



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

**December 11 & 12, 2023**

**Pólo da Mitra, Universidade de Évora**

## **Book of abstracts**

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

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Dear participants,

It is our great pleasure to welcome you to the **VIII PhD Students Meeting in Environment and Agriculture**, held in Évora on the 11<sup>th</sup> and 12<sup>th</sup> of December 2023. We have put together a two-day program with the aim of encouraging scientific discussion. This Meeting represents an excellent opportunity for young researchers to exchange ideas and to explore new challenges in research regarding Environmental and Agricultural Sciences.

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

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

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

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

Welcome to Évora!

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

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### Monday, December 11

09:00	Registration
09:45	Opening session
	<b>Rui Salgado</b> Director of the Institute for Advanced Studies and Research (IIFA)
	<b>Fátima Baptista</b> Director of the Mediterranean Institute for Agriculture, Environment and Development (MED)
	Moderator: Tiago Marques University of Évora, MED & CHANGE
10:00	<i>Portuguese consumers' perceptions of the agri-food sector's social responsibility policies</i> <b>Fernando Teixeira</b> University of Trás-os-Montes and Alto Douro, University of Évora and Rewilding Portugal
	<i>The potential of ants as biocontrol agents towards <i>Bactrocera oleae</i> in super-intensive olive groves: The effect of a seed mixture</i> <b>Ana Rita Azedo</b> University of Évora, MED & CHANGE
	<i>The importance of vegetation structure for bats: bridging remote sensing with occupancy models</i> <b>Frederico Martins</b> University of Évora, MED & CHANGE
	<i>Mediterranean temporary pond restoration increases bat species richness but not bat activity</i> <b>Joana Silva</b> University of Évora, MED & CHANGE
11:00-11:30	Coffee break and poster session
11:30	<b>Plenary Lecture</b> <i>Enhancing Understanding of Insect Decline through Extensive Monitoring of Azorean Forest Arthropods over Time (2012-2023)</i> <b>Paulo Borges</b> University of Azores, CE3C
12:30 – 14:30	Lunch break

	<p>Moderator: Catarina Campos University of Évora, MED &amp; CHANGE</p>
14:30	<p><i>Colistin resistance colonisation in companion animals and their cohabiting Humans</i> <b>Juliana Menezes</b> University of Lisbon, CIISA, AL4AnimalS</p>
	<p><i>Threat or opportunity? Interactions between ungulates and invasive plants in Mediterranean Ecosystems</i> <b>Mónica Roldão Almeida</b> University of Coimbra, CFE &amp; Polytechnic Institute of Coimbra</p>
	<p><i>Exploring Territorial Capital through the lens of Social Capital in two rural areas in Albania - the case of Gjirokaster and Puke district</i> <b>Elda Muco</b> University of Montpellier III, CIHEAM-IAMM</p>
	<p><i>On the sustainability of agri-food systems: getting the concepts right</i> <b>Luís Duarte</b> University of Évora, MED &amp; CHANGE</p>
	<p><i>Can power line pylons be used to promote patches of Mediterranean vegetation in an agricultural landscape?</i> <b>Mariana Pucarinho Fernandes</b> University of Évora, MED, cE3c &amp; CHANGE</p>
15:45 – 16:30	Coffee break and poster session
16:30	<p><i>Economic impact of business model change on farms: A modeling approach in montado silvopastoral systems</i> <b>Nour Fatahi</b> University of Évora, MED &amp; CHANGE; IAMM</p>
	<p><i>Environmental remediation: Poly(ionic liquid)-based aerogels for CO2 capture and conversion</i> <b>Raquel Valadares Barrulas</b> NOVA University Lisbon, i3N Cenimat</p>
	<p><i>Flocculation of microplastics with biofloculants from lignocellulosic materials</i> <b>Solange Magalhães</b> University of Coimbra, CIEPQPF</p>
	<p><i>Determining Factors Influencing Local Communities' Perceptions of Ecosystem Services in Divjakë-Karavasta National Park, Albania</i> <b>Zenepe Dafku</b> Universite Paul Valery Montpellier 3, CIHEAM IAMM</p>
17:30	Ending of the first day of the VIII PhD Students Meeting in Environment and Agriculture

**Tuesday, December 12**

	<p>Moderator: Cláudia Vicente University of Évora, MED &amp; CHANGE</p>
9:30	<p><i>Near Infrared Spectroscopy (NIRS) classification of sliced Duroc dry-cured ham under various packaging systems and storage temperature and time</i> <b>Lucía León Silva</b> CICYTEX-La Orden</p>
	<p><i>Portuguese Viticulture Thrives: Insights from Somatic Embryogenesis in National Grapevine Cultivars</i> <b>Catarina Estevão</b> University of Évora, MED &amp; CHANGE</p>
	<p><i>What do local stakeholders think about novel food processing technologies? A case study in Greece and Malta</i> <b>Aybike Bayraktar</b> CIHEAM-IAMM</p>
	<p><i>Characterization of production systems and agricultural activities in the area of the Living Lab of Luxor, Egypt</i> <b>Christos Galanis</b> CIHEAM-IAMM</p>
	<p><i>Phytotoxic effects of sesquiterpene lactones enriched fractions against weeds and its possible use as a bioherbicide</i> <b>Daniela Rosa</b> CEBAL, MED &amp; CHANGE; University of Cádiz</p>
10:45-11:30	Coffee break and poster session
11:30	<p><b>Plenary Lecture</b> <i>Reviving legume production in the MENA region... between hope and utopia</i> <b>Hatem Belhouchette</b> CIHEAM-IAMM; UMR ABSys</p>
12:30-14:30	Lunch break
	<p>Moderator: <b>Elsa Lamy</b> University of Évora, MED &amp; CHANGE</p>
15:15	<p><i>Economic valuation of the apiculture value chain in France. Identification of the potential impacts and diffusion conditions of an innovative traceability system within the industrial chains and territories in the Occitany region</i> <b>Elie Abou Nader</b></p>

	<p>CIHEAM-IAMM</p> <p><i>Why is CRISPR/Cas13d the best choice for engineering interference against RNA plant viruses?</i></p> <p><b>Joana Amaro Ribeiro</b> University of Évora, MED &amp; CHANGE</p> <p><i>From arid wild legume shrubs to food legume: Application of non-rhizobial bacterial endophytes to improve Cicer arietinum L. growth under salinity</i></p> <p><b>Roukaya Ben Gaied</b> Arid Lands Institute of Medenine</p> <p><i>Different inter-row management practices on a vineyard: Implications on soil microbial activity</i></p> <p><b>Vanessa Amaral</b> University of Évora, MED &amp; CHANGE</p> <p><i>(Bio)Conversion of organic acids to electricity as a form of detoxification of lignocellulosic hydrolysates in a biorefinery context</i></p> <p><b>Alonso Arroyo-Escoto</b> CEBAL, MED &amp; CHANGE</p>
15:45-16:15	Coffee break and poster session

	<p>Moderator: <b>Bruno Medronho</b> University of Algarve, MED &amp; CHANGE</p>
16:15	<p><i>Development and quality assessment of cured meat sausage with different salt reduction strategies</i></p> <p><b>Patrícia Bernardo</b> University of Lisbon, CIISA</p> <p><i>Genetic Signatures of Selection in Mozambique's Landim Pigs Unveil Regions for Meat Quality and Reproductive Traits</i></p> <p><b>Fábio Teixeira</b> University of Évora, MED &amp; CHANGE; University José Eduardo dos Santos</p> <p><i>Edible coatings as a natural alternative for the storage of 'Crimson' seedless table grapes</i></p> <p><b>Sara Ricardo Rodrigues</b> University of Évora, MED &amp; CHANGE</p>
17:00	<p>Closing Session</p> <p>Best Poster Prize</p> <p>Solange de Oliveira Prize (MED)</p> <p>Mário Carvalho Prize (MED)</p>

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

## BEST POSTER AWARDS



### Ana Paulino

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

*“RNA-Seq analysis at two different developmental stages of Cynara cardunculus”*

On behalf of the Organising Committee,

**Fátima Baptista**  
Director of MED



### Mariana Patanita

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

*“Are there any genes responsible for grapevine resistance to trunk diseases?”*

On behalf of the Organising Committee,

**Fátima Baptista**  
Director of MED





## Inês Silva

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

*Unveiling Microbiota within the Rocaille-Adorned Garden Grotto at  
Condes de Basto Palace, Évora, Portugal*

On behalf of the Organising Committee,

**Fátima Baptista**  
Director of MED



## Mónica Marques

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

*Establishment of a callogenesis protocol of Ceratonia siliqua L. to  
further explore its applicability for metabolites production.*

On behalf of the Organising Committee,

**Fátima Baptista**  
Director of MED





# Plenary Lectures



## **Long-term monitoring of the Azorean native forest arthropods: The application of an Index of Biotic Integrity for future monitoring**

P.A.V. Borges<sup>1,2</sup>, S. Lhoumeau<sup>1</sup>, N. Tsafack<sup>1</sup>

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<sup>2</sup>IUCN SSC Mid-Atlantic Islands Invertebrate Specialist Group, 9700-042 Angra do Heroísmo, Azores, Portugal.

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Since 2012, a comprehensive long-term study (LTER) has been underway in the forests of the Azorean Islands, monitoring the population of arthropods. Known as the "SLAM - Long Term Ecological Study of the Impacts of Climate Change in the natural forest of Azores," this project aims to investigate the drivers of biodiversity erosion and their effects on the distribution, abundance, and diversity of native arthropods in the Azores. Specimens were sampled over seven Azorean Islands during the 2012-2022 period.

Island ecosystems globally are witnessing a concerning decline in biodiversity, and forest biodiversity is particularly vulnerable to various drivers of biodiversity erosion. This distressing situation emphasizes the pressing need for conservationists to devise more precise and efficient tools for assessing site quality. In response to this challenge, our study is dedicated to the development of two biological integrity indices (IBI) that employ arthropod communities as indicators to evaluate the quality of forest sites. Building upon previous research demonstrating species diversity stratification, we have created an IBI for the canopy stratum (IBI-canopy) and an IBI for the forest understory (IBI-SLAM).

For the purpose of comparison, we calibrated both indices based on seven parameters, taking into account a previous epigeal IBI. Both indices incorporated percentages of endemic, native non-endemic, and introduced species richness and abundance. As anticipated, disturbance had a negative correlation with species richness and abundance of endemic species, leading to their inclusion in both IBIs. This study underscores the limitations of relying solely on individual measurements to detect various sources of pressure, advocating for a multi-measurement system to gain a more comprehensive understanding of overall system conditions. Our efficient and accessible indices confirmed the low preservation status of Flores Island when compared to Terceira and Pico, aligning with previous empirical studies. Our methodology has been successfully developed and tailored to suit the unique arthropod communities found in the Azores forests. Although it may not be directly applicable to random forest sites, it serves as a valuable inspiration for the development of arthropod-based IBIs in other islands worldwide, where standardized measurements of endemic and exotic species richness and abundance can be obtained.

This work was funded by National Funds through FCT under the Project FCT-UIDB/00329/2020-2024 (Thematic Line 1 – integrated ecological assessment of environmental change on biodiversity).

## **Reviving legume production in the MENA region... between hope and utopia.**

H. Belhouchette <sup>1,2</sup>

<sup>1</sup>UMR ABSys - Agrosystèmes Biodiversifiés

<sup>2</sup>CIHEAM-IAMM - Centre International de Hautes Etudes Agronomiques Méditerranéennes - Institut Agronomique Méditerranéen de Montpellier

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Growing food legumes has several advantages: it improves soil structure, breaks the cycle of diseases, especially in monoculture rotations, and reduces nitrate pollution by decreasing nitrogen inputs in the rotation. As far as food is concerned, legumes are an important source of plant protein and fibre, which are very useful for human health. In the current context of the Middle East and North Africa (MENA) region, essentially marked by a significant degradation of natural resources and an increasing inflation of food prices, legumes can appear to be an interesting solution to mitigate the effects of agricultural intensification on biodiversity, and to (at least partially) replace animal proteins that are increasingly expensive and inaccessible to disadvantaged population groups. Unfortunately, the reality in the MENA region is quite different: it has undergone a significant reduction in total legume production over the last 15 years. This is mainly due to a decrease in the areas dedicated to these crops and a stagnation of, or even a decrease in their yield. A direct consequence of this drop in production is a considerable increase in the import bill for legumes. The aim of this study is to present a preliminary assessment of the impact of different levers expressed as strategic scenarios over the Mediterranean region by considering a wide range of farm dataset collected over five different countries of the South and the North of the Mediterranean region. Data of existing representative 10 farming systems (representing more than 10,000 real farms) were selected by using the PCA over six distinct study sites existing in Lebanon, North Tunisia, South Tunisia, Algeria, Morocco and France. Interviews with farmers, over all study sites, were conducted to collect a set of biophysical, socioeconomic and nutritional data describing their farms. This wide range of collected data allowed covering different Mediterranean contexts as a function of several variables such as the climate (arid to semi-arid regions), resource endowment (irrigated area per farm, farm income ...), intensification production (as a part of production cost) and production goal (crop orientation, self-consumption...) (El Ansari et al. 2020). A static bio-economic model (Belhouchette et al. 2012) was developed in order to assess the farm behaviour in response to the strategic scenario of increasing water dedicated for irrigation in the Mediterranean region. Based on the model simulations, it became clear that for many farmers, legumes are not a priority, especially for those with access to irrigation. Their low price and low and variable yields obviously play a very important role in the decline of these crops. The path followed by the different irrigated areas created in the MENA region since the 1980s makes this very clear: these areas initially cultivated with cereal/legume rotations have progressively been intensified with market gardening and arboriculture, as soon as access to water was made possible. A direct consequence of this intensification is a significant decrease in the area dedicated to legumes, which are grown more for rotation needs than for the production itself. There are many reasons for this failure, but they all converge on one major observation: the lack of a real determination on behalf of public authorities to organise this sector and its different components. This lack of political will reflects, above all, a real debate and an "almost consensus" which states that giving the environment a more important place in agricultural policies by encouraging, for example, the cultivation of legumes, could only be done at the expense of cash crops with high added value and that generate wealth and employment in rural areas.





## **Portuguese consumers' perceptions of the agri-food sector's social responsibility policies**

F. Teixeira<sup>1</sup>, A. Marta-Costa<sup>2</sup>, A. Paço<sup>3</sup>, L. Vacas-de-Carvalho<sup>4</sup>

<sup>1</sup>University of Trás-os-Montes and Alto Douro (UTAD), University of Évora (UE) and Rewilding Portugal, Portugal;

<sup>2</sup>University of Trás-os-Montes and Alto Douro (UTAD) and Center for Transdisciplinary Development Studies (CETRAD), Portugal

<sup>3</sup>University of Beira Interior (UBI) and NECE – Research Center for Business Sciences, Portugal

<sup>4</sup>University of Évora and Center for Advanced Studies in Management and Economics (CEFAGE), Portugal

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The agri-food sector is giving increasing importance towards the adoption of corporate social responsibility (CSR) measures and, above all, the way in which these measures are perceived by the public. Consumers are paying increasing attention to the selection of products and brands based on these practices. The implementation of CSR practices has been widely researched, mainly from the point of view of companies in terms of the CSR measures they adopt and communicate. However, the research process has rarely focused on consumers and their preferences regarding the direction of CSR practices. As of the publication date, there has been no published study in Portugal on CSR in the national agri-food sector prior to this research. With this in mind, the aim of this study is to design a survey to be applied of Portuguese consumers which will allow us to identify the current perceptions of Portuguese consumers to identify their current perceptions of the agri-food sector's social responsibility policy. The questionnaire is based on an up-to-date review of the state of art. This study will allow the construction of a solid and well-founded data collection methodology regarding the CSR perception of the agri-food sector in the general public. This, in turn, will enable future cross-checking of information between consumers and companies in the agri-food sector, which has never been done before.

ACKNOWLEDGMENTS: This work is financed by national funds through FCT (Portuguese Foundation for Science and Technology, IP), under the projects UIDB/04011/2020, UIDB/04630/2020 and UIDB/04007/2020.

## Smart farming uptake and innovation ecosystems: some critical aspects

Ana Maria Ventura<sup>1</sup>, Cristina Cruz<sup>2</sup>, Ludwig Lauwers<sup>3</sup>, Abdul Mouazen<sup>3</sup>

<sup>1</sup>PhD Student: Biology (FCiências- U.Lisboa) and Biosciences Engineering –Socioeconomics (Dep. of Environment- UGent)

<sup>2</sup>cE3c-PSE Group/ FCiências- U.Lisboa

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**The aim of this presentation** (as part of the PhD work) is to signalize some critical factors that influence the Smart Farming (SF) uptake in agriculture sector, considering that there are internal (inner to the farm and to the farmer) and external (region, entrepreneurial culture) ones.

**The objectives of the presentation** are:

- i) to highlight the possible connections and associations between the critical factors identified until now (respecting SF adoption) and
- ii) to foresee the eventual role of innovation ecosystems in counteracting the found obstacles and in potentiating the positive trends.

**Basic concepts:**

**1) Smart farming (SF)** is the application of information and data technologies for optimizing complex farming systems. The focus is on access to data and the application of these data – how the collected information can be used in a smart way. SF involves not just individual machines but all farm operations and available technology, where smartphones and tablets allow to a quick access to large data basis to take more informed decisions.

**2) Innovation ecosystems (IE)** thinking is based on the assumption that there is a need to foster innovation environments where scientists, policymakers, producers, endusers and entrepreneurs can mobilize their collective knowledge to innovate. They seek to explore innovation niches and sustainability, what can be particularly relevant in the agriculture sector. “**Living Lab**” is a common form of IE nowadays; it is an user-centered open innovation ecosystem based on a systematic user co-creation approach, integrating research and innovation processes in real-life communities and settings. It engages the main actors of Quadruple Helix Model, namely: citizens, government, industry and academia.

**Methodology:**

Until now, the research is mostly based on a critical literature review, taking as main sources the Google Scholar and the Web of Science. The fieldwork (talks) in Portugal, Belgium and Israel, so far, are in the direction of the confirmation the results posted below, even with sensitive differences among the countries here considered. Their deep analysis is an ongoing process.

**As results**, until now, it can be stated that:

1. There is a correlation between the acquisition-use costs of the SF equipment (economic dimension) and time consumption to its understanding and use. They are related to the technical and economic support, farm dimension and structure and educational and skills level.



## Agribusiness

2. The opportunities may emerge when advisement services, association path and openness from farmers' side are coupled, in a given time and space. The regional dimension and productive system are clear facilitators or, on the opposite, are a negative influence in SF uptake.

The innovation ecosystems, in a form of LL, may work in favour of extended SF use, in the overcoming of the barriers connected with credit facility, farm dimension and structure and lack of adapted skills and management tools.

## **Elaias Guineensis Palm Oil and the Environment-Food-Energy-Water Nexus in the Brazilian Amazon**

Manuel António Henriques dos Reis Lopes

PhD student in Agribusiness and Sustainability; University of Évora/University of Trás-os-Montes and Alto Douro - Polo MED (Mediterranean Institute for Agriculture, Environment and Development) Évora, Portugal.

The article deals with the water footprint of oil palm cultivation in the Brazilian Amazon. Oil palm is an important commodity that contributes to the livelihoods of many communities, to the GDP of governments and to the achievement of several sustainable development goals (SDGs), biodiversity and climate change. However, its continuous cultivation and expansion, due to high and growing demand, has led to many negative effects and subsequent calls to make production sustainable. The preservation of forests will contribute to the minimisation of climate change worldwide, and the vulnerability of these sectors is associated with a wide spectrum of risks: the suppression of biodiversity in the Amazon, which is essential for the carbon cycle; the alteration of hemispheric hydrological systems that are essential for climate and rainfall. There is a correlation between climate and production in palm oil: the climate favorable to oil palm cultivation is of the tropical or humid equatorial type, with a rainfall of more than 1,800mm and the water deficit does not exceed 300mm. In climatic terms, the elements that most affect oil palm production are air temperature, hours of sunshine and rainfall, with the monthly distribution of rainfall and the occurrence of water deficit being the elements that have the greatest effect on crop growth and production. The major limitation of the study is related to the lack of systematic and credible data from the life cycle inventory (LCI) of oil palm, namely baseline data on: fertilizers, herbicides, pesticides and water resources (from birth to grave) used in the production cycle. The aim of this study is to quantify the water footprint (MAP) for the various stages of production along the system boundary from the production of fresh CFF bunches in the plantation, crude palm oil in the palm oil mill, palm kernel in the palm oil mill and crude palm kernel oil (kernel), in the Brazilian Amazon. The methodology used for the impact assessment was the Water Vulnerability and Accounting Assessment Method (*WAVE*). This method analyzes the water depletion index (*WDI*) based on the local scarcity of blue water. The method uses a water accounting model where the share of atmospheric evapotranspiration recycling is accounted for along with freshwater consumption and wastewater discharges. The method also assesses the regional vulnerability of watersheds to freshwater depletion. A palm oil palm tree has an evapotranspiration rate of 6.2 thousand/m<sup>3</sup> of water to produce one ton of palm oil. The water used by the crop has the most significant impact at the oil palm plantation stage. Data for this cradle-to-gate study was collected from the nursery, plantation, palm oil mill, and *kernel crushing plant (KCP)*. The climatic data were acquired at the Center for Weather Forecasting and Climate Studies - *cptec/inpe* in the city of Thailand-Pará-Brazil. It is hoped that the level of water degradation in this oil palm agribusiness plantation can be identified to provide guidelines to the government, plantation companies, as well as developers for the development of sustainable palm oil plantation in the Brazilian Amazon.

Keywords: water footprint, blue water, palm oil, palm oil

## **Understanding and Addressing Food Waste in Portugal: A Multidimensional Approach**

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

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This presentation aims to share the key aspects of our research project, which seeks to comprehend and tackle food waste in Portugal. The study endeavors to identify the causes of food waste and develop effective strategies for its reduction, as well as explore opportunities for the valorization of discarded food.

To achieve these objectives, we will adopt a multidimensional approach, including conducting a comprehensive literature review, gathering data through consumer surveys and interviews with retailers, and developing a statistical model.

In the literature review phase, we will explore existing literature on food waste and the implemented public policies. This will allow us to consolidate the current knowledge on the subject and identify research gaps.

In the data collection stage, we will use surveys to obtain information regarding consumer practices and attitudes towards food waste. This approach will be complemented with interviews with retailers, which will provide insights into food management practices in the retail sector in Portugal. Qualitative analysis of interview data and quantitative analysis of survey data will offer detailed information about the causes of food waste.

Additionally, we will develop a statistical model based on the findings from the preceding stages. This model will explore the complex relationships between various factors that impact food waste in Portugal. The model will help identify areas for more effective intervention and valorisation.

We anticipate that this study will yield practical recommendations for reducing food waste and valorising food residuals in Portugal. Furthermore, the research will provide a deeper understanding of the underlying causes of food waste and how they relate to consumer behaviour. This multidimensional approach is innovative and will contribute to a more sustainable and responsible society, aligning with the increasing concerns of consumers regarding sustainability.

In summary, this presentation aims to share the key aspects of the research project, emphasizing its multidimensional approach and the methodological steps used to understand and address food waste in Portugal.







## The potential of ants as biocontrol agents towards *Bactrocera oleae* in super-intensive olive groves: The effect of a seed mixture

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The olive fruit fly *Bactrocera oleae* (Rossi) is a major pest in Mediterranean olive groves, causing great economic loss. Arthropods can provide the pest control service in agroecosystems. *Bactrocera oleae* pupate in the soil and soil arthropods can help control the pest in this life cycle stage. Previous studies on *Bactrocera oleae* pest control, showed that some management practices, such as herbaceous cover maintenance, can enhance its biological control, providing shelter and food to natural enemies. Several studies validate that ants are predators of other fruit flies in different cultures, namely larvae and pupae, but more studies are needed to fully understand their role in agricultural ecosystems.

We examined the influence of herbaceous cover in south Portugal super intensive olive groves in functional biodiversity of ant community, and in ants' biocontrol potential towards olive fruit fly in the pupae stage. The study was carried out in winter and spring of 2023, in 10 olive groves in the region of Évora and Beja, Portugal. Each olive grove had two plots, one with spontaneous vegetation between rows (control plots) and the other was enriched with sowing seed mixture of nine plant species (sowed plots). In the winter period, we assessed the herbaceous cover and the ant community. To disclosure if ants were responsible for pupae predation, observation protocols (cafeteria experiments) were carried out in one of the olive groves. Interactions were observed, displaying 160 pupae on the control and sowed plots for one hour and ants' behavior was registered.

Ants outnumbered other arthropod groups in olive groves and there is a positive influence of the sowing enrichment on diversity and abundance of ant species. Ants' functional groups abundance was higher in sowed plots. Fabaceae family had a major influence on functional groups abundance. Cafeteria experiments showed that pupae predation is performed by different ant species in both control and sowed plots, but sowing slightly increased the predation potential of the ant Community towards the *B. oleae* pupae. The enrichment of between rows herbaceous cover, with Fabaceae species, could be beneficial for ant communities, increasing pest control in super intensive olive groves.

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### The importance of vegetation structure for bats: Integrating new remote sensing data on occupancy models

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At a global scale, significant land cover changes have ensued due to anthropogenic activities. These changes often negatively impact biodiversity, yet the extent of such impact on the distribution pattern of species depends on the landscape composition and configuration at local and landscape levels. To understand this impact at a landscape scale, we evaluated how the land use and vegetation structure influenced the bat species occurrences while considering imperfect species detection. We hypothesized that intensification of anthropogenic activities, like agriculture, reduces heterogeneity of land use and vegetation structure and thereby limits bat occurrence and species diversity. To investigate this, we acoustically sampled bats across 177 sites in southern Portugal, embedded in the project POSEUR; Livro Vermelho dos Mamíferos de Portugal Continental. We derived fine-scale vegetation structural metrics (vegetation height) by combining spaceborne LiDAR (GEDI), RADAR (Sentinel-1 and ALOS/PALSAR-2), and multispectral (Sentinel-2) data. Additionally, we included fine-resolution climatology data from CHELSA. Our findings unveiled a strong relation between forest-associated bat species' occupancy and vegetation parameters, particularly with canopy height in western barbastelle and lesser Horseshoe bat occupancy. Moreover, the proportion of forest and shrubland were the main composition gradients determining bat species responses. All species' best-ranking occupancy models included at least one climatic variable (*e.g.*, potential evapotranspiration), demonstrating the importance of climatologies when predicting bat distribution. Furthermore, we observed that the detection probability of all species was lower than one and dependent on nightly environmental conditions. These findings underscore the importance of accounting for imperfect detection for highly vagile and elusive species like bats. Our results demonstrate the effectiveness of using fine-resolution vegetation and landscape metrics derived from remote sensing data to model species distribution in the context of biodiversity research and monitoring.

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

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Climate change scenarios predict a continued decrease in rainfall across the Mediterranean regions in the next years, emphasizing concerns about water scarcity. The ongoing destruction, modification, and drainage of wetland habitats further intensifies this risk on the landscape. Ponds are vulnerable aquatic habitats that supply essential 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, there is a scarcity of long-term studies in aquatic habitats, and none was carried out in ponds. Here, we aim to assess the responses of bat activity and species richness to restoring temporary ponds. Such restoration actions involved the digging or deepening Mediterranean temporary ponds aimed at re-establishing the terrain depression to enhance the water holding capacity. We hypothesized that these actions promote overall bat activity and species richness in the long term. To evaluate this impact, we monitored the bat acoustic activity for five years (three before and two after the restoration actions) in 16 ponds (6 restored and 10 control ponds) along the southwestern coast of Portugal. Bat sampling was performed during the spring season, using bat detectors (Pettersen D500x) to passively detect and record echolocation pulse sequences. Results from the Before-After-Control-Impact (BACI) analysis revealed a positive impact of restoration on species richness: there were up to 2 more species in the intervened ponds after restoration actions. While many species increased their activity in both pond types, *Eptesicus serotinus* / *E. isabellinus*, *Myotis myotis* / *M. blythii*, *Nyctalus leisleri*, *Pipistrellus kuhlii* and *Tadarida teniotis* demonstrated increases in activity exclusively in the restored ponds. This response was observed two years after the implementation of the restoration actions. Overall bat activity also benefited from pond restoration, although its increase is slight deeming a non-significant change. These outcomes highlight that restoring Mediterranean temporary ponds promotes a win-win scenario for bat conservation, even considering the ability of bats to use the wider landscape.

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## Colistin resistance colonisation in companion animals and their cohabiting

### Humans

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The increasing reports of plasmid-mediated colistin resistance worldwide raises concern towards the treatment of MDR Gram-negative bacteria nosocomial infections. Our aim was to investigate the possible sharing of colistin-resistant *Escherichia coli* recovered from faecal samples of companion animals and their household members in Portugal.

Between February 2018 and February 2020, faecal samples from healthy and sick dogs and cats ( $n=102$ ) and their owners ( $n=125$ ) were plated onto SuperPolymyxin medium. Species identification was performed by PCR. Susceptibility tests were performed by broth microdilution (Sensititre<sup>TM</sup> FRCOL, Thermo Fisher Scientific and for MicroScan<sup>®</sup> Neg MIC Panel Type 44, Siemens). All isolates were screened for the presence of colistin resistance genes (*mcr-1* to *mcr-9*) by PCR and sequenced generating libraries of WGS paired-end with 150 bp. Assembly genomes were performed and genetic relatedness between animal and human strains estimated.

Colistin-resistant *E. coli* strains harbouring the *mcr-1* gene, were recovered from faecal samples of companion animals (7.8%,  $n=8/102$ ) and humans (3.2%,  $n=4/125$ ). In two households from dogs diagnosed with skin and soft tissue infection, had resistant strains in both animal and owner. Relatedness analysis of assembled genome suggests the occurrence of transmission within the households. All *mcr-1* positive strains were MDR.

The identification of similar *mcr-1*-positive strains in companion animals and humans is of great concern. These results demonstrate that dogs and cats may act as reservoirs of plasmid-mediated colistin resistance, allowing the spreading of these clinically important genes into the community. Reinforcing the importance of an active monitoring and hygiene practices.

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## Threat or opportunity? Interactions between ungulates and invasive plants in Mediterranean Ecosystems

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Invasive alien species (IAS) are one of the main threats to biodiversity, profoundly modifying ecosystem services, and having additional negative impacts on economic activities, food security and human health. In Portugal, several invasive alien plants (IAP) have been introduced in the past and their numbers continue to increase. Ungulates, known as ecosystem engineers, may be contributing to that expansion, but in Mediterranean ecosystems, the interactions between common ungulates and widespread IAP have been poorly explored. In this PhD thesis, I will evaluate the possible herbivory or avoidance of selected IAP, and the destruction or dispersion of their seeds by common ungulates, which may translate into ecosystem services and/or disservices provided by these animals. This will be done in areas heavily invaded by IAP in the Centre of Portugal. Additionally, I will study the impact of such IAP on the habitat use by ungulates. This work will be accomplished using a multidisciplinary approach with different areas of expertise in ecology, field monitoring, manipulative experiments and ecological modelling. Specifically, I will study the interactions between red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) and domestic goat (*Capra hircus*) with silver wattle (*Acacia dealbata*), Australian blackwood (*Acacia melanoxylon*), bushy needlewood (*Hakea decurrens*) and willow-leaved hakea (*Hakea salicifolia*). Ingestion of these IAP and food preferences of red deer and domestic goat will be assessed in cafeteria-style trials; viability of IAP seed will be assessed through experimental feeding trials with red deer, wild boar and domestic goat; these experiments will be conducted in the Biological Park of Lousã. Field monitoring will be conducted in the Lousã Mountain to assess consumption and dispersion of the selected IAP in wild conditions, along with the analysis of ungulates distribution in invaded habitats; in this case, the four ungulate species will be used. Finally, the possible services and/or disservices provided by the ungulates regarding IAPs will be modelled using the information obtained in the previous tasks.

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**RNA-Seq analysis at two different developmental stages of *Cynara cardunculus***

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The inflorescence in *C. cardunculus* plays a relevant role in human consumption and cheese manufacture, due to the milk-clotting capacity of cardoon pistils. Therefore, it is important to understand the complex process of the cardoon flower development, which involves sophisticated molecular and cellular activities. A differential gene expression study between the vegetative and reproductive phases was conducted using a comparative transcriptome analysis. A total of 23.5 million reads were generated, per sample, using high-quality sequencing, using a DNBseq platform. About 552 differentially expressed genes (DEG)s were produced by differential gene expression analysis, of which 321 and 231 had higher levels of expression in the vegetative and reproductive, respectively. A total of 88% of DEGs had a functional annotation, while the remainder of proteins had unidentified activities. Genes involved in phenylpropanoid biosynthesis (*PER42*, *poxN1*, *BGLU12*, *BGLU24*, *BGLU24*), the anther/pollen development (*RLP50*, *RLP46*, *YLS3*), and jasmonic acid signalling (*TIFY5A*, *BHLH14*, *WRKY4*, *BHLH118*) as well as transcription factors, constituted most of the developmental stage-related DEGs. The identified proteins, such as MADS-box family, play an essential role in meristem identity and flowering period regulation. The molecular regulatory mechanisms behind the vegetative-to-reproductive transition in *C. cardunculus* is crucial for maintaining the species' biodiversity and improving the quality and productivity of cardoon products and their derivatives.

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## Functional characterization of ShK domain-containing protein in the plant-parasitic nematode *Bursaphelenchus xylophilus*

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Plant pathogens cause severe economic and ecological problems in a wide range of crops and forestry plant species worldwide. The migratory plant parasitic nematode *Bursaphelenchus xylophilus* (pinewood nematode, PWN) is a European quarantine organism which causes economic and ecological damage to the forestry industry leading to the death of the tree. The interactions of plant-parasitic nematodes with their hosts are mediated by parasitism-related proteins: secreted proteins produced by the pathogen that modify the host to their benefit and to protect itself against host defenses. Several proteins have been identified in *B. xylophilus* as important during the interaction with the host, including toxins, venom-like proteins, and other peptides which their function is unknown. From the previous PWN transcriptomic dataset, we have identified 96 transcripts encoding ShK domain-like proteins. The ShK domain-like protein is a 35/37-residue peptide toxin capable of blocking the potassium channels, originally found in sea anemones. The ShK superfamily is characterized by a small and conserved domain (ShKT) distributed across plant and animal kingdoms, involving small toxic peptides and larger multifunctional proteins with several functions.

This study aimed to characterize and understand the functional role of ShK-domain like proteins in *B. xylophilus* biology. To achieve these objectives, we used different approaches such as *in silico* analysis, *in situ* hybridization to validate the spatial expression of the transcripts in the nematode tissues, and an oxidative stress assay to understand the nematode response to the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). From the results of a sequence similarity analysis, we have selected nine candidate genes with a predicted ShK domain-like protein that are only found in *B. xylophilus*, have a predicted signal peptide and, most of them, are highly expressed during infection of the host. The results showed that those genes are expressed in different nematode tissues, such as in the pharyngeal gland cells (a parasitism-related tissue), in the intestine and nerve cords, suggesting a diversity of functions in the nematode. Furthermore, eight genes have an upregulation the expression levels in response to H<sub>2</sub>O<sub>2</sub>, suggesting that they might be involved in the ROS scavenging and possibly have a protective role against host defenses enabling a successful parasitism. Understanding the role of ShK-domain like proteins may increase our knowledge of how nematodes modulate hosts during infection and develop new target molecules for nematode control.

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## Oral biology and orthodontics devices interaction between diet type and saliva characteristics

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The use of orthodontic brackets, in particular, has become common in recent decades, and several systems are employed to connect orthodontic archwires, including stainless steel ligatures and elastomeric ligatures. The use of any orthodontic device installed intraorally for a prolonged period must ensure that no significant release of toxic ions will occur, which is why this assessment is particularly important due to the increasing use of these devices in the general population. Orthodontic appliances and their components are exposed to a variety of intraoral conditions, namely contact with saliva and its interactions with ingested and chewed food. Thus, its degradation can potentially result in the leaching of its components, resulting in unintended human exposure. Despite this fact, little is known about the composition of the salivary protein film formed on orthodontic brackets. Therefore, it is important to investigate whether these devices can release potentially toxic elements during use and whether they constitute a relevant source of exposure to metals. Some previous studies have already evaluated the release of selected elements for various orthodontic appliances composed of alloys containing Fe, Cr, Ni, Si, and Mo. In vitro studies have shown that in the case of stainless-steel appliances, elements of concern include Ni and Cr. In vivo observations confirmed some of these results, indicating that Ni, in particular, is released at detectable levels in blood and urine in humans. Both Cr and Ni have been recognized as cytotoxic, mutagenic, and allergenic; however, little is known about the potential release of elements from elastic bandages. There is consensus, however, that increased and long-term exposure to all of these elements can have adverse effects on human health, including systemic and organ-specific toxicity. The objective of this review, which is part of a broader approach, was to analyze whether the interaction between the type of diet and the characteristics of saliva can contribute to exposure to toxic elements due to the corrosion of orthodontic devices.

## Genome-wide identification of Auxin Biosynthetic YUCCA Gene Family in *Olea europaea* L.

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The main natural auxin, indole-3-acetic acid (IAA), plays a key role in regulating numerous plant growth and developmental events, including apical dominance, embryogenesis, root morphogenesis, and floral patterning. YUCCA (YUC) flavin monooxygenase (FMO) enzymes catalyze the rate-limiting step for endogenous IAA biosynthesis via the indole-3-pyruvic acid (IPyA) pathway, established as the prevailing and highly conserved route in the plant kingdom. The YUC gene family has been characterized in several species but has never been explored in the olive tree (*Olea europaea* L.), the most valuable oil crop in the Mediterranean basin. By searching the olive genome databases, 24 YUC genes were identified. Translated OeYUC sequences were used in a phylogenetic analysis integrating YUC sequences from six Magnoliopsida species. Based on the topological organization of the phylogenetic tree, the OeYUCs were grouped into seven subfamilies and named according to their similarity with *Arabidopsis thaliana*'s YUC sequences (OeYUC2, OeYUC3, OeYUC4, OeYUC6, OeYUC8, OeYUC10 and OeYUC11). Composition of each subfamily appears to be highly variable. The gene structure examination showed that olive YUCs exhibit two to five exons, with an exon-intron configuration relatively preserved within each subfamily. Further, the assessment of protein features revealed that OeYUCs length ranged from 303 to 455 aa, with an average molecular weight (Mw) of 44.56 kDa. At least five of the six conserved domains reported for YUC-like FMOs were identified in all the OeYUC sequences, including the FAD-binding and NADPH-binding motifs considered crucial for YUC activity. Most of the OeYUCs were predicted to be located in the cytoplasm. Nevertheless, a few members were predicted to localize in different organelles: nucleus/cytoplasm (OeYUCCA3c and OeYUCCA4c), chloroplast (OeYUCCA4a and OeYUCCA8f), or endoplasmic reticulum (OeYUCCA8e). These results provide a basis for further investigation into the biological functions of olive YUCs.

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**Environment, Landscape and  
Sustainability**

## **Exploring Territorial Capital through the lens of Social Capital in rural areas in Albania**

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Territorial capital is a recent concept, firstly used by the European Commission in regional development policies. Although in literature this concept is taken into account in regional context, it can also be applied to smaller scales such as rural areas. Territorial capital explores the ability of local communities to draw development strategies and generate new territorial resources that can be exploited in development strategies by combining three dimensions of territory: the tangible assets, intangible assets and the social capital fostered among individuals. This approach assumes that territories provide development opportunities that, through social interventions, can be transformed into economic assets or sources of value. In our case, we examine territorial capital within the context of specific agricultural products (non-timber products) associated with forest resources (natural capital) that require a form of territorial governance, local know-how (human capital), and coordination networks between governmental and non-governmental actors (social capital) in rural areas in Albania. We used interview guides and semi-structured questionnaires to examine the access on natural capital, the characteristics and evolution of local know-how, and the willingness for cooperation and collective action between community members, government bodies and associative actors. The results shown that social capital shape the natural and human capital. Forest resources in Albania are managed through a combination of legal regulations and customary rights of access which complexifies the social capital and its effectiveness on both the use of natural capital and the performance of associated local value chains. Local traditions linked to the use of resources for different purposes in each area are mainly rooted in traditional knowledge inherited and passed from generations. Human capital is built through this inheritance and with support from external actors. The duality of access requires the mobilization and coordination of local stakeholders to effectively exploit the abundant resources in each region. Indeed, territorial capital is conceived as a theoretical and methodological way of linking forest resource management, know-how and the mobilization of local stakeholders around these resources.

Keywords: Territorial capital, forest resources, know-how, social capital, rural areas, Albania

## On the sustainability of agri-food systems: getting the concepts right

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Multiple, and frequently conflicting, views and approaches currently co-exist around the concepts of healthy and sustainable agri-food systems. Quite often, isolated interventions are touted as sustainable when they clearly contribute far too little to the wider goal of achieving effective healthy food systems. Initiatives like installing photovoltaic panels or planting shrubs on the farm's edges are clear examples of practices that are positive but insufficient. This presentation intends to contribute to the clarification of these concepts, and thus its main goal is to contribute to raising awareness of frameworks regarding sustainable food systems. It is based on a critical scientific and grey literature review and also on extensive social networking. Therefore, we provide an overview of relevant concepts (and buzzwords) commonly used in sustainable agri-food narratives.

We commence with a brief incursion through the need to go beyond sustainability, promoting regenerative approaches for environmentally responsible design. Then follows the framework advanced by Gliessman (2016) of a five-level food system change toward sustainability, based on which we differentiate between the deepening levels of any management action or policy. In addition, the framework permits to position of these initiatives in their context, differentiating between the agroecosystem level and the whole food system level. Under this analytical framework, concepts like bio-diversified agriculture, regenerative agriculture, organic agriculture, alternative food networks, localized agri-food systems, and agroecology are clearly differentiated, organized, and systematized. A description for each is provided allowing for a better understanding of the concepts and the avoidance of too frequent misinterpretations. Afterward, these concepts are updated to current scientific (and socio-political) trends, like agroecological transition, values-based territorial food networks, or agroecology-based localized agri-food systems. Food sovereignty is also lately becoming a core challenge, considered a fundamental notion around sustainable food systems for contemplating aspects of self-determination, democratic participation, co-creation of knowledge, etc. Complementing the previous reflections, we will also present further findings resulting from testing the capacity of territorial approaches to promote sustainable food systems.

We conclude by presenting the projected future steps to be followed in this doctoral research which will focus on disentangling the key drivers behind the emergence of agroecological initiatives at a regional level.

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**Can power line pylons be used to promote patches of Mediterranean vegetation in an agricultural landscape?**

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Power lines are a widespread linear infrastructure essential for humankind. They cross various landscapes, including farmland, with varying degrees of grazing and agricultural intensity. As the bases of very high-tension power lines are negligible for agricultural or livestock production purposes, they are usually left unmanaged, providing an opportunity to promote habitat patches in the landscape, provide refuge for plants and animals, and function as a feeding habitat for fauna. Our work aimed to analyse the potential of power line pylon bases to become suitable habitat patches by assessing the effect of three management practices (reference/without intervention, fenced, and fenced and sown) on plant community structure and diversity. The study occurred around Évora (South Portugal; Mediterranean climate) in 15 power line pylons (plots with ca. 48m<sup>2</sup>) distributed in semi-natural areas grazed by cattle. The plots were intervened in autumn 2017: five were used as reference (without intervention), five were fenced (to prevent livestock grazing and promote natural regeneration), and five were fenced and sown with a seed mix of native species (herbaceous and shrubs). The complete absence of grazing in fenced plots (sown and not sown) increases the amount of dry plant biomass and can favour the dominance of competitive species. In 2021, a second intervention was performed to reduce these effects by cutting and removing the dry plant biomass on half of each fenced plot (sown and not sown), mimicking the effect of extensive grazing. The effect of these management practices was evaluated by assessing the abundance and diversity of flora species and the vegetation structure before (spring 2017) and after the intervention (spring 2018 to 2023). Globally, intervened plots showed differences regarding the vegetation community structure, with the plots fenced and sown tending to register an increase in flora richness, diversity, and vegetation height. However, the results can be affected by storks nesting in the pylons – a common situation in Portugal – decreasing plant richness and diversity regardless of management practices. Fencing and sowing power line pylons seem to be the most suitable management to improve a vegetation structure that provides refuge for plants (including pollinator-promoting wildflowers) and animal communities in agricultural landscapes.

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## **Economic impact of business model change on farms: A modeling approach in montado silvopastoral systems**

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Ecosystems worldwide face threats from unprecedented climate change, which is altering their functioning and sustainability. The Montado, a traditional agro-silvopastoral system in southern Portugal within the Mediterranean area, is also experiencing increasing arid conditions due to higher temperatures, reduced rainfall, and other factors like the globalization of markets and technologies, alongside intensified and specialized farming practices. Our objective is to identify alternative business models for Montado farms that can be more economically, environmentally, and socially sustainable.

To achieve this goal, our methodology comprised four main steps:

i) The selection of three farms for interviews based on a preexisting network of farmers in the Alentejo region. Three contrasting types were considered, depending on the degree of intensification, the number of components, and the size of the farm.

ii) We developed a detailed questionnaire to collect data on farm components, inputs, and outputs.

iii) Using this data, we created a conceptual model for each farm type, comprising the active environment (representing external drivers like climate, economical and institutional environment), the silvopastoral system (illustrating different components and interactions), and the passive environment (representing system outcomes assessed through indicators such as economic, environmental, and social).

iv) Simple scenario testing like changes in input prices, was performed.

Following close interaction with farmers, the results provide a detailed description of the model through conceptual frameworks. This highlights the complexity of the Montado's silvopastoral system and explains the conflicts in decision-making within the system. The scenario tested reveals differences in income and resilience to market fluctuations among the different types of farms.

The next step involves discussions with various stakeholders, including farmers, to collaboratively formulate a set of scenarios that they estimate most significant. These scenarios include external factors like climate change and strategies for adaptation, such as changes in the farm's dependence to the market or in subsidies levels. Based on this, a numerical model will be written with the mathematical programming software GAMS (The General Algebraic Modeling System). This model aims to quantify and assess the impact of these scenarios on the farm using a set of indicators like farm income and labor hours.

Keywords: Economic modeling, silvopastoral system, Montado.

## Environmental remediation: Poly(ionic liquid)-based aerogels for CO<sub>2</sub> capture and conversion

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The increasing demand for climate change mitigation necessitates advancements in CO<sub>2</sub> capture methodologies [1]. In this study, an optimal CO<sub>2</sub> sorbent/catalyst was conceptualized by integrating high porosity and surface areas, thus enhancing both CO<sub>2</sub> sorption and conversion capabilities. This material was synthesized through the fabrication of aerogels using chitosan and poly(ionic liquids) (PILs), with a dual focus on morphological attributes and the chemical nature of the CO<sub>2</sub> sorbent/catalyst [2,3]. PILs are derived from ionic liquid (IL) monomers composed of organic cations and organic or inorganic anions, which offer tailorable properties suitable for various applications. PILs encompass the unique features of ILs within a macromolecular framework and have garnered significant attention across several applications [2]. Aerogels are lightweight, nanostructured materials characterized by their high porosity and specific surface area. They can be fabricated from biopolymers like chitosan, utilizing biomass residues as feedstock [4,5]. *AEROPILs*, presented in bead form, were successfully synthesized, demonstrating high porosity and surface areas. These materials were introduced for the first time as CO<sub>2</sub> sorbents and catalysts for CO<sub>2</sub> valorization. *AEROPILs* exhibited highly promising catalytic activity in the context of CO<sub>2</sub> addition to epoxides, even under relatively low-pressure conditions and in the absence of solvents and co-catalysts.

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## Flocculation of microplastics with bioflocculants from lignocellulosic materials

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Plastics are widely used, leading to an exponential consumption growth during the last decades. However, most plastics are not biodegradable and can exist for hundreds of years<sup>1</sup>. To tackle this ecological disaster, we studied and characterized novel greener approaches. Lignocellulose based flocculants capable to efficiently remove microplastics from aqueous media, especially from waters that have already been processed in wastewater treatment plants, were developed. Cellulose was subjected to modification strategies (cationic and hydrophobic modifications) which allow to obtain materials with different degrees of substitution, molecular weight and hydrophobicity. The obtained derivatives will be tested in effluents contaminated with microplastics and their flocculation performance will be evaluated by laser diffraction spectroscopy (LDS), coupled with optical and scanning electron microscopy. The bioflocculants developed in this study were found to successfully aggregate and remove model microplastics from aqueous media. Moreover, the flocculation kinetics were observed to increase when adding the bioflocculation agent<sup>2</sup>. Overall, this work demonstrates that “greener” approaches based on bio-based flocculants can be promising solutions for removing microplastics from aqueous media and minimize their potential negative effect on aquatic environments.

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## **Determining Factors Influencing Local Communities' Perceptions of Ecosystem Services in Divjakë-Karavasta National Park, Albania**

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Ecosystem services are integral to environmental preservation and the promotion of sustainable development. These services rely heavily on the perceptions of local residents to enhance ecosystem functionality. Our research is dedicated to exploring the perspectives of local communities regarding the preservation of ecosystem services and their contribution to the sustainable management of Divjakë-Karavasta National Park. Ecosystem services, as classified by the Millennium Ecosystem Assessment in 2005, encompass four types: provisioning, cultural, regulating, and supporting. These services are essential for the well-being and livelihoods of neighboring communities. Divjakë-Karavasta National Park is a rich source of ecosystem services and plays a significant role, not only locally but also at regional, national, and international levels. To ensure the continuity of ecosystem services and promote sustainable development, it is crucial to establish a strong connection with and garner a broad perspective from the local community. Our study involves conducting surveys with 201 respondents to assess local perceptions, knowledge, and attitudes related to ecosystem services within the national park. Furthermore, our research focuses on how these perceptions affect community involvement in park management, conservation initiatives, and the sustainable utilization of natural resources. Our primary findings indicate that proximity to the park and local economic activities significantly influence the perception of ecosystem services. These findings contribute to an elaborate relationship between local communities and the natural environment in this protected area, offering valuable insights for community engagement, sustainable development, and the preservation of the park's natural resources.

**Keywords:** ecosystem services, perception of the local community, preservation, proximity, Divjakë-Karavasta National Park



## Valorization of agroforest residues: Lignin extraction and modification to produce natural hair conditioners

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Conditioning agents are incorporated in hair care formulations to improve the physical and aesthetical features of hair. Some of the conventional conditioners are produced from non-renewable resources, are toxic to aquatic organisms and poorly biodegradable. These problems, and the increasing preference for natural products with low environmental impact, have motivated the research on renewable feedstocks for the development of cosmetic formulations. Natural biomass-derived compounds, such as lignin, have a great potential as future efficient and sustainable ingredients due to their high abundance, biodegradability, and low toxicity<sup>1</sup>. In this context, this work aims to use lignin as a resource to prepare natural conditioning agents that can repair damaged hair without compromising the environment or human health. The development of safe and efficient cosmetic formulations is dependent on the properties of its ingredients. So, the extraction of highly pure and valuable lignin from the biomass is a crucial step to consider. In this work, we developed a new extraction method using natural deep eutectic solvents (DES) capable of extracting all the lignin present in wood and with high purity. The extracted lignins were then successfully chemically modified to produce sustainable cationic hair conditioning agents that are expected to interact with human hair and efficiently repair it.

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## The presence of heavy metals in PM10 samples from Alentejo region

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Air quality represents one of the prime environmental and social challenges and depends upon the level of air pollution, changing the natural composition of the atmosphere. Poor air quality is associated with several respiratory and skin diseases and with environmental concerns, challenging both ecosystem services and public health. Deleterious impacts and mitigation strategies depend on the quantity and physico-chemical nature of the air pollutants originating from natural or human activities. Despite being commonly monitored, particulate matter (PM) composition, particularly its content of heavy metals, is scarce.

The aim of this work was to assess the heavy metals' concentration in PM10 samples collected at an urban site in the Alentejo region.

A total of 9 samples (PM10) were collected from October to December 2022 in Evora (38,5677375, -7,9113906) using a high-volume sampler (Digitel DH77), with a constant sampling flow, and quartz fiber filters. For heavy metal determination, microwave digestion of the filters with nitric acid and chloric acid was performed, followed by ICP-MS quantification. Chromium, iron, zinc, nickel, lead, cadmium, and arsenic were determined.

Total PM10 mass varied in the range 11,83 – 41,5  $\mu\text{g}/\text{m}^3$ , all samples were below the daily maximum limit (50  $\mu\text{g}/\text{m}^3$ ). The metals quantified in the highest concentrations were Cr and Fe, and in the lowest were As and Cd. The concentration of the compounds was influenced by sampling time, weather conditions, and the topography of the location. In general, As, Cd, Ni, and Pb concentrations were within the legal limits (5, 6, and 20  $\text{ng}/\text{m}^3$  and 0,5  $\mu\text{g}/\text{m}^3$ , respectively).

In summary, for most of the samples, regardless of the weather phenomenon, the legal limits for these pollutants were not exceeded. Knowing the concentration of PM10 and its physico-chemical composition, together with meteorological conditions, is very important for predicting and mitigating the effects on human health and the environment.

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**(Bio)Conversion of organic acids to electricity as a form of detoxification of lignocellulosic hydrolysates in a biorefinery context**

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For lignocellulosic biomass conversion into bioethanol, it is imperative to break down the polysaccharides enclosed in by applying a pre-treatment to allow their harnessing in the alcoholic fermentation process. During this step, several metabolic and enzymatic inhibitors are generated, resulting in affected fermentation efficiency. Options to eliminate these damaging compounds from the liquors that are subjected to be transformed through microbial metabolism, is normally accompanied by sugar depletion, and subsequently lower productivity. *Geobacter sulfurreducens* possess a heterotrophic oxidative respiration metabolism, mostly based on volatile fatty acids (e.g., acetic, formic, levulinic acid) degradation; considered inhibitors in our process. Fortunately, this metabolism carries out with no interest for fermentable sugars, basic for alcoholic conversion. This project aims to evaluate *G. sulfurreducens* capabilities to contend with fermentation inhibitors produced by acid hydrolysis of olive pomace under ideal growing circumstances while maintaining sugars presence. Once established *G. sulfurreducens* limiting development conditions, its conversion into electric current and inhibitor concentration depletion rate will be determined. This information allows implementation of approximate conditions to real liquid phase from acid hydrolysis pre-treatment and its subsequent optimization and standardization. Finally, process applicability will be assessed for fermentation boosting, demonstrating the detoxifying capabilities of the process.

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## Portuguese Viticulture Thrives: Insights from Somatic Embryogenesis in National Grapevine Cultivars

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Grapevines (*Vitis vinifera*) face challenges from emerging diseases to climate change, threatening vine health and grape production. To mitigate these issues and ensure the sustainability of viticulture, innovative techniques such as somatic embryogenesis (SE) are continually explored. SE is a process based on totipotency of plant cells, by which somatic cells are reprogrammed to form embryos that, in turn, can develop into complete plants. Particularly valuable for grapevines, SE provides an efficient alternative for breeding by rapidly producing large numbers of uniform plants with specific traits of interest, helps reducing the risk of disease transmission by producing disease-free plants and grapevine conservation by propagating rare or endangered cultivars, and facilitates genetic engineering, by allowing the introduction, modification, or expression of specific genes in the embryos to confer desirable characteristics. To establish a plant regeneration system for grapevine, SE was induced from 14 Portuguese cultivars. Carpels were aseptically excised from flower buds according to Cardoso et al. (2010) and cultured in induction medium (NN basal medium (Nitsch & Nitsch, 1969)) supplemented with 5µM 2,4-D and 1µM BAP) for one month under dark conditions. From the 14 cultivars, 12 were able to develop calli. Callogenesis rate ranged from 6,4% to 71,4%, achieved in the most responsive cultivar, 'Trincadeira'. After the induction period, the calli were transferred to expression medium (NN fresh medium lacking growth regulators) and kept under 16h photoperiod. Nine cultivars exhibited the differentiation of somatic embryos. On those cultivars the embryogenesis rate ranged from 0,7%, to 13,4%. Somatic embryos were isolated at the torpedo stage and converted to plants. Currently we have plants from eight cultivars which were successfully acclimated to greenhouse conditions. Further analysis will be performed to evaluate the presence of somaclonal variations and determine the applicability of the protocol on conservation and improvement of national grapevine cultivars.

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## **From seed to solution: carob by-products as novel biomaterials for a more sustainable agriculture**

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Limited resources, coupled with a rapidly growing population, create an urgent need to find sustainable alternatives that do not irreversibly degrade ecosystems. In this context, agroforestry by-products are emerging as an important source of valuable natural compounds that can contribute to minimizing the severe impacts of non-sustainable fossil-based products. In the Portuguese context, a particularly relevant agroforestry product is carob; Portugal ranks first in its global production, with up to 42,000 tons in 2018. Indeed, the exploitation of carob can be strategic for the Portuguese economy, becoming an interesting renewable raw material for the development of new biomaterials. However, carob is underexploited, as the economy is almost exclusively focused on the commercialization of the endosperm to produce locust bean gum (LBG). In addition, LBG processing generates significant quantities of two carob seed by-products: carob germ, which has a low market value, and the seed hull, which is discarded. The low utilization of locust bean co-products is mainly due to their low solubility in the solvents commonly used in the industry, which hinders their incorporation into novel bio-based materials. The application of LBG can be further expanded by circumventing some of its critical aspects, such as its neutral chemical structure, low aqueous solubility, and high molecular weight. This project presents novel and sustainable approaches for the utilization of carob seed by-products and LBG, with the ultimate goal of valorizing the carob fruit. This strategy will provide innovative solutions that could help mitigate important problems currently facing the agricultural sector, such as the misuse of agrochemicals and, more importantly, the problems of drought and water scarcity to which Portugal is dramatically exposed. In doing so, we also expect to pave the way for the creation of new local business opportunities that could help boost the Portuguese economy.

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## Establishment of a callogenesis protocol of *Ceratonia siliqua* L. to further explore its applicability for metabolites production.

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Plants can produce a broad spectrum of compounds with important biological activities, some of which are involved in plant defense, protecting plants from various biotic and abiotic stress factors. The identification of these compounds, mainly secondary metabolites, has been of interest in different fields. High-scale production of these compounds has shifted towards specialized *in vitro* systems with significant advantages (e.g., consistent yield and quality of plant-derived compounds, shorter production cycles, and greater biosafety). The carob tree (*Ceratonia siliqua* L.), a traditional species in Mediterranean agronomical systems, is currently considered one of the most prized fruit and forest trees in different sectors due to the high nutritional and medicinal value of its fruits. Despite its high interest, few studies have been conducted on the characterization of the metabolome composition of different organs across the high diversity of Portuguese cultivars. The limited studies available report a strong antimicrobial action of various compounds present in extracts of carob leaves, namely tannins, phenolic acids, flavonoids, and flavonoid glycosides, myricitrin and gallic acid, (-)-epigallocatechin-3-gallate and isoquercetin, which could be explored as a promising alternative to control pathogens, contributing to more sustainable agriculture. To investigate the potential of *C. siliqua* cells to establish a green cell factory for valuable metabolite production, a callogenesis protocol was established using leaf explants from the Portuguese cultivars 'Cardeira' and 'Pé Comprido', and the Spanish cv. 'Ramillete'. Explants were collected from plants growing under *in vitro* conditions and inoculated into Petri dishes containing MS solid culture media supplemented with three different combinations of the auxins IBA (indole-3-butyric acid), and 2,4-D (2,4-dichlorophenoxyacetic acid), and the cytokinin BAP (6-Benzylaminopurine). Different responses were observed, which will allow the selection of a specific growth media composition to proceed with further optimizations that includes different growing conditions. Characterization of metabolome from *in vitro* cell cultures will be performed using Liquid Chromatography coupled with a linear ion trap (LC-MS; Thermo Scientific).

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## Biotechnological Sustainable Production of Killer Yeast Toxins for Cultural Heritage Safeguard

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The issue of biodeterioration in the preservation of Cultural Heritage has gained significant attention, underscoring the necessity for novel approaches and advancements in this field. To tackle this issue, there is a pressing demand for innovative solutions. While synthetic polymers are frequently employed to combat biodeterioration in heritage items, they exhibit drawbacks such as high toxicity, short-lived effectiveness, and the rapid development of resistance among various microorganisms to these substances [1]. Instead of using conventional products, biotechnological methods have emerged as an alternative to produce environmentally friendly biocides. These biocides are composed of bioactive compounds synthesized by microorganisms as a natural defense mechanism, often referred to as “killer toxins.” Once these killer toxins are produced and purified, they can be employed to regulate the growth of microorganisms in heritage assets. The goal is to provide more efficient and sustainable alternatives that are safe for both human health and the environment, without causing any detrimental effects on the assets themselves [2]. This study was carried out within the scope of the ART3mis Project (2022.07303.PTDC) with the aim of producing killer toxins from yeast strains and evaluating their antimicrobial activity against different species of microorganisms isolated from Cultural Heritage. The range of antimicrobial effects exhibited by the metabolites generated from specific killer yeast strains was assessed. This assessment was carried out in both solid and liquid growth media, targeting bacterial strains like *Methylobacterium extorquens*, *Gordonia alkanivorans*, *Microbacterium foliorum* and *Bacillus firmus*. Additionally, the antimicrobial activity of these metabolites was also tested in solid medium against biodeteriogenic fungi such as *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., and *Fusarium* sp. The results obtained are encouraging in the development of new ecological biocides, which effectively suppress the biodeterioration caused by a wide range of microorganisms commonly found in various Cultural Heritage materials. These outcomes pave the way for the implementation of novel, green, safe, and sustainable solutions derived from fast and cost-effective biotechnological processes.

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**Micrografting of *in vitro* produced walnut (*Juglans* sp.) rootstocks**P. Tavares<sup>1</sup>, R. Nobre Pires<sup>1</sup>, L. Rodrigues<sup>1</sup>, H. Cardoso<sup>2</sup>, A. Peixe<sup>3</sup>

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Most of the walnut plants produced worldwide derive from grafting over seedling rootstocks. The use of this rootstocks leads to problems of lack of homogeneity in the orchards, hindering the mechanization of the culture. The optimization of micropropagation protocols allowed recently the production of clonal rootstocks and self-rooted cultivars, but, the grafting methods, still remain dependent of traditional nursery techniques. This work aimed on evaluate the possibility of introducing the micrografting technique, into an already optimized protocol for micropropagation of 'Paradox' rootstocks (*Juglans hindsii* x *Juglans regia*) cl. 'Vlach'. Factors like the grafting type, the developmental stage of the rootstock and the cultivar used, which normally condition the success of the technique, were evaluated. Two cleft grafting types were carried out (top and side), with the rootstocks in three distinct development stages (pre-rooting, post-rooting and post-acclimatization), using scions collected from *in vitro* cultured plants of the cvs. 'Chandler' and 'Howard'. Data shows that for both cleft grafting methods (top and side), as well as for the cultivar type ('Howard' and 'Chandler'), no statistically significant differences were observed. Conversely, between the different phases in which micrografting was carried out statistically significant differences were observed, with the best results, 66.33% of grafting success rate, being obtained with the cv. 'Howard', grafted by side cleft method, when performed in the pre-rooting phase of rootstock development. The success grafting rates obtained, when a specific factors combination was used, allow to conclude that *in vitro* micrografting, although it needs to be improved, may considered as an alternative to conventional walnut grafting processes.

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**Exploring the crosstalk between signalling molecules and auxin metabolism during adventitious root formation in walnut tree (*Juglans* spp.)**

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In vitro propagation of walnut cultivars (*Juglans regia* L.) using microcuttings appears as an efficient alternative to the conventional ex vitro methods used for clonal propagation allowing large-scale production of plants with genetically desirable agronomic traits. Nevertheless, the difficulty on the development of adventitious roots when followed the conventional methods of hardwood or semi-hardwood cuttings (recalcitrance), can also be seen in some genotypes under in vitro conditions. Understanding the molecular mechanisms that define this phenotypic difference may help find solutions not only for the in vitro system but also for the ex vitro system. Adventitious rooting is associated with plant response to two abiotic stresses, the wounding associated to stem cutting during microcutting preparation, and the application of an auxin, the indole-3-butyric acid (IBA), to the culture medium that will work as rooting inducer. The perception of these two stress factors involves a complex signaling network linked to the perception and homeostasis of reactive oxygen species (ROS), as well to perception to changes on Ca<sup>2+</sup> level. Enzymes involved on further homeostasis of those molecules have been implicated on adventitious rooting across different plant species. Its involvement in AR efficiency will be investigated by comparing its expression in genotypes behaving differently upon the AR stimulus ('Paradox' hybrid cl. 'Vlach' (*Juglans regia* x *Juglans hindsii*), known as easy to root genotype, and the cv. 'Chandler' known as difficult-to-root. IBA is used in a wide range of species as inducer of adventitious rooting. However, plant cells must convert this synthetic auxin to its active form, the indole-3-acetic acid (IAA). Enzymes involved on IBA metabolism, that includes its transport, and its conversion have been demonstrated as highly associated to AR efficiency. Here it will be studied different enzymes involved in the different pathways of IBA biosynthesis, including sensing, transport and conversion. This study may lead to important insights that can be applied to optimize the propagation process of recalcitrant genotypes.

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## Somatic Embryogenesis as a Plant Regeneration System: A comparative study considering the cvs. 'Galega vulgar', 'Cobrançosa' and 'Arbequina'

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Somatic embryogenesis (SE) is an in vitro technique that allows the regeneration of new plants from somatic cells, without the occurrence of gamete fusion. This approach not only enables the large-scale production of clonal plants with agronomic interest but can also contribute to the development of new genotypes through genetic engineering approaches. In this context, the development of efficient plant regeneration protocols via SE is crucial, allowing the regeneration of the plant from a single modified cell. In order to establish SE protocols for different cultivars, the Portuguese cv. 'Galega vulgar' and cv. 'Cobrançosa' common on the traditional orchards, and the Spanish cultivar 'Arbequina', highly used in the new orchard's plantation, were selected. Zygotic embryos were used as initial explants and the procedure to induce SE was followed as described by Pires et al. (2020). Embryogenic *calli* were maintained in liquid olive ECO medium by monthly subcultures. After three subcultures embryogenic *calli* were characterized by inoculating 200 mg in solid OMc expression medium. Results showed that cv. 'Arbequina' is the most responsive to the embryogenic stimulus ( $22.9 \pm 2.2$  % of embryos/*calli*), and 'Cobrançosa' is the least responsive ( $13.6 \pm 2.6$  % embryos/*calli*). Further, the conversion of the somatic embryos to plants, usually one of the most difficult steps in an SE protocol, was also evaluated. Embryos at different developmental stages (globular, torpedo, and cotyledonary) taken from the three cultivars were inoculated in OMc medium devoid growth regulators and kept at  $25 \pm 1^\circ\text{C}$  with a photoperiod of 16 h and a light intensity of  $40\text{--}45 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Results indicated that the cv. 'Arbequina' exhibited the highest conversion rate, followed by the cv. 'Galega vulgar'. However, a high number of abnormal embryos across the three cultivars was verified, being probably responsible by the low conversion rates. This work demonstrated the high dependency on the genotype in olive and allowed the selection of lines with different abilities to differentiate somatic embryos for further studies.

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## **What do local stakeholders think about the novel food processing technologies? A case study in Greece and Malta**

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In recent years, novel food processing technologies have been the focus of innovative food processing research. Novel food processing technologies offer a range of benefits covering various aspects of the food industry, from production and distribution to consumption, including improved quality and nutritional values, reduced food waste, increased efficiency and productivity, sustainability due to efficiency in the use of resources, economic benefits and market competitiveness. However, increasing the adoption of novel food processing technologies is necessary to ensure their potential benefits are utilized more. Adopting novel food processing technologies can face various technological, economic, regulatory and societal obstacles. Therefore, it is crucial to explore the opinions of local stakeholders in adopting novel food processing technologies. In this article, we used Q-methodology to identify potential barriers to adopting novel food processing technologies by investigating the perceptions of various actors in Greece and Malta, including local and supply chain stakeholders. Fifty-one stakeholders, twenty-seven from Greece and twenty-four from Malta, were asked to rank from strongly agree to strongly disagree with forty statements regarding the social, economic, environmental and governance aspects of novel food processing technologies. Factor analysis was used to interpret the results, and three different factors, each representing different perceptions, were determined for Greece and Malta. Surprisingly, these three perceptions were represented by various stakeholder groups, and the analysis results revealed that not all respondents from the same sector shared the same perception. One of the most important results of the study is that the stakeholders in both countries focus on the benefits of the novel food processing technologies in different aspects. For example, it was determined that the Greek stakeholders attach more importance to the social and environmental benefits of the novel technology, while the stakeholders in Malta attach more importance to the economic benefits. Stakeholders' concerns about cost, lack of knowledge and understanding about novel food processing technologies, resistance to change, and technical expertise may be considered barriers. However, at the same time, the social, economic and environmental benefits mentioned by the stakeholders may provide an opportunity to adopt novel food processing technologies.

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## **Characterization of production systems and agricultural activities in the area of the Living Lab of Luxor, Egypt**

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In North Africa agriculture is a significant sector influencing the lifestyle of the population. The current dominant form of cultivation is what is called conventional agriculture, which relies on the use of great amounts of inputs to achieve the desired outputs of production. Especially in the area of Luxor, Egypt, more than 30% of the population has a professional relation to agriculture. Moreover, its agricultural systems are vulnerable to exogenous factors, such as droughts, limited rainfall, booming population, and land fragmentation. Thus, there is the need to fortify those systems and establish their longevity over time by increasing their resilience. As a mean to do so, the study argues that the addition of agroecology, which includes participatory methods and practices as, low tillage, crop rotation and low inputs is the most suitable way to achieve resilience in Luxor. This study aims at the classification of the different food production systems based on their agricultural activities, with the parallel aim of adding agroecology as a proposed solution to the issues. In this context, in the area of Luxor, Egypt three producing systems were identified. These stemmed from meetings with stakeholders and action takers of the area, while in total twenty-five farmers participated in one-on-one interviews, for data to be collected. For the scenario generation, a bioeconomic model (DAHBSIM) will be used. The results of these studies provided a characterization of each, while at the same time were able to identify possibilities that with some further research, there is room for change to more optimal agroecological pathways to production. These findings highlighted the path for the adoption of agroecological practices by the local farmers by assessing the main farmers determinants in their adoption process, while the demonstrated their technoeconomic efficiency towards the resilience of the north-African agricultural systems.

## Phytotoxic effects of sesquiterpene lactones enriched fractions against weeds and its possible use as a bioherbicide

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Sesquiterpene lactones (SL) are allelopathic compounds with high expression in *Cynara cardunculus* (cardo) leaves ( $\approx 95\text{g/kg}$  dry weight) [1], [2]. Because of their wide-ranging phytotoxic activity, SL are a promising option as bioherbicide in sustainable weed management [3]. In our previous work, SL-enriched fractions (SL-EF) obtained through membrane processing technology revealed an improvement on phytotoxic activity against the weed species *Portulaca oleracea*, specifically on shoot growth. The aim of this study was to assess the phytotoxic potential of SL-EF obtained through SuezGH2000 diaultrafiltration of cardoon leaves extract against a panel of Mediterranean weed species in pre-emergence, as well as the effect on morphological and biochemical parameters on *P. oleracea*'s post-emergence state. For that, cardoon leaves initial extract (CcLEi), SL-EF and the commercial herbicide Stomp®Aqua (HBC) were tested at concentrations ranging from 100 and 800ppm against a panel of 8 weed species. After incubation, germination rate, root and shoot length were evaluated. For *P. oleracea* post-emergence bioassay, CcLEi, SL-EF and HBC were added to the plant's roots, in a hydroponic system, at 800, 600 and 400ppm. After 12-day trial, the plants were harvested, and phytotoxic potential assessed using morphological analysis, as well as measurements of total protein, malondialdehyde (MDA), photosynthetic pigment, and superoxide dismutase inhibition activity. Results demonstrated that CcLEi and SL-EF had inhibitory effect on the weed panel used, especially on root growth. Furthermore, SL-EF inhibited root growth more effectively than CcLEi on 6 of the 8 weed species tested, evidencing the SL purity enhancement on the fractions. According to the results regarding post-emergence bioassay, both CcLEi and SL-EF had an influence on plant morphology by causing chlorotic leaves and leaf fall especially at 800ppm, with SL-EF having a more evident effect (23,70% decrease of photosynthetic pigments content). Oxidative stress was also induced, as proved by MDA levels 45.27% in comparison with control. This study demonstrated the effectiveness of SL-EF on root growth inhibition of several weed species in their pre-emergence stage, suggesting the possibility of applying these fractions through irrigation. Also, SL effects on plants metabolism biochemical parameters were evidenced, by affecting photosynthetic pigments and inducing oxidative stress.

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**Economic valuation of the apiculture value chain in France. Identification of the potential impacts and diffusion conditions of an innovative traceability system within the industrial chains and territories in the Occitany region**

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The apiculture industry is a significant commercial sector in the European Union (EU). It offers a diverse range of products including honey, royal jelly, pollen, propolis, and beeswax worth €14.2 billion per year (European Commission, 2022), as well as crucial ecosystem services to the agriculture sector, given that 76% of European food crops (Lonsdorf et al., 2011) and 80% of European wild plants (Ollerton et al., 2011) depend on pollination. However, European bee populations have declined due to the spread of diseases, pesticides, loss of plant diversity, climate change, and wildfires (Goulson et al., 2015). If no action is taken, the European pollinator numbers may collapse with severe economic and environmental consequences on human welfare (European Commission, 2016). In France alone, the economic value of pollination services supplied by bees varies between €2.3 and €5.3 billion per year (Leonhardt et al., 2013). Regional data on the economic value of pollination services is currently unavailable despite national-level data being available. Therefore, there is a need to evaluate the economic value of pollination services on a regional level to develop effective local public policies. This leads us to the Occitania region, where we calculate the crop production value (CPV) and pollination service value (PSV) for seven regional crops (melon, tomato, apple, peach, cherry, sunflower, and strawberry) using accurate regionally sourced data from 2021, through the dependence ratio method. The combined CPV of the crops is 1132.13 million euros, while the PSV is 712.16 million euros, meaning that pollination contributes to 62% of the studied crop production. This ratio suggests that action is needed to support regional sustainable development, including pollinator conservation and ecologically intensified farming practices.

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## **Why is CRISPR/Cas13d the best choice for engineering interference against RNA plant viruses?**

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Plant viruses represent a major threat for crops and food security worldwide, causing significant quality and yield losses and being very difficult to control. Virus-resistant plants have been developed over the past years as a way of reducing these losses, using a wide range of molecular technologies. One of the most promising technologies is based on an adaptative immune system against viruses found in prokaryotes, the Clustered regularly interspaced short palindromic repeats and associated proteins (CRISPR/Cas). The first CRISPR/Cas systems studied were very useful to target DNA. More recently, other types that can specifically cleave single-stranded RNA (ssRNA) in eukaryotic cells have been identified, representing a major breakthrough in plant virology, since most plant viruses have RNA genomes. CRISPR/Cas13 systems consist of single RNA-guided Cas13 effector nucleases that solely target ssRNA in a programmable manner, without altering the DNA. The Cas13 protein family comprises four subtypes (a-d) and recent studies point out Cas13d as the most effective, with higher efficiency and specificity in cleaving targeted RNA. Additionally, Cas13d has shown structural and functional advantages over the other Cas13 variants, suggesting it as the best choice for viral RNA interference, engineering and editing. This work brings together the most up to date information about CRISPR/Cas systems components and immunity steps, focusing on the advantages of the use of Cas13d. A detailed characterization and previously reported applications of Cas13d, such as targeting and cleaving plant RNA-viruses are presented. The deep knowledge on these systems will allow a variety of RNA manipulations, including the production of virus-resistant plants, as well as the development of rapid molecular diagnostic tests, which are valuable tools towards a sustainable agriculture.

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## From arid wild legume shrubs to food legume: Application of non-rhizobial bacterial endophytes to improve *Cicer arietinum* L. growth under salinity

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Chickpea (*Cicer arietinum* L.) is an important grain legume with a high nutritional value. Being the third-largest food legume produced worldwide, chickpea is mainly cultivated in developing countries where crop farming suffers the impacts of various biotic and abiotic constraints such as heat, salinity, and drought. In such vulnerable areas, the increase of soil salinity results in an annual loss of 8-10% of chickpea yield. Salt stress induces not only anatomic and biochemical changes in chickpea plants, but also inhibits the symbiotic interaction with *Mesorhizobium* resulting in impaired biological nitrogen fixation. In this context, the present study evaluates the effects of salinity on the early molecular events of the *Mesorhizobium*-chickpea interaction and presents the use of non-rhizobial bacterial endophytes, isolated from wild legume shrubs, as a biological alternative to mitigate the negative impacts of salinity on chickpea growth and symbiosis.

As expected, salinity had a negative effect on both symbiotic partners. In chickpea, a significant decrease of 44% of seeds germination was registered under 0.2% NaCl. In addition, a significant reduction of the plant growth was observed in uninoculated plants supplemented with synthetic nitrogen. Similarly, results showed a significant decline in the symbiotic performance of the *Mesorhizobium* strain under salt stress. Both *in vitro* and *in vivo* assays revealed a significant negative impact of salinity on i) the expression of specific symbiotic genes and, ii) the formation of nodules in chickpea plants inoculated with *Mesorhizobium* alone. Interestingly, the combination of chickpea microsymbiont with different non-rhizobial bacterial endophytes consortia showed to promote i) the plant growth under salinity and ii) the *Mesorhizobium*-chickpea symbiosis in co-inoculated plants submitted to salt stress. The present study highlights the sensitivity of the early signalling molecular events between both partners to salt stress and reveals the potential application of non-rhizobial bacterial endophytes from wild legume shrubs as plant-growth promoting bacteria in salinized soil.

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## Different inter-row management practices on a vineyard: Implications on soil microbial activity

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Wine grape production is an important economic activity in Portugal, as in most Mediterranean countries; however there are several risks related with unbalanced soil management, and loss of biodiversity, that lead to a decrease in grapevine yield and grape quality. The importance of soil health to guarantee the quality of the grape led several wine growing regions worldwide to adopt soil sustainable management practices. The effects of soil on wine quality, when associated to biophysical parameters and grapevine variety are encompassed in the concept of terroir. The soil microbiome is crucial in order to maintain healthy soils and contribute to biodiverse and resilient ecosystems [2]. The soil microbial community is a reliable bioindicator that reports on soil health and fertility, and which is highly influenced by agronomic practices [1]. The use of cover crops can increase soil and plant health and also contribute to the conservation and ecosystem services provision. Our objective was to evaluate two soil management practices: absence and presence of cover crop in inter-row with *Vitis vinifera* L. cv. Alicante Bouschet and Arinto through their impact on soil microbial activity, using several parameters: soil basal respiration; enzymatic activities of Dehydrogenase, Arylsulfatase,  $\beta$ -glucosidase; and the number of culturable bacteria and fungi. Soil samples were collected at different growing seasons of the vineyard: flowering and veraison in 2022 and budding in 2023. The results showed that the different soil management practices had the most significant effect on the increase of dehydrogenase activity over time. In general, the presence of cover crop increased the activity of the analysed microbial activity and also favored culturable bacteria in Alicante Bouschet and fungi in Arinto inter-rows. Additional research on this topic is ongoing to better understand the effects of the cover crops in the microbial communities.

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## **Fungal community present in symptomatic and asymptomatic almond trees in the Alentejo region**

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In the context of climatic changes, the increment of plant diseases can be boosted in the Mediterranean regions, triggering changes in the interactions between host and microorganisms. Also, new pathogenic strains can emerge, leading to the appearance of new diseases. Although the almond tree is a traditional culture in the north and south of Portugal, new commercial orchards have been introduced in the Alentejo region. Some phytosanitary problems related to fungal pathogens have already been observed. Fungal pathogens are the main cause of disease that can lead to decreased productivity and death of the orchards in almond trees mainly by the formation of branch and trunk cankers. Understanding the fungal community is an important step toward the development of effective strategies for plant protection. In this work, we identified and characterized the fungal community from two different orchards in Alentejo region (Mora and Ferreira do Alentejo). Samples of leaves and trunks from symptomatic and asymptomatic trees were analyzed by molecular techniques, targeting the internal transcribed spacer (ITS) region of nuclear rDNA for PCR amplification and Sanger sequencing. Irrigation water samples from the two orchards were also analyzed by the same methodologies. Similar pathogenic fungi agents were obtained in symptomatic and asymptomatic trees. The analysis of the water demonstrated the importance of phytosanitary treatment. The water treated presented lower number of fungal species as compared to the non-treated water. In the future, we will develop comparative transcriptome studies of symptomatic and asymptomatic almond trees to understand the molecular mechanisms behind the tolerance to plant disease. The knowledge obtained with this work will allow the designing of new strategies for disease management and precision plant breeding.

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## Quick and accurate diagnostic test for field detection of anthracnose and brown rot diseases in almond trees

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Almond tree (*Prunus dulcis*) has become one of the most important agricultural sources of income in Portugal and Spain and is now a trait of the Mediterranean landscape. In the Alentejo region, there is an increasing interest in intensively produced almonds in fields previously occupied by other crops, which inevitably poses new challenges associated with the plants' phytosanitary conditions. The emergence of new pathogenic species and the adaptation of old ones from other crops, namely pathogens responsible for anthracnose and brown rot diseases, are causing severe diseases and losses in almond orchards that are not yet fully understood and there is little to no available treatment.

The main purpose of this work is to develop a diagnostic test for the early and rapid field detection of anthracnose and brown rot associated fungi, using the 'Doctor Vida' equipment, a portable and reusable PCR-like device, which allows a rapid response on the infection rate and the treatment opportunity. For that purpose, pure cultures of pathogens were already isolated from symptomatic almond plants and fruits from different orchards located in the Alentejo region, and genomic DNA extracted. For fungal genetic identification, the ribosomal internal transcribed spacer (ITS) region was amplified through PCR using ITS1 and ITS4 primers, and sequenced. Sequence homology was explored at the NCBI database using the BLAST algorithm. Specific primers for target pathogens, based on LAMP technology, will then be designed and/or identified in the available literature and the protocol will be optimised for the rapid field detection of these pathogens. This study will allow the early detection of the target pathogens associated with almond trees using a sensitive diagnostic method, avoiding production losses and unnecessary chemical interventions, for a more productive, competitive, and sustainable agricultural system.

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## Effect of mobilization systems in several biomes of the Montado: analysis of soil microbial activity

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The Montado is a multifunctional agro-silvo-pastoral system shaped by human activity. These areas were originally occupied by Mediterranean forests and today they are mainly made up of cork oaks (*Quercus suber*), holm oaks (*Q. rotundifolia*) and pastures that grow in very poor soils. This is a biodiverse system, providing several important ecosystem services. Therefore, preserving and improving its potential should be a priority. Sustainable management systems can play an important role in this context, as is the case of no-till, which in many aspects favors soil health.

Soil enzymatic activity is a good indicator of soil quality, as it rapidly responds to both environmental and management changes.

The objective of this study was to evaluate the impact of two tillage systems (no-till and conventional tillage) in the soil microbial activity of the different biomes found in the Montado ecosystem (thickets, scrubs and vernal pools). This study was carried out at Herdade do Mouchão, which adopted no-till technique a year ago, and the soil sampling took place in February. Arylsulfatase (sulfur cycle), betaglucosidase (carbon cycle), phosphatase (phosphorus cycle) and urease (urea cycle) were the selected enzymes for analysis.

In general, the results obtained for the different enzymes are in line with what was expected, since we found higher activity values in no-till plots than in the mobilized ones. In the thickets biome, the differences were significant for 3 of the 4 enzymes studied, namely phosphatase, betaglucosidase and urease. Although the no-till technique was only applied one year ago, it is already possible to perceive the differences between the two tillage systems. No-till preserves the soil structure, favoring soil microbial activity, and these differences are expected to increase over time.

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**Effect of different types of grazing and the application of dolomitic limestone, on the height and floristic composition of pasture, and their relationship with the preferred locations for grazing sheep, in the Montado ecosystem**

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The Montado is an agro-silvo-pastoral ecosystem, typical of the Alentejo region. The acidity of its soils and manganese toxicity limits the quality of the pastures. The use of pastures by grazing animals is influenced by height, floristic composition, and quality. The objective of this work was to study the relationship between pasture height and floristic composition, and the locations preferred by grazing sheep. A 4ha field was divided into 4 plots, with the following treatments: Plot 1- without application of dolomitic limestone and continuous grazing (P1UC; 7 sheep/ha); Plot 2- without application of dolomitic limestone and deferred grazing (P2UD; 16 sheep/ha); Plot 3- application of dolomitic limestone and deferred grazing (P3TD;16 sheep/ha); Plot 4- application of dolomitic limestone and continuous grazing (P4TC;7 sheep/ha). The experimental work took place between March and June 2021, at Mitra farm - University of Évora. In each plot, 12 sampling points, representing the plant communities of the pasture, were identified. During the test period, 6 animal observation dates were carried out (biweekly, on two consecutive days, every 10 minutes), from sunrise to sunset, where the animals' activity (rumination, idleness, grazing) was recorded. On the day before each observation date, the pasture height was measured at each sampling point. The identification of botanical species, at each sampling point, was carried out in winter, peak of spring, and early summer. As for the height of the pasture, there were significant differences among dates and for the plots. The amplitudes of the pasture heights shows that there was selectivity in all plots. The low locations of the plots were preferred for grazing in all treatments. Next to the road, fewer observations of grazing were recorded. The importance of locations, close to the drinking trough, for grazing, increases with increasing temperatures and photoperiod. The floristic composition of the pasture did not seem to have been decisive for the choice of grazing locations, nor for its changes throughout the observation period. The type of grazing and the treatment with dolomitic limestone did not seem to change the grazing pattern between treatments.

## A new tool to protect olive trees against *Collectotrichum* spp., the causal agents of olive anthracnose

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Olive anthracnose is one of the main limiting factors of olive production in Portugal and is responsible for significant yield losses and poor fruit and oil quality. It is caused by several *Colletotrichum* species and variations in its virulence depend on the interaction between the pathogen and the host. Symptoms appear with the first autumn rains and the damage can be devastating when humidity and temperature conditions are favorable. The disease is normally controlled through a combination of appropriate cultural practices and preventative applications of copper fungicides. However, when conditions are particularly favorable for the disease development, fungicide applications become insufficient or ineffective. In addition, European Community directives restrict the use of copper-based products, so there is a need to find alternatives to combat this disease.

The goal of this research program is the use of a viral vector, previously developed, with an antimicrobial peptide (AMP) that is effective against *Colletotrichum* spp., which will be a tool to inoculate into young olive plantlets, giving them protection against *Colletotrichum* spp. The first step of the work will be to obtain and characterize various isolates of *Colletotrichum* spp. from symptomatic fruits. After that, their pathogenicity will be tested by inoculating *in vitro* olive plantlets, to warranty their healthy status. Among others, 'Galega' variety will be used as target plants, once it is the most widespread national cultivar and the most susceptible to anthracnose disease. Subsequently, for each of these isolates the AMP viral vector and its antagonism will be tested. The third step will be to test the stability of viral+AMP in *Nicotiana benthamiana* plants and then inoculated in olive plantlets propagated *in vitro*. Finally, to test the working hypothesis, "Colletotrichum protected" olive plants will be inoculated with isolates of *Colletotrichum* spp. We emphasize the importance of this study to incorporate new sources of resistance of olive trees to anthracnose for the promotion of sustainable management strategies.

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## **Application of *Opuntia ficus indica* extracts on grapevine to mitigate the effects of climate change**

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Many commercial products made of kaolin and silicium have been used in vineyards to mitigate the effect of high temperatures and intense radiation. The objectives of this study were to compare the use of kaolin and silicium commercial products (Humigel Kaolin<sup>®</sup> and Humigel Plus<sup>®</sup>) and *Opuntia* extracts on plant's physiological performance. The final goal is of this work is to develop sustainable practices, using natural products against environmental stresses.

Seven treatments were tested, including commercial products based on silic and kaolin in different doses as well as *Opuntia ficus indica* extract (OFI), and compared to a control group without any treatment. The application occurred in June and samples were collected in 3 dates until harvest. It was observed, comparing varieties, a lower stomatal conductance in Aragonês and a reduction of water loss through transpiration in the last sampling date. Among treatments there were no significant differences in the relative water content (RWC) however the OFI extract treatment showed a higher of RWC comparing with other commercial substances. Regarding specific leaf area (SLA), the last sampling date showed the lower values as expected, which is in accordance with the increase in dry weight. No significant differences were observed in leaf chlorophyll index, photosynthesis performance index (Plabs), and maximum quantum efficiency of photosystem II (Fv/Fm) between treatments, only among grape varieties, in fact, Aragonês showed higher values for leaf chlorophyll index and Plabs. These results are important to redirect the trial in the next year the, which means that the treatment doses need to be adjusted using a higher concentration.

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## Development of *Vitis vinifera* cultivars resistant to powdery and downy mildew – Presentation of a Portuguese breeding program

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Grapevine (*Vitis vinifera* L.) is widely recognized as one of the most important perennial fruit crops grown in Europe. Downy mildew (caused by the oomycete *Plasmopara viticola*) and powdery mildew (caused by the fungus *Erysiphe necator*) are two devastating diseases affecting *V. vinifera* cultivars, leading to significant losses in productivity and wine quality. To combat these diseases, high amounts of fungicides are applied every year to vineyards, raising concerns about the sustainability of viticulture from the perspectives of producers, consumers, and environmental impact. In response to this problem, several wine-producing countries implemented conventional breeding programs years ago, focusing on the selection of hybrids resulting from crosses between resistant genotypes and elite *V. vinifera* cultivars. Several new cultivars have already been developed and are available in European countries for new vineyard plantations. In Portugal, a breeding program following the same approach has been implemented at the Viveiros PLANSEL Lda. company (Montemor-o-Novo, Portugal). The goal is to develop resistant genotypes that exhibit the introgression of different resistance loci without a negative impact on productivity and wine quality. Under this program, various *V. vinifera* elite Portuguese cultivars, such as 'Touriga Nacional,' 'Alvarinho,' and 'Fernão Pires,' were used in controlled crosses with resistant genotypes (cvs. 'Defensor,' 'Regent,' 'Calardis Blanc,' 'Floreal,' 'Artaban,' and the hybrids 2004-43-21 and 2014-92-51, generously provided by Dr. Olive Trapp of the Julius Kühn-Institut - JKI, Germany). The hybrids were selected based on SSR (Simple Sequence Repeats) associated with the loci *Ren3*, *Ren9*, *Run1*, *Rpv1*, and *Rpv3.1*. Over the years, the Portuguese collection of hybrids carrying resistant loci has expanded, and two experimental fields were planted to produce enough fruits for wine analysis. The selection of multi-loci hybrid genotypes with good organoleptic characteristics will enable the continuation of the breeding program through controlled crosses with different traditional Portuguese cultivars. This presentation provides an overview of the breeding program and the achievements obtained after several years of work.

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## LIDAR sensor applied to olive trees

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In this study, we address a methodology for measuring olive tree canopies using LIDAR sensors. The orchard is managed in a super high-density system (SHD) with the Picual cultivar, located in Monforte, North of the Alentejo Region. The orchard was part of an experimental pruning trial, where, in the first treatment, the canopy was cut conventionally, and in the second treatment, selective pruning was implemented, sparing the new branches and the most flexible ones. This resulted in an expectedly dense and large canopy.

The sensor used was a fixed one that took several collections at different locations in all the space, which were then merged. Applying LIDAR technology enables detailed measurements of the weight and length of all sections of a tree, as well as the density of leaves and branches. This facilitates a comparison between the two treatments and their adaptability to the harvest machine.

Data were processed in CloudCompare (open source software), where the trees were normalized with respect to the ground points with those subsequently removed as they were unnecessary and too dense and heavy to work with. The outcome of this experiment is a point cloud in a LAS file, comprising nearly 2.5 million points with the x, y, z dimensions and corresponding georeferenced coordinates.

The results include the superficial area, volume, and limit points around the canopy. These parameters provide insights into the performance of the pruning and, in the future, the behaviour of different cultivars and biomass in olive orchard canopies.

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## Effects of combined mulch and *biochar* application on soil carbon cycle in vineyards of the Alentejo region

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According to the European Environment Agency (2019), 80% of greenhouse gas (GHG) emissions are CO<sub>2</sub>. In the context of global warming, there is an urgent need of finding new ways of storing more carbon. Plant biomass can be transformed to *biochar* and store a big amount of carbon in the soils. *Biochar* is the product of the pyrolysis of biomass. This product can be used for various purposes, including increasing soil moisture, improving soil habitat or increasing soil organic carbon. The capacity for increasing the soil organic carbon is very variable, being dependent on the type of biomass used, the pyrolysis temperature, the application rate, soil characteristics, climate, among other factors. The knowledge and correct use of different types of *biochar* may be one of the solutions to mitigate the effects of global warming, make agriculture more sustainable and still have a positive effect on the economic viability of agricultural companies. The aim of this research, within the scope of the Master's dissertation in Conservation Biology, is to evaluate the effect of mulch and *biochar* to the delivery of soil ecosystem services, such as erosion control, water storage, carbon storage and soil habitat in vineyards in the Alentejo region.

Laboratory rainfall simulations were carried out to assess the erosion and water storage capacity in the untreated, mulch, *biochar* and different combinations of mulch-*biochar*. Soil habitat is being assessed through phytotoxicity tests with different types and concentrations of *biochar* using *Lactuca sativa* L. seeds. Plots were installed in the field and untreated, mulch and mulch+*biochar* treatments are being monitored in Alentejo vineyards. Adicionally, CO<sub>2</sub> rings will be added to the field to monitor soil respiration using a LI-COR device (model LI-7810).

Preliminary results with rainfall simulations and phytotoxicity tests indicate that the combination of straw and *biochar* reduces significantly runoff water and erosion and that *biochar*, in low concentration, up to 3 %, does not affect soil phytotoxicity.

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**Keywords:** erosion, phytotoxicity, sustainability, agriculture, climate change

## Effect of biochar application to soil habitat on lettuce germination (*Lactuca sativa* L.)

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Soil is the support of life on the planet and is essential to preserve and recover it. The intensification of agricultural activity has contributed to soil degradation, increasing erosion, and favoring desertification. Measures for soil conservation are essential, to help improve its characteristics, namely increasing the organic matter content. Biochar is a product that results from the pyrolysis of different biomasses. Its application to the soil can be one of the ways to increase the organic matter content, improve physical and chemical properties and contribute to greater microbial diversity. Biochar, depending on the raw material and manufacturing process, may present phytotoxicity, making it necessary to evaluate it.

Seven types of biochar were tested, coming from different raw materials. The phytotoxicity of each biochar was evaluated, mixed with soil from an olive grove, at concentrations of 1.5%, 3% and 5%, as well as pure biochar and soil, in a total of 22 modalities. Distilled water was added to each mixture to reach the saturation point, and the soil was distributed in Petri dishes and covered with filter paper. Germination tests were carried out in an incubation chamber, at a constant temperature of 25°C and in the dark. Phytotoxicity was tested using *Lactuca sativa* L. seeds, which were placed on filter paper. 5 Petri dishes were prepared, with 10 seeds each, for each study modality. After 72 h, the following parameters were calculated: germination percentage, germination index, root growth and total growth. Additionally, the content of 32 major Polyaromatic hydrocarbons (PAH), pH, OM% were measured for each biochar and soil.

It was found that pure biochars did not favor germination or seedling growth. However, biochar mixed with soil at 1.5 and 3% contributed to an increase in germination, greater growth of roots, as well as shoots. Preliminary results shows that each mixture of biochar-soil has its own optimum application rate. More studies are needed in the search for more knowledge about the effect of biochar application on soil properties. This work was funded by National Funds through FCT under the Project SOLVO (2022.06004.PTDC) and PRR Project - Vine and Wine Portugal (Solvit).

**Keywords:** phytotoxicity, sustainability, agriculture

## **A new approach to detect *Alternaria* spp., an emerging pathogen on olive groves**

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In recent years, a very intense and precocious fall of the fruits before maturation has been observed in several cultivars of olive groves in the Portuguese regions of Alentejo and Ribatejo. Internally, the affected fruits, still green, showed necrosis in the peduncle's insertion zone extended to the stone, with serious consequences in olive production. Also, an increase in the acidity content of the oil obtained from these olives was observed, leading to its depreciation. The group of the Micology Lab (MED, UÉvora) already identified through morphological and molecular analysis *Alternaria alternata* as the fungi responsible for the described symptomatology. In this context, the development of a methodology that enable to get an accurate and reliable detection of *Alternaria* sp. in olive groves is for sure of high interest. In the present work, a TaqMan MGB (Minor Groove Binder) specific assay for *Alternaria* sp. detection, previously established for a different purpose, was here applied as a molecular-based tool to detect *Alternaria* sp. in olive trees genomic DNA (gDNA) and the qPCR procedure was successfully established. To determine the reliability of the assay, the detection and quantification of *Alternaria* sp. was successfully performed in symptomatic and asymptomatic olive fruits, leaves and branches from different cultivars. Also, *Alternaria* sp. fungal isolates obtained from symptomatic plants, identified through amplification of the ribosomal internal transcribed spacer (ITS) region of nuclear rDNA, were used as qPCR positive control. The established methodology allowed a reliable, sensitive, and reproducible estimation of *Alternaria* accumulation in infected olive plants, gaining new insights for disease control.

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## Transcriptomic profiling of grapevine cultivars reveals candidate genes associated with trunk diseases symptomatology

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Grapevine trunk diseases (GTDs) have become a major concern to viticulture worldwide, causing a high economic impact on yield and vineyard longevity in all growing areas. The prevalence of GTDs is increasing and, together with the absence of effective treatments, is currently one of the major challenges for viticulture sustainability. Given the urgent need to identify and develop new sustainable and effective protection strategies, a better understanding of grapevine defense mechanisms is essential to develop more tolerant plants and provide valuable insights for disease management. This study aimed to explore the transcriptomic profile of GTDs symptomatic and asymptomatic plants of two cultivars with different levels of susceptibility, 'Trincadeira' and 'Alicante Bouschet', using next-generation sequencing technology. RNA-seq yielded 1 598 differentially expressed genes (DEGs) between cultivars, 622 overexpressed in 'Trincadeira' and 976 in 'Alicante Bouschet'. Major differences were found between both asymptomatic groups, with a total of 1 628 DEGs. The fewest differences were found between groups with different symptomatology, regardless of the cultivar, with only 64 DEGs, 59 upregulated in symptomatic and 5 upregulated in asymptomatic plants. In these ones, the significantly enriched pathways are related to peroxidase activity and response to oxidative stress. Transport, transmembrane transport, proteolysis, and lipid metabolic process were revealed as the main biological processes involved, generally activated in 'Alicante Bouschet', the most susceptible cultivar, suggesting that the DEGs associated with these processes may play an important role in disease development. Contrarily, genes involved in peroxidase activity and response to oxidative stress (such as *PER42*) may explain the expression of GTDs symptoms. Our results can help explain the different susceptibilities of grapevine plants to GTDs and, based on the identification of promising candidate genes, it may turn possible the development of strategies involving the activation or inhibition of potential plant response regulators, offering sustainable and effective alternatives to successfully manage GTDs.

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## The need to develop tailored pest management strategies in insect mass rearing-facilities

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*Hermetia illucens* (Black Soldier Fly, BSF) is increasingly recognized for its potential in bioconverting organic waste, providing nutrient-rich animal feed and contributing to increase sustainability efforts. Due to its versatility and capability to bioconvert different wastes into protein, BSF is used in large-scale production for waste management and livestock feed.

As with other livestock production systems, unwanted species may occur in insect farming and can pose a challenge to the successful rearing of BSF larvae. The larvae, the type of substrates, and egg batches can attract other living organisms. Among the most common occurrences in BSF rearing facilities, are rodents, fungi and different types of arthropods, such as, ants (Hymenoptera), flies (Diptera) and mites (Arachnida), with more or less effects in larval development. In the past couple of years, the BSF rearing pilot unit of Coimbra Agriculture School (ESAC-IPC) had occasionally been infested with some of already reported pests, and we further report booklice (Psocoptera).

The presence of pests and their pernicious effects in insect mass rearing facilities underpins the need to apply preventive and pest management control measures. However, similarities between arthropod pests and *Hermetia illucens*, makes this more challenging. Therefore, is highly recommended to increase research on the development of effective pest management strategies in BSF as well as other insect mass rearing facilities.

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## Influence of cardoon seed in lamb diets on animal performance and carcass and meat quality

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*Cynara cardunculus* L. (Cardoon) is a perennial herbaceous species from the Asteraceae family, particularly well adapted to Mediterranean climatic conditions. Due to its chemical composition and nutritional value, the cardoon seed can be an alternative to conventional raw materials in animal feed. The objective of this work was to evaluate the effect of including cardoon seed in lamb diets on growth performance and carcass and meat quality, comparing with a diet without lipid supplementation and with a diet supplemented with a conventional oilseed (sunflower seed). The experimental procedures respected the EU Directive 2010/63/UE and were approved by ORBEA Ethics Committee of INIAV. Twenty-one lambs of approximately 60 days of age and live weight of  $23.5 \pm 2.00$  kg (mean  $\pm$  s.d.) were housed individually and divided into three diets (7 lambs/diet): C – without lipid supplementation; SS –supplemented with sunflower seed (4.7%); and CS – supplemented with cardoon seed (10%). Diets with the oilseeds were formulated to achieve the same ether extract level. Diets were offered *ad libitum*. After an adaptation period of 7 days, the weight of the lambs was assessed weekly and the feed intake daily over 5 weeks. After slaughter the carcass and meat traits were evaluated. In meat, colour and lipid stability were evaluated over 7 days of refrigerated storage. The CS diet reduced the average daily gain ( $P < 0.001$ ) compared with other diets (182 vs. 253 g/day). Diets with both oilseeds reduced the dry matter intake, with a more pronounced reduction in CS diet ( $P < 0.001$ ; 1467, 1324 and 1135 g/day in C, SS and CS diets). Neither carcass or meat quality parameters were affected by diets. Moreover, concerning the refrigerated raw meat stability over storage time, lower lipid oxidation levels were found for SS and CS diets ( $P = 0.002$ ). Cardoon seed did not compromise the carcass and meat quality. However, the negative impact on animal growth indicates that the use of cardoon seed in the diet of growing lambs needs to be adjusted.

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**Total mercury levels in duplicate diet samples**S. Coelho<sup>1,2,3,4</sup>, S.D. Coelho<sup>3</sup>, R. Barros<sup>4</sup>, R. Pastorinho<sup>2,4,5</sup>, A.C. Sousa<sup>1,2,4</sup><sup>1</sup>Department of Biology, University of Évora, Évora, Portugal<sup>2</sup>Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal<sup>3</sup>Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Aveiro, Portugal<sup>4</sup>NuESA-Health and Environment Study Unit, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal<sup>5</sup>Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, PortugalEmail: [susana.coelho@uevora.pt](mailto:susana.coelho@uevora.pt)

Ingestion of contaminated food, particularly seafood, is considered the most important exposure pathway to mercury. Most studies focus on the evaluation of mercury levels in seafood, and few reports are available on the total mercury levels in duplicate diet samples. In this sense, the objective of this work was to analyse the mercury concentrations in duplicate diet samples. Forty volunteers, recruited at University of Aveiro academic community, collected a duplicate sample of all food items ingested for one week. The samples for each volunteer were pooled together, homogenized, freeze-dried, and stored at -20°C until analysis. Quantification of total mercury was performed in triplicate by atomic absorption spectrometry with thermal decomposition and gold amalgamation in a NICMA-3000 analyser (Nippon Instruments Corporation, Japan). Mercury concentrations ranged from  $2.63 \times 10^{-3} \mu\text{g.g}^{-1}$  wet weight (ww) to  $80.4 \times 10^{-3} \mu\text{g.g}^{-1}$  ww, with an average value of  $10.1 \times 10^{-3} \mu\text{g.g}^{-1}$  ww. These values correspond to an estimated weekly intake (EWI) between  $0.052 \mu\text{g kg body weight (bw)}^{-1}$  per week (w)<sup>-1</sup> and  $15.789 \mu\text{g kg-bw}^{-1} \text{ w}^{-1}$  (values calculated considering the average adult body weight of 70 kg). The average EIW of  $1.993 \mu\text{g kg-bw}^{-1} \text{ w}^{-1}$ . Is higher than the tolerable weekly intake value (TWI), defined by the European Food Safety Authority (EFSA) of  $1.3 \mu\text{g.kg}^{-1} \text{ bw}$ . Furthermore 40% of all diet samples were above this value, indication potential risk for consumers.

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## **Conventional vs organic agriculture farming: are there stress responsive genes linked to grapevine trunk diseases in different vine varieties?**

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Grapevine trunk diseases (GTDs) are a disease complex composed by several fungi with similar life cycles and epidemiology, which pose a significant threat to the productivity and longevity of vineyards worldwide. The fungi within this complex colonize the plants xylem, reducing sap circulation and causing substantial economic losses for producers. The absence of an efficient control protocol highlights the importance of early identification and prevention measures against GTDs to curb their further spread. The study here presented focuses on identification of GTDs stress induced genes in two grapevine varieties, ‘Alicante Bouschet’ and ‘Trincadeira’, which exhibit different susceptibilities to those diseases. The experiment was performed in two selected vineyards in which presence of GTDs is demonstrated: one vineyard following a conventional farming system and another using organic farming procedures. Grapevine leaves were sampled, RNA extracted, and target-gene expression levels were evaluated following a qPCR procedure. Target genes selection was based on previous knowledge on their involvement in plant-pathogen interaction. Gene expression was compared between varieties and production modes in order to determine whether the type of phytopharmaceutical products used influences the expression levels of target genes associated with GTDs. This study offers valuable insights into the identification of stress responsive genes of different grapevine varieties in two different farming systems, facilitating genetic engineering efforts to incorporate new sources of resistance for protection against pathogens and are of major importance for sustainable plant-disease management, namely the ones relying on the plants innate immune mechanisms in view of plant breeding.

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## **Near Infrared Spectroscopy (NIRS) classification of sliced Duroc dry-cured ham under various packaging systems and storage temperature and time**

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The selling and consumption of dry-cured products in sliced format is becoming more important in the market compared to the traditional sales format as a whole piece, due to sociodemographic changes and consumer lifestyles. However, this format requires more control and traceability. Therefore, the feasibility of near infrared spectroscopic (NIRS) in combination with partial least squares discriminant analysis (PLS-DA) was studied for the classification of sliced Duroc dry-cured ham according to storage temperature ( $4^{\circ}\text{C} \pm 2$  vs.  $20^{\circ}\text{C} \pm 2$ ) and for the prediction of storage time (0, 3 and 5 months). Two types of packaging were considered, vacuum ( $n = 133$ ) and modified atmosphere packaging ( $n = 133$ ). One spectrum per package was taken with the MicroNIRTM 1700 OnSite-W (VIAVI) by direct contact with the surface of the unopened package (MicroNir Pro v2.2 software (VIAVI Solutions, Inc., San Jose, California, USA). The mean spectra had similar shapes and showed similar peaks and valleys over the entire spectral range studied (1000-1700 nm) regardless storage temperature and time but differed in absorbance intensity at different wavelengths. The statistics results of the PLS-DA models obtained after external validation were successful for the discrimination of both storage temperature and time. showing an accuracy of 100 % for both packages (vacuum and MAP) in the case of storage temperature, and an accuracy of 100 % and 94 % for vacuum and MAP, respectively, for storage time. Thus, the results suggest that NIRS technology could help to support the traceability of storage conditions of sliced Duroc dry-cured ham.

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## Development and quality assessment of cured meat sausage with different salt reduction strategies

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The excessive intake of sodium is responsible for several health problems, mainly cardiovascular diseases. The WHO aims to reduce the world's salt consumption by 30% by 2025, and the demand for food products containing less sodium is increasing. The present study aims to develop a low sodium cured meat sausage (CMS), using KCl and oleoresins as sodium replacers, and to assess its quality attributes. Three batches of each CMS formulation were produced: C1- Control with 1.5% NaCl; F1- CMS with 1% NaCl and 0.5% KCl; F2- CMS with 1% NaCl and 0.5% encapsulated oleoresins with 0.3% KCl; F3- CMS with 1% NaCl and 0.5% encapsulated oleoresins. Analysis was performed on the mixture, after fermentation, final product and during shelf life in refrigeration (2, 4 and 6 months). Microbial analysis was performed according to ISO Standards: Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS), *Enterobacteriaceae*, and *Listeria monocytogenes*. Water activity ( $A_w$ ), pH, and TBARS were evaluated. The  $L^*a^*b^*$  colour was measured with a Konica Minolta CR-400/410 (Konica Minolta, Japan) illuminant D65. There were no significant differences between CMS formulas regarding  $A_w$  and pH. CMS final product, and during shelf-life, presented  $A_w$  values between 0.92 and 0.93 and pH values between 5.3 and 5.5. Different formulations significantly ( $p < 0.0001$ ) affected instrumental red colour, being F2 ( $a^* = 15.79$ ) the redder one. The effect of treatment was highly significant ( $p < 0.0001$ ) on TBARS, with sausages prepared with oleoresins (F2 = 0,46 mg MDA/kg and F3 = 0,39 mg MDA/kg) presenting a lower oxidation indicator than the control (0,77 mg MDA/kg). *Listeria monocytogenes* counts were always below 10 cfu/g. The effect of different formulas was not significant ( $p > 0.05$ ) for *Enterobacteriaceae*, LAB, or CNS. Technological CMS microbial populations presented an increase in the course of time. The use of plant oleoresins in CMS delayed lipid oxidation, probably due to their phenolic content. Moreover, when combined with KCl (F2), oleoresins improved the desirable red color of CMS. Results from this study show that formulation with 1% NaCl and 0.5% encapsulated oleoresins with 0.3% KCl might be a good solution to reduce sodium intake associated to the consumption of meat products.

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## Edible coatings as a natural alternative for the storage of 'Crimson' seedless table grapes

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Table grapes are a highly perishable commodity that requires postharvest treatment to maintain their quality and safety. In recent years, edible coatings based on chitosan and *Aloe vera* have been considered as effective options for this purpose. The current study aimed to evaluate the postharvest behavior of 'Crimson' seedless table grapes stored at 1°C and 95% relative humidity, with the application of edible coatings based on chitosan and *A. vera* for 35 days. Six treatments were tested: 1) control, 2) 0.125% *A. vera* gel, 3) 0.25% *A. vera* gel, 4) 0.8% chitosan, 5) 0.8% chitosan + 0.125% *A. vera* gel, 6) 0.8% chitosan + 0.25% *A. vera* gel. Analysis was performed at 0, 7, 14, 21, 28, and 35 days of storage, and the following parameters were evaluated: weight loss, color, texture, soluble solids content, titratable acidity, phenolics compounds, and antioxidant content. The material harvested had quality characteristics indicative of marked ripeness. The aim was also to characterize the edible coatings based on chitosan and *Aloe vera* through the evaluation of surface tension and rheological behavior. The use of chitosan coatings improved the preservation of grapes, in respect of texture and antioxidant capacity. The formulations exclusively with *A. vera* did not perform the intended coating function due to their physical characteristics. However, these *Aloe vera* solutions showed to be of extreme interest because they were very effective in inhibiting the proliferation of fungus. The chitosan treatment has proved to be successful in storing the grapes for 14 days with good characteristics of quality. Nevertheless, for longer periods of storage, it is advisable to use treatment modalities composed of chitosan and *A. vera*, enhancing their complementary effects. The characterization of the edible coatings has shown that they should only be applied by immersion for low concentrations of chitosan because it is a Newtonian fluid. It should be noted that the addition of *A. vera* to some of the chitosan solutions caused an increase in surface tension, which will make more difficult the film formation on the surface of the fruit.

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## **Assessment of the Efficacy of Starch-Based Edible Coatings on *Fragaria x ananassa* Strawberries**

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Consumers are becoming increasingly discerning in their food choices, not only regarding the products they consume but also in terms of their production and post-harvest preservation. Starch is emerging as an attractive solution due to its cost-effectiveness and environmental friendliness for preserving fruits. The primary objective of this research was to investigate the impact of starch-based edible coatings, in combination with plasticizers and surfactants, on the post-harvest shelf life of strawberries. To ensure consistency, only fruits with uniform color and no visible defects were chosen. These fruits were immersed for a period of 5 minutes, dried, and subsequently stored in containers within a controlled environment.

In addition to the surfactant treatment, a second control group was established for comparison. The evaluation criteria encompassed the color quality of the fruits, the total soluble solids content, acidity levels, and an assessment of the fruits' respiratory rate by monitoring changes in oxygen and carbon dioxide levels. The analysis of the gathered data leads to the conclusion that starch-based edible coatings exhibit desirable properties, such as a reduction in respiration and prevention of water loss. By slowing down the fruit's metabolic processes, it became possible to minimize the oxidation of phenolic compounds and maintain an attractive color for consumers. Starch-based films present a promising avenue for enhancing food preservation while also reducing the environmental footprint. Future research could explore various formulations of these coatings to optimize their efficacy further.

## Analysis of free varietal volatile compounds in grapes using comprehensive two-dimensional gas chromatography

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Terpene compounds and C<sub>13</sub>-norisoprenoids are the main compounds responsible for varietal aromas and are the compounds that have been most studied in grapes in recent years. The volatile varietal compounds, characteristic of each variety, are present mainly in the skin, but also in the pulp of the grapes. Exploring varietal volatile compounds is important in the differentiation of the viniferous varieties, allows tracing the characteristics of each grape variety, allows the identification of the origin of the grapes and allows the evaluation of the potential of each variety for wine production. This work focuses on the development of a methodology using HS-SPME-GC×GC-TOFMS, with a flow modulator in the analysis of free varietal volatile compounds in grapes from Trincadeira, Cabernet Sauvignon, Syrah, Castelão and Tinta Barroca from 2021 and 2022. To achieve this, it was necessary to optimize the sample preparation methodology and extraction conditions, and after optimizing some parameters it was found that the largest quantity of compounds was obtained using 4 g of grape, 2 g of NaCl and 2 mL of H<sub>2</sub>O, with extraction for 40 minutes at 60 °C. The fibre used was a triple carboxen/divinylbenzene/polydimethylsiloxane fibre. The analytical conditions were also optimized so that it was possible to separate the analytes. With the optimized methodology it was possible to identify 52 free compounds of which, 17 monoterpenes, 28 sesquiterpenes and 7 C<sub>13</sub>-norisoprenoids. Results showed that there are some compounds in only one variety, for example β-myrcene, hotrienol and β-citronellol are only present in the TB variety, as well as β-elemene, cedrene and aromadendrene in the Sy variety and finally in the Trinc variety, isocaryophyllene. It was observed that in the year 2021 it was possible to identify more free varietal volatile compounds than in the year 2022. Comparing the varieties for the year 2021, Tinta Barroca was the variety with the highest total relative area. In the year 2022, Trincadeira was the one with the largest relative area. According to the results obtained through linear discriminant analysis, the volatile varietal signature of the grape is significantly different between varieties and between years.

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**Sweet music modulates the sensory and hedonic evaluation of foods**D. Guedes<sup>1</sup>, M. V. Garrido<sup>1</sup>, E. Lamy<sup>2</sup>, & M. Prada<sup>1</sup><sup>1</sup>Iscte - Instituto Universitário de Lisboa, CIS\_Iscte, Portugal<sup>2</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.Email: [dhfgs@iscte-iul.pt](mailto:dhfgs@iscte-iul.pt)

The presence of music during eating can change the way foods are perceived. A growing body of research now suggests that music can be selectively manipulated to improve the evaluation of taste and hedonic attributes in foods. One hypothesis is that the crossmodal associations between audition and taste (e.g., “sweet” music) enhance the corresponding gustatory sensations in foods. However, music is also an expression of emotion, and its influence on mood might help explain why music changes taste perception. In this context, the modulation of sweet taste sensations has been particularly challenging since music more associated with sweetness is usually also rated high in valence. This work presents two experiments where participants tasted different foods while listening to two pairs of music varying in sweetness (Experiment 1a) and valence (Experiment 1b). In both experiments, participants evaluated foods with varying sugar content and healthiness levels (e.g., cucumber, chocolate). We evaluated the effects of music condition on the evaluation of sweetness, liking, valence, and probability of future consumption. The results show that the higher (vs. lower) sweetness soundtrack significantly increased ratings in all dimensions. In contrast, no differences were observed in any of the dependent measures when listening to the higher valence (more positive) versus the lower valence (less positive) soundtrack. These findings support the hypothesis that crossmodal correspondences between music and tastes can contribute to modulating the multisensory eating experience and improve the evaluation of healthier foods. However, these conclusions are still complicated by the non-negligible correlations between sweetness and valence. This work contributes to understanding the role of affect in multisensory taste perception and informs future scientific and practical efforts toward improving the acceptance of healthier food alternatives.

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## Improving red fruits shelf-life with edible coatings based on *Opuntia ficus indica*

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Red fruits, such as raspberries have been gaining popularity for their nutritional value and for their versatility in culinary applications. However, raspberry short shelf life poses major challenges in terms of storage and distribution. Rapid post-harvest deterioration, resulting from the fragility of raspberries and their susceptibility to microbial contamination, underscores the need for effective storage solutions to meet the growing demand for this fruit. The present study addresses raspberry preservation through the application of three distinct edible coating formulations made from prickly pear cactus. In this context, the research aims to assess the impact of these edible and natural coatings on the quality and shelf life of raspberries during their storage on cold conditions (2 °C and 90% R.H).

Throughout the experiment, various quality parameters were monitored over the storage period. Weight loss was recorded to assess transpiration in raspberries. Changes in color and texture were measured to understand potential visual and textural alterations during preservation. Additionally, the content of total soluble solids and titratable acidity were analyzed to evaluate the preservation of the raspberry's natural flavor and acidity. This study also investigated the evolution of total viable microorganisms, as well as the presence of molds and yeasts, with the aim of assessing the effectiveness of the coatings in inhibiting microbial growth and, consequently, product preservation. In addition to the physicochemical aspects, the sensory quality of raspberries was evaluated throughout the storage period, to check if the formulations of the edible coating affect the acceptance of the fruits, through evaluation by trained tasters. Although the application of edible coatings causes some visual downgrade, all the formulations reduce weight loss.

The results obtained will provide valuable insights for the producers and may contribute to the development of more effective storage strategies for raspberries and other red fruits, ensuring the availability of fresh and high-quality products for consumers.

## Optimization of immunohistochemistry assay for the Identification of *Listeria monocytogenes* in animal tissues

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Listeriosis, according to “The European Union One Health 2021 Zoonoses Report”, is one of the foodborne infections with the highest mortality rates in the European Union. One of the transmission routes of *Listeria monocytogenes* to humans involves animals, mainly cattle. Cattle and human listeriosis can manifest mainly in two clinical pictures, one related to abortions and the other to encephalitis. There is evidence that these clinical manifestations might be linked to different virulent strains of *L. monocytogenes*. The goal is to assess the presence of *Listeria monocytogenes* in tissues and characterize the lesions observed, correlating with the virulence of the strains isolated using a collection of encephalon tissues of ruminants and other animals with clinical signs of listeriosis. To achieve this goal, it is necessary to have an optimized method of immunohistochemistry (IHC) detection for *Listeria monocytogenes*. Two positive controls from ruminants with lesions in the encephalon linked to listeriosis, with confirmed isolation of *L. monocytogenes* were selected. The encephalon lesion-free tissue from a ruminant was considered a negative control. For nonspecific binding control of the visualization system, the primary antibody was omitted. From each paraffin-embedded tissue block sections of 4 µm were run in the IHC protocol. The protocol was divided into several steps: deparaffinization and rehydration, inactivation of endogenous peroxidase (3%), blocking step with normal goat serum, primary antibody anti-*Listeria* (1:200) incubation, EnVision detection system/peroxidase, development with chromogen (3,3'-Diaminobenzidine), counterstaining, dehydrating and stabilizing with mounting medium. In the first assay, it was possible to detect the bacteria within the lesion profile, however, there was a high presence of background, and as such a second assay was performed. To reduce the non-specific binding of the first assay, the normal goat serum concentrations were changed from 1.5% to 10%, and the Tris-buffered saline (TBS) washing buffer was added 0.1% of Tween 20. With these conditions, it was possible to reduce the background and have a clearer identification of the bacteria and their presence in several encephalon regions. With the protocol optimized, it will be possible to continue the investigation to be able to link serogroups and particular lesions in ruminants.

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## How the differences in salivary proteome might induce changes in the release of odorants from commercial food flavours.

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Aroma plays a key role in food/beverages acceptability and thus influences consumers' choices and purchases. However, the aroma that we perceive during food consumption (retronasal aroma) is quite different from the one perceived by smelling the food (ortonasal aroma) before tasting. This is due (in part) to the oral processing of food during ingestion, in which aroma molecules from food can interact with oral physiology, modifying their release behaviour, and ultimately their perception.

Among oral factors affecting aroma compounds, recent studies highlight the role that salivary proteins can play in the release behaviour of odorants through different interactions (e.g. bindings, metabolism). As a result, diverse effects have been described, as a retention effect of the odorants by salivary proteins (e.g., mucins), which may contribute to the aroma persistence; or the metabolism of aroma molecules by salivary enzymes that give rise to different degradation products, which may also be associated with very different aroma attributes. Therefore, the salivary protein-aroma interactions might have an impact on retronasal aroma perception. Despite this, the role of other salivary proteins (e.g., PrPs) on the in-mouth behaviour of odorants is still largely unknown.

This study aims to investigate if saliva with different protein profiles might affect, in different ways, the release behaviour of aroma compounds, with potential impact on retronasal aroma perception.

For that, unstimulated saliva samples from different individuals, and therefore with different composition will be collected. Then, each saliva will be mixed with different commercial flavours (e.g., strawberry, orange) and incubated (37°C, 20min). The release of aroma compounds from saliva-flavour systems will be assessed by headspace solid-phase microextraction two-dimensional gas chromatography time-of-flight mass spectrometry (HS-SPME-GCxGC-ToF/MS). Additionally, the protein profile of saliva samples will be determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis (2DE).

The results will allow us to better comprehend the interactions occurring between salivary proteins and aroma compounds and their impact on aroma release. Although this approach do not exactly represent the oral conditions during ingestion, these findings will contribute to the understanding of the retronasal release behavior of odorants during tasting.

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## Microbial diversity in meat products – A metagenomic approach

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Resistance to antimicrobials is considered an emerging global problem, especially in the food industry. Although the administration of antimicrobials in food-producing animals is now banned in Europe, the consequences of its use for years may still be found. In particular, the presence in food of bacteria resistant to antimicrobials of “last-resort”, namely carbapenems, and third generation cephalosporins (1, 2). Since the pig is one of the biggest reservoir of antimicrobial resistance (AMR) (3), this study was performed in pork meat. The aim of the current study was to investigate the microbiome of pork meat (obtained from the *latissimus dorsi* muscle) to analyze the microbial community, and the presence/absence of antimicrobial resistance genes. First, total DNA was extracted using the FOOD DNA Kit (E.Z.N.A.). Quality control of the extracted DNA was carried out by electrophoresis on a 0.8% agarose gel and quantified using the Nanodrop equipment. Finally, the sample was sequenced using the MinION equipment (Oxford Nanopore Technologies) to optimize the methodology and identify both the microorganisms present in the sample, and the presence of antimicrobial resistance genes. After sequencing, the results were analyzed with two workflows on the EPI2ME platform (Metricor) - What's In My Pot (WIMP) and Fastq Antimicrobial Resistance. The first preliminary results showed that the most prevalent bacterial species all belonged to the *Enterobacteriaceae* family, the most abundant bacteria being *Escherichia coli*, followed by *Salmonella enterica* and *Escherichia marmotae*. It may be possible that these result from cross-contamination from human handling during the evisceration step, since they were not found in other studies. Regarding the Fastq Antimicrobial Resistance workflow, a fluoroquinolone resistance gene (*gyrB*) from the bacterium *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 was detected. Although the *gyrB* gene is not an AMR-specific gene, it can confer resistance to fluoroquinolones (a class of antimicrobials that act on nucleic acid synthesis), through mutations within quinolone resistance-determining regions. Recent studies have characterized this resistance in *Salmonella* spp. isolated from retail meat, which is a serious public-health threat (4, 5). The comparison of our preliminary results with those obtained in other studies have shown that the methodology used could be applied to microbial metagenomic studies (6-10). However, it is necessary to use more samples of different meat and meat products to test the reproducibility of the method.

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## Genetic Signatures of Selection in Mozambique's Landim Pigs Unveil Regions for Meat Quality and Reproductive Traits

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Selection for adaptation and productive traits of African local pigs is expected to have left distinctive marks in the swine genome. The use of whole genome sequencing (WGS) with 10x depth coverage increases the accuracy of identifying signatures of selection. These findings aid in identifying candidate genes that can be used to develop novel genomic tools for breeding programs. We have generated genome-level data from Landim pigs from Mozambique and compared them with the genomes of other European, African, and Asian pig breeds, including Duroc, Large White, Landrace, Pietrain, Iberian, Meishan, and Angolan pigs. To identify candidate regions, we employed two different approaches, a within-breed approach, the integrated haplotype score (iHS) method, and a pairwise-breed approach, the Cross Population Extended Haplotype Homozygosity (XP-EHH) method. Our results suggest that Landim pigs display unique mutations related to innate immune response and have undergone selection for fat deposition and reproductive traits. This contribution represents the first genetic characterization of Mozambique's local pigs using whole genome sequencing and establishes the foundation for future conservation initiatives.

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**Keywords:** LANDIM PIGS; WHOLE-GENOME SEQUENCING; SIGNATURES OF SELECTION; AFRICA; SNPs.

## Use of almond hulls in lamb diets – Effect on the fatty acid composition of intramuscular fat

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Some *trans* fatty acids (FA) from ruminant fat are associated with beneficial effects on human health, such as vaccenic (*t*11-18:1) and rumenic (*c*9,*t*11-18:2) acids. However, concentrated diets rich in cereals create favourable ruminal conditions to produce *t*10-18:1 instead *t*11-18:1 (known as *t*10-*shift*), resulting in lower levels of *t*11-18:1 and *c*9,*t*11-18:2 in ruminant fat. The replacement of cereals with raw materials rich in sugars in high-forage diets has been proposed as a strategy to prevent the *t*10-*shift* and, simultaneously, ensure high productive performances. Almond hulls (AH), which consist of the green outer covering of the almond, contain high sugar content. So, the objective of this work was to evaluate the effect of partial replacement of cereals with increasing levels AH in the diet of growing lambs on the total lipid content and FA profile of intramuscular fat. The experimental procedures were approved by ORBEA Ethics Committee of INIAV, respecting the European Union Directive 2010/63/UE. Twenty-four ram lambs were individually housed and randomly assigned to the three diets (8 lambs/diet), with feed offered *ad libitum*. The cereals in diets were stepwise replaced by AH, reaching 0% of dry matter (DM) (AH0), 9% DM (AH9), and 18% DM (AH18) of AH in diets. The trial lasted 6 weeks after an adaptation period of 7 days. Samples of *Longissimus lumborum* muscle were collected. Total lipids were extracted with dichloromethane:methanol (2:1, v/v), transesterified into FA methyl esters and then analysed by gas chromatography with flame ionization detection (GC-FID). The intramuscular fat content was not affected by dietary treatments ( $P > 0.050$ , 16.9 mg/g meat). The dietary replacement of cereals with increasing levels of AH did not affect the *t*10-18:1 content ( $P > 0.050$ , 1.33 mg/g total FA). Conversely, the contents of *t*11-18:1 ( $P = 0.049$ ) and *c*9, *t*11-18:2 ( $P = 0.004$ ) increased linearly with increasing levels of AH in the diets (*t*11-18:1 – 3.34, 4.66, and 5.03 mg/g total FA and *c*9,*t*11-18:2 – 1.08, 1.41 and 1.54 mg/g total FA for AH0, AH9 and AH18 diets, respectively). The partial replacement of cereals with increasing levels of AH improved the nutritional value of lamb meat, increasing its contents in health beneficial FA.

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## Water Quality in Dairy Cattle Farms: Impact on production, reproduction and animal health

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The estimated increase in the world's population growth rate suggests that in the future there will be greater demand for food, which in turn will have a direct effect on the use of water for agricultural purposes (Mancosu et al., 2015). Furthermore, as a result of increased water scarcity and drought due to climate change (Jiménez et al., 2014), the extensive use of water for irrigation is expected to occur in the context of growing competition between agriculture and other sectors of the economy. Potable water is a scarce resource in many regions of the world. Water is an essential medium for transferring nutrients, excreting waste products resulting from metabolism and regulating the body temperature of all living organisms. It helps in the initiation and regulation of all physiological processes and thus plays a vital role in living organisms for the maintenance of life (Golher et al., 2021). On dairy farms, the use of quality water is essential to maximize the animals' milk production. The aim of this study was to verify the importance of water quality on intensive dairy farms in Portugal. The farms (n=285) were selected based on their location, using the databases of the Portuguese Association of Friesian Breeders (APCRF) and the Dairy Cattle Support Station (EABL). The questionnaires aimed to collect information: (1) the importance of water attributed by producers; (2) water monitoring, storage and quality; (3) production, reproduction and animal health. The preliminary results of the questionnaires indicated that 51% of farms consider that the quantity of water alone is the most important factor on dairy farms; 98% of producers say that, in the context of climate change, water scarcity is very worrying; 91% of farms use their own water; 40% of farms DO NOT carry out water quality analyses; 86% of farms DO NOT monitor water consumption; 88% of farms DO NOT carry out any water treatment. The main water quality problem was associated with microbiological quality, followed by the presence of iron, nitrates and manganese. With regard to the impact of water quality on dairy farms, in the case study, it was found that the excess of manganese present in the drinking water affected reproduction (increased inseminations) and production (reduced the quantity of milk) and animal health (increased incidence of kidney and liver diseases).

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## How our salivary proteome changes over time in response to the smell of strawberry - a methodological approach

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As soon as our senses detect a food (e.g., smell of foods), a cascade of different physiological responses are triggered, including a rapid release of saliva into the oral cavity. This increase in saliva - termed as cephalic-phase salivary responses - helps our body to be prepared for the subsequent food ingestion and digestion. Although salivation increases, less is known about how saliva composition changes. Previous studies suggest changes in saliva proteome with smell stimulation, but they were made using saliva collected through some minutes of constant stimulation. To better capture what is really happening it is necessary to define the best methodologies to assess salivary responses. While our brain processes and reacts to a food stimulus in a short time, an adequate amount of saliva, needed for several biochemical analysis, takes longer to be produced. For most of the studies on salivary proteome, saliva samples are collected by passive drooling/spitting for a total of 3 to 5 minutes. However, it is not known if this period is representative of the real effect of the food smell or whether a saliva that represents a combination of 3-5 minutes secretion masks the initial effect of smell. The present study sought to address these questions and investigate how salivary protein profile changes at each minute of a 5-minute stimulation. To achieve this, a total of 12 volunteers (7 women; 21-46 years) took part of this study. Whole saliva samples were continuously collected, at intervals of 1-minute, for different tubes, immediately before (t=0, no odour), and for 5 minutes exposure to a strawberry aroma (t=1, t=2, t=3, t=4, t=5). To assess if the existing changes were reverted, a new saliva collection was performed 10 minutes after the first moment of exposure (t=10, no odour). Additionally, participants were asked to smell the strawberry aroma again and collect the total amount of saliva produced for 5 minutes to a collection tube. Changes on salivary responses through the time were evaluated in terms of salivation, amylase enzymatic activity and variations in protein composition (SDS-PAGE). Main results will be presented and discussed in terms of the best period to collect saliva to better capture salivary cephalic-phase responses triggered by olfactory stimulation. These findings are important contributors to methodology development and understanding physiological processes involved in eating behavior.

### Keywords:

Salivary proteome; salivary cephalic phase responses; food aroma exposure; saliva sampling methods

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### Ethical statement:

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Commission of the University of Évora for human studies (no.GD/29823/2022). A signed written informed consent was obtained for each participant.

## Unveiling Microbiota within the Rocaille-Adorned Garden Grotto at Condes de Basto Palace, Évora, Portugal

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Biodeterioration of Cultural Heritage is the consequence of interactions between living organisms, material support and environmental conditions. In stone materials, deterioration by fungi is mainly the result of mechanical, chemical, and physical processes, consisting essentially of penetration of hyphae, production of fruiting bodies, production and release of destructive extracellular organic acids, enzymes, and metabolites, as well as the development of biofilms and chemical reactions with inorganic compounds.

Eugénio de Almeida Foundation's Casa de Fresco is a heritage site of inestimable historic-artistic value that exhibits developments of biofilms due to growth of microorganisms in the stone and in the rocaille materials. The biodeterioration noticeable in this local were investigated within the scope of the Conservation and Restoration Project. Through advanced high-throughput DNA analysis, we successfully characterized the microbial population inhabiting the site. Our findings revealed the presence of various lichens or lichenized fungi, including genera like *Variospora*, *Verrucaria*, *Circinaria*, *Caloplaca*, among others. Additionally, we also identified some bacteria associated with the presence of these lichens. To address this microbiological challenge effectively and prevent rapid fungal recolonization, we tested commercial antimicrobial agents. Thus, the ongoing action is precisely aimed at ensuring the conservation of Casa de Fresco as an architectural, decorative, and functional element essential to the harmonization of the entire set formed by the garden. This work will be an opportunity to deepen knowledge about the historical, symbolic, and material dimension of this heritage.

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**Xerostomia: symptomatology and salivary biochemical properties**L. Carreira<sup>1</sup>, J. Zamora<sup>2</sup>, J. Nunes<sup>2</sup>, J. Coromina<sup>2</sup>, C. Cordeiro<sup>3</sup>, E. Lamy<sup>4</sup>

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Xerostomia is a medical condition characterized by dry mouth sensation that can be related to decreases in salivary flow rate or not. This medical condition has different symptoms that affect well-being (e.g., difficulty in speaking and swallowing), oral food perception and intake. This can be due to autoimmune diseases (e.g., Sjogren syndrome), radiotherapy in the head and neck area, multidrug usage, or unknown causes.

Saliva is a biological fluid that coats and protects the oral cavity and helps in oral digestion and food perception and the decrease in some proteins (e.g. lactoferrin, IgA, and IgG) in xerostomia patients has been reported. The changes in salivary proteome can be associated with changes in saliva functions, like lubrication and the protection capacity of saliva. In fact, xerostomia patients complain about food sensory characteristics, with some foods being harder or crunchier, leading to lower acceptance by them. Saliva biochemical composition can be related with symptoms of xerostomia patients, but, at the same time, can give information about what which pathways are affected in this disease.

We aim to investigate the relationship between salivary biochemical properties and xerostomia symptoms. Furthermore, the existence of an association between symptoms and protein profile obtained with bidimensional electrophoresis will be assessed, as well as saliva metabolome of this patients will be monitored.

Methodologically, we have samples from xerostomia patients, diagnosed in Teknon Medical Center after ethical approval (GD 22368/2021), before (time 0) and after 3, 6, 9, and 12 months of starting treatment with pilocarpine. At time 0 we collected unstimulated and pilocarpine acutely stimulated samples. Total protein quantification, alfa-amylase enzymatic activity determination, protein separation (by SDS-page and bidimensional electrophoresis), immunodetection of amylase, cystatins, and mucin 5B by western blot and FT-ICR assay were performed. Also using FT-ICR, we performed analyses that allowed us to evaluate the metabolome of these patients.

In this study, we will present the results obtained with the approaches described previously, making a critical analysis of them. A hypothesis will be developed that explores the possibility of how the influence of variations in salivary biochemical properties can affect oral food perception.

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**Proteome analysis by 2-DE electrophoresis in leaves of *Olea europaea* L.  
during adventitious root formation**

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Olive tree (*Olea europaea* subsp. *europaea* var. *europaea* L.) holds great significance in the Mediterranean region mainly attributed to its fruits used for oil production. Olive is conventionally propagated by semi-hardwood cuttings but some traditional Portuguese cultivars that mark the typicity of Portuguese olive oils are recalcitrant to that propagation system due to the difficulties to develop adventitious roots.

Adventitious rooting (AR), the process of adventitious roots formation, is influenced by various internal and external factors, including two abiotic stress factors: the wounding and the immersion of the cutting in a solution containing the auxin IBA (indole-3-butyric acid). Molecular mechanisms inherent to the efficiency of adventitious roots formation have been investigated in order to identify markers and/or regulators in this process. However, most of the research has been focused on transcript analysis, and proteins that could be used as biomarkers of AR efficiency are scarce. Proteomics is the large-scale study of proteins, including their structure, function and interactions within a biological system and can provide valuable insights into the proteins that are involved in different stages of AR process. In this study, the two-dimensional electrophoresis (2-DE) technique was applied to analyse the protein profile of olive leaves at early stages of AR (induction phase). For the establishment of 2-DE protocol, leaves of cv. 'Galega vulgar' were collected from in vitro growing plantlets after treatment with IBA. Plantlets not treated were used as control. Samples were collected at 72 hours. Four replicates were considered per condition (control and IBA-treatment). After homogenization of plant material using liquid nitrogen, proteins were extracted following acetone precipitation protocol and 60 µg of protein was separated by 2-DE. After protein profile analysis by SameSpots, TotalLab programme, some spots, corresponding to differentially expressed proteins comparing control and IBA-treated groups, were identified.

This knowledge could contribute to identify new molecular biomarkers that help to understand the process of adventitious roots formation.

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## Revealing Animal-Origin Parchments: An Integrated Study Using Physico-Chemical and Metagenomic Approaches

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Parchment, an age-old writing material, derived from animal skin, mainly sheep, calf and goat, has been instrumental in preserving human knowledge and cultural heritage for centuries. Its main component, collagen, is the subject of extensive studies since its state of degradation, either through denaturation, oxidation or hydrolysis, is a good indicator of the conservation status of these artefacts. Although the chemical and structural degradation of collagen fibers, reflected by the loss of mechanical properties of documents, is a hot topic in conservation studies, little is addressed about the differences in this pivotal protein between the different animal species used for parchment production. However, proteomic analysis applied to heritage materials, such as peptide mass fingerprinting, are being implemented to help determine the hides animal species by analyzing the molecular weights, not only of collagen but also of the other peptides present in a sample.

In the present study, several parchment samples of known animal origins were investigated for the discovery of protein biomarkers that could help predict and/or confirm parchment raw materials, non-invasively. Overall, the focus was to deepen our understanding regarding molecular biomarkers occurring in parchments crafted of different animal species, resorting to non-invasive techniques, such as the Fourier Transform Infrared analysis with the attenuated total reflectance (FTIR-ATR) and Raman spectroscopy. To strengthen the analysis, the parchments microbiome was also analysed by High-Throughput sequencing (NGS).

Although this is just a preliminary study in the creation of a database of biomarkers for historic parchment, it was possible to identify different microbiome patterns among parchments originating from different animals and detect molecular proteic biomarkers.

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## Wood Microbiota in Min-Zhe Wooden Arch Bridges: Insights, Interactions, and Preservation

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Min-Zhe wooden arch bridges in Fujian and Zhejiang Provinces of China are a unique type of woven wooden arch bridge whose traditional building craftsmanship is listed in the UNESCO List of Intangible Cultural Heritage in Need of Urgent Safeguarding.

These bridges are mainly composed of wood whose composition comprises cellulose, hemicellulose, and lignin, all of which act as fertile grounds for microorganisms, including bacteria and fungi. The colonization of these microorganisms can lead to the degradation of wood, resulting in a consequential loss of value, a matter of particular concern when dealing with heritage artifacts, where the historic significance is at risk.

This study was envisaged to understand the appearance and proliferation of large biofilms on one of these precious historical bridges and was developed under the framework of the China-Portugal Joint Laboratory for Heritage Conservation Science promoted by the Belt and Road Initiative. We meticulously investigated the microbiota inhabiting this bridge, employing both semi-invasive and non-invasive sampling techniques. Among the species identified in our analysis, *Mycobacterium botniense* (from the Mycobacteriaceae family), *Talaromyces rotundus*, and *Penicillium steckii* (from the Trichocomaceae and Aspergillaceae family) emerged as the predominant inhabitants of the studied samples. Notably, our examination unveiled a diverse composition of microbial communities, including microorganisms capable of forming vibrant biofilms, such as Actinobacteria, Proteobacteria, Cyanobacteria, and Firmicutes Phyla, as well as Ascomycota and Basidiomycota fungi. These microorganisms were associated with potential aesthetic and structural damage to the wood.

The primary goal of this study is to offer valuable insights into the colonization of wood microbiota in tropical environments. We aim to deepen our understanding of the mechanisms by which these microorganisms operate and to raise awareness of the intricate interactions between prokaryotic and eukaryotic communities within wood. Ultimately, this research lays the foundation for the development of effective mitigation strategies aimed at preserving these invaluable cultural assets.

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