acid-based deep eutectic solvents (DES) have shown promising results, especially when combining two different acids to form ternary mixtures with tuned properties¹. These mixtures have shown a great selectivity for lignin, being capable of extracting lignin with high purity degree while presenting low cellulose dissolution. DES have also some other advantageous inherent properties, such as low cost, ease of preparation, and high thermal stability. In addition, DES can be prepared with natural ingredients and be easily recovered at the end of the process and reused without compromising the extraction efficiency¹.

In this work, novel natural deep eutectic solvents were prepared, characterized, and screened for the fractionation of wood residues to infer on their suitability for an efficient and selective extraction of lignin. A remarkable dependency of the DES viscosity on their composition was observed. The extraction capacity was also observed to be greatly affected by the DES composition, i.e., its components and their molar ratio, and by the extraction conditions, namely the extraction temperature. The new DES composed of choline chloride and two acidic hydrogen-bond donors resulted in solvents with enhanced properties and superior extraction performance. The superior performance of the prepared DES and its “green” features makes the process highly appealing for biomass fractionation.

This work was supported by funding from the Portuguese Foundation for Science and Technology (FCT) through the projects PTDC/ASP-SIL/30619/2017, the Strategic Research Centre Project (UIDB/00102/2020) and the researcher grant CEECIND/01014/2018. C. Fernandes acknowledges FCT for the PhD grant with reference 2021.05991.BD. L. Alves acknowledges FCT for the research grant 2021.00399.CEECIND.

(1) Fernandes, C.; Melro, E.; Magalhães, S.; Alves, L.; Craveiro, R.; Filipe, A.; Valente, A. J. M.; Martins, G.; Antunes, F. E.; Romano, A.; et al. New deep eutectic solvent assisted extraction of highly pure lignin from maritime pine sawdust (*Pinus pinaster* Ait.). *Int. J. Biol. Macromol.* 2021, 177, 294-305. DOI: <https://doi.org/10.1016/j.ijbiomac.2021.02.088>.

Mapping colluvial mesovoid shallow substratum habitats: a case study in karst (Arrábida Natural Park, Portugal)

Eusébio, R.P.^{1*}; Fonseca, P.E.²; Rebelo, R.¹; Mathias, M.L.³ & Reboleira A.S.P.S.^{1,4}

¹ Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

² Instituto Dom Luiz (IDL), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749–016, Lisboa, Portugal

³ Centre for Environmental and Marine Studies (CESAM) and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

⁴ Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen East, Denmark

*Email: fc58762@alunos.fc.ul.pt

Colluvial mesovoid shallow substratum (MSS) is a system of air-filled spaces between disaggregated rocky fragments which accumulate on steep slopes. As it can be a permanent habitat or a climatic refuge for several species, from an ecological point of view, colluvial MSS is a very important and widespread habitat. The current method for locating these habitats consists of *in situ* visual scouting, which is time and resource consuming. We developed a method for locating, mapping, and estimating the geographic distribution of colluvial MSS habitats. Five main geological criteria for habitat genesis were created. The method can be applied in four steps: 1) Define a geological area to map, 2) Select potential locations using satellite imagery, 3) Combine the potential sites with geological maps, in order to apply the five criteria, 4) Perform an *in situ* verification of the sites that met all the criteria. The karstic area of the Arrábida Natural Park (Portugal) was selected as the study site, where 24 potential locations were initially selected, 11 of which met all the criteria, considerably reducing the *in situ* scouting area. Of these, 8 were confirmed *in situ* as colluvial MSS. Biodiversity was also assessed in order to verify habitat suitability for invertebrate communities. This new mapping method provides not only a more efficient way of locating colluvial MSS habitats but also an approach to estimate potential MSS habitat extension. We also show that the habitats found using this mapping method are suitable for invertebrate fauna. This method can be utilized in non-karst study areas, expanding the current knowledge on the distribution of colluvial MSS habitats worldwide.

This work was supported by the Portuguese National Funds through “Fundação para a Ciência e a Tecnologia” (FCT) within the cE3c Unit funding UIDB/00329/2020, CESAM (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020), PhD grant (2021.04868.BD), and by the VILLUM FONDEN (research grant 15471). All specimens were collected under the Instituto de Conservação da Natureza e das Florestas (ICNF) permit, number 013/2022/CAPT.

Nature-based solutions as climate change adaptation measures in Mediterranean watersheds

M. Silva Rodrigues¹, L. Filipe Dias¹, J. Pedro Nunes^{1,2}

¹cE3c (Center for Ecology, Evolution and Environmental Changes) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Edifício C2, 5º Piso, Sala 2.5.46, Campo Grande, 1749-016 Lisboa, Portugal.

²Soil Physics and Land Management Group, Wageningen University, Droevendaalsesteeg 4, 6708PB Wageningen, The Netherlands

Email: miguel-silva-rodrigues@edu.ulisboa.pt

Global climate change impacts have reinforced the need for adaptation measures. The Mediterranean basin is particularly vulnerable to increasing pressures on water resources due to climate and land-use changes and growing water abstractions. In this work, we aim to assess the effectiveness of Nature-based solutions (NBS) as climate change adaptation measures for the Mediterranean basin. Due to its Mediterranean climate, Algarve constitutes the use case for this project. We will start by analysing NBS effectiveness for land management and regulation of eco-hydrological processes. To estimate Algarve's future water balance, we will consider the impacts of climate change, land use, and irrigation on the regional water availability until 2100 with the SWAT+ eco-hydrological model. We will evaluate the effectiveness of NBS for flow and water quality regulation at the watershed scale through a thorough analysis of NBS to the regional water balance. The produced results will be adaptable to other Mediterranean watersheds.

This work is funded by National Funds through FCT under the Scholarship 2021.04535.BD. M. S. R. acknowledges FCT (SFRH/BD/04535/2021).

Spatiotemporal analysis of marginalization drivers in Gennargentu-Mandrolisai inland areas

Pietro Todde^{1*}, Marta Debolini^{2,3}, Pier Paolo Roggero¹ and Vittoria Giannini¹

¹Department of Agricultural Sciences, University of Sassari, Viale Italia 39A, 07100 Sassari

²UMR 1114 INRAE/Avignon University, EMMAH, Avignon, France

³CMCC Foundation – Euro-Mediterranean Centre on Climate Change, IAFES Division, Viale Italia 39, 07100 Sassari, Italy

* corresponding author: p.todde@studenti.uniss.it

The Gennargentu-Mandrolisai district, located in central Sardinia, is part of the national strategy for inland regions intended to foster the economic growth of marginal areas. After the Second World War, the massive abandonment of cereals and orchards, combined to overgrazing or abandonment, has caused the degradation of grasslands or their ecological succession to woodlands. The present research aims to understand the marginalization process integrating a direct approach with the main stakeholders (farmers, public bodies) combined with quantitative spatial analysis. Qualitative data from local actors were collected through questionnaires about the main drivers that led to the abandonment of rural areas. A diachronic analysis of land use from Corine Land Cover maps on four different dates over thirty years (1990, 2003, 2008 and 2018) was used to understand the main changes. In total, thirty farmers from six municipalities were interviewed. Their farms cover 1602 ha in total between two municipalities in the mountain area (670 ha in Desulo and Tonara) and four municipalities on the hill area (932 ha in Atzara, Samugheo, Sorgono, Ortueri). The spatial analysis allowed to highlight relevant differences among municipalities caused by different geo-morphological characteristics, social attitudes, and historical decisions of the former and current local government. The interviews revealed some recurrent marginalization factors such as: unprofitability (50% of answers), property atomization (37%), the distance of the fields (27%), stringent legislation (10%). Some 93% of the interviewed have undertaken operation of recovery, regarding viticulture, pasture, forage production and orchards. In particular, the analysis of the fragmentation pointed out that the average farm has 10 fields, ranging from 1 to 63. The analysis of these factors of marginalization will lead us to hypothesize new models and innovative systems to promote agricultural activities in the territory.

Integrated assessment of ecological and human dimensions in the functional dynamics of Atlantic mangroves: studies in the Tarrafes do Rio Cacheu Natural Park (Guinea-Bissau)

Arthur Fernando Veronez de Sousa, Helena Freitas e António C. Gouveia

Centre for Functional Ecology - Science for People & the Planet, Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal.

E-mail: asousa@student.uc.pt

Atlantic mangroves are crucial for the well-being of societies and demand sustainable long-term adaptive capacity from human actions to maintain their conservation status and help sustain the livelihoods of communities living in, around and deriving ecosystem services from these areas. The main objectives of this PhD project are to produce knowledge on the functioning and conservation of Atlantic mangroves, focusing on the Tarrafes do Rio Cacheu Natural Park (PNTC) in Guinea Bissau as a case study, assessing the ecological integrity of mangrove ecosystems (natural and restored), using macroinvertebrates as bioindicators, and their correlations with the provision and sustainable use of key services. The project comprises four general tasks: produce knowledge on the functioning and conservation of Atlantic mangroves (through systematic review of published studies and data), considering natural and human dimensions; assess the ecological integrity of mangrove ecosystems (natural and restored), through the use of macroinvertebrates as bioindicators, and their correlations with the provision and sustainable use of key ecosystem services (ES) and nature contributions to people (NCP); help inform conservation and restoration strategies of mangrove ecosystems of the Atlantic coasts (Africa, Brazil and Portugal); and finally, contribute to conservation, sustainable development and ES/NCP valuation within the PNTC by designing participative activities, translating the scientific knowledge into the needs and aspirations of the resident communities of the PNTC and foster science communication through activities of environmental education and socioeconomic-environmental methodologies to diverse stakeholders. The preliminary result obtained from the systematic review of published studies related to Atlantic mangroves (Brazil and West African Portuguese-speaking countries), including the characterization of ES/NCP provided by these works, reveal knowledge gaps such as: the creation of reliable databases, studies on specific biodiversity groups (invertebrates), community-based management, cultural services valuation (cultural heritage, spiritual values, environmental education) and provision services alternatives.

This doctoral fellowship was awarded by the Atlantic International Research Centre (AIR Centre) and funded by FCT [PRT/BD/153040/2021].

Adoption of digital technologies in agricultural trade in Portugal - current situation and future strategies

L.M.G. Faria¹, R. Quaresma², C. Fonseca^{1,3}

¹UTAD (Universidade de Trás-os-Montes e Alto Douro), Quinta de Prados, 5000-801 Vila Real, Portugal.

¹CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia), Palácio do Vimioso (Gab.224), Largo Marquês de Marialva, n.º 8, 7000-809 Évora, Portugal.

²CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.

Email: luisgomesf8@gmail.com

Agriculture is going through a digital revolution, opening new possibilities for the use of new technologies. Online agricultural markets have attracted increasing interest due to their potential transformation of supply chains, from the origin of products to their commercialization, which enables not only the development of market capitalization agility but also the agility of operational adjustment of the rural world. The development of online rural commerce is significantly driven by the local industrial base and neighborhood effects, currently, agricultural logistics infrastructure has been improved and many dispersed small-scale agricultural production modes have been effectively integrated, which has built a favorable condition for the expansion of online agricultural trade. However, there is still a long way to go when it comes to facilitating communication and interaction with your customers, even if farmers benefit from online sales. For governments, must not only understand the positive impacts of online commerce for the development of the agricultural sector, but also recognize the premise and logic of how online commerce can play a prominent role in agriculture. In turn, established companies, like agricultural producers, feel increasing pressure to change or adapt their business models to improve the management system for marketing channels for agricultural products. Otherwise, there is a risk of being left behind and finally being forced out of the market. A qualitative approach will be adopted through the case study method to analyze the degree of penetration and importance of digital technologies related to online trade in the Portuguese agricultural sector. This approach will provide analytical insights into the impacts of market power, heterogeneity, farmers' availability of information and related knowledge such as online commerce. This will help to build scenarios and develop solid recommendations in the agricultural sector, an important contribution, considering that, during the research carried out to date, we didn't found academic attempts to link this aspect of literature in Portugal.

Agri-food Sustainable Consumption and Implications to Agribusiness

E. Venâncio¹, M.R. Lucas², A. Marta Cota³

¹CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia, Universidade de Évora, Portugal),
Email: eunycassia@gmail.com

²CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia, Universidade de Évora, Portugal),
Email: mrlucas@uevora.pt

³CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro,
Portugal
Email: amarta@utad.pt

The increase in industrialization and purchasing power are factors that influence consumerism, which has motivated the adoption of practices that encourage responsible and sustainable consumption attitudes. The bet on a more sustainable choice by the consumer, has implications for agribusinesses that respond with products and services also more sustainable. This research has as objectives: to analyze the factors and determinants of purchase intention of sustainable agri-food products by final consumers, and its implications for agribusiness. In order to achieve the objectives, the work will be done in three distinct stages: Initially, it will be done exhaustive research on important concepts to consider, as well as factors and determinants already identified in the literature, in order to identify existing gaps on the factors and determinants of the intention to purchase products agri-food, that is, what has not been investigated, which will allow us to have a general idea of the state of the art on the subject; then, an inquiry will be made to the final consumers, about the factors (internal and external) associated with the practice of consumption lead to choices more sustainable food/more responsible consumption, which could allow the identification of different groups of food consumers; and finally it will be an interview was carried out with representatives of different agribusiness companies, about their knowledge of these identified consumer groups, and how this impacts their companies.

This work is funded by National Funds through FCT under the PhD grant with reference PRT/BD/152339/2021).

Sustainable consumption of agri-food: analysis of scientific production

E. Venâncio¹, M.R. Lucas², A. Marta Cota³

¹CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia, Universidade de Évora, Portugal),
Email: eunycassia@gmail.com

²CEFAGE (Centro de Estudos e Formação Avançada em Gestão e Economia, Universidade de Évora, Portugal),
Email: mrlucas@uevora.pt

³CETRAD (Centro de Estudos Transdisciplinares de Desenvolvimento), Universidade de Trás-os-Montes e Alto Douro,
Portugal
Email: amarta@utad.pt

Sustainability is one of the biggest challenges that agricultural supply chains have been facing. The increase in industrialization and purchasing power are factors that influence consumerism, which has motivated the adoption of practices that encourage responsible and sustainable consumption attitudes. Sustainable consumption is one of the requirements for sustainable development. The study seeks to highlight the evolution of scientific production on sustainable consumption of agri-food products, developing an analysis on the main publications and topics addressed. The methodology adopted consists of a bibliometric literature review with citation-based analysis with support from the software RStudio and Rbibliometrix, using a database consisting of articles from the Web of Science and Scopus. Eighty-five articles were analyzed. An evolution of publications over the years was verified. North America presented the highest number of duplicates. There is still little collaboration between authors who investigate this theme. In general, the approach of the articles corroborates the fact that it is more and more important to adopt adequate policies for agri-food sustainability and that consumers are more and more willing to bet on food products that caused less impact in their production. This study can be used as a starting point for future research, allowing us to have an agenda on the subject under study. Future studies are necessary to deepen this theme and thus allow a more real knowledge of the progress of research on agri-food sustainability.

This work is funded by National Funds through FCT under the PhD grant with reference PRT/BD/152339/2021).

Capillarity natural dye recovery application to improve wool dyeing process

A.I. Arroyo-Escoto^{1,2,3}, F. Carvalho^{3,4}, M.C. Fernandes^{1,2}

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

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

³Departamento de Tecnologias e Ciências Aplicadas, Instituto Politécnico de Beja (IPBeja), Ap 158, 7801-902 Beja, Portugal

⁴FiberEnTech, Fiber Materials and Environmental Technologies, Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal

Email: maria.fernandes@cebal.pt

Textile industry is one the most polluting in terms of its water effluents. During the different processes involved, the dyeing stage entails the biggest water contaminant percentage. Textile dyes are aromatic molecules normally obtained from biological origin with the particular feature to be capable to interact with the selected fibre and light to produce the desired colour. The growing textile demand has forced the production of other synthetic molecules with similar capabilities. Nevertheless, these molecules have shown to be highly environmental recalcitrant, and unfortunately create several health problems on the population in contact (wastewater and textiles). The biggest world textile dyers use low recovering technology which ends up in environmental discharge with poor or non-dye recovery, losing more than 80 % of the applied concentration in the process. Attending this situation in terms of technology and economy, it has been proposed a capillarity recovering technic envisioning the wastewater detoxification and subsequent dye reflux back into the system. To achieve this objective, it has been created a rudimentary system based on applied pressure and fibre catching that allows its replication at any location to trap and liberate the dye at will, with the advantage of recovering the material to reuse it in another detoxification cycle. Fibres like cotton and filtering paper are being tested for this porpoise, focusing on its environmental implications.

A. I. Arroyo-Escoto thanks to Fundação para a Ciência e a Tecnologia (FCT) for the grant UI/BD/153579/2022. This work is done under GO -Tinturaria natural - Utilização dos corantes naturais em fibras naturais project (REF: PDR2020-101-031963), cofinanced by Fundo Europeu Agrícola de Desenvolvimento Rural (FEADER). MED, CHANGE, and FiberEnTech are funded by UIDB/05183/2020, LA/P/0121/2020, and UID/Multi/00195/2020 projects, respectively.

Non-volatile solvents as a promising strategy to improve indoor air quality

Ricardo T. Pais¹; Liliana P. Silva¹; Ana C. Sousa²; Pedro J. Carvalho¹

¹CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

²Department of Biology and Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal

Email: ricardotp@ua.pt

According to the World Health Organization, access to clean air is considered an essential human right. However, air pollution is a serious threat to human health, being responsible for around 7 million deaths per year. This mortality is associated to several pollutants with both ambient and household emissions, such as gases, particulate matter, and volatile organic compounds. As poor air quality is responsible for various diseases, improving indoor air quality (IAQ) must be a priority. Despite the existing guidelines to guarantee IAQ, other strategies should be employed whenever possible, including the reduction of pollutants at their source, air ventilation, and, if necessary, indoor air treatment. Although several technologies can be considered for indoor air treatment, these possess some disadvantages such as high energy consumption, waste generation and harmful by-products production. Therefore, new indoor air technologies are required to overcome these limitations. Among several new technologies and solvents, due to their high sorption capacity, unique thermophysical properties, easily tuneable by the simple combination of anions and cations, ionic liquids (ILs) and eutectic solvents have gathered special attention. On top of their inherent characteristics, one can further enhance gas physisorption and transport properties by preparing mixtures with positive deviations to ideality and, therefore, positive excess volumes. In a previous work, we have shown that mixtures of the aprotic IL $[C_4C_1im][DMP]$ or $[C_4C_1im][NTf_2]$ with carboxylate-based protic ILs present high excess volumes, standing thus, as mixtures with high potential for gas separation. Thus, in this work, we will present the sorption capability of $[C_4C_1im][DMP]$ with carboxylate-based protic ILs, evaluated through their CO_2 solubility measurement using the isochoric method in the pressure and temperature range of 0.1-0.45 MPa and 303.15-343.15 K, respectively. The results obtained highlight the potential to take advantage of the fine-tuning of these solvents with mixtures that present positive excess molar volumes and, ultimately, it is possible to envisage this approach with high potential for IAQ improvement.

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Ricardo T. Pais acknowledges FCT for his PhD scholarship (2020.07796.BD) and Liliana P. Silva acknowledges FCT for her PhD Grant SFRH/BD/135976/2018.

Risk Assessment of pesticides quantified in the hydro-agricultural area of Brinches-Enxoé (Alqueva Project)

A. Catarino^{1,2}, I. Martins¹, C. Mourinha¹, J. Caturra¹, P. Palma^{1,2}

¹Escola Superior Agrária - Instituto Politécnico de Beja. R. Pedro Soares S/N, 7800-295 Beja, Portugal.

²Instituto de Ciências da Terra (ICT). Universidade de Évora, Évora, Portugal.

Email: adriana.catarino@ipbeja.pt

The use of pesticides plays an important role in the quality of agricultural crops, providing several benefits for increased production. Despite that their excessive use can induce the development of harmful effects in terrestrial and aquatic ecosystems. The hydro-agricultural area (AHA) Brinches-Enxoé is one of the 21 areas of the Alqueva irrigation plan, part of the Multipurpose Development of Alqueva (EFMA—Empreendimento de Fins Múltiplos de Alqueva). The Lage and Montinhos reservoirs, and 8 irrigation hydrants (H5, 6, 7, 16, 21, 22, 23 and 33) are part of the AHA, all of which are monitored within the scope of the FitoFarmGest project. The objective of this study was to evaluate the environmental risk of the pesticides quantified in these irrigation waters in 2 years (2018 and 2019). For this, 46 pesticides were quantified and studied. The risk assessment was calculated based on the risk quotient (RQ) method, that is, the ratio between the MEC (measured environmental concentration) and the PNEC (expected no effect concentration) ($RQ = MEC/PNEC$). The levels of concern reported were: $RQ \geq 1$, high risk; $0.1 \leq RQ < 1$, medium risk; $0.01 \leq RQ < 0.1$, low risk (Sanchez-Bayo *et al.*, 2002). The results revealed that the highest amounts of pesticides were observed in July 2019. Unlike the Lage reservoir, where the concentration of total pesticides was higher in October 2019 (714.9 ng/L) and in the H5, H22, H23 and H33 with the highest concentrations reaching in May 2018 (495.5, 986, 358.8 and 163.5 ng/L). The bentazone and terbuthylazine were the pesticides found in the highest concentrations in all the sampling points. Irganol presented $RQ > 1$ at all locations, imidacloprid was not present in all locations, but when detected, it occurred in concentrations that could induce environmental risk to the aquatic ecosystem ($RQ > 1$; in the Lage reservoir). Azhinhos ethyl is the compound that showed the highest RQ values (Lage reservoir in 2019 and H23 in 2018). Through the analysis of the results, it was possible to conclude that several pesticides were quantified in concentrations that could induce environmental risk, most of which are already banned from commercialization in Portugal.

Acknowledgments: The study is co-financed by the European Union through the European Regional Development Fund, included in COMPETE 2020 through the project Institute of Earth Sciences (TIC; UID / GEO / 04683/2013) with reference POCI-01-0145- FEDER-007690 and by the European Agricultural Fund for Rural Development through the FitoFarmGest Operational Group (PDR2020-101-030926).

Tannin-furan and lignin-furan based foams for insulation and wastewater treatment – removal of methylene blue and mechanical characterization

Hugo Duarte¹, João Brás¹, Anabela Romano¹, Bruno Medronho^{1,3}

¹MED - Mediterranean Institute for Agriculture, Environment and Development, University of Algarve, Faculty of Sciences and Technology, Laboratory of Plant Biotechnology, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal

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

Email: hmduarte@ualg.pt

The continuous increasing demand for energy and the increase in energy prices directly affects families and companies, both benefiting from the energy saving from proper building insulation. The most common insulation materials available as polyurethanes, polystyrene, glass or rock wool present many desirable features, however, nowadays more sustainable and natural alternatives are often demanded¹. Tannin-furanic foams are known to be fire resistant, have low thermal conductivity and high water and chemical stability. Nevertheless, these have continued in constant development of its desirable characteristics with the aim of becoming suitable candidates for the replacement of less sustainable materials. The use of natural renewable feedstocks has increasingly attracted attention, such as the use of vegetal polyphenolic material as tannins or lignin, in which both can be recovered from residues or side products from industries¹⁻³. The process for acquiring the first tannin-furanic-formaldehyde foams was based on a heat-generated expansion, initiated by a blowing agent evaporation coupled with an acid-catalysis self-condensation of furfuryl alcohol, with or without a crosslinker to ensure the foam would not collapse². Tannin-based rigid foams have been modified, for example, with nanocellulose, enhancing their mechanical properties, fire retardancy and even removing the need for chemical crosslinking⁴. But as material science is becoming a more interdisciplinary field of knowledge, and modern world presents new challenge and necessities, the need for multipurpose materials rises. Therefore, rigid foams have also been tested as material suitable as adsorbents for wastewater treatment⁵. With the ongoing work, we present tannin and lignin-furanic based foams with several desirable characteristics. These have successfully been tested in the removal of methylene blue from water and new foams were produced by reducing the amount of pentane used, replacing it with sodium bicarbonate as blowing agent and producing more lightweight foams with the addition of controlled amounts of surfactant. The produced foams are promising materials that can be useful in construction and water treatment.

References

1. Tondi, Gianluca; Link, Martin; Kolbitsch, Christian; Gavino, Johannes; Luckeneder, Paul; Petutschnigg, Alexander; Herchl, Richard & Van Doorslaer, Charlie. Lignin-based foams: Production process and characterization. *BioResources* 2016, 11, 2972–2986.
2. Chen, Xinyi; Li, Jinxing; Pizzi, Antonio; Fredon, Emmanuel; Gerardin, Christine; Zhou, Xiaojian & Du, Guanben. Tannin-furanic foams modified by soybean protein isolate (SPI) and industrial lignin substituting formaldehyde addition. *Ind. Crops Prod.* 2021, 168,.
3. Pizzi, Antonio. Tannin-based biofoams-A review. *J. Renew. Mater.* 2019, 7, 477–492.

Environment, Landscape and Sustainability

4. Missio, André Luiz; Otoni, Caio G.; Zhao, Bin; Beaumont, Marco; Khakalo, Alexey; Kämäräinen, Tero; Silva, Silvia H. F.; Mattos, Bruno D. & Rojas, Orlando J. Nanocellulose Removes the Need for Chemical Crosslinking in Tannin-Based Rigid Foams and Enhances Their Strength and Fire Retardancy. *ACS Sustain. Chem. Eng.* 2022, 10, 10303–10310.
5. Sánchez-Martín, J.; Beltrán-Heredia, J.; Delgado-Regaña, A.; Rodríguez-González, M. A. & Rubio-Alonso, F. Optimization of tannin rigid foam as adsorbents for wastewater treatment. *Ind. Crops Prod.* 2013, 49, 507–514.

Aknowledgments: Bruno Medronho and Hugo Duarte acknowledge the financial support from the Portuguese Foundation for Science and Technology (FCT) via the projects PTDC/ASP-SIL/30619/2017, UIDB/05183/2020, and the researcher grant CEECIND/01014/2018.

The purpose of the Delphi methodology on a participatory approach for the assessment of the Social Dimension of Sustainability in Agribusiness

J. Massaça¹, A. Marta-Costa², R. Lucas³

¹Universidade de Trás-os-Montes e Alto Douro and Universidade de Évora

²Universidade de Trás-os-Montes e Alto Douro

³Universidade de Évora

Email: jose.massuca@gmail.com

After a thorough literature review, that investigated which social indicators are incorporated in sustainability assessment models applied to agribusiness, and from which the main result was an inventory of more than a thousand of "*different*" social indicators, it was perceived that these indicators are applied without uniformity or standardization in the implementation of different sustainability evaluation models. Thus, the need for harmonization regarding the desirable comparability of social sustainability results among agribusiness was obvious. During this research it was also evident that the need to identify which social indicators are truly important for stakeholders of a specific agribusiness is key.

Valuing these guidelines, it was decided to apply the Delphi Methodology as a tool to support the process of identifying which are the most relevant social indicators integrating the sustainability assessment models found in the literature review. On the Delphi method, the participation of agribusiness stakeholders is a foremost condition because this method is based on a strong group interaction. This method is structured to identify and combine individual opinions, of a group of "*experts*", aiming consensus, reaching agreement, and identifying which indicators are most important to be considered on a context.

This methodology naturally requires a previous identification of the most important and relevant stakeholders for the agribusiness under analysis, as well as an establishment of balanced working groups, ensuring the existence of harmony between the necessary heterogeneity of participants roles and the need of freedom to express themselves. Desired consensus will only be possible if the working group is well structured and guided. It is also necessary to do some previous role-play sessions, and it is also essential to design good questionnaires for the different stages of the methodology, together with the implementation of a good application for data loading, handling, analysis and presentation of results.

After this process of identification of the "*best*" social indicators to incorporate in a model for assessing agribusiness sustainability, it will be necessary to apply a second methodological tool to hierarchize and sustainably measure the indicators identified. For this purpose, the methodology AHP (*Analytic Hierarchy Process*) will be used next.

The role of local sustainable agri-food systems in enhancing the resourcefulness and well-being of communities – experiences from the Portuguese Oeste region

Luís Duarte

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.

Email: luis.duarte@uevora.pt

This communication aims to present a PhD research project in its early stage. It intends therefore on discussing the main questions posed, the main goals established, and the main methodologies foreseen in order to foment a debate about its merits and in this way allow further improvements to be made while still in the designing phase of the research. The research project here presented intends to shed light on current sustainable farming practices that are established within alternative agri-food systems and to analyze how these practices (re)connect to the territory. In other words, it aims to help understand how these environmentally friendly farming practices interact with their socio-economic and cultural contexts, and how can local communities benefit from their emergence. It should be pointed out that the existing literature examining the “empirical foundations for place-based agriculture” is still very scarce. And on the other hand, sustainable farming practices’ studies are most frequently focused either on the biotechnical functioning of the systems or on the social-political movements they represent, but “they do not explicitly consider how farming systems interact with their socio-economic environment”. Regarding the methodology for this project, a mixed methods research strategy is envisaged, integrating both qualitative and quantitative methods but still keeping the main focus on inductive and interpretative approaches. Being the main goal of this research to expand the current understanding of the territorial integration of agroecological practices, a case-study instrument, supported by personal interviews, will be used. Later, an action-research method will be applied in order to debate and prioritize the most relevant activities that could be undertaken toward an agroecological transition and therefore toward the improvement of communities’ well-being. The area for the empirical research will be the Portuguese Oeste region. Outcomes are expected in the form of governance guides for decision-makers and practitioners, and for academia, in the form of scientific research papers providing a deeper understanding of the territorial integration of alternative sustainable farming practices.

Endophytic bacteria associated with spontaneous legumes in arid zones of Tunisia: Genetic diversity, metabolic functionalities and potential application to mitigate the impact of climate change

R. Ben Gaied¹, C. Brígido², I. Sbissi¹, M. Tarhouni¹

¹Arid Lands Institute of Medenine, Pastoral Ecology Laboratory University of Gabes - Tunisia

²MED – 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: roukaya.bengaied@gmail.com

Legumes naturally adapted to harsh climate environments represent a new source of plant growth promoting (PGP) bacteria that can be used to improve crop resilience to climate change. However, the diversity and functionality of endophytic bacteria associated with endemic wild legumes in Tunisia are almost unknown. To study the taxonomic and functional diversity of these bacteria, we conducted a study on root nodules of 15 spontaneous legumes, some of which studied for the first time, growing in three different Tunisian bioclimates (arid, semi-arid and Saharan). More than 210 strains, corresponding to 78 different phylotypes, were isolated. Sequencing of the 16S rDNA revealed the presence of rhizobial isolates belonging to the genera: *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Neorhizobium*, *Agrobacterium*, *Phyllobacterium*, *Cupriavidus* and *Burkholderia*. Other non-rhizobial bacteria assigned to *Microbacterium*, *Pseudomonas*, *Paenibacillus*, *Starkeya*, *Kocuria* among others, were also obtained. In addition, the nucleotide similarity of housekeeping genes suggested the presence of new species in our collection. Indeed, the genomic sequencing analysis identified a new species of *Mesorhizobium* (PRJNA800673), a microsymbiont of *Retama raetam*, which is one of the most important Tunisian shrubs. Regarding PGP-screening abilities, several strains stood out for possessing several PGP traits, phosphate solubilization, indole acetic acid and siderophores production, along with high tolerance to abiotic stress (> 45°C; > 0.4M NaCl). For rhizobia, whole genome sequencing of the Saharan isolate *Sinorhizobium meliloti* IRAM:0087 (PRJNA842649) revealed the existence of several gene clusters coding for different PGP activities, rhizosphere competitiveness and stress tolerance, some of which were confirmed *in vitro* assays. Based on the evaluation of the PGP potential of non-rhizobial strains, three bacteria *P. salinitolerans*, *P. turukhanskensis* and *Starkeya* sp. were selected to improve *Pisum sativum*, a cold season crop, tolerance to a heat stress event. Our preliminary results suggest that endophytic isolates from arid legumes represent a promising resource of biofertilizers/biostimulants to increase plant resilience to heat stress. Future studies to explore the adaptative responses of these endophytes to different types of stress will allow to better understand their stress tolerance mechanisms and contribute to select strains for nature-based solutions towards the establishment of new agricultural technologies in drylands.

This work was carried out within the framework of the PhD mobility grant supported by the University of Gabes (FSG) and the Ministry of Higher Education and Scientific Research of Tunisia, and the Tunisian-South African project AFRITRUF.

CB acknowledges a CEECIND2018 contract (CEEIND/00093/2018) from FCT.

HSI modelling derived from GPS tracking data, shows spatial variability and significant historic suitability loss in a declining grassland bird

Tiago Mendes¹, Francesco Valerio², Ana Teresa Marques², Ricardo Pita¹, Sérgio Godinho³, João Paulo Silva²

¹Unidade de Biologia da Conservação, 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.

² CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-601 Vairão, Portugal

³ Institute of Earth Sciences (ICT), Universidade de Évora, Rua Romão Ramalho, 59, 7002-554, Évora, Portugal

Email: d46173@alunos.uevora.pt

Classified as vulnerable in Europe, the little bustard (*Tetrax tetrax*) has faced a continuous population decline in recent decades. The main objective of this work was to model the species current and historical distribution, understanding the factors that caused its decline. To achieve this, a high resolution (250m) Species Distribution Model (SDM) was developed for the region of Extremadura (Spain) for each of the phenological phases of the species (winter, breeding, post-breeding), which were later projected for the period between 2005 and 2021. This allowed a precise understanding of its distribution at a spatial (within the year) and temporal (between years) level. Presence data was used as the response variable and was obtained from a GPS telemetry dataset of 60 individuals captured in Extremadura and Alentejo between 2009 and 2020. Remote sensing data and topographical variables were used as explanatory variables. A variant of Random Forests (Boruta) was employed as the algorithm for the SDMs. At the spatial level, the SDM revealed a high similarity of suitability between the winter and breeding phases, with the most suitable areas being found mostly within the Special Protection Areas (SPAs). Conversely, in the post-breeding phase, the most suitable areas were found outside the SPAs. Although the average suitability values are higher within the SPAs, mainly in the winter and breeding phases, most of the suitable area for the species, both in the breeding and post-breeding phases, is outside the SPAs. At a temporal level, between 2005 and 2021 there were large fluctuations in the availability of suitable habitat, mainly in the breeding and post-breeding phases. In general, during this period there were gains in the area suitable for the species in the winter phase and losses in the breeding (-24%) and post-breeding (-15%) phases, the last two being the most critical phenological phases for the species. This study contributes to identify possible factors that impacted the species and highlights the need to rethink the management of protected areas as well as the need to include external areas in conservation actions, emphasizing the usefulness of the SDM developed as a management support tool.

TM was funded by FCT doctoral grant SFRH/BD/145156/2019.

Rewilding and rewiring animal-plant interactions in road verges: measuring the provision and regulation of ecosystem services in a Green Infrastructure

C. Silva¹, S. Santos², N. M. Pedroso³, A. Belo⁴

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

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

³MED (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.

⁴MED (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: carmoms@uevora.pt

Road and highways verges represent a significant extent of territory that remains generally unused or are usually managed for safety-only purposes. Although roads are a major cause of habitat loss, fragmentation and degradation, verges form a network of potential habitats benefiting species as a refuge or for dispersing events. Consequently, the conditions offered by road verges, expressed by the structure of the vegetation and the availability of resources, can largely determine ecosystem service (ES) provision. The extent to which the trade-offs between road impacts, landscape context and verge conditions offset the rewiring of the networks of animal-mediated processes remains largely unknown. The main goal of this work is to evaluate the potential of road verges to provide and regulate ES involving plant-animal interactions, while determined by the management of vegetation strips, and framed within the surrounding landscape. Specifically, we aim to explore ES based on two types of plant-animal interactions: plant-seed disperser mediated by carnivore mammals and plant-pollinator spill-over into the surrounding areas.

The study area will be embedded within Central Alentejo (Évora district), in Southern Portugal. Seed dispersal mediated by carnivore mammals will involve the placement of feeding stations inside and apart on both sides of the road verges. Roads, road verges, dirt roads and cattle paths will be afterwards census to find carnivore scats. Spill-over effects on pollination into the matrix and road-crossing exchanges will be measured with the application of a unique fluorescent dye particles as pollen analogues on flowers of both road verges. After identifying road verge stretches that can be managed to provide ES into the surrounding landscape, the projection of ES will be modelled to the Central Alentejo region considering the characteristics of the roads and verges, and the surrounding landscape context, as provided by remote sensing data. The resulting outputs intend to provide a tool for managers and policy-makers by identifying spatially the potential of each verge to deliver ES, and thus allowing efficient management practices or territorial planning to be employed in order to promote it.

CS is funded by National Funds through FCT under the PhD scholarship 2021.08556.BD.

Natural Remnant Habitats – a key for biodiversity conservation in Montado agroecosystems

Erika Almeida¹, João Carlos Azevedo², Gabriela Gomes³, Cláudia Lima³, Carla Pinto-Cruz⁴

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

²CIMO-Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5300-253 Bragança, Portugal

³Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

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

Email: erikaroldao@gmail.com

The Portuguese Montado, known for its high levels of biodiversity, is under threat by land-use changes (intensification or abandonment) resulting in strong habitat loss and fragmentation, resulting in loss of biodiversity. This way, the presence of natural habitats patches and corridors, with distinct ecological conditions from the matrix (natural remnant habitats – NRH), are determinant for maintaining the structural and functional diversity of the Montado landscape. In this perspective, this PhD thesis aims to identify and quantify the ecological value of NRH through the establishment of a biodiversity evaluation system based on biological importance, habitat suitability for fauna species and habitat connectivity. This will be achieved through: identification of different NRH in the field (small forest and shrub patches, rocky outcrops, temporary/ permanent ponds, and riparian gallery, with a minimum of 30 sample units by NRH typology); identification of its specificity and conservation status based on typical plant and lichens species; analysis of the relationship between vegetation data and different management-related variables; analysis of selected fauna species preferences for NRH with different characteristics; identification of minimal optimal density and compositional diversity of NRH in montado through structural and functional connectivity analysis; integration of the results into a final ecological value for each NRH. The study area includes several estates in Central Alentejo (southern Portugal), in Montado areas held by different landowners. With this approach we expect to contribute to the conservation, restoration, and re-creation of NRH.

This presentation will address data collected until November 2022, namely the selection of the study areas (estates, n=17), field identification of NRH (so far n= 170) and field data collection regarding flora and vegetation (species identified so far: plants – n=260, from more than 1000 specimens; lichens – n=50 species, from more than 400 specimens). Also, under this PhD a final course project (graduation in Biology) has been conducted regarding some of the flora and vegetation data, a Master's degree is ongoing regarding amphibians' preferences for HNR, and two other Master's topics also addressing fauna have been proposed.

Biology and Biochemistry

This thesis is funded by National Funds through FCT under the PhD scholarship 2020.07109.BD. and by FCT – Foundation for Science and Technology under the Project UIDB/05183/2020.

Soil fungal community structure is shaped mostly by edaphic properties and cultivated crops in the subtropical and temperate agroecosystems

M.C.O. Oliveira^{1,3*}, M.Â.A. Pinheiro de Carvalho^{1,2}, A. Alves³

¹ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira (UMa)

²Research Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB).

³CESAM – Centre for Environmental and Marine Studies, Biology Department, University of Aveiro

*Cristina Oliveira, mcooliveira@ua.pt

Agricultural soils are responsible for ecological functions and services that include primary production of food, fibre and fuel, nutrient cycling, carbon cycling and storage, water infiltration and purification, among others. Soil organisms are drivers of most of those ecosystem services. Fungi, due to their ability to produce a wide variety of extracellular enzymes, are able to decompose all kinds of organic matter regulating the balance of carbon and nutrients. Besides, they can act as biocontrol agents, for example they can be antagonist of other organisms that are pests or cause diseases, and can interact either positively or negatively with plants roots or above ground plant components. Exploring the community composition and diversity of soil fungi in agroecosystems has important implications for understanding how to manage the soil in a sustainable way. In this study, we analysed, through ITS amplicon metagenomic approach, soil fungal communities from 18 agroecosystems: 6 vineyards, 6 banana plantations and 6 horticulture plantations. We hypothesised that the structure of soil fungal communities is predominantly determined by management practices and edaphic properties. Results showed significant correlations between diversity indices (Richness, Shannon-Wiener, Evenness and Simpson) and soil parameters, mainly the degree of saturation, pH and calcium. The management practices didn't influence the diversity of fungal communities, but the cultivated crops had an impact on the most abundant genera found in the agroecosystems. The pairwise ANOSIM R based on weighted UniFrac distance metric showed significant differences among soil fungal composition of the three types of agroecosystems. Thus, we can conclude that soil fungal community diversity is mainly shaped by edaphic properties, but the composition also depends on the type of cultivated crops.

Acknowledgements:

PhD grant supported by the Agência Regional para o Desenvolvimento da Investigação, Tecnologia e Inovação, Portugal 2020 and the European Union through the European Social Fund [M1420-09-5369-FSE000002, ARDITI]. Field trips and analysis were also supported by Programa Operacional Madeira 14–20, Portugal 2020, and the European Union through the European Regional Development Fund, grant number M1420-01-0145-FEDER-000011 [CASBio], and by Interreg MAC 2014-2020 Cooperation Program, grant number MAC2/3.5b/307 [VERCOCHAR].

Alagoas (Brazil) red propolis antimicrobial and anticariogenic potential

Rosana S. Tajra^{1,*}, Elsa Lamy¹, M. Ramiro Pastorinho², Marizia D. Pereira³, F. Capela e Silva¹

¹ 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.

² Department of Medical and Health Sciences, School of Health and Human Development & CHRC-Comprehensive Health Research Center, University of Évora, 7002-554 Évora, Portugal.

³ Department of Landscape, Environment and Planning. MED - Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento (Membro Colaborador).

* Email: rosanastajra@gmail.com

Caries is a public health problem, representing the most important and prevalent oral health problem in Brazil, being the most studied oral disease worldwide. This disease has a multifactorial etiology, manifesting itself as enamel demineralization. The ingestion of diets rich in carbohydrates and with cariogenic potential appears as a determining element in the process associated with the onset of carious lesions. This is mainly due to the installation of microorganisms associated with the development of caries, with emphasis on the importance of *Streptococcus mutans*, resulting in decreases in salivary pH. Propolis is a mixture of resinous substances from various plants and beeswax produced by honey bees (*Apis mellifera*) and other bee species, which leads to variable chemical composition, according to the geographic region of the world where it is being produced. Despite this variability, several similar biological activities are reported in the literature, such as antimicrobial, anticariogenic, cytotoxic, anti-inflammatory, immunomodulatory, antioxidant and antitumor, which lead to its use by mankind since antiquity (~300 BC) in mummification rituals, popular medicine and, more recently, in the food and beverage industry, cosmetics, mouthwashes, and dentifrices. A new type of propolis from the mangrove region of the State of Alagoas, red in color, had its botanical origin identified as *Dalbergia ecastophyllum*, being classified as the 13th type of Brazilian propolis and has demonstrated several biological activities in *in vitro* assays. Recent studies have shown that Brazilian red propolis has high antibacterial activity against *Staphylococcus aureus* (similarly to *S. mutans*, also a gram-positive bacteria) and high antioxidant activity. The objective of this work is to analyze the antimicrobial property of red propolis and its anticariogenic capacity. The effects of its use on saliva composition and possible secondary interactions will also be evaluated.

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

Biochemical fight between bacterial strains and the plant pathogenic fungi *Magnaportheopsis maydis*

Matos, D.¹; Cardoso, P.¹; Molinero-Ruiz, L.²; Figueira, E¹

¹Department of Biology & CESAM-Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal;

²Institute for Sustainable Agriculture, Av. Menéndez Pidal, 14004 Córdoba, Spain.

Email: dianarmatos@ua.pt

Fungi constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases. These diseases are a major threat to crop production and cause severe economic losses.

Magnaportheopsis maydis causes late wilt in maize plants, which can lead to severe economic losses. Maize grain production has increased more than eight-fold in the past century, and by 2050, it is estimated that it will contribute more than half of the increased demand for cereals. So a disease with such a high influence in productivity will have a huge impact in food security and will be an obstacle to meet the need of food demand.

The fungus infects the roots at an early stage, but wilt symptoms usually develop when the plants approach the flowering stage. Late wilt is frequently associated with infection by secondary plant parasitic fungi causing the stem symptoms to become more severe and even higher losses in crop production.

There is an urgency in effective alternatives to mitigate the effects of late wilt and biocontrol emerges as an environmental sustainable alternative. This approach was followed in the present study, by isolating bacteria from the roots of symptomatic plants with different degrees of infection. The antifungal ability of bacterial isolates delivered 8 strains able to inhibit the fungus over 50% and some of them were able to work not only against *M. maydis* but also against *Fusarium spp.*. Additionally, some of these bacteria were also able to promote plant growth and fitness. So it is of great interest to understand what are the biochemical alterations that these bacteria are able to induce in the fungus, but also what alterations the fungus induce in these bacterial strains.

With the aim to potentiate of developing a successful methodology to minimize the effects of the fungi, the most promising bacterial isolates can be tested in different combinations helping to reduce the effects of the disease and to reduce the economic losses.

References

Degani, O., Chen, A., Dor, S. *et al.* (2022) Remote evaluation of maize cultivars susceptibility to late wilt disease caused by *Magnaportheopsis maydis*. *J Plant Pathol* **104**, 509–525. <https://doi.org/10.1007/s42161-022-01039-9>

Dor, Degani (2019) Uncovering the Host Range for Maize Pathogen *Magnaportheopsis maydis*. *Plants* **8**, 259. <https://doi.org/10.3390/plants8080259>

Ortiz-Bustos, C.M., López-Bernal, A., Testi, L., Molinero-Ruiz, L. (2019) Environmental and irrigation conditions can mask the effect of *Magnaportheopsis maydis* on growth and productivity of maize. *Plant Pathol.* **68**, 1555–1564. <https://doi.org/10.1111/ppa.13070>

This research was funded by FCT through the financial support to the H₂O value project (PTDC/NAN-MAT/30513/2017) also supported by FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership agreement and Compete 2020 (CENTRO-01-0145-FEDER-030513). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020) through national funds.

Development of new *Vitis vinifera* genotypes resistant to powdery and downy mildew – a Portuguese breeding program

J. Costa¹, M. Silvério¹, J. Böhm², L. Rodrigues, C. Campos, D. Tavares², A. Peixe³, H. Cardoso⁴

¹Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²Viveiros PLANSEL Lda., Apartado 2, 7050-909 Montemor-o-Novo.

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

⁴MED—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.

Email: hcardoso@uevora.pt

The grapevine (*Vitis vinifera*) is one of the most important fruit crops worldwide. Their cultivars are highly susceptible to pathogens such as *Plasmopora viticola* and *Erysiphe necator*, which cause the fungal diseases respectively known as downy and powdery mildew. Those pathogens were introduced in Europe in the XIX century and their control is still made nowadays by the use of fungicides. This situation besides having a high economic impact, also present a negative effect on the environment and public health, being mandatory to develop new resistant genotypes contributing for a safer and more sustainable viticulture. The development of resistant genotypes following conventional breeding programs, based on hybrids selection resulting from crosses between resistant genotypes (American and Asiatic *Vitis* species) and *V. vinifera* elite cultivars, has been the approach followed by plant breeders in several wine producing countries. This approach already contributed to the development of new genotypes being registered as *V. vinifera*, exhibiting high levels of fungal resistance, due to the introgression of different resistance *loci*, and presenting good wine quality. In Portugal, resulting from the collaboration of the Viveiros PLANSEL Lda. company (Montemor-o-Novo, Portugal) and the University of Évora, a breeding program based on this strategy is currently running. Different Portuguese elite cultivars have been considered on controlled crosses with resistant hybrid genotypes (pollen gently provided by Dr. Oliver Trapp from the Julius Kühn-Institut (JKI) – Germany). Molecular markers (SSR – Simple Sequence Repeats), associated with resistance *loci* were used at the University of Évora, aiming on efficiently select the hybrid plants exhibiting the introgression of a highest number of resistance *loci*. The results from this evaluation will now be presented. Fragment analysis revealed that all plants analysed were hybrids, confirming the efficiency of the procedure followed for pollination. The introgression of the *loci* was higher when the resistant genotype was used as the female progenitor. The simultaneous presence of three *loci* on some hybrid plants, lead to the selectin of 15 genotypes as progenitors for further crosses in this breeding program.

This research was funded by National Funds through the Projeto PDR2020-784-042746 “Programa de conservação e melhoramento genético da videira” and FCT - Foundation for Science and Technology under the Project UIDB/05183/2020 and the fellowship given to J. Costa under the program “Verão com Ciência – MED2022”.

Exploring the relationship between the salivary Amylase (AMY1) Gene Copy Number and the levels of α -amylase in saliva in response to sucrose stimulation

Carla Simões¹, Inês Caeiro¹, David Guedes², Hélia Cardoso¹, Elsa Lamy¹

¹MED - University of Évora; Pólo da Mitra, 7006-554 Évora, Portugal

²ISCTE - University Institute of Lisbon, Avenida das Forças Armadas, 1649-026 Lisboa, Portugal

E-mail: d51413@alunos.uevora.pt

Salivary α -amylase is one of the most abundant proteins in saliva and it is responsible for initiating starch digestion in the mouth^{1,2,3}. The concentration and activity of this enzyme in saliva varies greatly among individuals, and in the same individual, depending on several factors^{2,4}, including food sensory stimulation⁵. Although salivary α -amylase has been observed to be associated with sweet taste (e.g.,^{6,7}), the way this salivary enzyme changes in response to sweet stimulation is not clear. Besides the type of stimulation, the genetic background of an individual's may also be a source of variations in the levels of amylase present in saliva. The *AMY1* gene is one of the genes in the human genome, with higher copy number variation with a reported range from 1 to 18 copies^{3,1,8}. These differences have been linked, by some authors, to body mass index⁹, glucose homeostasis¹⁰ and consequently different overall nutritional status³. The fact that an individual has a greater number of copies of the *AMY1* gene suggests that one may present a higher level of this protein, at least when subjected to stimulation. However, to our knowledge, this direct association has been little explored.

The objective of the present work is to investigate whether sweet stimulation, with sucrose, induces variations in the levels of the protein α -amylase in saliva and if the number of the copies of the *AMY1* gene influences this potential effect. To achieve this, saliva samples from 20 adults, both sexes (18 to 65 years old) was collected before and immediately after 3 times stimulation with 8 concentrations of sucrose (0.55 – 20.00g/L). After the extraction of the DNA genomic from the saliva samples the number of copies of the *AMY1* gene was estimated by quantitative real-time PCR (qPCR). For saliva protein analysis, enzymatic activity was determined using p-nitrophenol as substrate and α -amylase relative quantification through Western blot. Main results will be presented and discussed.

This work was funded by National Funds through FCT under the PhD fellowships with the reference numbers 2021.06485.BD and SFRH/BD/145929/2019.

References

1. Abigail L., P. A. S. M., Catherine, P. des G., Kimberly L., P., Suzanne, A. & Breslin. Individual differences in *AMY1* gene copy number, salivary α -amylase levels, and the perception of oral starch. Preprint at <https://doi.org/10.1371/journal.pone.0013352> (2010).
2. Carpenter, D., Mitchell, L. M. & Armour, J. A. L. Copy number variation of human *AMY1* is a minor contributor to variation in salivary amylase expression and activity. *Hum Genomics* **11**, 1–6 (2017).

3. Yang, Z. M. *et al.* The roles of AMY1 copies and protein expression in human salivary α -amylase activity. *Physiology and Behavior* vol. 138 173–178 Preprint at <https://doi.org/10.1016/j.physbeh.2014.10.037> (2015).
4. Yang, Z. *et al.* Influences of AMY1 gene copy number and protein expression on salivary alpha-amylase activity before and after citric acid stimulation in splenic asthenia children. *Journal of traditional Chinese medicine = Chung i tsa chih ying wen pan / sponsored by All-China Association of Traditional Chinese Medicine, Academy of Traditional Chinese Medicine* **35**, 266–272 (2015).
5. Carreira, L., Castelo, P. M., Simões, C. & Capela, F. Changes in Salivary Proteome in Response to Bread Odour. 1–18 (2020) doi:10.3390/nu12041002.
6. Rodrigues, L. *et al.* Salivary proteome and glucose levels are related with sweet taste sensitivity in young adults. *Food Nutr Res* **61**, 1389208 (2017).
7. Lamy, E. *et al.* Saliva Protein Composition Relates with Interindividual Variations in Bread Sensory Ratings. *Starch - Stärke* **n/a**, 2000052 (2020).
8. Pinho, S., Padez, C. & Manco, L. High AMY1 copy number protects against obesity in Portuguese young adults. *Ann Hum Biol* **45**, 435–439 (2018).
9. Marcovecchio, M. L. *et al.* Low AMY1 gene copy number is associated with increased body mass index in prepubertal boys. *PLoS One* **11**, 1–9 (2016).
10. Farrell, M. *et al.* Effect of AMY1 copy number variation and various doses of starch intake on glucose homeostasis: data from a cross-sectional observational study and a crossover meal study. *Genes Nutr* **16**, (2021).

Forward-looking on new microbial consortia: combination of rot fungi and rhizobacteria on plant growth-promoting abilities

Bruno Carneiro ¹, Paulo Cardoso ^{1,2}, Etelvina Figueira ^{1,2}, Isabel Lopes ^{1,2}, Cátia Venancio ^{1,2}

¹Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

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

Email: catiavenancio@gmail.com

With the world population constantly growing, the need for food will increase over time. Yet, climate change-induced threats to agricultural activities undermine food security. Abiotic stressors like drought and salinity, are increasing every year the amount of degraded soil all over the world, rendering those soils incapable of being used for crop production. The use of microbes, like rot fungi and rhizobacteria, have the potential to act as inducers of climate-resilient soils, and to increase crop productivity and health, providing plants with protective effects in drought and salinity contexts, that usually take a large toll in the efforts to grow crops at profitable yields. Here a consortium of a rhizobacteria and a fungus is proposed. This work highlights how their combination may achieve higher positive effects by combining the plant growth-promoting abilities of both organisms, recycling, mobilization of nutrients, the production of phytohormones, nitrogen fixation and the restoration of soil physicochemical properties. Gains include the reduction in the application of chemical fertilizers, which can higher autonomy from fertilizer exporting-countries and the reduction of carbon footprints by decreasing energy expenses for fertilizer production and transportation. Another feature potentially related with this consortium is helping with climate-change-mitigation by promoting carbon storage in the soil. It is also envisaged their use in the overall improvement of crop productivity. The ameliorating effect on soil properties and functions enable the recovery of damaged soil and the amendment in its general performance. In addition, the impacts of biotic and abiotic stresses may be reduced, and soil-borne plant pathogens may be controlled by the application of this consortium. Considering all the benefits mentioned above, this type of consortium can be a driver to, not only promote global raise in food production, but on a smaller scale, promote local and regional development, with increased income for small farmers.

CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) received financial support from FCT/MEC through national funds (PIDDAC), and co-funding by FEDER (POCI-01-0145-FEDER-00763) within the PT2020 Partnership Agreement and Compete 2020. This work was also financially supported by the FCT projects SALTFREE (PTDC/AAC-CLI/111706/2009) and SALTFREE II (POCI-01-0145- FEDER-031022) and by LabEx DRIIHM—Dispositif de Recherche Interdisciplinaire sur les Interactions Hommes-Milieus and OHMI—Observatoire Hommes-Millieux International Estarreja through funding the project “CLEAR - Resorting to microbial Consortia to restore metal contaminated soils for the area of EstArReja”.

Harnessing soil microbiota: ameliorative effect of rot fungi exudates application in mildly salinized soils

João Borges¹, Isabel Lopes², Paulo Cardoso², Etelvina Figueira², Cátia Venâncio²

¹Chemistry Department, University of Aveiro, 3810-193, Aveiro, Portugal

²CESAM & Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal.

Email: catiavenancio@gmail.com

Soil salinization has a significant impact on crop revenues, which may be counteracted through the exploitation of the soil microbiota and the potential that its biodiversity harbours.

Therefore, this work was developed with the main objective of exploring the application of the rich-polysaccharide fungi exudates of two white rot fungi (*Trametes versicolor* and *Pleurotus sajor caju*) and its ameliorative potential regarding the salinity-induced stresses on the performance of *Lens culinaris*. For that, a 96-h cross-design experiment was sought using two different soils (a silvopastoral agroforestry soil and LUFA 2.2), three salinity levels (8, 16, and 26 mS.cm⁻¹), and three levels of exudates (0%, 1%, 6%, and 12%) from each fungal species. Seed germination was checked daily, and by the end of the exposure period (96-h), seedlings radicle and hypocotyl were measured, and plantules fresh and dry weights determined, as well as the soils phosphatases activity.

The application of each fungus exudates in non-saline conditions did not evidence effects on the germination and seedling development of *L. culinaris*. Salinity alone, induced a dose-effect response in all *L. culinaris* endpoints, confirming that conductivities above 8 mS.cm⁻¹ are detrimental for many crop species. The application of fungi exudates on salinized soils, improved germination rates at the salinity levels of 16 and 26mS.cm⁻¹ comparatively to the respective salinity treatment without fungi exudates, but mainly in assays carried out in natural soil, with simultaneous increase of the radicle and hypocotyl lengths of the seedlings. Furthermore, under the same conditions (26 mS.cm⁻¹ and 1 to 12% fungi exudates), there was an increase on the natural soil acid phosphatases activity. These results demonstrate that the application of rot fungi exudates and consequently its efficacy on the amelioration of salinized soils is dependent on the type of soil, nonetheless, demonstrating potential as a sustainable measure to reclaim and restore salinized soils, as well as its productivity, accordingly to the current soil protection policies.

CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/ 0094/2020) received financial support from FCT/MEC through national funds (PIDDAC), and co-funding by FEDER (POCI-01-0145-FEDER-00763) within the PT2020 Partnership Agreement and Compete 2020. This work was also financially supported by the FCT projects SALTFREE (PTDC/AAC-CLI/111706/2009) and SALTFREE II (POCI-01-0145- FEDER-031022).

Graphene oxide influence in soil bacteria is dose dependent and changes at osmotic stress: growth variation, oxidative damage, antioxidant response and plant growth promotion traits of a Rhizobium strain

Lopes, T¹; Cardoso, P²; Matos, D²; Rocha, R¹; Pires, A²; Marques, P³; Figueira, E²

¹Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

²Department of Biology & CESAM-Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

³Department of Mechanics & TEMA-Centre for Mechanical Technology and Automation, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

Email: tslopes@ua.pt

Climate change events, such as drought, are increasing and soil bacteria can be severely affected. Moreover, the accumulation of emerging pollutants are expected to rapidly increase, and their impact on soil organisms, their interactions, and the services they provide is poorly known. The use of graphene oxide (GO) has been increasing due to their enormous potential for application in several areas and it is expected that concentration in soil will increase in the future, potentially causing disturbances in soil microorganisms not yet identified. Here we show the effects that GO nanosheets can cause on soil bacteria, in particular those that promote plant growth, in control and 10% polyethylene glycol (PEG) conditions. Low concentrations of GO nanosheets did not affect the growth of Rhizobium strain E20-8, but under osmotic stress (PEG) GO decreased bacterial growth even at lower concentrations. GO caused oxidative stress, with antioxidant mechanisms being induced to restrain damage, effectively at lower concentrations, but less effectively at higher concentrations, and oxidative damage overcame. Under osmotic stress, alginate and glycine betaine osmoregulated the bacteria. Simultaneous exposure to PEG and GO induced oxidative damage. Plant growth promotion traits (indole acetic acid and siderophores production) were increased by osmotic stress and GO did not disturb these abilities. In a context of climate change our findings might be relevant as they can form the premises for the implementation of crop production methodologies adapted to the new prevailing conditions, that include the presence of nanoparticles in the soil and more frequent and severe drought.

This research was funded by FCT through the financial support to the H2O value project (PTDC/NAN-MAT/30513/2017) also supported by FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership agreement and Compete 2020 (CENTRO-01-0145-FEDER-030513). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020) through national funds.

How parasitism genes are regulated: a *motif* to search for genes regulators in the plant parasitic nematode *Bursaphelenchus xylophilus*

Madalena Mendonça¹, Cláudia Vicente^{1,2}, Pedro Barbosa¹, Isabel Velada¹, Paulo Vieira³, Jorge Faria², Margarida Santos², Filomena Nóbrega², Sebastian Eves-van den Akker⁴, Maria de Lurdes Inácio², Manuel Mota¹, Margarida Espada¹

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

² INIAV – Instituto Nacional de Investigação Agrária e Veterinária, Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal.

³ United States Department of Agriculture (USDA), Agricultural Research Services (ARS), Mycology & Nematology Genetic Diversity & Biology Lab, Beltsville, MD 20705, USA.

⁴ Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom.

Email: maria.silva@uevora.pt

Plant-parasitic nematodes threaten global agricultural and forestry systems. The search for new control strategies in line with the EU's sustainability goals highlight significant knowledge gaps. Like all other plant pathogens, plant-parasitic nematodes deliver several parasitism proteins (effectors) into the host plant to cause disease. NemaWAARS project focuses on mechanism(s) of regulation and gene control expression of parasitism genes in pinewood nematode, *Bursaphelenchus xylophilus*. From the previous transcriptomic data derived from the pharyngeal gland cells (considered a specialized tissue potentially related to parasitism) we have identified a non-coding DNA motif - STATAWAARS - associated in the promotor region of highly abundant and secreted expressed genes. Given that this non-coding genetic signature unifies many sequences of unrelated parasitism genes, it implies the existence of a potential major regulator(s), that binds to this sequence to control the expression of downstream genes. We hypothesize that by disrupting this regulator(s), it would be possible to simultaneously disrupt the expression of many associated parasitism-related genes. To test the hypothesis the project aims to identify proteins (or complex of proteins) that bind in the promoter regions of parasitism-related genes (*in vivo*) or identify other regulatory candidates for master regulators of parasitism-related genes expression that are enriched in the pharyngeal gland cell tissues. For the best candidate regulatory proteins, an RNAi approach will target the selected gene candidates and evaluate the regulatory role in effector genes expression and in interaction with the host (*in planta*). Under an ongoing national and international collaborative network, the strategy in NemaWAARS will include innovative approaches to explore the regulators that govern effector gene expression applied in *B. xylophilus* research.

Funding NemaWAARS project is funded by National Funds through FCT - Foundation for Science and Technology under the Project PTDC/ASP-PLA/1108/2021 and CEEC'18 for M. Espada (CEECIND/00066/2018) and C. Vicente.

Mediterranean temporary pond restoration increases bat species richness but not bat activity

J. Ribeiro-Silva¹, C. Pinto-Cruz^{1,2}, J. T. Marques¹

¹MED (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.

²MED (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, 7002-554 Évora, Portugal.

Email: joanasilva.ctx@gmail.com

Climate change scenarios predict that in the next years a continuous decrease in rainfall is expected across the Mediterranean regions. This concern, combined with the ongoing destruction, modification, and drainage of wetland habitats, increases the risk of water scarcity on the landscape. Ponds are vulnerable aquatic habitats that supply several ecosystem services and provide foraging and drinking resources to bats. To enhance the integrity of degraded habitats, restoration actions have been widely implemented, demonstrating positive effects for bat conservation. However, few studies showed long-term results in aquatic habitats, and none was carried out in Mediterranean temporary ponds. Here, we will present an assessment of the responses of bat activity and species richness to the restoration of temporary ponds. The restoration actions applied here were the pond digging or deepening aimed at re-establishing the terrain depression to increase the water holding capacity. To assess this impact, we monitored the bat acoustic activity for five years (three before and two after the restoration actions) in 15 ponds (7 restored and 8 control ponds) along the southwestern coast of Portugal. Results from the Before-After-Control-Impact designs (BACI) analysis showed an impact of restoration on species richness: there was 1-2 more species in the intervened ponds after restoration actions. In contrast, bat activity increased only slightly over restored ponds, deeming a non-significant change. Therefore, the restoration of mediterranean temporary ponds has direct positive impacts on the bat community despite the ability of bats to use the wider landscape.

This work was funded by National Funds through FCT under the Project LIFE12NAT/PT/997 J. R-S. acknowledges FCT (2021.04651.BD).

More is better: higher numbers of bat species and individuals increase suppression of PPM

A.M. Augusto¹, H. Rebelo², D. Russo³, O. Bina¹

¹ICS (Instituto de Ciências Sociais), Universidade de Lisboa, Av. Professor Aníbal de Bettencourt, 9, 1600-189 Lisboa, Portugal.

²CIBIO/InBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto), Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão, Portugal.

³WRU (Wildlife Research Unit, Laboratorio di Ecologia Applicata, Sezione di Biologia e Protezione dei Sistemi Agrari e Forestali, Dipartimento di Agraria), Università degli Studi di Napoli Federico II, via Università 100, I-80055 Portici, Napoli, Italy.

Email: ana.margarida.santos@ics.ulisboa.pt

Coniferous forests make a significant contribution to Portuguese GNP, yet they have been suffering severe losses since the late 1990s due to the plague of the invasive pine processionary moth (PPM). The fight against the PPM consists essentially of using pesticides, whose excessive use has several negative impacts on public health and the environment. The risks associated with pests and the impacts of climate change tend to exacerbate the gap in the potential of forest exploitation. It is necessary to develop a support tool for forest management to promote the action of natural agents in pest control. Bats are voracious predators of insects, eating half their weight in prey every day. The main goal of this study was to evaluate which bats are providing a processionary suppression service to pine stands and how that pest consumption is affected by bat community composition and abundance. Mountain environments such as Serra da Estrela, provide a great variability of environmental conditions, thus allowing to sample bat species with different biogeographic affinities. Moreover, the steep variation in environmental conditions will also cover the ecological niche of PPM. The field work included a sampling scheme and mist-net effort. The laboratory methodology combines metabarcoding data and bat food-web analyses. GLMs analysis was applied to evaluate the relationship of a) bat diversity, b) bat relative abundance and c) bat diet diversity with the frequency of PPM consumption. This analysis shows that none of the evaluated parameters was significantly influenced by the year of sampling. In all analysed models the signal tendency was positive, meaning that higher the number of a) bat species, b) bat relative abundance and c) bat diet diversity, the higher the PPM consumption. Overall, this research show that the intensity of the PPM consumption is independent of the diversity of insects in that place but benefiting from a diverse and abundant bat community. This study contributes to a better understanding of the pest suppression services by bats at pine stands and contribute as a basis for future studies. The aim is to produce maps and guidelines that may improve the resilience of these services considering predicted climate change.

This work was funded by National Funds through FCT under the Project BatPine AAC/02/SAICT/2017/31731. A.M.A. acknowledges FCT (PD/BD/150555/2019).

Obesogenic potential of consumer products in *Daphnia magna* - preliminary results

S. Coelho^{1,2,3,4}, I. Lopes⁴, M. R. Pastorinho^{2,5}, A. C. A. Sousa^{1,2}

¹Department of Biology, University of Évora, Évora, Portugal;

²Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal;

³NuESA-Health and Environment Study Unit, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal;

⁴Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Aveiro, Portugal;

⁵Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal

Email: d51244@uevora.alunos.pt

Obesogenic compounds are environmental contaminants that induce lipid accumulation and/or overweight in *in vitro* and *in vivo* models. More than 50 compounds with obesogenic potential have already been described, including tributyltin, metals, pesticides, and plasticizers. However, data regarding exposures under realistic scenarios (particularly for mixtures) is still scarce. These scenarios include, for example, chronic exposure or exposure to complex mixtures. Bearing in mind such limitations, this study aims to evaluate the impact of long-term exposures to potential obesogens present in consumer products using the model test species *Daphnia magna*. This aquatic invertebrate, recognized by regulatory agencies as a standard organism for ecotoxicological testing, due to the establishment of protocols by international standardization bodies (i.e., OECD, ASTM, and ISO), is relatively easy to maintain in the laboratory. Due to its short life cycle and parthenogenic reproduction, it allows studying of the impact of long-term exposures over a short period of time. Furthermore, daphnids have a transparent body allowing the easy identification of lipid accumulation (upon application of 106 appropriate staining techniques).

Daphnids were exposed for 48 h and 21 days to the model obesogen tributyltin and to leachates obtained from costume jewelry, to evaluate the acute and chronic toxicity, respectively. After 48 h, immobilization and lipid accumulation were evaluated. At the end of the chronic test, the following endpoints were evaluated: mortality, reproduction, growth, and lipid accumulation.

After 48 h of exposure, the daphnids subjected to the highest concentrations (80%) were all immobilized, demonstrating the high toxicity of the leachate. Concerning lipid accumulation, daphnids exposed to the highest concentration (80%) exhibited higher accumulation of lipids when compared to the model obesogen TBT (ca. 200% increase). After 21 days of exposure, full mortality had occurred, except to the daphnids exposed to the lowest concentration tested (12.5%). Thus, results for growth, reproduction and lipid quantification were only obtained for this concentration. A decrease in reproduction and an increase in lipid accumulation were observed when comparing these animals with those enrolled in the control. Overall, our results demonstrate that both short- and long-term exposures to leachates obtained from consumer products have a deleterious effect on daphnids in terms of immobilization and reproduction, being also responsible for an increased accumulation of lipids.

Acknowledgments: This work was developed under the scope of the Comprehensive Health Research Centre (CHRC) project (UIDP/04923/2020). S. Coelho acknowledges FCT for the PhD fellowship 2021.08027.BD.

Plant growth-promoting traits of maize bacteria isolated from different water regimes

Sá, Carina¹; Figueira, Etelvina¹; Girbés, Carmen²; Cardoso, Paulo¹

¹Department of Biology & CESAM-Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

²Institute of Environmental Assessment and Water Research (IDAEA-CSIC), c/ Jordi Girona 18-24, 08034, Barcelona, Spain.

Email: carinafsa@ua.pt

Alterations in precipitation and soil moisture, along with temperature increase in the past decade, are threatening agricultural productivity in the Iberian Peninsula and rest of the world. Drought is considered a significant abiotic stress in agriculture, due to the high consumption of water require to produce high crop yields. Thus, there is urgent need for sustainable crop production more resilient to water deficit and at the same time economically viable. To accomplish this goal new agricultural methodologies must be explored. Maize is a principal source of food for millions of people worldwide, however, is highly susceptible to drought. Consequently, promoting maize resilience to drought is a significant step towards food security and adaptation to climate change. The use of microorganisms capable of promoting plant growth in drought environments is a potential approach to mitigate crop losses. Plant growth-promoting bacteria help plants survive in stressed environments by promoting faster germination and development. Here we describe the isolation of surface and endophytic bacteria from maize plants roots growing in INOVMIHO-ANPROMIS (Santarém, Portugal) exposed to three different water regimes (100%, 50% and 0% of irrigation) in two different stages of their life cycle (vegetative and reproductive). Plants biochemical status was assessed, to understand if the plants were under stress, and to serve as a basal data for further inoculation studies with specific isolates. Isolates were typed using BOX-PCR to screen for isolates with unique fingerprints. This allowed us to obtain 405 strains. These strains were screened for the ability to tolerate osmotic stress using 15% of polyethylene glycol 6000. The isolates displaying the higher ability to tolerate osmotic stress were tested for bacterial plant-growth promoting traits, namely the ability to produce siderophores, indol-3-acetic acid and phosphate solubilization, in the presence and absence of osmotic stress. The isolation of bacteria from different water availabilities can help to select bacteria effective in the improvement of crop resilience to drought-affected areas, helping in the management of climate change impacts by enhancing plant resilience to drought.

This research was funded by FCT through a PhD scholarship awarded to Carina Sá (2020.05872.BD). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020), through Portuguese national funds. We also thank INOVMIHO-ANPROMIS for the support in the field work.

Relationship between salivary protein profile and symptomatology in xerostomia patients

L. Carreira¹, J. Zamora², J. Nunes², J. Coromina², C. Cordeiro³, E. Lamy⁴

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

²Dry mouth institute, Teknon Hospital, 08022 Spain

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

Email: lrec@uevora.pt

Saliva is a biological fluid with an important role in coating and protecting surfaces of oral cavity, helping in oral digestion and food perception. Patients with diagnosed xerostomia have been described as having a reduced whole saliva flow rate. Besides the reduced salivary flow rate, that can vary among individuals, or even not to exist for some, saliva composition can also occur in these patients. Decreases of lactoferrin, salivary peroxidases, IgA and IgG have been reported. Changes in salivary protein levels may be associated to changes in saliva function of these patients, affecting lubrication and/or protection capacity of saliva. Besides this, xerostomia is also known to affect food consumption, food acceptance and food choices. Since some salivary proteins have been reported as directly related with taste sensitivity (such as the case of cystatins, for bitterness, or amylase, for sweetness), changes in the levels of these proteins in xerostomia patients need to be considered.

Our aim was to investigate the existence of a relationship between salivary protein profile and xerostomia symptomatology. Moreover, the existence of association between xerostomia symptoms and alpha amylase and/or cystatins, both proteins related with sweet and bitter taste, respectively, will be assessed.

Saliva samples were collected, to xerostomia patients, in three different situations: 1) Control - first appointment, before the beginning of treatment; 2) first appointment, immediately after the first acute pilocarpine stimulation; 3) during the treatment (from 3 to 9 months after the begging of treatment) and immediately after acute pilocarpine stimulation. Samples were centrifuged to remove insoluble material. Total protein quantification, alfa-amylase enzymatic activity determination, protein separation (SDS-PAGE) and immunodetection of amylase and cystatins, by western blot, were performed. Salivary parameters were compared among the three different collection periods and both absolute amounts, at each time point, and variation through time, were correlated with symptoms described by patients (e.g. dry mouth, dry lips, swallowing difficulties, taste abnormalities, etc.).

In this work, the results will be presented and critically discussed. Hypothesis about how variations in salivary proteome may affect taste perception and subsequent food acceptance, will be drawn.

This work was funded by National Funds through FCT under research contract CEECIND/04397/2017 to Elsa Lamy and doctoral scholarship (SFRH/BD/06273/2021) to Laura Carreira. This work is funded by National Funds through FCT - Foundation for Science and Technology under the Project UIDB/05183/2020.

RNA-Seq reveals differential expression profiles and functional annotation of genes involved in cynaropicrin synthesis in cardoon

A. Paulino^{1,2*}, R.C. Pires¹, I. Fernandes², J. Santos^{1,4}, T. Brás^{1,4}, D. Rosa^{1,3,4}, O.S. Paulo², M.F. Duarte^{1,4} and L. Marum^{1,4}

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

²cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Computational Biology and Population Genomics Group (CoBiG2), Campo Grande, 1749-016 Lisboa, Portugal

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

⁴Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

*Email: ana.paulino@cebal.pt

Cynara cardunculus L. (Cc) is a versatile crop representing a natural source of sesquiterpene lactones, namely cynaropicrin [1]. Portugal has a great natural variability of this plant at morphological, biochemical, and genetic levels [2]. Previously, great variability in cynaropicrin content was obtained in plants from different genotypes and different collection periods [3]. This work aimed to identify differentially expressed genes (DEGs) in Cc plants with different levels (high *versus* low) of cynaropicrin, to further understand the molecular mechanisms underlying this sesquiterpene lactone synthesis.

For transcriptome analysis, total RNA was successfully extracted from Cc leaves, using RNAqueous™-4PCR Total RNA Isolation Kit (Invitrogen™). cDNA libraries were prepared for transcriptome sequencing. Stranded paired-end sequencing was performed on Illumina Sequencers. The quality analysis of sequencing data was performed using FastQC and MultiQC. The high-quality reads were mapped to the assembly of the Cc reference genome of Cc downloaded from The Global Artichoke Genome Database using STAR. DESeq2 method was used for DEGs analysis. High-quality sequencing generated an average of 24 million reads, of which an average of 91% were mapped to the Cc reference genome. An average of 24 thousand genes were expressed in all plants, either with high or low production of cynaropicrin. Differential expression analysis generated a total of 36 DEGs, with an average of 86% of DEGs being annotated according to the reference genome, which was mainly downregulated. The results suggest that the production of cynaropicrin has a regulation mainly related to environmental factors. However, DEGs involved in stress and genes related to photosynthesis and cellular respiration were also found. We can highlight the *HSP70* and *HSP17.3-B* genes involved in increasing tolerance to abiotic stresses, including salt, drought, heat, and cold. Among the expressed genes related to cellular respiration, we can focus on *ATPA* and *COX1*. Related to photosynthesis, *MT-CYB*, and *psaB* are examples of differentially expressed genes.

This study reveals new insights into the transcriptional regulation of cynaropicrin biosynthesis in Cc, being a necessary step for better conservation of the wild cardoon gene pool, and more efficient use for future Cc breeding programs.

Biology and Biochemistry

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 also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), PhD grant to A. Paulino (SFRH/BD/145383/2019) and D. Rosa (SFRH/BD/143845/2019), and Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and Project LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

[1] Ramos, P. A. et al. *J. Agric. Food Chem.*, 2013, 61(35), 8420-8429.

[2] Castro M. M. et al. *PLoS ONE*, 2021, 16(6): e0252792.

[3] Paulino, A. et al. *Biol. Life Sci. Forum*, 2022, 11, 60.

Single cell protein production using local wastes – a boost to circular economy

N. Coelho¹, S. Raposo², I. Soares-Silva^{3,4}, T. Brás^{5,6}, M.F. Duarte^{5,6}, R. Barros², A. Romano¹, B. Medronho^{1,7}

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Universidade do Algarve, Ed. 8, 8005-139 Faro, Portugal

²Center for Marine and Environmental Research - CIMA, Universidade do Algarve - Campus de Gambelas, 8005-139 Faro, Portugal

³Centre of Molecular and Environmental Biology (CBMA), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

⁴Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

⁵Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL) / Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.

⁶MED & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal

⁷FSCN research Center, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

Email: ncoelho@ualg.pt; bmedronho@ualg.pt

As human population rapidly grows, conventional procedures to generate protein might not be sufficient in the future to meet all food and feed demands worldwide. Production of single-cell proteins (SCP) utilizing agricultural wastes is an alternative solution to meet the global protein shortage and minimize pollution problems. Due to its high protein content, SCP can be used as food supplement to humans and animal feed. Utilization of several agricultural wastes to produce SCP via fermentation using *Saccharomyces cerevisiae* and *Cyberlindnera jadinii* was studied in the present work. The waste materials used were originated from carob, cardoon, peanut husk, pine bark and pine needle, after being submitted to an extraction procedure using levulinic acid or a mixture of levulinic acid and formic acid. Prior to fermentation, to release the sugars still present in the residues extracted (and in the raw residues, for control), these were pre-treated with sulfuric acid and enzymatically hydrolyzed with different enzyme mixes. The hydrolysates with higher sugar content were used to prepare the fermentation media. For instance, for cardoon residue extracted with levulinic acid and treated with HTec2 enzymes mix for 72h, the sugar concentration of the hydrolysate was 10.33 g/L. Biomass production with *S. cerevisiae* ranged from 1.43 to 3.20 g/L. The highest value, 3.20 g/L, was obtained using the hydrolysate derived from the residue extracted from peanut husk with the mixture levulinic acid:formic acid. Using a different approach, a mixture of all extracted residues, *C. jadinii* was able to produce 4.54 and 3.64 g/L of dry biomass with residues extracted with levulinic acid and the mixture levulinic acid:formic acid, respectively, as substrate. Considering these preliminary tests, the substrates used yielded considerable biomass production and promising results are

expected in scale-up experiments. Ultimately, this study will contribute to improve local waste management and consequently, boost local circular economy.

This research was funded by National Funds through FCT—Foundation for Science and Technology under the Project PTDC/ASP-SIL/30619/2017, the researcher grant (Bruno Medronho) CEECIND/ 01014/2018, Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute, Project LA/P/0069/2020 granted to the Associate Laboratory ARNET and UID/00350/2020 CIMA. 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).

The role of *UPBEAT1* in adventitious root formation in olive (*Olea europaea* L.)C. Morais¹, C. Campos¹, L. Rodrigues¹, A. Peixe², H. Cardoso¹

¹MED (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.

²MED (Mediterranean Institute for Agriculture, Environment and Development), Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: hcardoso@uevora.pt

Olive tree (*Olea europaea* L.) is a woody plant species with high importance in the Mediterranean region due to its fruit, mainly used for olive oil production. Olive cultivars are usually vegetatively propagated by semi-hardwood cuttings, being IBA (indole-3-butyric acid) the auxin used as the inducer of adventitious roots (AR) development. Adventitious rooting, the morphogenic process of AR formation, depends on a diversity of endogenous and exogenous factors in which are included two abiotic stress factors, the wounding and the immersion of the cutting in the auxin solution. Nevertheless, if there are cultivars exhibiting high rooting rates, there are others exhibiting a recalcitrant behavior. Some traditional Portuguese cultivars that mark the typicity of Portuguese olive oils are representative of this recalcitrant behavior. Molecular mechanisms underlying the efficiency of AR formation have been investigated at the University of Évora and recent findings highlight the involvement of diverse families of transcription factors in the rooting process. Among those, the *UPBEAT1*, a subfamily of the *Basic Helix-Loop-Helix (bHLH)*, are regulators of the expression of a set of genes that encode enzymes directly associated with the maintenance of the balance of oxidative oxygen species (ROS), typical of the transition from cell proliferation to differentiation during the process of root formation. Although associated with rooting, there is no reference to the involvement of *UPBEAT1* in the formation of adventitious roots, a process characterized by dedifferentiation for the reacquisition of meristematic characteristics and *de novo* differentiation. Here it will be presented the results achieved on the involvement of the *OeUPBEAT1* encoding genes on AR formation, and their role in ROS homeostasis through regulation of enzymes from ROS-scavenging mechanisms. From the five *OeUPBEAT1* genes, two are upregulated upon IBA treatment, exhibiting high transcript levels at an early stages (6 and 24 h post IBA-treatment). The interplay between these two genes and the transcript pattern of genes encoding for peroxidases will be here presented and discussed.

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 to Virginia Sobral by the help on the establishment of the *in vitro* trials.

Transcript analysis of *Plastid Terminal Oxidase (PTOX)* reveals a putative role in adventitious roots and somatic embryos development in *Olea europaea* L.

C. Mendes¹, R. Pires¹, C. Morais², D. Marques², L. Rodrigues¹, C. Campos¹, A. Peixe³, H. Cardoso¹

¹MED (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.

²Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³MED (Mediterranean Institute for Agriculture, Environment and Development), Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: hcardoso@uevora.pt

The Plastid Terminal Oxidase (PTOX) is a non-heme diiron quinol oxidase located at the non-appressed region of the thylakoid membrane of chloroplasts. PTOX is responsible for the oxidization of plastoquinol and reduction of O₂ to H₂O, being involved in different biological processes, like carotenoid biosynthesis, plastid development, and chlororespiration. Its involvement in plant abiotic stress response has been also reported, proposed to act as a safety valve by protecting the plastoquinone pool from overreduction. The present study intends to explore the putative involvement of *PTOX* during the processes of adventitious roots and somatic embryo development in olive tree (*Olea europaea* L.). Both processes have been defined as morphogenic responses upon abiotic stresses. To study the involvement of *OePTOX* in the development of adventitious roots, *in vitro* growing explants of cv. 'Galega vulgar' were used to establish a rooting assay. The indole-3-butyric acid (IBA) was used as the root induction factor. Rooting induction was performed according to Macedo et al. (2013) and Velada et al. (2020). Samples were collected at 0 hours (before the establishment of the *in vitro* trial), and at 6, 24 and 72h, and 8 and 12 days post-induction, from IBA-treated and control microshoots (non-IBA treated). Samples consisted of basal segments taken from both IBA-treated and non-treated microcuttings (10 shoots/biological sample), and leaves collected from the same microcuttings. Furthermore, to investigate the involvement of *OePTOX* on embryo differentiation, two embryogenic cell lines exhibiting different abilities to differentiate somatic embryos, previously obtained from zygotic embryos taken from fruits of open-pollinated cv. 'Galega vulgar' trees, were inoculated in expression medium devoid of growth regulators (Pires et al. 2020). Samples were collected at 0, 4, 24 hours, and 6 days post-inoculation in expression medium. Transcript quantification performed through RT-qPCR revealed an involvement of *OePTOX* in both morphogenic processes.

References:

Santos Macedo E. et al. (2012) Plant Cell Reports 31 (9): 1581-90.

Velada I. et al. (2020) Plants 9(2): 185.

Pires R. et al. (2020) Plants 9 (6): 758.

This research was funded by National Funds through FCT—Foundation for Science and Technology under the Project 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 to Virginia Sobral by the help on the establishment of the *in vitro* rooting experiment.

Unravelling bat species' response to environmental factors and patterns of occupancy across the southern Portuguese landscape

Martins, F.C.¹, Godinho, S.², Guiomar, N.¹, Medinas, D.³, Mira, A.^{1,3}, Amorim, F.⁴, Rebelo, H.⁴, Barros, P.^{5,6}, Cabral, J. A.^{5,6}, Segurado, P.⁷, Marques, J.T.¹

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

²Department EaRSLab—Earth Remote Sensing Laboratory, University of Évora, Évora, Portugal

³Conservation Biology Lab, Department of Biology, University of Évora, Évora, Portugal

⁴CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vila do Conde, Portugal

⁵Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

⁶Laboratory of Fluvial and Terrestrial Ecology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

⁷CEF, Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017, Lisboa, Portugal

Email: d49669@alunos.uevora.pt

Due to their ecological diversity, bat species have variable responsiveness to the ecosystem's structure. Therefore, to better understand the relationship between the presence/absence of bats and the landscape ecology in southern Portugal, we implemented spatial occupancy models with relevant environmental factors at different scales. Occupancy models allow inferences from occurrence data while considering that bats are not always detected in each survey occasion, thus increasing our ability to estimate the species' potential distribution or response to environmental variables more accurately. Bats were detected acoustically in 59 locations selected within the Natura 2000 protected areas in southern Portugal, and each location had three spatial replicates. We investigated the landscape structure by remotely extracting the enhanced vegetation index and the canopy height across the study region. The composition component of the landscape was studied through the proportion of forest and agroforestry systems and wetland areas. Human disturbance was inferred using the distance to urban areas and the distance to roads.

Bat species' occupancy responded variably to the different components of the landscape, and the considered scale was also a significant factor. Generalist bat species from the *Pipistrellus* genus showed low responsiveness to the landscape components, likely to the fact that these were widespread. However, two species' occupancy was positively predicted by distance to roads. On the other hand, we found a significant relationship between the enhanced vegetation index and forest-associated species occupancy. For the remaining investigated species, we could not model occupancy with the available landscape factors. Thus, for some species, site-use variables may be more appropriate to model than landscape ones. Across all species, survey date was a significant factor in informing detection probability, demonstrating that this modelling approach can help research seasonal and elusive species, such as bats.

This work was supported by Foundation for Science and Technology (FCT) funding FCM via the national PhD research studentship (2020.05448.BD) (<https://www.fct.pt/>). Fieldwork was funded by: Programa Operacional Sustentabilidade e Eficiência no Uso de Recursos, POSEUR 2020.

Plenary Lecture Speakers

Name	Email
Andreia Amaral	andreia.fonseca@uevora.pt
Manuela Moreira da Silva	msanti@ualg.pt
Helena Freitas	hfreytas@uc.pt
Manuela Oliveira	moliveira@fmv.ulisboa.pt

List of Participants

Name	Email
Adonir Both	d44601@alunos.uevora.pt
Adriana Catarino	adri.catarino@hotmail.com
Agostinho Gonçalo	d49855@alunos.uevora.pt
Agostinho Serranito	agostinho_p_29@hotmail.com
Alonso Escoto	alonso8623@gmail.com
Ana Ventura	amventura@uevora.pt
Ana Paulino	ana.paulino@cebal.pt
Ana Faustino	ana.faustino@cebal.pt
Ana Elisa Rato	aerato@uevora.pt
Ana Margarida Pontes Augusto dos Santos	ampaugusto@gmail.com
Ana Patrícia Cebola Poeiras	ana.poeiras@hotmail.com
Ana Rita Azedo	d51406@alunos.uevora.pt
André Albuquerque	d40926@alunos.uevora.pt
André Jorge	d52876@alunos.uevora.pt
Andreia Silva	andreia.silva@cebal.pt
Antonino Kamutali	ninokamutali@gmail.com
António Chen	antonioyuchen@hotmail.com
António Jandir	d44120@alunos.uevora.pt
Arthur Veronez	asousa@student.uc.pt
Beatrice Tadeu Querido	btadeu@fmv.ulisboa.pt
Bruno Guimarães Borges	d55856@alunos.uevora.pt
Cândida Jerónimo	candida.pt@sapo.pt
Carina Sá	carinafsa@ua.pt
Carla Simões	d51413@alunos.uevora.pt
Carla Varanda	carlavaranda@uevora.pt
Carmo Silva	carmoms@uevora.pt
Carolina Morais	20carolinam@gmail.com
Cássia Barbosa	d49783@alunos.uevora.pt
Catarina Campos	mccampos@uevora.pt

Catarina Fernandes	csfernandes@student.uc.pt
Cátia Venâncio	catiavenancio@gmail.com
Clárisse Brígido	ccb@uevora.pt
Cristina Mateus Alfaia	cpmateus@fmv.ulisboa.pt
Cristina Mendes	cristina.ib.mendes@gmail.com
Daniel Bailote	m48156@alunos.uevora.pt
Daniela Farinha	dff@uevora.pt
Daniela Filipa Firmino Rosa	dffr@uevora.pt
David Guedes	dhfgs@iscte-iul.pt
Diana Matos	dianarmatos@ua.pt
Eduardo Ferreira	ferreira@uevora.pt
Elsa Lamy	ecsl@uevora.pt
Emanuel Carreira	d41989@alunos.uevora.pt
Erika Roldão Lemos de Almeida	d49668@alunos.uevora.pt
Eunice Venâncio	eunycassia@gmail.com
Fábio Teixeira	fteixeira@fmv.ulisboa.pt
Filipa Cabecinhas	d53947@alunos.uevora.pt
Flávio Silva	fsilva@uevora.pt
Frederico Martins	d49669@alunos.uevora.pt
Hélder Tavares	helderdbtavares@tecnico.ulisboa.pt
Helia Guerra Cardoso	hcardoso@uevora.pt
Hugo Manuel Matias Duarte	hmduarte@ualg.pt
Ibrahim Prazeres	gibaedy@gmail.com
Inês Mansinhos	ifmansinhos@ualg.pt
Inês Mira	d50831@alunos.uevora.pt
Ivo Lourenço Dias	d54251@alunos.uevora.pt
Joana Amaro Ribeiro	d55735@alunos.uevora.pt
Joana Silva	joanasilva.ctx@gmail.com
Joana Oliveira	joliveira.joana@gmail.com
Joana Costa	joanaleitao2016@gmail.com
João Canaria	joao.canaria@gmail.com
João Batista Guerreiro	d48177@alunos.uevora.pt
Jordana Lopes	jordana.lopes@uevora.pt
José Alves	alvesmateus@gmail.com
José Massuca	jose.massuca@gmail.com
Juliana Raeder	juliana.raeder@edu.ufes.br
Latifa Hajri	hajrilatifa145@gmail.com
Laura Carreira	lrec@uevora.pt
Lénia Rodrigues	lrodrigues-88@hotmail.com
Letícia Fialho	leticia.fialho@cebal.pt
Liliana Cachucho	117iguel117.cachucho@cebal.pt
Luís Duarte	luis.duarte@uevora.pt
Luís Gomes	lag@uevora.pt
Luís Miguel Faria	luisgomesf8@gmail.com
Madalena Mendonça	maria.silva@uevora.pt
Maria Feliciano	mcarmo.vet@gmail.com
Maria Fernandes Félix	mrff@uevora.pt
Maria Beatriz Mendes	mbmendes@uevora.pt

Maria Cristina Ornelas Oliveira	maria.oliveira@staff.uma.pt
Maria Doroteia Campos	mdcc@uevora.pt
Maria Inês Rouxinol	mir@uevora.pt
Mariana Pucarinho Fernandes	mpfernandes@uevora.pt
Mariana Abreu	m.abreu@campus.fct.unl.pt
Mariana Patanita	d46100@alunos.uevora.pt
Maria Pedro Ribeiro Teixeira	mariaribeiro.3995@gmail.com
Mavudi Maleka	mavudimaleka@gmail.com
Miguel Ferro	d43463@alunos.uevora.pt
Miguel Rodrigues	miguelsilvarodrigues@edu.ulisboa.pt
Miguel Advinha	118iguel.fm.advinha@gmail.com
Miguel Rodrigues	l33453@alunos.uevora.pt
Milton Gussule	d39583@alunos.uevora.pt
Natacha Coelho	nrcoelho@ualg.pt
Nicasio Morillo	ntjm@uevora.pt
Nicolas Garrido de la Osa	nicolas.osa@uevora.pt
Nuno Pedroso	nmpsp@uevora.pt
Patricia Vicente	patricia.silva@iniav.pt
Patrícia Bernardo	patriciarbernardo@gmail.com
Pedro Barbosa	pbarbosa@uevora.pt
Pedro A. Salgueiro	pas@uevora.pt
Pietro Todde	p.todde@studenti.uniss.it
Ricardo Vieira Santos	d51301@alunos.uevora.pt
Ricardo Rocha	ricardorocha16@ua.pt
Ricardo Teles Pais	ricardotp@ua.pt
Rita Pereira Eusébio	rita6eusebio@gmail.com
Rosa Pires	rmpires@ualg.pt
Rosana Tajra	rosanastajra@gmail.com
Rosário Silva	mrosariovs@gmail.com
Roukaya Bem Gaied	ex16781@alunos.uevora.pt
Sandra Ferreira	sandracmferreira@hotmail.com
Sandra Martins	scpintomartins@gmail.com
Sara Conceição	d44723@alunos.uevora.pt
Sara Ricardo Rodrigues	sirr@uevora.pt
Sérgio Prats Alegre	sergio.prats@uevora.pt
Sofia Trindade	d50890@alunos.uevora.pt
Sofia Lavado Eufrázio	srle@uevora.pt
Susana Coelho	d51244@alunos.uevora.pt
Susana Lucas	susanalucas1@gmail.com
Teresa Saraiva	teresa.saraiva@ecosativa.pt
Tiago Lopes	tslopes@ua.pt
Tiago Crispim Mendes	d46173@alunos.uevora.pt
Vanessa Amaral	s.schnitzervanessa@gmail.com
Vânia Resende	vjgr33@gmail.com
