VI Encontro de Estudantes de Doutoramento em Ambiente e Agricultura

9 e 10 de dezembro 2021

VI PhD Students Meeting in Environment and Agriculture

9th and 10th December 2021

Pólo da Mitra, Universidade de Évora

Book of abstracts

Title: VI PhD Students Meeting in Environment and Agriculture

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Published:

UE – Universidade de Évora

ISBN: 978-972-778-224-6

Dear participants,

It is our great pleasure to welcome you to the **VI Encontro de Estudantes de Doutoramento em Ambiente e Agricultura (EEDAA) / PhD Students Meeting in Environment and Agriculture**, held in Évora on the 9th and 10th of December 2021. 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 and IIFA – Institute for Advanced Studies and Research, University of Évora and supported by UNIMED – Mediterranean Universities Union.

The EEDAA focuses on four main areas: (1) Biology and Biochemistry, (2) Veterinary Sciences and Animal Production, (3) Agricultural Sciences and Food Sciences, and (4) Environment, Landscape and Sustainability. The meeting includes four invited plenary lectures and several presentations selected from the abstracts submitted by PhD students. In addition, all authors that were not selected for oral communication, will present their work as posters displayed throughout the meeting.

This meeting intends to stimulate the interaction between PhD students, to streamline scientific discussion and highlight the ones who will become the researchers of the future.

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

Welcome to Évora!

The Organising Committee,

Marta Laranjo, MED

Ana Alexandre, MED

Bruno Medronho, MED

Cláudia Marques, IIFA

COMMITTEES

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Teresa Pinto Correia, Universidade de Évora – MED

Organised by

Program

Thursday, 9th December 2021

09:00-09:45	**Registration**
09:45	**Opening Session**
	António Candeias Diretor of *Instituto de Investigação e Formação Avançada* (IIFA) Vice-Rector of Universidade de Évora
	Teresa Pinto Correia Diretor of MED-*Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento*

10:15	**Plenary Lecture** *Lactonas Sesquiterpénicas – extração e bioatividades, Cynara cardunculus como caso de estudo* **Fátima Duarte** Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) – MED
11:00	**Coffee break and poster session**

Biology and Biochemistry	
	Chair: Carla Varanda Universidade de Évora - MED
Presentations selected from the submitted abstracts	
11:30	*Salivary proteome of patients with xerostomia – a pilot study evaluating the effect of pilocarpine* **Laura Carreira** Universidade de Évora – MED
	Single Phage Suspensions and Phage Cocktail in the Inactivation of E. coli and S. Typhimurium **Pedro Santos da Costa** Universidade de Aveiro – CESAM
	Acoustic monitoring and occupancy models a systematic review with insights for future monitoring programs **Frederico Martins** Universidade de Évora – MED
	Morphological characterization of Cynara cardunculus L. (cardoon) in southern Portugal **Ana Paulino**

	Universidade de Lisboa – cE3c e CEBAL
	Influence of food smell emotional response in saliva biochemical composition: fundamental research with potential for further applied interventions on food acceptance **Carla Simões** Universidade de Évora – MED
12:45 – 14:30	**Lunch**

Environment, Landscape and Sustainability	
	Chair: Sara Santos Universidade de Évora - MED
	Presentations selected from the submitted abstracts
14:30	*Ecotoxicological responses used in the assessment of the potential ecological status of the Lage reservoir* **Adriana Catarino** Instituto Politécnico de Beja
	Re-use of waste materials from the pulp and paper industry in mine soil recovery: chemical and ecotoxicological assessment **Clarisse Mourinha** Universidade de Évora
	A participatory approach to the measurement of Social Sustainability in Agribusiness: The case of Alvarinho in the Monção and Melgaço sub-region **José Massuça** Universidade de Trás-os-Montes e Alto Douro e Universidade de Évora – CEFAGE
15:15	**Coffee break and poster session**

Veterinary Sciences and Animal Production	
	Chair: Rui Charneca Universidade de Évora - MED
	Presentations selected from the submitted abstracts
16:00	*A DNA barcode reference library of Portuguese mosquitoes* **Sara Madeira** Universidade de Lisboa
	Evaluation of the birth impact in the newborn dairy calf using ethophysiological measurements **Flávio Silva** Universidade de Trás-os-Montes e Alto Douro – CECAV e MED

16:30	**Plenary Lecture** *Técnicas reprodutivas: sua aplicação em projetos de investigação; investigação de campo versus Investigação de laboratório* **Elisa Bettencourt** Universidade de Évora – MED
17:15	Closing of the first day of the VI EEDAA

Friday, 10th December 2021

Agricultural Sciences and Food Sciences	
	Chairs: Elsa Lamy e Nuno Pedroso Universidade de Évora - MED
	Presentations selected from the submitted abstracts
9:30	*The use of a chromatographic-based approach to reveal the yeast modulation potential on the chemical profile of Arinto white wines* **Catarina Mendes** Universidade de Évora – MED
	Suitability of Citrus × sinensis and Citrus × limon as hosts of Trioza erytreae **Tomás Abranches de Magalhães** Universidade do Algarve – CEOT, MED e CIMO
	Response of Mediterranean aromatic plant species (Lamiaceae) to contrasting environmental conditions **Inês Mansinhos** Universidade do Algarve – MED
	Evaluation of the Potential of Opuntia Ficus-Indica Cladodes as a Natural Flocculant for Wastewater Treatment **Sofia Trindade** Universidade de Évora
	Grapevine responses to trunk pathogens: potential genes involved in plant-pathogen interactions **Mariana Patanita** Universidade de Évora – MED
10:45	**Coffee break and poster session**

11:15	**Plenary Lecture** *Vírus de Plantas: de agentes causadores de doença a protetores de plantas* **Maria do Rosário Félix** Universidade de Évora – MED

12:00-14:00	**Lunch**

14:00	**Plenary Lecture** *Adaptação dos bosques e florestas do semiárido às alterações climáticas* **Cristina Branquinho** Universidade de Lisboa – cE3c

Presentations selected from the submitted abstracts	
14:45	*Influence of Fe availability in the composition of root's grass exudates* **Teresa Saavedra** Universidade do Algarve – MED
	Effect of dolomitic lime application and grazing type on growth, protein value and floristic composition of natural pastures under Montado **Emanuel Carreira** Universidade de Évora – MED
	Antimicrobial Photodynamic Therapy as an innovative approach for the inactivation of pathogenic bacteria on fish fillet **Cátia Vieira** Universidade de Aveiro – CESAM
15:30	**Coffee break and poster session**
16:15	*Cynara cardunculus leaves extract fractioning and phytotoxic evaluation* **Daniela Rosa** Instituto Politécnico de Beja – CEBAL, MED
	Phages for Escherichia coli and Salmonella Typhimurium inactivation in milk **Márcia Braz** Universidade de Aveiro – CESAM
	The taste of music: Taste and emotional associations in a set of musical stimuli **David Guedes** Instituto Universitário de Lisboa – CIS_Iscte
17:00	**Closing session** **Best Poster Prize**
	António Candeias Diretor do Instituto de Investigação e Formação Avançada (IIFA) Vice-Reitor da Universidade de Évora
	Teresa Pinto Correia Diretora do Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento (MED)

Note: The posters will be displayed throughout the Meeting.

BEST POSTER AWARD

Erika Roldão Lemos de Almeida

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

"Natural Remnant Habitats
- a key for biodiversity conservation in Montado agroecosystems"

On behalf of the Organising Committee,

Teresa Pinto Correia
Director of MED

Plenary Lectures

Plenary Lecture 1

Lactonas Sesquiterpénicas – extração e bioatividades, Cynara cardunculus como caso de estudo

Fátima Duarte (CEBAL, MED)

Principal Investigator at CEBAL- Centre of agronomic and Agro-Industrial biotechnology of Alentejo.
Coordinator of Bioactive Compounds Research Group.

Her research is centered on the characterization of natural extracts, or pure compounds, and on the subsequent study and optimization of extractive processes and their biological activity.

More information at https://orcid.org/0000-0002-2223-7784

Plenary Lecture 2

Técnicas reprodutivas: sua aplicação em projetos de investigação; investigação de campo versus Investigação de laboratório

Elisa Bettencourt (University of Évora, MED)

Associate Professor at the Department of Veterinary Medicine, School of Science and Technology, University of Évora.
Director of the Department of Veterinary Medicine.
Deputy clinical director of the HVUE, responsible for the Clinical Unit of the *Coudelaria de Alter* and for the *Centro de Reprodução de Equinos da Coudelaria de Alter*.

Reproductive biotechnologies in horses and ruminants, namely with regard to their application in conservation and genetic improvement programs: artificial insemination, cryopreservation of semen and embryos.

More information at https://orcid.org/0000-0003-0022-666X

Plenary Lecture 3

Vírus de Plantas: de agentes causadores de doença a protetores de plantas

Maria do Rosário Félix (University of Évora, MED)

Associate Professor in the Department of Crop Sciences, University of Évora

PhD in Agricultural Sciences concerning olive viruses (2008). Working for about 25 years on olive pathogens, with main focus in olive affecting viruses, characterizing these pathogens molecularly, designing and improving molecular diagnostic tests such as conventional and multiplex RT-PCR, RT-qPCR, studying the relationships between viruses and their vectors, and the use of virus as a tool for plant protection.

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Plenary Lecture 4

Adaptação dos bosques e florestas do semiárido às alterações climáticas

Cristina Branquinho (University of Lisbon, CE3C)

Associate Professor at the Department of Plant Biology, Faculty of Sciences, University of Lisbon. Researcher at the Centre for Ecology and Environmental Changes.

Her research is focused on studying early indicators of climate change and desertification, especially in southern Portugal. The aim is to analyse changes in ecological indicators of the structure and functioning of ecosystems because of global changes, climate change, pollution by nitrogen, metals and organic compounds.

More information at https://orcid.org/0000-0001-8294-7924.

ABSTRACTS

1. Biology and Biochemistry

Oral Communication 1

Salivary proteome of patients with xerostomia – a pilot study evaluating the effect of pilocarpine

L. Carreira [1, 4], J. Zamora [2], J. Nunes [2], J. Coromina [2], C. Cordeiro[3] , E. Lamy [4]

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Saliva is a biological fluid composed by water, electrolytes, proteins and metabolites, which constitution will change with dietary habits and pathologies. Saliva has an important role in coating and protecting surfaces of oral cavity, defending and lubricating them, and helping in oral digestion, with salivary proteome being important in these functions.

Xerostomia is a subjective dry mouth sensation, which can be a true hyposalivation or not. Xerostomia causes oral discomfort, loss of taste and difficulties in chewing that affect food choices and diet. Nowadays, the most common treatment, pilocarpine administration, tries to alleviate symptoms. This agonist of parasympathetic nervous system is thought as inductor of fluid, without affecting protein composition, however its effects in salivary proteome have been few explored.

Our objective is to access the effect of pilocarpine treatment in salivary biochemical parameters of xerostomia patients.

Saliva samples were collected in a specialty appointment in three different stages: 1) Control, first appointment, before treatment initiation; 2) first appointment, before treatment initiation but immediately after acute pilocarpine stimulation; 3) in the appointments during the treatment (until 9 months after the begging of treatment) after acute pilocarpine stimulation. These samples were subjected to protein profile analysis, after SDS-PAGE protein separation and alfa-amylase enzymatic activity determination.

Pilocarpine resulted in an immediate effect in Band B (decrease) and F3 (increase) expression levels, total protein concentration (decrease) and amylase enzymatic activity (decrease). In medium-term (4 months) it is observed that amylase bands (F, F1) increase and a non-identified protein decrease, in their relative amounts. The pH and amylase enzymatic activity also presented increases after 4 months pilocarpine treatment. For long term (9 months) the higher pH is also observed, although less significant differences can be observed, probably due to the limited number of individuals with the 3 time-points collections (N=6).

We may conclude pilocarpine treatment has medium-long-term effects in salivary proteome of xerostomia patients, which may be related to the improvement of symptoms. Moreover, some of the differences are in proteins related to oral food perception, such as amylase. Therefore, these preliminary results reinforce the relevance of saliva proteome for understanding xerostomia-related symptoms.

This work was supported by FCT – Portuguese Science Foundation, through research contract CEECIND/04397/2017 to Elsa Lamy and doctoral scholarship 2021.06273.BD to Laura Carreira.

Oral Communication 2

Single Phage Suspensions and Phage Cocktail in the Inactivation of *E. coli* and *S.* Typhimurium

Pedro Costa[1], Ana T P C Gomes[1], Carla Pereira[1] and Adelaide Almeida[1]

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Enterobacteriaceae *Escherichia coli* and *Salmonella enterica* serovar Typhimurium strains are among the main foodborne pathogens responsible for a variety of diseases and are a major public health burden worldwide, in part because they frequently present resistance to antibiotics. As treatment of Enterobacteriaceae infections is empiric, using the same antibiotics to treat *E. coli* and *Salmonella* infections, the same concept can be applied with phages. Phage therapy has already some food, veterinary and environmental applications but those applications focus in the prevention and control of one pathogen. The use of different phages combined in cocktails, frequently used to circumvent the development of phage-resistant mutants, also allows for the treatment of multiple pathogens, broadening the phage`s action spectrum. As such, the aim of this study was to evaluate the efficiency of a cocktail of two phages (ELY-1, produced on *E. coli* and phSE-5, produced on *S.* Typhimurium) to control *E. coli* and *S.* Typhimurium. Phages ELY-1 and phSE-5 were effective against *E. coli* (maximum reductions of 4.5 and 3.8 log CFU/mL, respectively), *S*. Typhimurium (maximum reductions of 2.2 and 2.6 log CFU/mL, respectively), and the mixture of both bacteria (maximum reductions of 2.2 and 2.0 log CFU/mL, respectively). The cocktail ELY-1/phSE-5 was more effective against *S*. Typhimurium and the mixture of both bacteria (maximum reduction of 3.2 log CFU/mL for both) than the single phage suspensions and as effective against *E. coli* as its specific phage ELY-1 (maximum reductions of 4.5 log CFU/mL). The use of both the phage cocktails, as well as the single-phage suspensions, however, did not prevent the occurrence of phage-resistant mutants. Overall, the results indicate that the application of the phages in the form of a cocktail show their potential to be used presumptively, that is, prior to the identification of the pathogens, paving its use to control *E. coli* or *S.* Typhimurium.

Thanks are due to FCT/MCTES for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020) through national funds. Pedro Costa was supported by a PhD grant (PD/BD/150360/2019) financed by the Portuguese Foundation for Science and Technology (FCT). Carla Pereira was supported by a Junior Research contract (CEEC Individual/03974/2017), Thanks are also due to the Department of Biology and University of Aveiro, where this research work was carried out.

Oral Communication 3

Acoustic monitoring and occupancy models: a systematic review with insights for future monitoring programs

Martins, F.C[1]; Segurado, P[2]. & Marques, J.T.[1]

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2CEF, Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017, Lisboa, Portugal

Wildlife monitoring programs are implemented to infer spatiotemporal changes in the presence or abundance of species and communities of organisms. Advances in monitoring of species have been increasingly facilitated by both technological advancements in data collection methods and new statistical tools for data analysis. Such technological advancements include small sized wildlife acoustic recording devices that can record a wide range of sound frequencies. Data provided by such devices are especially suitable to be analysed by recently developed statistical tools such as occupancy models, which have the major advantage of accounting for variations in species detectability.

We revised 107 research papers that use both acoustic monitoring and occupancy modelling, to synthesise recent research on species monitoring and discuss the outcome and potential of combining these two methodologies in future monitoring studies.

We found that 80% of these studies limit their analysis to single-season and single-species models, despite involving the collection of recordings of multiple species and sampling periods extending across several seasons. This limitation in the analysis hinders the full information retrieval from available datasets because more complex models, such as the multi-species occupancy models, provide more robust occupancy parameters for both commonly and rarely detected species. We argue that dynamic models are an essential part of monitoring programs to keep track of species presence fluctuations. A recent development in occupancy models is the inclusion of false-positive detections, but it was only applied in 11 studies. This modelling approach seems particularly under-used as many species, particularly bats, are hard to distinguish solely based on acoustic data. Finally, we noted a geographical bias in the implementation of acoustic methods with occupancy models towards research in North America.

Coupling low-cost PAM with a diversified set of occupancy models is a scalable methodology that can help to implement standardized protocols for regional scale monitoring programs. The long-term programs that can inform habitat management to promote bat conservation will be critical for bat conservation in an increasing anthropogenic landscape.

Oral Communication 4

Morphological characterization of *Cynara cardunculus* L. (cardoon) in southern Portugal

A. Paulino[1,2], J. Santos[1,3], M. Pereira[1], T. Brás[1,3], D. Rosa[1,3,4], O.S. Paulo[2], L. Marum[1,3], M.F. Duarte[1,3]

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Cynara cardunculus (Cc) is a perennial plant well adapted to Mediterranean climatic conditions. Cc as a multipurpose crop has been described with a wide spectrum of applications.
Previous studies from our research team evidence an interesting natural variability of Cc within Alentejo region. This variability when translated into biomass production gives quite different productivities, and therefore different economic values.
Within the present study we evaluated a group of 23 F1 cardoon plants, with different chemical profiles, over a period of 4 months (March, April, May and June 2021) in order to achieve morphological and biomass productivities traits, throughout the evaluation of the main morphological parts (leaves; stalks and inflorescence) in all plants.
The obtained results underlined a great morphological variability, concerning the genotype and collection period. Cardoon plants presented a remarkable variation in height, being the min 12cm - max 144cm. Notable differences within leaves number (from 1 to 49 leaves) and leaves dimensions (a maximum value of 127cm/80cm and a minimum value of 19cm/9cm) were found. The observed variability in regard to leaves size can affect the ability of these plants to capture light, competing with neighbouring plants, affecting their own development.
The inflorescences started to appear in the month of April in small quantity, in the month of May there was an increase in the number of inflorescences. The maximum number of inflorescences found in a single plant was 18, and a very high number of inflorescences that did not develop (abortions) were found in all studied plants.
Cc is a relatively complex crop in terms of morphology and growth. During the study, the plants varied in size, number of leaves and increase in leave size, as well as increase in stem size and development of inflorescences. The wide range of morphological parameters observed within the sampled population is within the highly heterozygotic nature of this species.
This study aims to provide information about the variability in cardoon populations. It is therefore necessary to associate this morphological information generated with genetic and chemical data (work in progress), for a better understanding of cardoon population behaviour.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), PhD grant to A. Paulino (SFRH/BD/145383/2019) and D. Rosa (SFRH/BD/143845/2019), and Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED).

Oral Communication 5

Influence of food smell emotional response in saliva biochemical composition: fundamental research with potential for further applied interventions on food acceptance

Carla Simões[1], Inês Caeiro[1], Laura Carreira[1], David Guedes[2], Elsa Lamy[1]

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Pre-ingestive stimuli, as vision or olfaction, allow the anticipation of the food nutritional and oral sensory features that will be perceived. This anticipation can result in emotions with variable arousal (intense or weak) and valence (positive or negative), depending on the type of food and individuals' characteristics. The responses to pre-ingestive food signals, denominated cephalic phase responses, consist in several physiological processes (e.g. hormone release) that intend to prepare the body to the subsequent ingestion of food. These responses can modulate the final perception of the food and, consequently, its acceptance/preference.

Pavlov, that introduced the concept of cephalic phase of ingestion (Wood, 2004), showed that anticipation of food results in salivation. Moreover, all of us have the experience of "watering mouth" when smelling a favorite food. Despite this knowledge, only recently it was demonstrated that, besides flow rate, the biochemical composition of saliva is changed in response to food smell: bread odor resulted in some changes in salivary proteome that are like the ones induced by the mastication of this food, but not by the mastication of a different food (Carreira et al., 2020). Moreover, some of these changes appeared to be dependent on the type of emotions elicited by smell. Salivary amylase, for example, was observed to be negatively associated with positive emotions. Since this protein was previously observed to be linked to sweet taste sensitivity (Rodrigues et al., 2017); Lamy et al., 2021), it is possible that changing saliva through food smell may also change oral taste perception.

The objective of the present study was to evaluate the effect of different olfactory stimuli (vanilla, almond and lemon) on emotional response and salivation (salivary flow rate and salivation). Vanilla and lemon induced more positive emotions than almond, with lemon smell eliciting energy and inducing much higher salivary flow rates than vanilla. Variations in salivary proteome will be presented and discussed based on the different sensations elicited by the different smells. In conclusion, pre-ingestive signals change oral medium, being potential agents of oral food perception modulation, what can be of interest for interventions aimed at promoting healthy and sustainable food acceptance.

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Acknowledgements:

The FCT–Portuguese Science Foundation, supported the study through UID/AGR/00115/2019 and the research contract CEECIND/04397/2017 to Elsa Lamy and the PhD fellowship 2021.06485.BD to Carla Simões.

Poster 1

Comparison indigo extraction from *Isatis tinctoria* L. with low-cost technology methods

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The most of dyeing substances available for textile industry come from chemical synthesis sources[1]. Its consumption has been encouraged due to cheaper generation and lower dependency on natural production irregularities. The variant quantity of natural sources and dyeing processes available, makes the use of natural dyeing on a full-scale manner at least complicated[2]. Besides, the good fastness properties on different required substrates by the fashion industry generates a problematic situation when process needs to be implemented on industrial facilities and there is a gap of adequate reported techniques. Nevertheless, the advantages of lesser toxicity, better biodegradability, and easier waste-water treatments when using natural instead of chemical dyes are far bigger than the cost of focus on the complexity of achieving the level of knowledge that synthetic dyeing processes already have. In this work we present a comparison of physical against biochemical methods to produce indigo dye to be implemented on wool with the advantages to be lesser expensive, more eco-friendly, and efficiently produced[3]. The selected processes were improved and generated acceptable results even with almost a tenth of the reported needed biomass to produce the desire product[4]. Fast and mid thermal, aerobic, and anaerobic extraction were the selected methods of this work.

This work is taking place at GO - Natural Dyeing - Use of natural dyes in natural fibers (REF: PDR2020-101-031963), co-financed by the European Agricultural Fund for Rural Development (EAFRD) through the Rural Development Program PDR2020. MED and CEF, are funded by the Foundation for Science and Technology (FCT) from Portugal under the projects UID/AGR/00239/2020 and UIDB//05183/2020, respectively.

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Poster 2

Establishment of a protocol for proteome and metabolome analysis on leaves of *Olea europaea* L. associated with adventitious rooting

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The olive tree (Olea europaea L.) is one of the most important fruit species in the Mediterranean basin. This culture is of high economic interest, due to the increase in oil consumption worldwide. However, some olive cultivars have difficulty in being propagated (recalcitrant) due to the difficulty in developing adventitious roots by following the traditional method of semi-hardwood cuttings. The traditional Portuguese cv. 'Galega vulgar' is well known for its recalcitrant behavior when propagated by semi-hardwood cuttings. An in vitro culture system has been developed and is currently used for molecular and biochemical studies associated with adventitious rooting ability. The present study aims to describe the establishment of a protocol to simultaneously isolate proteins and metabolites to further investigate proteomic and metabolic changes occurring at the aerial plant tissues that could be associated with the formation of adventitious roots. For the establishment of this protocol, leaves of cv. 'Galega vulgar' were collected from in vitro growing plantlets. After homogenization of plant material using liquid nitrogen, proteins and metabolites were extracted following four different multiphase protocols, two based on acetone precipitation, one in methanol/chloroform and another in Methyl tert-butyl ether/methanol precipitation. Six biological replicates (bulked sample of leaves taken from ten microshoots) were considered per protocol. The ideal extraction method should reproducibly capture the most content of proteins and metabolites, and simultaneously minimize degradation and contamination. Results based on total protein concentration and protein profile (analysed by SDS-PAGE electrophoresis) allowed the selection of the TCA/Acetone method as the most appropriate method for protein extraction. Metabolites were collected at different steps of each protocol, allowing the identification of polar and non-polar metabolites. Metabolome profile was achieved by 1H Nuclear Magnetic Resonance (NMR) spectroscopy. Results achieved through the four different methods indicate the TCA/Acetone method as the most reliable method for metabolome analysis. The present research allowed the establishment of a method for the simultaneous extraction of high-quality metabolites and proteins samples, allowing further analysis by high-throughput platforms.

This work was funded by National Funds through FCT under the Project UIDB/05183/2020 and through FEDER funds under the Program Alentejo 2020 with the project GESCERTOLIVE - ALT20-03-0246-FEDER-000058. The authors are grateful to Virginia Sobral for technical support.

Poster 3

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

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The Portuguese Montado is a traditional production system broadly classified as High Nature Value with management practices and intensity of use that promote high levels of biodiversity of conservation interest. However, the Montado system is under threat by land-use changes resulting in strong habitat loss and fragmentation. In this perspective, natural remnant habitat (NRH) patches and corridors, such as small woodlands or riparian forest, are determinant for maintaining the structural and funcional diversity of the Montado landscape, crucial for the preservation of their high levels of biodiversity through increasing habitat and landscape heterogeneity and connectivity. This PhD thesis aims to identify and quantify the ecological value of NRH through the establishment of a biodiversity evaluation system based on biological importance, habitat suitability for fauna species and habitat connectivity. This will be achieved through: identification of different NRH in the field, namely small forest patches, shrub patches, rocky outcrops, temporary and permanent ponds, and riparian gallery (with a minimum of 30 sample units by NRH typology); identification of its specificity and conservation status based on typical plant and lichens species/ assemblage and vegetation; analysis of the relationship between vegetation data and different management-related variables, that will be translated to different vegetation quality classes; analysis of selected fauna species preferences for NRH with different specifications and/or on isolation/connectivity; identification of minimal optimal density and compositional diversity of NRH in montado through structural and functional connectivity analysis; integration of the results into a final ecological value for each NRH. This thesis it will also produce a NRH conservation and management guide for landowners and policy makers. The study area includes several estates in Central Alentejo (southern Portugal), in Montado areas held by different landowners. This contact is being facilitated by the MED R&D institute. With this project we expect to contribute to the conservation, restoration, and re-creation of NRH.

This thesis is funded by National Funds through FCT under the PhD scholarship 2020.07109.BD.

Poster 4

Tocopherol biosynthesis dynamics in almond kernel development

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Almond is one of the most important tree nut crops, due to its high nutritive value, namely in lipids and tocopherol richness. Its production in Portugal has been increasing during the last years, as traditional varieties have been substituted by commercial varieties in the newly-established orchards. Tocopherol (Vitamin E) is an antioxidant that prevents the peroxidation of unsaturated fatty acids, increasing almond storage-life. As an antioxidant, it is highly effective preventing cardiovascular diseases by inhibiting platelet aggregation. Genes involved in its biosynthesis have been characterized in other species [1] but no studies have been made in almonds. This work aims to study tocopherol profile of fruits (collected at different developmental stages), and to quantify and characterize candidate genes involved in the synthesis of vitamin E from different almond cultivars, and along almond kernel development.
The almond cultivar Soleta was used for kernel developmental study and its fruits were morphologically characterized from March to August. Fruits collected from the other cultivars (Gama, Rogel, João Dias, Fura Sacos, Bonita de São Brás and Verdeal) were morphologically characterized only at maturity stage. The tocopherol profile was analyzed and quantified by HPLC, using fluorescence detection and a normal-phase silica column. The differential expression level of candidate genes involved in tocopherol synthesis (*tocopherol cyclase - PdVTE1, homogentisate phytyltransferase - PdVTE2, tocopherol O-methyltransferase - PdVTE4*) was characterized using RT-qPCR. For this analysis, total RNA extractions of almond kernels were optimized, followed by synthesized cDNA. The sequences of candidate genes *PdVTE1, PdVTE2* and *PdVTE4* obtained from *in silico* studies and the transcripts amplified were confirmed by Sanger sequencing. The transcripts abundance analysis was carried out in a LightCycler 480 (Roche) using SYBR Green I Master (Roche).
Almond cultivar Fura Sacos stood out for its high concentration in α-tocopherol. It was also possible to observe an increase in α-tocopherol content along kernels development over the months, with higher increments between May and June. Given the relevance of vitamin E in almond quality determination, we are performing the transcriptional analyses of the target candidate genes by RT-qPCR, aiming to obtain new insights into the tocopherol biosynthetic pathway.

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This work was funded by Inov-Amendo-AL: Microenxertia in vitro de amendoeiras selecionadas para a promoção do amendoal no Alentejo (ALT20-03-0246-FEDER-000068) supported by Program Alentejo 2020, through the European Fund for Regional Development (ERDF), within the scope of the Collective Action Support System - Transfer of scientific and technological knowledge. Domain of Competitiveness and Internationalization. Authors also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018) and FCT for UIDB/05183/2020 and UIDB/04551/2020 (UIDP/04551/2020) projects.

Poster 5

CynaraTeC - a technology transfer strategy towards cardoon economic valorization

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Cynara cardunculus, commonly known as cardoon, is an endogenous resource well-adapted to the territory, with great economic potential. Current scientific knowledge underlies its multifunctionality, being the cardoon flowers widely used as a coagulating for cheese production, the leaves a source of bio-ingredients for food and pharmaceutical industries, and stalks a source of biomass for a wide range of industrial applications.

CynaraTeC is a strategy for scientific knowledge and technology transfer for cardoon valorization, focus on flowers and leaves, towards its economic add-value. Among the various actions planned within CynaraTeC project, it was developed a National Technological Roadshow entitled “Integrated Cardoon Valorization”, with intervention in the 6 regions of Mainland Portugal with PDO cheese production: Azeitão, Serpa, Évora, Nisa, Castelo Branco and Serra da Estrela. Aiming the training the different economic agents, and other stakeholders involved in cardoon production, from planting, to harvesting the different parts of the plant, and its subsequent transformation into multi-sector applications.

In a period of 12 months, in online format, 17 Roadshow actions were carried out, with a total of 515 participants, national and international, from different sectors, namely: 33,98% higher education students, 28,35% research and development, 12,04% agricultural production, 5,83% food industry, 3,88% cheesemakers, 2,52% local development associations and 13,4% participants from other sectors (7,38%) or undefined sectors (6,02%). The performance indicators also allowed us to assess that 78% of the business public revealed an intention to learn more about cardoon regarding its economic valorization. In the same period, 4 collaboration agreements were established with agriculture producers, whom are installing or currently producing cardoon.

The results show impact of the developed action, providing a tool for dissemination of new knowledge and technology for cardoon enhancement. In the future, a web thematic portal is foreseen, to host all the presentations made during the RoadShow, as well as a list of frequently asked questions, and respective answers, to streamline the sharing and access of knowledge.

"CynaraTeC - Transfer of Technology for the Valorization of Cardo" (ALT20-03-0246-FEDER-000067), is a project co-financed by the European Regional Development Fund (FEDER) through the Regional Operational Program of Alentejo – Alentejo 2020, within the scope of Support System for Collective Actions – Transfer of Scientific and Technological Knowledge – Domain of Competitiveness and Internationalization Thanks to FCT for funding UIDB/05183/2020 to the Mediterranean Institute for Agriculture, Environment and Development (MED).

Poster 6

Characterization of the *PIN* auxin carrier gene family in *Olea europaea* L. and analysis of its expression patterns in response to wounding and infection with *Verticillium dahlia*

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The PIN-FORMED (PIN) protein represents the most important polar auxin transporter in plant tissues, assuming a key role in the distribution of auxin and in the control of multiple biological processes. The *OePIN* gene family in olive tree was characterized by analysing its composition and radiation pattern, gene intron-exon structure, and protein structural features. A total of 17 members of the *PIN* gene family were identified at the olive genome database, available for the var. *europaea* (cv. Farga) at the http://denovo.cnag.cat/olive. Information on phylogenetic relationships considering PIN gene members belonging to different subfamilies from different eudicot plant species revealed that the olive *PIN* family is composed by members belonging to six subfamilies, named *OePIN1*, *OePIN2*, *OePIN3*, *OePIN5*, *OePIN6,* and *OePIN8*. Differences in the pattern of gene radiation were observed across the subfamilies. RNA-seq data retrieved from http://denovo.cnag.cat/ was used to investigate the differential expression patterns of all *OePIN* genes in association to wounding and infection with the pathogenic fungus *Verticillium dahlia* at 2- and 7-days post inoculation (dpi). Trimmed reads were mapped against the *O. europaea* cDNA RefSeq. The *edgeR* package was employed to identify differential expressed genes (DEGs) from raw counts using TMM as normalization method. The KEGG Orthology-Based Annotation System (KOBAS) was used to perform a functional classification and pathway assignment of the up- and down-regulated DEGs using OmicsBox software. To obtain an overview of the functions of *OePIN* genes a Gene Ontology enrichment analysis was further performed. A complementary weighted gene co-expression network analysis (WGCNA) was used to characterize the *OePIN* gene family. Changes in *PINs'* expression were observed regarding both stress factors. Most of the *OePIN* members were down-regulated at the early time-point (2dpi) and increased at 7 dpi. *OePIN1b* appears as the single member upregulated upon both stress conditions, visible already at 2 dpi. Significant differences between wounded and fungus infected plants were only detected on *OePIN2a*. This gene was also identified by WGCNA in a co-expressed module tightly correlated with wounding (P = 0.002).

This work was funded by National Funds through FCT under the Project UIDB/05183/2020.

Poster 7

The role of apoplastic fluid in driven somatic embryogenesis efficiency – establishment of a workflow allowing proteome and metabolome analysis

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Somatic embryogenesis (SE) is used for mass propagation of several important agronomical plant species, with advantages in comparison with the use of microcuttings and semi-hardwood cuttings due to the high rates of direct regeneration, direct embryo conversion, and sanitation. However, it has not been routinely used in the propagation of *Olea* spp., and one of the main reasons is the recalcitrant behaviour associated with the use of adult tissues as initial explants. In cv. 'Galega vulgar' an efficient SE protocol was established using somatic tissues (radicle and cotyledons) taken from zygotic embryos. However, because seeds arise from open pollination, each embryo used presents a unique genetic background different from the mother plant elite cultivar. Due to the non-conservation of the genetic background, this system is far from optimal. Different attempts were made to overcome the SE recalcitrance response in adult tissues. The role of different factors has been considered, that includes the type of explant taken for the establishment of in vitro cultures, its development and physiological stage, the growth conditions, and the chemical composition of the culture media. Another important factor that has been highlighted as affecting SE response, but not always taking the required attention, and never studied in olive, is the release of organic bioactive molecules by the explants into the culture medium. The influence of secreted molecules on SE efficiency has been observed for a long time through the use of nurse cells culture. Whitin the secreted molecules that demonstrated to induce, or modulate, SE response, there are proteins and a diversity of metabolites. To identify extracellular bioactive molecules that could be further used to promote SE efficiency in olive adult tissues, a research line has been established considering two different omics, able to identify/quantify proteins and metabolites with a key role in SE efficiency. Here it will be presented the workflow already developed to isolate both proteins and metabolites allowing further proteome and metabolome analysis through LC-MS/MS and NMR platforms, respectively.

This work was funded by National Funds through FCT under the Project UIDB/05183/2020 (MED exploratory project ID 10_2021).

Poster 8

SENSEPREDICT – "Predict the taste outside the mouth" An entrepreneurship project

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Tannins constitute a large family of polyphenols and are associated to astringency and some degree of bitterness (Mateus, Pinto, Rua, & Freitas, 2004). This organoleptic sensation, accompanied by a shrinking or drawing feeling in the mouth, is characteristic of some products, such as red wine (Rinaldi & Moio, 2020). Astringency can lead to rejection of the wine, being economically important, but not fully understood. It is usually accessed through taste panels, which involves subjectivity, representing a great analytical challenge in wine industry (Rinaldi & Moio, 2020). To give answers to these aspects and create value for the business market, we are developing an entrepreneurship project aimed at developing a test to quantify the astringency of wines, *in vitro*, "outside the mouth". Wines have levels of tannins that can lead to different perceptions of astringency by different consumers. This is particularly due to the differences in salivary proteome, among individuals. The test is based on this knowledge, particularly by monitoring salivary proteins*wine interactions. Being independent on subjective individual report, this technology will provide a quantifiable result of astringency intensity, which can be used by producers to compare wines and/or monitor the wine sensory quality through different phases of the process. In order to have objective and standard results, an artificial saliva is being developed, with the salivary proteins mainly involved in astringency development. The interaction between this saliva and wine will mimic, *in vitro,* what happens, *in vivo*, in the mouth and can be monitored through in-gel proteomics-based approaches. We will present our preliminary results, showing the potential of this test to quantify wine astringency, complementing sensory evaluation and/or wine chemistry analysis. We observed that increasing concentrations of tannins results in increased precipitation of particular salivary proteins. Moreover, they induce alterations in saliva protein profile correlated with the intensity of astringency perceived. Both these observations allowed the identification of the proteins most involved in astringency process. In addition to artificial saliva, this test can be further used with individuals' saliva, allowing to predict the level of astringency perception by individual consumers, helping in tailored wine choices.

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Poster 9

Expression of manganese transporters in wheat varieties with different tolerance to manganese toxicity.

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Manganese (Mn) is an essential micronutrient for plant development and growth, and it participates in several physiological mechanisms. It serves as cofactor for many enzymes and is a component of the oxygen-evolving complex in photosystem II, serving as cofactor in the water-splitting reaction for producing oxygen and providing electrons to the photosynthetic electron transport chain. However, once in excess it can induce deleterious effects in plants such as brown spots in the leaves, interveinal chlorosis and potentially necrosis. In plants, there are several genes encoding for manganese transporters which can contribute for the regulation of metal imbalances. In the present study we have evaluated the molecular response of 6-week-old wheat (*Triticum aestivum* L.) plants subjected to two different levels of Mn in the soil (control and high-Mn). Two wheat varieties with different tolerances to Mn toxicity were used: Ardila (non-tolerant) and Norquay (tolerant). The levels of expression of three different manganese transporters – *MTP8.1*, *CAX2* and *NRAMP3* – were measured in wheat leaves by Real-Time qPCR. The relative expression analysis revealed differences in gene expression in wheat leaves amongst varieties and Mn treatments. The expression of *MTP8.1* in the Norquay variety was significantly higher in the high-Mn soil ($P < 0.05$) than in the control. Similarly, the addition of Mn increased the expression of *NRAMP3* in both Ardila and Norquay varieties relatively to the control soil ($P < 0.01$ and $P < 0.001$, respectively). The *NRAMP3* and *CAX2* genes also showed differences between Ardila and Norquay varieties for the control treatment, being the expression significantly higher in Ardila ($P < 0.05$). Our results confirm a higher tolerance of the Norquay variety comparing to Ardila regarding Mn toxicity, since there was a more pronounced increase in transporters' expression under Mn stress. This study contributes to the understanding of the regulation of Mn transporters in wheat and their role regarding the mitigation of metal toxicity.

This work was funded by European Regional Development Fund through ALT20-03 -0145-FEDER-000039 and Fundação para a Ciência e a Tecnologia (Portugal) through SFRH/BPD/108734/2015.

Poster 10

Polyethylene, polyvinyl chloride and polyamide microplastics as potential cytotoxic particles to human intestinal cells, human hepatocytes and rat dopaminergic neurons

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Microplastics (MPs) are plastic particles with sizes between 100 nm and 5 mm, being considered an emerging class of contaminants with deleterious effects on the environment and human health. They result from the release of the plastic particles that are part of dailylife objects or through the degradation of larger objects. The presence of toxic substances in the plastics themselves and the ability to function as chemical sponge by absorbing or adsorbing such substances is particularly relevant. When MPs are in contact with biological fluids, the environmental contaminants are released and become bioavailable, which may exert toxic effects. Since MPs are ubiquitous in the environment, humans are continuously exposed, being ingestion the most frequent pathway. Thus, it is fundamental to evaluate MPs potential toxicity. In this work, the cytotoxicity of three common MPs: Polyethylene (PE), Polyvinyl Chloride (PVC) and polyamide (PA-commonly known as nylon) was evaluated towards human intestinal epithelial cells (Caco-2), human hepatocytes (HepG2) and rat dopaminergic neurons (N27). The results obtained showed that for the tested concentrations, the toxicity of the three MPs for intestinal epithelial cells was negligible, excepting for the highest concentration of PE, when compared with the control. In contrast, for the HepG2 cell line, both PA and PVC reduced significantly the cell viability, while PE were not toxic. For the N27 cell line only the highest concentrations of PE and PVC significantly reduced the cell viability.

This work was developed under the scope of the Comprehensive Health Research Centre (CHRC) project (UIDP/04923/2020) and CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Further funding was provided by project ICON (Ref. CENTRO-01-0145-FEDER-000013, project funded by FEDER funds through POCICOMPETE 2020) and funds from the Health Sciences Research Center (CICS-UBI) through National Funds by FCT - Foundation for Science and Technology (UID / Multi / 00709/2019)

Poster 11

The alternative respiratory pathway revealed a key role in *Pisum sativum* L. germination

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Seed germination is a vital stage in plant development and an important factor for successful agricultural production. Considering that seed germination involves the activation of several metabolic pathways, including cellular respiration to provide the required energy to embryo germination and further development, the involvement of the Alternative Oxidase (AOX) was investigated by following transcript, protein and metabolic/respiratory analysis during pea (*Pisum sativum* L.) seeds germination. Alternative oxidase (AOX) is a key enzyme in the alternative respiration pathway, playing a crucial role in regulating cell-reprogramming by improving metabolic transitions related to the cellular redox state and the flexible carbon balance. To assess the involvement of AOX on *P. sativum* L. seeds germination, seeds of two cultivars ('Torta de Quebrar' and 'Maravilha d'América') were imbedded for 16h in a Rotenone solution (inhibitor of cytochrome pathway, CYT) and compared with seeds only imbedded in sterile tap water. Calorespirometry was used to accesses metabolic/respiratory changes by using a Multi-Cell Differential Scanning Calorimeter (MC-DSC, TA Instruments) running in isothermal mode at 25°C. For validation of calorespirometric parameters, a seed germination trial was conducted in parallel using sterile tap water and inhibitor of CYT pathway (Rotenone). The involvement of AOX was evaluated at the transcript and protein level. Transcript level accumulation of the three pea *AOX* genes (*PsAOX1*, *PsAOX2a* and *PsAOX2b*) was assessed by RT-qPCR. At the protein level, analysis of AOX expression was performed through Western blot. The results achieved demonstrate the existence of a relationship between calorespirometric parameters, *PsAOX* transcript accumulation and PsAOX expression. At 16h of water and Rotenone imbibition, the cultivar characterized by low seed viability (cv. 'Maravilha d'América') exhibited higher levels of calorespirometric parameters Rq and RCO2, coincident with significantly higher transcript accumulation of the two most responsive genes (*PsAOX1* and *PsAOX2d*), and PsAOX protein expression level. These results demonstrate the involvement of AOX on seed germination, strongly related with seed viability.

Acknowledgments: This work was supported by the EU project LIVESEED - Improve performance of organic agriculture by boosting organic seed and plant breeding efforts across Europe funded by the European Union's HORIZON 2020 research and innovation programme under the Grant Agreement no 727230, and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090; and by National Funds through FCT under the Project UIDB/05183/2020. Authors are very thankful to IIFA for the fellowship given to Lénia Rodrigues. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the EC and the Swiss government. Neither the European Commission/SERI nor any person acting behalf of the Commission/SERI is responsible for the use which might be made of the information provided on this document.

Poster 12

In vitro neurotoxicity of house dust extracts

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Indoor contaminants are potentially toxic and can negatively affect human health. Considering that in modern society people tend to 90% of their time indoors, it is important to study the potential toxicity of these indoor contaminants. The evaluation of the indoor environment contamination has been increasingly performed using dust. This matrix is an important reservoir and repository of chemicals that are protected from the degradation processes occurring naturally in the external environment. Most of the available studies are based on chemical analyzes that do not allow to evaluate the toxicity in an integrated way.

In this work, we evaluated the levels of mercury, a well-known neurotoxicant, in house dust extracts from Covilhã, Portugal. The cytotoxic potential of these dust extracts was also evaluated in the dopaminergic neural cell line N27. Mercury was detected in all analysed dust samples, with values ranging from 43 to 1135 $ng.g^{-1}$. Also, the neurotoxic potential of house dust varied widely between houses, with the most cytotoxic extract corresponding to the sample with higher mercury levels. Overall, the obtained results disclose a moderate neurotoxic potential of the different dust extracts analyzed and a statistically significant correlation between cell viability and mercury concentrations ($p<0.05$, $r=0.900$).

This work is developed under the scope of the Comprehensive Health Research Centre (CHRC) project (UIDP/04923/2020) and CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Further funding was provided by project ICON (Ref. CENTRO-01-0145-FEDER-000013, project funded by FEDER funds through POCI-COMPETE 2020). Rafael Barros acknowledges funding from the project "Endocrine disruptors and precocious menopause: study of the effect of plastics and cosmetics on menopause age", funded by the Portuguese Society of Endocrinology, Diabetes and Metabolism (Dr. Maria da Conceição Barbas Endocrinology Scholarship).

Poster 13

Selection of reference genes for gene expression analysis in *Pisum sativum* L. seeds germination

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Pisum sativum L., commonly known as pea, is nowadays recognized as one of the most important grain legume species. The development of more resilient cultivars, able to efficiently cope and adapt to environmental constraints, has been recognized as a key strategy to contribute to more sustainable agricultural practices. Molecular studies aiming to gain insight into the function of genes involved in stress response during seed germination and seedlings' establishment are of high interest in this research field. Reverse transcription quantitative PCR (RT-qPCR) is widely used in gene expression studies across a diversity of plant species and biological systems. However, the accuracy of this methodology strongly depends on several factors, making necessary the use of reference genes (RGs), genes that show a stable expression across all samples contributing to reducing/eliminating technical variations and further achieving accurate target genes' expression measurements. To date, there is no consensus on the use of systematically validated RGs across different genotypes even if a similar biological system is used. As far as we know, no studies are available reporting the evaluation and selection of the best RGs for normalization of expression data in *P. sativum*. Aiming to further proceed to gene expression studies in pea seed germination, in the present study, gene expression stability was evaluated in nine candidate RGs: *PsPOB*, *PsPP2AA1*, *PsSAR1*, *PsTIP41*, *PsUBI*, *PsGAPDH*, *PsACT*, *PsTUB*, and *Ps5.8S rRNA*. A germination experiment was established using the cv. 'Torta de Quebrar' and the cv. 'Maravilha d'América'. The expression stability was determined by three different software, the geNorm, NormFinder, and BestKeeper. The choice of the best RG from the nine candidates, separately by both cultivars and considering all samples, was consistent between software's. Nevertheless, a clear dependency on the cultivar was observed. According to the data achieved, normalization requires the use of two RGs when considering both cultivars and when using a single cultivar. *PsUBI* and *PsSAR1* appear as the most suitable for cv. 'Maravilha d'América', while *PsPOB* and *PsSAR1* were selected for cv. 'Torta de Quebrar'. The genes *PsUBI* and *PsSAR1* can be proposed as the most appropriate set of RGs to be used in studies involving both cultivars.

This work was supported by the EU project LIVESEED - Improve performance of organic agriculture by boosting organic seed and plant breeding efforts across Europe funded by the European Union's HORIZON 2020 research and innovation programme under the Grant Agreement no 727230, and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090; and by National Funds through FCT under the Project UIDB/05183/2020. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the EC and the Swiss government. Neither the European Commission/SERI nor any person acting behalf of the Commission/SERI is responsible for the use which might be made of the information provided on this document.

Poster 14

ADIPOHSITE – ADvancIng the obesogen hyPOtHeSIs Through biomonitoring, observation, and Experimentation

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The obesity epidemic is one of the most important public health challenges of the 21st century. Scientific evidences suggest that exposure to a particular class of environmental contaminants, the so-called obesogens, might be implicated in the obesity epidemic. However, even with the increasing scientific evidences available, there are still unanswered questions and there are some doubts whether the associations found might just be chance findings. We propose to provide further evidences in support of the obesogen hypothesis by performing doseresponse experimental studies with animals from different taxonomic groups under realistic exposure scenarios and by implementing biomonitoring studies to provide exposure assessment data. In order to achieve the main goal of this proposal, a set of specific objectives were established: (1) To study the associations between metals and overweight/obesity in humans; (2) To study the effects of exposure to obesogens under realistic conditions in crustaceans, including the evaluation of transgenerational effects; (3) To study the effects of the exposure to the model obesogen TBT, in gastropods from wild populations and animals from laboratory experiments, and (4) To study the effects of exposure to obesogens under realistic conditions in Amphibians. By using such a multi-tiered approach it will be possible to understand the real impact of obesogens on weight gain not only in experimental and wild animal models but also in humans and thus to contribute to deepen our understanding on the obesity drivers.

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

2. Environment, Landscape and Sustainability

Oral Communication 6

Ecotoxicological responses used in the assessment of the potential ecological status of the Lage reservoir

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Alentejo is located in a region with a Mediterranean climate, classified as one of the most vulnerable in the world to climate change, with an increase in air temperature and a decrease in precipitation levels, which induce a greater hydrological stress on aquatic ecosystems. In countries where water scarcity has become an issue, reservoirs represent one of the most important forms of water storage and supply. The Lage reservoir is a small reservoir included in the Alqueva project (Southern of Portugal). Agriculture is the predominant activity in the drainage basin of the Lage reservoir, with a prevalence of vineyards and olive groves. The aim of this study was to assess the effectiveness of the use of biological responses, as ecotoxicological endpoints, in the detection of pollution induced by agricultural activities in the Lage Reservoir. Water quality was analysed during the years 2018 and 2019 (march, july, october and november) using: i) support parameters: pH, dissolved oxygen (DO; mg O_2/L), total phosphorus (TP; mg P/L); total nitrogen (N; mg N/L) and nitrates (N; mg NO_3/L) (APHA, 1998); ii) priority substances (pesticides from several classes of action: triazines, phenylureas, organophosphorus compounds, acid compounds); and iii) ecotoxicological bioassays with the bacteria *Vibrio fisherie,* the crustacean *Thamnocephalus platyurus* and the microalgae *Pseudokirchneriella subcapitata*. All support parameters are within the limits established for a good ecological potential of the water body (INAG, 2009). The pesticides that achieved the highest concentrations were Imidacloprid (133.3 ng/L; november 2018), fluroxypyr (89.3 ng/L; october 2019) and diflufenican (81.3 ng/L; october 2019). The month with the highest amounts of pesticides was october 2019 (714.9 ng/L). *Vibrio fisherie* was more sensitive to water samples from March and November 2018 (30 min-EC_{50} of 38.87 and 50.69%, respectively). The results showed a marked growth inhibition of the green microalgae when exposed to the Lage reservoir water. The ecotoxicological results showed that although the water from the Lage reservoir has shown support parameters within the guide levels and amounts of pesticides below the values for good chemical status, it can still compromise the balance of the aquatic ecosystem, in certain periods of time.

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

Oral Communication 7

Re-use of waste materials from the pulp and paper industry in mine soil recovery: chemical and ecotoxicological assessment

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The aim of this study was to evaluate the use of wastes from the pulp and paper industry (biomass ash and biologic sludge from the industrial wastewater treatment plant) to improve the quality of soils degraded by mining activities, using chemical and ecotoxicological indicators. The materials were tested in a pilot plot, installed in São Domingos Mine, with soils from different mines of the Iberian Pyrite Belt (Aljustrel, Lousal and São Domingos). The following waste-based materials were prepared before application to soil: biomass ash granules (AG); AG and composted sludge (AG+CS); and a mixture of the fresh materials, without stabilization (A+S). Results obtained for the Aljustrel's soil 1 month (T1) and 25 months (T4) after the application of the materials were evaluated. The results included basic soil chemical characteristics (e.g., pH and organic matter content (OM)), and soil ecotoxicological assessment: (i) soil phytotoxicity, evaluated using germination and growth tests with watercress (*Lepidium sativum*), and barley (*Hordeum vulgare*); (ii) soil habitat function, evaluated with the *Eisenia fetida* mortality test; and (iii) soil retention function, using soil-water extracts and aquatic organisms representative of different trophic levels (growth inhibition of the green microalgae *Pseudokirchneriella subcapitata*, mortality of the crustacean *Thamnocephalus platyurus* and *Daphnia magna* immobilization). The selected endpoints were quantified by calculating the EC_{50} (concentration of the materials under study that produce an effect on 50% of the test organisms).

There was a marked improvement of the soil physico-chemical properties, with a significant increase in soil pH from initial acid values (4.0-4.3) to values close to neutrality (6.9-7.5) at T1 and T4, with all the treatments, and in the OM content from 0.5% to 1.6%-2.9%, at T1 and T4, in the plots with AG+CS and A+S application. The increase in OM was lower with the application of AG, reaching only 0.6% at T4. The phytotoxic response (germination and growth) of both plant species decreased markedly, compared to the non-amended soil, with better results in the plots with AG+CS and A+S application. The non-amended soils were toxic towards the organisms used in the bioassays, both at T1 and T4, with EC_{50} values ranging from 1.3 to 5.8 (%; v/v) for *T. platyurus*, from 1.3 to 9.4 (%; v/v) for *D. magna*, and from 42.7 to 87.5 8 (%; m/m) for *E. fetida*. The toxic responses for the same organisms decreased at T1 and reached non-quantifiable toxic responses at T4 in all the amended soils, irrespectively of the treatment. As for *P. subcapitata*, a total inhibition of growth was observed in non-treated soil, both at T1 and T4, and that inhibition was lower in the treated soils at T1 (31-66%) and even lower at T4 (12-35%).

The results suggested that the tested waste-derived additives are suitable to be used as amendments for soils affected by mining activities, allowing a stable acidity correction for at least 25 months, and a decrease in the toxic responses towards a battery of soil organisms.

This study was supported by the project Life No_Waste LIFE14 ENV/PT/000369 – "Management of biomass ash and organic waste in the recovery of degraded soils: a pilot project set in Portugal".

Oral Communication 8

A participatory approach to the measurement of Social Sustainability in Agribusiness: The case of Alvarinho in the Monção and Melgaço sub-region

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Studies on sustainability of agribusinesses often overlook the social dimension of sustainability. Out of the three traditional pillars of sustainability, the social pillar is clearly the least studied, and gaps in the classification and estimation of its indicators have been identified. Some social indicators lack scientific validity, others are most commonly limited to capturing intra-company realities.

This research aims to contribute to the definition and operationalization of a framework for better assessing the social dimension of agribusiness sustainability, incorporating the realities and priorities valued by a wide set of stakeholders in a robust and replicable methodological model.

The empirical component of research should answer the following central questions: (i) How is social sustainability defined by stakeholders? (ii) What is the most appropriate and feasible way to translate this definition into measurable indicators? (iii) How to model the use of these indicators to measure the social dimension of sustainability in agribusiness?

The investigation will begin by the necessary identification of the most used sustainability assessment models on the literature, on an agribusiness context, select the most relevant and common social indicators across the selected models and classify these social indicators according to the other sustainability scopes they combine. Following this framing we propose a participatory approach to determine and weight social sustainability indicators relevant to stakeholders, and subsequently apply them in an evaluation model, using the particular context of Alvarinho wine production in the Monção and Melgaço sub-region as an experimental field.

We anticipate to contribute with a set of tools to support the decision-making of agribusiness companies by allowing them to measure their level of resilience with regard to the social sustainability of the business.

Either by identifying how to measure the social pillar of sustainability, or by generating evidence that the social dimension spams all pillars and works as a master beam that secures balance to sustainable agribusinesses, this research aims at supporting the sustainable development of the sector from a social perspective.

Poster 43

LactoMTeC project - a technology transfer approach for circular valorization of effluents from the cheese sector

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In the Alentejo region, the cheese industry stands out for its significant impact on the rural economy. However, cheese production activity generates effluents of high complexity and great environmental impact. Over the years, CEBAL has combine R&D with knowledge and technology transfer activities, grading membrane technology, exploring in particular the use of nanofiltration as a green and innovative technology, and its application on the treatment and/or valorization of wastewater from different agro-industrial sources. CEBAL is currently equipped with five membrane units at different scales, including a semi-industrial one built as a car trailer. Given the challenges identified on the cheese sector in previously works, [1,2] a technology transfer project called LactoMTeC arise from the clear opportunity to transfer scientific and technological advances on membrane separation processes, since the technology allows the treatment of cheese wastewaters, the reuse of treated water and also the valorization of their by-products, such as protein or lactose, that can be an alternative source of functional ingredients on food or pharmaceutical industries.[3] Therefore, the project aims to mobilize cheese-making producers into dynamics considering (bio)circular economy perspectives at a broad interaction and sharing with researchers, as well as other players that can contribute to build a circular chain. The project is based on three crucial stages for its development: 1) roadmapping and characterization of the cheese sector; 2) organization of public events and technology demonstrations; 3) validation of the technology transfer through on-site experimentation actions. In the end of the project, the intention is to provide the basis for an effluent management model based adapted to the Region and the contexts, using membrane technology.

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“LactoMTeC – Tecnologia de Membranas na valorização sustentável de efluentes do sector dos Lacticínios” (ALT20-03-0246-FEDER-000073) was funded under the framework of the “Specific Regulation on Competitiveness and Internationalization” by the “System of Support to Collective Actions” on the scope of “Transfer of Scientific and Technological Knowledge”, supported by the Alentejo 2020 Regional Operational Programme through the European Regional Development Fund (ERDF). Carina Pissarra acknowledges a fellowship from the CEBAL.

Poster 44

New deep eutectic solvent assisted extraction of highly pure lignin from maritime pine sawdust (*Pinus pinaster* Ait.)

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Lignin is a natural aromatic polymer that has tremendous potential as a renewable and sustainable feedstock to produce fuels, chemicals and biomaterials. Its efficient extraction could expand the utilization of biomass and reduce the dependence on fossil fuels; however, the complex molecular structure of lignin makes its isolation from the other biomass components non-trivial. In this work, novel acidic deep eutectic solvents (DESs) were prepared and screened for the fractionation of maritime pine (*Pinus pinaster* Ait.) sawdust to infer on their suitability for an efficient and selective extraction of lignin. DESs show several favorable features such as biodegradability, low cost and tunability that encourage their use as extraction solvents[1].

The extraction capacity was observed to be greatly affected by the DES composition. New DESs composed of ChCl and two acidic hydrogen-bond donors were prepared to achieve solvents with enhanced properties and superior extraction performance. Using a DES composed of lactic acid, tartaric acid and choline chloride in a molar ratio of 4:1:1, allows the recovery of 95 wt% of the total lignin present in pine biomass with a purity of 89 %. Moreover, the developed DES can be recycled and reused without compromising its performance for, at least, two additional cycles. The superior performance of the prepared DES and its “green” features makes the process highly appealing for biomass fractionation.

This work was financially supported by the Portuguese Foundation for Science and Technology, FCT, via the projects PTDC/AGR-TEC/4814/ 2014, PTDC/ASP-SIL/30619/2017, UIDB/05183/2020 and the researcher grant CEECIND/01014/2018. R.Cwas funded through contract under the scope of project Des.solve (ERC consolidator), ERC-2016-COG 725034. E.M. is grateful for the PhD grant (SFRH/BD/132835/2017) from FCT. The CQC is supported by FCT through the project projects UID/QUI/ 00313/2020 and COMPETE. The Associate Laboratory for Green Chemistry-LAQV receives financial support from the FCT/MCTES national funds (UIDB/QUI/50006/2019). The Strategic Research Centre Project UIDB00102/2020, funded by FCT, is also acknowledged.

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Poster 45

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

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According to the World Health Organization, access to clean air is considered an essential human right. However, air pollution is a serious threat to human health, being responsible for around 7 million deaths per year. This mortality is associated with several pollutants with both ambient and household emissions, such as gases, particulate matter, and volatile organic compounds (VOCs). As poor air quality is responsible for various diseases, improving indoor air quality (IAQ) must be a priority. Despite the existing guidelines to guarantee IAQ, other strategies should be employed whenever possible, including the reduction of pollutants at their source, air ventilation, and, if necessary, indoor air treatment. Although several technologies can be considered for indoor air treatment, these possess some disadvantages such as high energy consumption, waste generation and harmful by-products production. Therefore, new indoor air technologies are required to overcome these limitations and non-volatile solvents appear as a promising alternative. Due to their unique thermophysical properties and adjustability of anion-cation/compound combinations, ionic liquids (ILs) and deep eutectic solvents (DES) are promising non-volatile solvents for gas and VOCs capture. Despite all these greener compounds' properties and potential for gas/VOCs separation, transport properties limitations have hampered the development of separation units and their use on target applications. Thus, to develop a technical and economically viable process for gas/VOCs capture, the pursuit on improving solubility, viscosity, and heat capacity must be addressed from an engineering perspective. Aiming at enhancing absorption and desorption processes, the use of a solid phase to immobilize non-volatile solvents can be considered. Membrane gas absorption stands as a mature technology where high specific surface area, independent controllable gas and liquid flow rates, compact and energy efficient separation units, and a linear scale-up design allows to envision its use for gas and gas/VOCs capture. The use of non-volatile solvents as absorbents with membrane technology improves the separation selectivity and the mass transfer driving force, allowing high membrane fluxes and low gas outlet concentration. This highlights the potential of the technology to take advantage of green solvents and, ultimately, stand as a technology with high potential for IAQ.

This work was funded through FCT PhD grant (2020.07796.BD). A.C.A. Sousa acknowledges the development of this work under the scope of the Comprehensive Health Research Centre (CHRC) project (UIDP/04923/2020) and P.J. Carvalho acknowledges the development of this work under the scope of the project CICECO – Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

Poster 46

The role of associations in the sustainability of the agricultural sector

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Agricultural sustainability increasingly depends on the choices made consciously by farmers, but also on the demands of the various stakeholders who intervene daily with farmers to meet the needs of populations. Associations play an important role in disseminating the latest information on all aspects and services to farming communities. However, it seems imperative to assess their contribution to the sustainability of agricultural systems. Although the consensus in the literature suggests that participation in collective agricultural organizations significantly affects agricultural production and its sustainability, there is inconclusive evidence to this effect. Assessing agricultural sustainability is a useful tool that can be used for broad purposes, such as improving the management of the agricultural sector and the marketing of food products.

This investigation aims to understand the role of associations in the sustainability of the agricultural sector. Several questions arise: is sustainability in the agricultural sector an obligation or a necessity? How are associations organized in Portugal? What is the importance of associations in agricultural sustainability? How do associations disclose sustainable practices to their members? This research will be important to understand the role of associations in agricultural sustainability in Portugal, to understand the types of existing associations, adapting a sustainability assessment model, identifying determinants and weighting of relevant indicators. In view of the new Common Agricultural Policy, associations will have a preponderant role in small producers, and it is necessary to understand their role in agricultural sustainability.

Poster 47

Contribution of land-based sources to microplastics increase in aquatic systems: The Portuguese case

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Microplastics (MP) have recently become a central discussion theme around the world mainly due to their potential harmful impact on different ecosystems [1]. Plastics are part of our society and since most of them don't biodegrade in any meaningful way, plastic waste can exist for hundreds of years. Moreover, up to now, only a small percentage of plastic waste is recycled being the rest dumped in landfills, incinerated or simply not collected. During all these processes, plastics undergo a reduction in size which not only impairs the cleanliness of the waters, but also causes marine animals several problems due to ingestion. Moreover, MP can appear in domestic and industrial effluents through textiles washing, cosmetics, among other sources. The distribution of MP within the water ecosystem, depends on particle density and environmental characteristics, such as winds, currents and turbulence [1].

Portugal is a coastal country located in the southwestern part of Europe with 92 212 km^2, 1230 km of Atlantic coast and an exclusive economic zone with 1 727 408 km^2, forming part of the Iberian Peninsula [2]. In 2011, Portugal registered about 10.5 million inhabitants, the majority of which lived in coastal areas and the highest densities found in the two metropolitan areas, Porto and Lisbon. In the present study, we characterize and identify different effluents (industrial and municipal treatment stations and industrial effluents) to analyze which MP predominate in Continental Portugal and which contribute most to environmental contamination. Overall, this work develops strategies for MP analysis in waste waters (effluents and treated waters), especially in the coastal zone, and maps the types of MP prevalent in Portugal. This data base, will allow us to create laboratory models which will be used to test new and green removal processes based either on flocculation by bio-flocculants or new membranes separation.

Acknowledgements: This work was financially supported by the Portuguese Foundation for Science and Technology, FCT, via the PhD grant (2020.07638.BD) and the Strategic Research Centre Projects UIDB00102/2020 and UIDB/05183/2020.

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3. Veterinary Sciences and Animal Production

Oral Communication 9

A DNA barcode reference library of Portuguese mosquitoes

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Mosquitoes are important vectors of pathogens and species identification is essential for the monitoring and surveillance of arboviruses. Classically, mosquito species are identified based on their morphological characteristics, but this method can be time-consuming, requires specialist knowledge and may not be able to identify damaged specimens or distinguish sibling species. Over the last years reliable molecular methods have been developed to overcome these limitations. DNA barcoding, which involves the use of a fragment of the mitochondrial Cytochrome Oxidase I (COI) gene, is one of the most widespread methodologies being used for species identification but a reliable and comprehensive reference database of verified sequences is required.

In this study, we aimed to generate a DNA barcode reference library for the identification of mosquito species from Portuguese mosquito fauna, including most relevant vector species, as there is only a reduced number of mosquito COI sequences from Portuguese origin in genetic databases.

Immature and adult mosquitos captured under the Portuguese vector surveillance programme (REVIVE), run by human health authorities, and previously identified by morphology, were subject to DNA barcoding. Samples were processed for DNA extraction, COI gene fragment amplification and sequencing.

Nighty-eight barcode sequences were obtained, representing 26 species and 6 genera. Genetic analysis included the creation of a neighbour-joining tree and the use of two different methods for molecular species delimitation, Automatic Barcode Gap Discovery (ABGD) and Barcode Index Number (BIN) assignment tool. These methods clustered COI sequences into twenty-five molecular operational taxonomic units (MOTUs), that agreed with previous phenetic classification.

This study generated a reference DNA barcode library for 26 mosquito species reported for Portugal and highlights the utility of DNA barcoding in the identification of Portuguese mosquito fauna, including some cryptic and sibling species, that can have different epidemiological and vectorial roles. Also, the results contribute to the international mosquito barcode initiative, with less common species, that will help on the surveillance and control of mosquitoes and mosquito-borne diseases.

This can help to improve current vector surveillance programs and also contributes for the detection of hidden diversity among mosquito species.

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

Oral Communication 10

Evaluation of the birth impact in the newborn dairy calf using ethophysiological measurements

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The calving moment is one of the most stressful events in the calf's life. Therefore, evaluating the level of the birth impact in the calf can provide valuable information for both nursery and research activities. The evaluation can be performed by measuring calf's vitality, or vigor, using ethologic and physiological parameters. However, there is a lack of general agreement regarding which measures are more suitable. In this paper, we aimed to evaluate the dairy calf's ethophysiological profile (EPP), taking into consideration calving time and calving difficulty. The EEP from twenty-five calves belonging to two dairy farms were evaluated after birth in a scoring system from 0 to 3, with 0 being the normal state and 3 being the most impaired condition. The EEP consisted in measuring six variables: i) meconium staining; ii) head and tong swelling; iii) intensity of response to a stimulus (straw in nostril; iv) sucking reflex; v) time until independent locomotion and vi) rectal temperature. All scores were combined into one global score, with higher scores representing a worse vigor condition. Oxygen-carrying capacity was assessed through hematocrit determination. Calf delivering time and calving difficulty were also registered. Total serum proteins (TSP) were measured 24h after birth for passive immune transfer (PIT) analysis. Descriptive statistics, Pearson correlations and principal component analysis (PCA) were performed to determine the possible association between all the parameters. The PCA showed that meconium staining, head and tong swelling and intensity of response to a stimulus were positively correlated with calving difficulty and calving time. Rectal temperature was inversely correlated with calving ease. Calves with delayed independent locomotion were associated with lower hematocrit, which can be related to hypoxia. TSP mean was 5.23 ± 0.69 g/dL, indicating a sufficient level of PIT. Occurrence of calves with better vigor was correlated with higher scores of TSP. Although further evaluations are required to enlighten some physiologic results, this trial showed that the ethophysiological traits measured in this preliminary study were feasible to evaluate the impact of the birth in the newborn dairy calf. Also, a more vigorous state at birth can lead to a better PIT.

Poster 15

Outcomes and mortality data of European hedgehogs (*Erinaceus europaeus*) in three Portuguese rescue centers

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The European hedgehog (*E.europaeus*) is a mammalian species widely distributed in western-central Europe, including Portugal. Hedgehogs are very adaptable to different habitats and one of the most common mammals admitted to rescue centres. The aim of this study is to analyse and interpreted hedgehogs' casualties and pathologies in rescue centres. From January 2019 to October 2021, a total of 561 hedgehogs (516 alive and 45 dead) were admitted to three Portuguese recognized rescue centres: CERVAS (north), LxCRAS (centre) and RIAS (south). From the 516 admitted alive, 261 were successfully recovered, and 231 died or were euthanized. 101 dead hedgehogs were necropsied: 15 from CERVAS, 23 from LxCRAS and 63 from RIAS. Estimated age, sex and weight were determined. All the observed data was interpreted and correlated with geographical provenance and clinical data when available. The most common causes for admission included unprotected orphans (28,7%;29/101), debility (12,9%;13/101), trauma (11,9 %;12/101) and diseases (11,9%;12/101). Strictly human causes (namely, roadkills, illegal captivity, accidental capture, poisoning) represented 10,9% of the cases (11/101). Approximately a half of these animals (51/101) presented at least one pathologic lesion. Respiratory tract lesions were the most common with 30,7% (31/101) of positive cases, followed by deep wounds (11,9%;12/101). No statistically significant correlations were found between our findings and clinical or geographical data, except that animals from densely populated parishes (NUTS 1; INE) tend to present diseases (as reason for admission) more frequently, comparing to those from less populated parishes (NUTS 3; INE) (p-value = 0.028), which may reveal a greater exposure to some risk factors (possibly of anthropogenic origin) in densely populated areas.
Our results are consistent with published literature on hedgehog pathology and mortality reports in different European rescue centres. More studies are needed, for instance using rescue centres data, to evaluate the true impact of each cause (as infectious agents or human activities) in hedgehogs and implement strategies to ensure healthy and stable wild mammal populations.

This work was funded by National Funds through FCT under the phD scholarship 2021.04520.BD.

Poster 16

Unravelling the genetic diversity and population structure of four Portuguese native sheep breeds

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Since their domestication, approximately 10,500 years before present, sheep accompanied humankind. In Portugal, native sheep are reared nationwide mainly in agrosilvopastoral systems. Merino Branco, Merino Preto, Campaniça and Bordaleira Serra da Estrela are among the most abundant local breeds. Merino and Campaniça are mainly raised in the Alentejo region to produce meat, dairy and wool. Bordaleira Serra da Estrela is the main Portuguese dairy breed, typically used to produce a high-value cheese with a Protected Designation of Origin. The lack of genomic studies is a major concern for the management of genetic diversity, thus the purposes of this study were to estimate genetic variation in these four Portuguese native sheep breeds and a population of crossed Merino, and describe their population structure in the context of worldwide sheep.

Whole-genome resequencing data were obtained from DNA extracted from 56 blood samples [Campaniça (n=6), Bordaleira Serra da Estrela (n=11), Merino Branco (n=10), Merino Preto (n=10), Merino Cruzado (n=19)]. Clean reads were mapped to the sheep reference genome (Oar_rambouillet_v1.0). High-quality SNPs were filtered (SNP quality (≥30), minimum depth coverage per genotype (≥7) and genotype quality (≥20), no indels and only bi-allelic variants) and categorized according to the functional effects and distribution across genomic regions. Filtered SNPs were used to estimate genetic diversity and infer the population structure trough principal component analysis and Bayesian clustering methods.

After filtering, 31,320,380 high-quality SNPs were obtained, of which 30,707,281 were located within intergenic (65.2%), intronic (33.4%) and exonic (0.7%) regions. Additionally, 120,172 (57.2%) and 80,882 (38.5%) SNPs found in coding regions were associated to synonymous and nonsynonymous effects, respectively. Population structure analysis separated these breeds in two clusters: one comprising Campaniça and Serra da Estrela together with transboundary dairy breeds (e.g. Leccese and Lacaune); and another of the well-differentiated multi-purpose Portuguese Merino sheep. Admixture analysis revealed a wide distribution of parental lineages and corroborated the PCA clustering results.

The results derived from this study will be useful to develop several genomic tools for these breeds, including genome-wide association studies, genetic diversity and traceability schemes.

Keywords: Native sheep, Whole-Genome Resequencing; Genetic diversity; Population structure

Acknowledgements: This work was co-financed by Program Alentejo 2020, through the European Fund for Regional Development under the scope “Gen-Res-Alentejo – Use of genomics methodologies to assist selection of sheep resistant to footroot and gastrointestinal nematodes in the Alentejo region” (ALT20-03-0145-FEDER-000037). The authors also acknowledge FCT for the contract grant 2020.02754.CEECIN (CG), for the PhD fellowship SFRH/BD/140168/2018 (DG), and for UIDB/05183/2020 (AU).

Poster 17

Myostatin gene mutation occurrence and expression in Portuguese "Preta" cattle

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In domestic production species there are high risks of loss of local genetic heritage. Meanwhile, sustainability requires valorization of local resources and national products. In the indigenous Preta cattle breed, at risk of extinction, the identification of animals with muscular hypertrophy related to the presence of genetic mutation nt821 in the myostatin gene or GDF8, at the level of chromosome II, may stimulate its conservation. In breeds studied, higher carcass yield and better organoleptic characteristics of the meat have been proven in the presence of the mutation, but also fertility problems are involved. This work aims to identify benefits of selecting for mutation in the Preta breed. The new generation of sequencing technologies allows accelerating the genetic characterization of local animal resources, a key condition for their preservation, improvement and valorization (Ramos, M., meeting SPREGA, 2012). In the first phase, a statistical treatment of the existing growth and fertility data was performed with GenPro program data. Animals from farms involved, genotyped for the mutation with phenotype data collected, were evaluated. We found significant differences between genotypes, wild type (+/+), heterozygous (mh/+) and homozygous (mh/mh) animals. Carcass yield is higher in the mh/mh genotype and does not differ between mh/+ and +/+ animals ($P<0.01$). The age at first calving differs between the three genotypes being the highest in the mh/mh animals and lowest in the +/+ ($P<0.01$). The calving interval is higher in the mh/mh genotype at the mean value close to 80 days and does not differ between the mh/+ and +/+ genotypes ($P<0.01$). We collected and analyzed meat samples from about 60 animals of the three genotypes. There are interim results for physical characteristics, color and instrumental texture. We are continuing analysis of the missing samples. Centesimal chemistry and sensory analyses are in process. In the next phase of the investigation, we intend to use genotyping techniques and gene expression study to identify the genes involved in the expression of hypertrophy, by identification of SNP's. The techniques used will be GWAS, signature selection, qPCR (real-time PCR), microRNA, RNA sequencing.

Poster 18

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

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Potable water is a scarce resource in several regions of the world. Water is essential for the life of animals, as it intervenes in several metabolic processes. Inadequate water supply could reduce animal health and performance. In dairy farms, the use of quality water is essential to maximize the animals milk production.
Farms use large amounts of water in their daily activities, which is why efficient use is increasingly important.
The objective of this work is to verify (1) the importance of water quality in intensive dairy farms and if (2) water quality affects the production, reproduction and health of animals.
Therefore, the project is organized into two parts:
1st Part: analyze, by searching, whether or not in intensive dairy farms in mainland Portugal:
(1) The water (as a natural and scarce resource) and its quality is a daily concern.
(2) Wich water capture methods are used.
(3) If there are water consumption monitoring systems (water efficiency).
(4) If there are water quality analyzes (if animals have access to drinkable water).
(5) Use water treatment systems to get potable water.
(6) If there are problems in the farm associated with the consumption of water (not drinkable water).

2nd part - Case study in dairy farm in Alentejo.
(1) If there is a preference of animals for a type of water - treated water or untreated water.
(2) If water quality has an impact:
(a) In food and how it occurs.
(b) In milk production and how it occurs.
(c) In reproduction and how it occurs.
(d) On the health of animals.
(3) What is the economic impact of water quality on a dairy farm.

4. Agricultural Sciences and Food Sciences

Oral Communication 11

The use of a chromatographic-based approach to reveal the yeast modulation potential on the chemical profile of Arinto white wines

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The importance of yeasts in aroma production during wine fermentation is a significant concern for obtaining a wine that appraises a broad number of consumers. For wine producers, wine aroma modulation is an essential issue where the yeasts used during the winemaking process represents a feasible way to improve the complexity and enhance wines specific characteristics. During the fermentation process of wines, yeasts convert grapes sugars into alcohol, carbon dioxide and many secondary metabolites, depending on yeast metabolism, affecting the wine composition, namely its aroma and amino acids (AAs) composition. So, the present work aims to study the effect of different Saccharomyces-type yeasts on the AAs composition and volatile profile of Arinto white wines. To pursue this goal, four white wines from Arinto grapes were fermented with three different commercial yeasts (*Saccharomyces bayanus* EC1118, *Saccharomyces cerevisiae* CY3079, *Saccharomyces bayanus* QA23) and one Native yeast. Arinto wines AAs composition was quantified by HPLC-DAD, after a derivatization step to obtain the aminoenone derivatives. The volatile content of Arinto wines was determined by GC/MS, after an HS-SPME extraction. Results showed significant differences among the AAs content and volatile profile in the Arinto wines. The higher AAs content was found in the Arinto wines fermented with the CY3079 yeast (470.74 mg/L), and the lowest content of AAs in the Arinto wines fermented with EC1118 yeast (343.06 mg/L). Native yeast results in wines with a volatile profile richer in esters compared to the other sample wines. Principal component analysis (PCA) obtained with combined data of AAs and volatile compounds, after normalization, for each Arinto wine samples, shows a clear separation of wines fermented with Native and CY3079 yeasts in relation to QA23 and EC1118 fermented wines. The first and second principal components are responsible for 44.40% and 32.20%, respectively, of the system's variance, which clearly showed a differentiation among wines.

This work was funded by National Funds through FCT and LAQV which is financed by national funds from FCT/MCTES [UID/QUI/ 50 0 06/2020]. This work has received also funding from the European Union's Horizon 2020 research and innovation program un- der the Marie Skłodowska-Curie grant agreement No. 778045. This research was also anchored by the RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology. The authors want to acknowledge FitaPreta for providing the wines and want to acknowledge Zeyton Nutraceuticals for funding the fellowship of the first author.

Oral Communication 12

Suitability of *Citrus* × *sinensis* and *Citrus* × *limon* as hosts of *Trioza erytreae*

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The african citrus psyllid *Trioza erytreae* (Del Guercio) has entered Europe and is present in Portugal and Spain, with recent sightings in the Algarve region, which is Portugal's main citrus producing region. This psyllid develops on young leaves, creating a gall in each point where a nymph grows, thereby affecting the photosynthesis of the infested tree. The main concern with the presence of *T. erytreae* is the high risk of introduction and rapid dissemination of huanglongbing (HLB), a serious bacterial disease not yet detected in Europe, which has this psyllid as a vector. *T. erytreae* has been described to have distinct preferences for different citrus hosts. The aim of this work was to understand how the development and survival of nymphs is affected when they are hosted by a lemon or a sweet orange tree. In the present study 8 *Citrus* × *limon* (L.) Burm. f. 'Eureka' and 8 *Citrus* × *sinensis* (L.) Osbeck 'Valencia midknight seedless' were used as biological replicates. Plants were infested with 10 adult *T. erytreae* (5 females and 5 males) and maintained under controlled temperature, humidity, and light conditions. Plants were monitored continuously until the emergence of the first new adults, registering nymph instars development and infestation intensity. Then the number of developed nymphs was recorded. A significant difference was found in the number of nymphs that attained the fourth and fifth instar, when comparing host species, with best results for *C.* × *limon*, with an average number of 301 nymphs per lemon tree and of 99 nymphs per orange tree. This work, performed in controlled conditions, demonstrates a better suitability of *C.* × *limon* (L.) Burm. f. 'Eureka' as a host for *T. erytreae* when compared with *C.* × *sinensis* (L.) Osbeck 'Valencia midknight seedless', in line with previous field studies. This information is very useful for the management of this pest, helping identify critical points of spread within a large area and identify potential trap crops to influence the movement of *T. erytreae* out of commercial orchards.

This work was funded by National Funds through Fundação para a Ciência e Tecnologia (FCT) under the PhD research grant 2020.07798.BD to T. Magalhães and by the European Union's Horizon 2020 research and innovation program under the grant agreement No 817526 (H2020-SFS-2018-2) PRE-HLB - "Preventing HLB epidemics for ensuring citrus survival in Europe"

Oral Communication 13

Response of Mediterranean aromatic plant species (Lamiaceae) to contrasting environmental conditions

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Climate change is affecting every region across the world, being the Mediterranean one of the most potentially affected. In this context, resisting environmental adverse conditions is a big threat faced by plants. The main goal of this project is to provide new insights into the response of Mediterranean aromatic plants from the Lamiaceae family to environmental alterations (e.g. drought, temperature, nutrient deficiency) with particular emphasis on the production of plant secondary metabolites and associated bioactivity. One of the aims of this project is to use green extraction procedures, so the effectiveness of ten natural deep eutectic solvents on the extraction of phenolic compounds from *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco was evaluated. Proline: lactic acid (1:1) mixture showed good results and it was selected to assess the composition and bioactivity of plant extracts in subsequent studies. *Thymus lotocephalus* G. López and R. Morales in vitro grown cultures were subjected to drought stress induced by 2, 5 and 7% polyethylene glycol (PEG). Drought triggered oxidative stress based on PEG concentration-dependent manner, increasing the levels of H_2O_2 and decreasing shoots growth, photosynthetic pigments, and secondary metabolites accumulation. The effect of temperature (15, 20, 25 and 30°C) was evaluated on micropropagated cultures and plants of *Lavandula viridis* L'Hér and *T. lotocephalus*. The temperature 30°C caused the greatest accumulation of phenolic compounds and their associated antioxidant activity in both species of the micropropagated plants. The access limitation to N, P, Ca, Mg and Fe was investigated in *L. viridis* in vitro cultures and it was observed that cultures subjected to P and Mg deficiencies showed an increased amount of phenolics without causing cultures oxidative stress. These studies will contribute to a better understanding of the impact of adverse environmental conditions on morphological, physiological, biochemical, and biological traits of two Lamiaceae species from the Mediterranean region.

This research was funded by the project INTERREG–MD.NET: When Brand Meets People and by National Funds through FCT—Foundation for Science and Technology under the Project UIDB/05183/2020. Inês Mansinhos (Grant SFRH/BD/145243/2019) and Sandra Gonçalves (under the Norma Transitória–DL 57/2016/CP1361/CT0022) are funded by national funds through FCT.

Oral Communication 14

Evaluation of the Potential of *Opuntia Ficus-Indica* Cladodes as a Natural Flocculant for Wastewater Treatment

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The *Opuntia ficus-indica* (L.) Mill cactus, known as prickly pear cactus, is a xerophyte plant widespread worldwide, growing in arid and semiarid zones. Considering the rising concern for the declining water resources and climate changes, this culture has been gaining relevance as an effective food production opportunity in Mediterranean areas. In addition to its use as food, the composition of the cladodes and its mucilage enables it to act as a natural flocculant. Cladodes have the ability to flocculate the suspended particles in water, so they can potentially reduce the use of the toxic aluminum sulfate in wastewater treatment. Nowadays there is a search for new alternatives to replace the harsh and expensive chemical methods of wastewater treatments because the common methods include the usage of chemicals that negatively affect the environment and human health. These cladodes have a great potential for use in innovative, cheap, renewable, and eco-friendly water treatment due to their flocculant properties. The objective of this study was to seek the most effective and simple methods for using prickly pear cladodes, or their mucilage, to treat wastewater. Therefore, the cladodes were prepared in different manners, such as fresh cladodes, lyophilized cladodes, and mucilage obtained through two different extraction methods. These prepared materials were added in different quantities to a solution of water with clay. For one hour, the turbidity was measured with a turbidimeter Canadawide Scientific Model 800, the results are presented in Nephelometric Turbidity Unit (NTU). As a complementary observation, photographs were taken with a camera LEICA EC3 and a LEICA MZ12.5 magnifying glass that provides a three-dimensional view of the sample with magnifications of 8x-100x. There was an important decrease in the turbidity in all cases, very noticeable when compared to the control. It was concluded that the use of 60 g of small pieces of fresh cladodes was the method with the best results, reaching the very low value of 4 NTU. This study confirmed the ability of *O. ficus-indica* (L.) Mill cladodes to promote the flocculation of clay in water, allowing the transformation of unused waste material into a useful raw material.

Oral Communication 15

Grapevine responses to trunk pathogens: potential genes involved in plant-pathogen interactions

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Grapevine trunk diseases (GTDs) have increased considerably in the last two decades and are currently considered the most spread and destructive diseases that affect grapevines, influencing the productivity and longevity of vineyards in all the major growing regions of the world. The simultaneous presence of multiple trunk pathogens in a single plant, together with the inconsistent expression of symptoms, their isolation in asymptomatic plants, and the absence of effective treatments, make these diseases extremely complex to early detect and eradicate, being currently one of the most relevant challenges for the sustainability of viticulture. Aiming to search for sustainable alternatives to limit their development, the present study intended to investigate plant molecular responses against GTDs following a real-time qPCR approach on a set of candidate genes, and associate this data with phenotypical differences. Samples were collected from GTDs asymptomatic and symptomatic plants of two cultivars with different levels of susceptibility to GTDs, 'Alicante Bouschet' and 'Trincadeira'. Target genes included defence-related genes, genes involved in sugar transport or metabolism, genes previously identified in grapevine response to pathogens, and other differentially expressed genes (DEGs), selected in a previous transcriptome analysis, totalling 18 genes. Several genes were found to be significantly overexpressed (p<0.05) in symptomatic plants namely *GIN2*, *TLP3*, *TLP8* and *PR1*. On the other hand, the genes *MAPKKK17* and *PER42*, already recognized as involved in responses to biotic stresses, were overexpressed in the asymptomatic ones. Similarly, *STS1*, *cwINV* and *HT5* were significantly overexpressed in the less susceptible cultivar, 'Trincadeira', and *PR3* was the only one overexpressed in the most susceptible cultivar, 'Alicante Bouschet'. Altogether, these results can help to explain the phenotypical differences that occur between plants and shed light into the underlying mechanisms of resistance and susceptibility of grapevines against these pathogens. However, further research is required to gain better knowledge of these diseases and their expression in order to limit their propagation and contribute to the development of effective protective methods, through the activation or inhibition of potential plant responses regulators.

This work is funded by Portuguese National Funds through FCT/MCTES under the PhD scholarship SFRH/BD/145321/2019, attributed to Mariana Patanita, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo, and under the project UIDB/05183/2020 (MED exploratory project ID 04_2019). It is also supported by the project *"Control of olive anthracnose through gene silencing and gene expression using a plant virus vector"* (ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017) and by the project *"Development of a new virus-based vector to control TSWV in tomato plants"* (ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017), both projects co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through the FCT, in its national component.

Oral Communication 16

Influence of Fe availability in the composition of root's grass exudates

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Higher plants develop physiological and morphological mechanisms as a response to iron (Fe) deficiency, which is frequently in calcareous soils. Monocotyledons (gramineous plants) utilize acomplexation strategy (Strategy II) for iron acquisition from the soil. This strategy is characterized by the secretion of several compounds such as organic acids and phytosiderophores (PSs) into the rhizosphere to increase metal bioavailability. The aim of this work was to identify the compounds in the root exudate of different grasses plants grown under Fe different levels. Seeds of *Poa pratensis, Lolium perenne* and *Festuca rubra* were germinated for 15 days in a glasshouse under natural photoperiod conditions and air temperature ≤ 30ºC. After germination, the seedlings were precultured in half-strength Hoagland's nutrient solution with adequate Fe supply (10 µM Fe (III) applied as FeDDHMA) for 2 weeks before starting the experiment. Gramineous seedlings with 5 cm tall were transferred to 12 liters containers with complete Hoagland's nutrient solution. Three treatments were imposed: Fe0 (0 µM Fe), Fe 1(1 µM Fe) and Fe10 (10 µM Fe). After the appearance of chlorosis symptoms, compounds were identified in root exudates by ESI- electrospray ionization mass spectrometry (ESI-MS). Considering the three species under study, some common compounds were found only under Fe deficiency. This work discusses the role of these compounds in Fe metabolism.

This work was funded by FCT. T. S. are thankful to for the grant SFRH/BD/144764/2019.

Oral Communication 17

Effect of dolomitic lime application and grazing type on growth, protein value and floristic composition of natural pastures under Montado

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Montado is a multifunctional agro-forestry-pastoral ecosystem characteristic of the Mediterranean region. The objective of this work was to evaluate the effect of dolomitic lime application and grazing type (continuous *vs* deferred) by sheep on the growth, protein value and floristic composition of natural pastures in Montado. The study was carried out in Mitra's farm at the University of Évora – between November 19, 2019, and May 31, 2020. One area with 4ha was divided into four sub-plots with 1ha each, subject to the following treatments: plot 1 - without dolomitic limestone application and continuous grazing (7 sheep/ha) (P1UC); plot 2- without dolomitic limestone application and deferred grazing (16 ewes/ha) (P2UD); plot 3- application of dolomitic limestone and deferred grazing (P3TD); plot 4- application of dolomitic limestone and continuous grazing (P4TC). The grazing days were counted in each plot for deferred grazing (P2UD and P3TD), defined as a function of the average height of the pasture (> 2.5cm). Pasture heights were evaluated on November 19, February 27 and May 26, at 12 locations per plot, representing previously identified plant communities. Composite samples of pasture were collected per plot to determine the quality and estimated yield on these dates. On May 26, the characterization of the floristic composition of the pasture in the four plots was carried out, identifying the predominant species. The vegetative vigour of the pasture was monitored by the time series of the NDVI (Sentinel-2). The average heights of the pasture were higher in the P4TC plot. The estimated total productivity was higher in P3TD, followed by P4TC, P1UC and P2UD. The number of dominant grass species was 15, 8, 12 and 15, in plots 1, 2, 3 and 4, respectively. The legumes were 12, 8, 8 and 5, in plots 1, 2, 3 and 4, respectively, and the composites were 12, 8, 11 and 7, in plots 1, 2, 3 and 4, respectively. The results suggest that the application of dolomitic lime, combined with deferred grazing, was the best strategy to improve pasture biomass production and maintain similar score condition in sheep.

Keywords: continuous grazing; deferred grazing; natural pastures; biotic pressure; floristic composition.

Oral Communication 18

Antimicrobial Photodynamic Therapy as an innovative approach for the inactivation of pathogenic bacteria on fish fillet

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Over the years, farmed fish consumption has increased, being an important worldwide source of protein and essential nutrients. However, the deterioration of fish products caused by the proliferation of microorganisms can cause fatal foodborne diseases in human. Few antimicrobial treatments are available to control these infections and their application can even promote the development of resistant strains, demanding for new strategies to decontaminate fishery products. Following this notion, the aim of this study was to investigate the efficiency of antimicrobial Photodynamic Therapy (aPDT) in the photoinactivation of pathogenic bacteria in fish fillet. To this purpose, studies on ***Escherichia coli*** and ***Vibrio parahaemolyticus*** were conducted considering the high pathogenicity of these bacteria to humans and their often transmission through contaminated fish. This study was performed in the presence of two photosensitizers (PSs), **5,10,15,20-tetrakis(1-methylpyridinium-4yl)porphyrin tetra-iodide** (**Tetra-Py(+)-Me**) and **Methylene Blue** (**MB**), under irradiation with white light (100 $mW.cm^{-2}$). The efficiency of the developed protocol was also evaluated in the photoinactivation of the microorganisms naturally present on fish fillet. *In vitro*, both PSs (0.5 μM) were efficient to reduce ***E. coli*** and ***V. parahaemolyticus*** viability until the detection limit of the method (~8 log of $CFU.mL^{-1}$) after 30-45 min of treatment. In fish fillet, **Tetra-Py(+)-Me** and **MB** (50 μM) promoted a similar photoinactivation profile of ***E. coli*** (ca. 2. log of $CFU.mL^{-1}$ after 60 min of treatment), while for ***V. parahaemolyticus*** a more pronounced inactivation was achieved with **MB** (> 3 log of $CFU.mL^{-1}$) than with the porphyrinic compound (ca. 2 log of $CFU.mL^{-1}$). Nevertheless, the photodynamic treatments were also efficient in the photoinactivation of the fish natural microorganisms. In this case, a bacterial reduction of 2.6 and 3.5 log of $CFU.mL^{-1}$ was achieved with **Tetra-Py(+)-Me** and **MB** (50 μM), respectively. These results reveal that the developed protocol was not only efficient to inactivate ***E. coli*** and ***V. parahaemolyticus*** in fish fillet, but also in the inactivation of the fish natural microorganisms. In summary, the results suggest that aPDT can be a promising approach to decontaminate fish fillet and provide safe food to the consumer.

Thanks are due to the University of Aveiro and FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), LAQV-REQUIMTE (UIDB/50006/2020) and CICECO-Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/50011/2020), to the FCT project PREVINE (FCT-PTDC/ASP-PES/29576/2017), through national funds and when applicable co-financed by the FEDER, within the PT2020 Partnership Agreement and "Compete" 2020. Thanks are also due to the Portuguese NMR and Mass Networks. SANAQUA project MAR02.05.01-FEAMP-0004 and I9+Proalga project MAR2020-16-01-03-FMP-0011. C.V., S.P., M.Q.M. and M.B thank FCT for the PhD grants SFRH/BD/150358/2019, SFRH/BD/132584/2017, SFRH/BD/112517/2015 and SFRH/BD/121645/2016, respectively.

Oral Communication 19

Cynara cardunculus leaves extract fractioning and phytotoxic evaluation

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In the face of synthetic herbicides negative effect in the environment and in human health, the search for an ecological alternative turned the attention to natural compounds and the allelopathy phenomenon[1], [2]. From a vast list of natural compounds with bioherbicidal potential, Sesquiterpene Lactones (SL) – Cynaropicrin (Cyn), Aguerin B and Grosheimin – are the main compounds present in *Cynara cardunculus* leaves extract (CCLE) [3]. Plant extracts fractionation and/or isolation of target compounds, is typically done using solvents with diverse selectivity and affinity to the desire compounds, using chromatographic processes and traditional operations such as distillation. In recent years, nanofiltration has been successfully applied for fractionation and concentration of different compounds [4].

The aim of the present study was the CCLE fractionation by membrane technology and subsequent SL enriched fractions phytotoxicity evaluation by Coleoptile Bioassay [5]. CCLE fractionation experiment was performed using GK membrane (MWCO-2000Da) on dia-ultrafiltration mode, at a transmembrane pressure of 16bar, till a 95% (m/m) SL removal. A total of eight permeate fractions were obtained, highlighting the first four permeate fractions (F1-F4) that showed, approximately, an increase of 50% of Cyn content when compared with CCLE (23% of Cyn). In terms of phytotoxic activity determined by Coleoptile Bioassay, only one of the fractions (F2) stood out from other fractions where IC_{50} observed (126 ppm) was better than CCLE (140 ppm). Results obtained confirms the applicability of membrane technology to produce SL-rich fractions with relevant phytotoxic activity. It is of our interest to evaluate phytotoxic effect in growth plants not only to determine the effect in morphology and physiology resultant as well as develop an effective formulation for further application.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors also acknowledge FCT for PhD grant to D. Rosa (SFRH/BD/143845/2019) and A. Paulino (SFRH/BD/145383/2019), Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED) and Project PID2020-115747RB-I00 / AEI / 10.13039/501100011033 to Agencia Estatal de Investigación - Ministerio de Ciencia e Innovacion

Oral Communication 20

Phages for *Escherichia coli* and *Salmonella Typhimurium* inactivation in milk

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Nowadays, food deterioration and foodborne illnesses are serious health problems worldwide, due to bacterial resistance to antibiotics. The persistent use of antibiotics, including in animal production, significantly increases the appearance of multiresistant bacterial strains, resistant to commonly used antibiotics. These bacteria are responsible for food deterioration and more important for foodborne illnesses. Consequently, it is crucial to develop new antibacterial alternative approaches to antibiotics that avoid food contamination and improve its security. Phages, viruses that only infect bacteria, have proved to be an effective strategy to inactivate bacteria, and producing fewer resistant mutants than the conventional antibiotics. Therefore, the main goal of this study was to inactivate important foodborne pathogens present in milk by using phage therapy. For this, milk was artificially contaminated with *Escherichia coli* or *Salmonella enterica* serovar *Typhimurium* and treated with the phages phT4A or phSE-5, respectively. Phage therapy for these two bacteria were first performed in Tryptic Soy Broth (TSB, *in vitro*). The TSB results showed that phage phT4A was effective to inactivate *E. coli* with about 6 log colony-forming unit per millilitre (CFU/mL) of bacterial inactivation. Phage phSE-5 was equally effective to inactivate *S. Typhimurium* with about 3 log CFU/mL of bacterial inactivation. In milk, a more complex matrix, a maximum inactivation of about 4 log CFU/mL was obtained for *E. coli* and *S. Typhimurium* inactivation in the presence of the phages phT4A and phSE-5. Although more assays with different pathogenic bacterial strains in food must be done, phage treatment seems to be a promising approach for the inactivation of food pathogens. It is expected that the application of this eco-friendly procedure for foodborne pathogens inactivation will prevent infectious diseases that may arise from eating contaminated food and will improve food safety.

The authors acknowledge the Portuguese Foundation for Science and Technology for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020) and to CICECO (UIDB/50011/2020+UIDP/50011/2020). Márcia Braz thanks FCT for the PhD grant (2020.06571.BD). Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Thanks are also due to Biology Department and University of Aveiro, where this research work was performed.

Oral Communication 21

The taste of music: Taste and emotional associations in a set of musical stimuli

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Eating is a multisensory experience. While the smell, visual appeal, and textural properties of foods and drinks are long acknowledged determinants of preference and choice, less is known about the role played by audition. Previous research suggests that individuals associate auditory attributes and basic tastes in a reliable way (e.g., sweet tastes and high-pitched sounds) and that auditory stimuli may influence how participants 'perceive' basic tastes.

In this study we tested the basic taste associations and emotional connotations of 100 musical stimuli varying in mood and genre. A sample of 163 Portuguese participants rated basic taste associations (sweet, bitter, sour salty) and subjective dimensions of familiarity, discrete emotions (joy, anger, sadness, fear, surprise), valence, and arousal of a subset of musical stimuli (each participant evaluated a set of 25 stimuli selected randomly). Additionally, participants completed self-report measures of mood and taste preferences, as well as the Goldsmiths Musical Sophistication Index (Gold-MSI).

The results of this study support the ability of music to communicate basic taste attributes. Several associations were found between basic tastes and affective dimensions, namely, between pleasant emotions (e.g., joy) and sweetness ratings and between unpleasant emotions (e.g., fear) and bitterness and sourness ratings. Sex and individual differences in musical sophistication and preference for specific basic tastes were generally unrelated to subjective ratings of the stimuli.

Overall, these findings support the adequacy of this set of musical stimuli to elicit different taste and emotion associations. This database could be a valuable tool for future research in the field of multisensory food perception, as well as for supporting evidence-based interventions for the promotion of healthier and more enjoyable food choices.

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

Poster 19

Postharvest conservation of the Autumn Crisp seedless grape variety using different methods

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Autumn Crisp is a seedless white grape variety with a late harvest, well known for its greenish-yellow skin, the larger sized berries and a characteristic very firm and crunchy pulp. For consumers, the variety is described as sweet with a juicy texture and a gentle Muscat aroma.

There was performed a conservation test with a length of approximately 80 days with two different modalities, one in a modified atmosphere packaging (MAP) and other with a sodium metabisulfite slow releasing mat (Sulfite modality) and a control without any packaging.

All the modalities (including control) were kept at 2ºC and 90% RH. Early harvest grapes with total soluble solids between 17 and 19 brix were studied. Every 15 days, samples were taken to proceed to post harvest tests, such as weight loss, color, texture, total soluble solids, titratable acidity, phenolic compounds, and antioxidant activity.

According to the results, the color at the 63rd day of conservation in the Sulfite modality lost its market viability. On the other hand, the control modality and MAP had their texture affected, losing their firmness at the 80th day, with a progressive decrease on texture.

During the study, the nutritional value of samples has changed, with significant differences from the 35th day of conservation. The total phenolic content and the antioxidant activity decreased progressively until the end of the experiment (possibly due to the loss of water), as well as in the total soluble solids and texture.

We can consider that 35 days is the top limit to preserve this grape variety according to the results.

This work was financed through the project “Investigação e desenvolvimento de novas tecnologias de conservação pós-colheita de uva de mesa sem grainha e na Promoção e divulgação dos resultados de I&D”, UID / AGR / 00115/2013 em colaboração com “Vale da Rosa Sociedade Agrícola Lda ”

Poster 20

Management of fungal diseases in almond trees - Biocontrol and genetic tolerance studies

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In Portugal, almonds are a traditional culture of Algarve, Douro and Trás-os-Montes. Recently, due to water availability and possibility of mechanical use, new orchards have been introduced in Alentejo and Beira Interior regions without previous studies of their adaptability to the regional edaphoclimatic conditions. The climate and the microbiota of these regions can influence the health of almond trees, productivity and storage time of almond kernels. Fungal pathogens are responsible for decline of productivity and consequently significant economic losses essentially by production of mycotoxins and formation of branches and trunk canker diseases. The economic losses caused by these pathogens encouraged new studies on effective actions that rely on cultural control, using resistant cultivars and/or biological control by antagonistic organisms. At the moment only few studies of molecular/genetic mechanisms associated with to fungus disease resistance in *Prunus* have been described [1,2,3] and only one study was performed in almond trees to evaluate the use of antagonistic endophytic and rhizosphere communities as biocontrol agents [4]. Using a combined strategy with the identification of plant resistance genes and endophytic fungi with antagonistic activities in crop protection, this project will contribute to understand and manage the fungus diseases in almond trees. Therefore, this work aims (1) to identify and distinguish pathogenic and endophytic fungi from fungal communities of commercial almond plantations, established in Alentejo; (2) to identify antagonistic activity of endophytic fungi and secondary metabolites active against almond tree diseases by inoculation assays, to assess the pathogenicity of almond-associated fungi; (3) to determine the susceptibility/tolerance of commercial/Portuguese varieties to the main pathogenic agents, by pathogenicity tests of plant-associated fungi; (4) to identify genes associated with the susceptibility/tolerance to fungus infection, using transcriptomic and virus-induced gene silencing (VIGS) approaches. The knowledge obtained with this work will allow the design of new strategies to future sustainable disease management and precision plant breeding.

This work was funded by Inov-Amendo-AL: Microenxertia *in vitro* de amendoeiras selecionadas para a promoção do amendoal no Alentejo (ALT20-03-0246-FEDER-000068) supported by Program Alentejo 2020, through the European Fund for Regional Development (ERDF), within the scope of the Collective Action Support System - Transfer of scientific and technological knowledge - Domain of Competitiveness and Internationalization. Authors also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), FCT for UIDB/05183/2020 and UIDB/04551/2020 (UIDP/04551/2020) projects.

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Poster 21

The use of X-Ray Micro Computed Tomography in cork porosity analysis

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Porosity is an important characteristic present in cork from *Quercus suber* L. which is responsible for the type of cork products developed. It is also related with other characteristics, such as density and cork thickness. The volume of lenticular channels defines porosity, which borders have thicker cell walls that showing higher density. To analyse the inner porosity of cork tissue the X-Ray micro computed tomography technology was applied. The principles are based on an attenuated X-ray beam that passes through the sample and allowed a 2-D and 3-D object reconstruction by a set of cork cross-sections and using mathematic algorithms. Individual pixels in each cross-section are the result of the attenuation coefficient distribution. The inner porosity and lenticular channels may be quantified. The lenticular channels are different according to the direction – radial or tangential. This is a nondestructive method without cell damage.

The objective of this analysis was to quantify and visualize the inner porosity by means of µCT technology, demonstrating the importance of this method to some specific cork samples analysis.

This technology was used in research submitted for publication, belonging to the author' PhD work.

Poster 22

Effect of manual and free pollinations on the fruit size of pitaya fruits

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The pitaya is one of the exotic fruit species whose demand in European markets has increased. This demand is justified by its numerous health benefits.

In Europe, it is the countries of the Mediterranean basin that have favorable soil and climatic conditions for its cultivation. With the aim of diversifying the fruit growing in the Algarve, the particularities of this culture under these conditions have been investigated.

The pollination of flowers seems to be the most relevant aspect regarding fruit production: each flower opens only once and at night, and its fertilization is dependent on nocturnal pollinating agents (scarce presence in the region), or on the activity of bees in the early morning hours. Floral morphology can also be compromising, as the male organs are positioned much lower than the female organs.

Bees, being so small compared to the flower, do not seem to be effective in depositing pollen in the stigma. Even so, when present in large numbers, the probability of brushing the pollen-covered body in the stigma increases. However, the amount of pollen deposited by this route can be much lower than the pollen inserted into the stigma when manual pollination is used, which could be reflected in the size of the fruit. Furthermore, *H. undatus*, the most common species at the commercial level, has a self-incompatibility mechanism, and fertilization only occurs if there is cross-pollination. So, when it comes to commercial productions, dependence on bees can be a risky alternative, so manual pollination is a fundamental practice, not only to ensure that all flowers are effectively pollinated but also to obtain superior quality fruit.

In field trials carried out this year, the two modalities of pollination were tested: free, by the action of bees; and manual, by collecting pollen from all flowers opened that day, and inserting it into the stigma. The effect of the two types of pollination on the weight and on the equatorial and longitudinal diameters of the fruits was determined.

The Fruta Dragão Operational Group is co-financed by the Rural Development Program PDR2020. Grant number PDR2020-101-FEADER-031201: Fruta Dragão: Validar a capacidade produtiva da pitaia vermelha.

Poster 23

Auxiliary arthropods in insect hotels in citrus orchards

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In plant protection systems, one of the most important things, especially if we want to be more ecological and sustainable, is to promote the increase of populations of auxiliary organisms, such as insects, arachnids or microorganisms that naturally control pests and diseases. One of the many ways to do this is to install the so-called "insect hotels" in orchards. These are structures, more or less complex, placed inside or on the field borders that provide protection and resources to certain auxiliary arthropods. These structures are usually made of wood and filled with a variety of materials such as small branches, perforated wooden blocks, pinecones, reeds, wood chips or straw. Depending on the materials the insect hotels are occupied by different organisms such as arachnids, wasps, earwigs, hoverflies, lacewings and ladybugs.

To better study the presence of auxiliary organisms and their material preferences in insect hotels, a trial was installed in a mandarin orchard in Ferreira do Alentejo. For the trial, 9 insect hotels were built using 30cm diameter PVC tubes cut into 15cm sections. These were filled with two types of materials: branches and reeds of various diameters. Thus, 3 modalities of 3 repetitions each was made: hotels with reeds only (R); hotels with branches only(B); and mixed hotels(M), with 50% of its area with reeds and 50% with branches. These hotels were randomly implanted inside the orchard and stuck on branches inside the trees in November 2020. One year after they were removed, disassembled and occupation was analysed.

As preliminary results, are present the main strategies of occupation of these hotels by auxiliary arthropods. Concerning the reeds, it will report the several types of materials used by wasps to colonise the reeds. Furthermore, we will try to establish a relation between reed diameter and its occupation. Regarding the branch hotels (B) it will be described the main arthropods groups that colonized this material.

This work was funded by European Union through the project Life Vida for citrus LIFE18 CCA-ES-001109.

Poster 24

Involvement of small RNAs in response to heat stress in mycorrhizal grapevines

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MicroRNAs (miRNAs) are non-translated RNA 21-24 nucleotide molecules that down-regulate their target genes by mRNA cleavage or transcription inhibition. They play crucial roles in plant developmental processes, in stress responses and physiological response/adaptation to the environment, as well as in regulating interactions with beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF). To determine if inoculation with different AMF species modulated miRNA expression in grapevine under heat stress (HS), an experiment using the Touriga Nacional variety was conducted. Grapevines inoculated with *Rhizoglomus irregulare* (Ri) or *Funneliformis mosseae* (Fm) were exposed to HS for 40 °C during 4h per day for a one-week period. Illumina miRNA-seq was conducted in leaves of mycorrhizal and non-mycorrhizal plants, subjected or not to HS, and 198 differentially expressed conserved miRNAs were found. GLM analysis using mycorrhization as primary experimental factor and temperature as secondary factor found 27 differentially expressed miRNAs. The miRNAs that were mostly expressed in mycorrhizal plants (Ri + Fm) under HS included miR393a/b, miR166a-3p, miR167a/l, miR3630-3p and miR156a, whereas miRNAs up-regulated in non-mycorrhizal plants included miR399a/b and miR156e. Analysis by AMF species showed that in Ri inoculated plants, miRNAs belonging to the miR156/miR529/miR535 superfamily, known to be involved in plant stress responses, were up-regulated by HS. However, while miR3640 was down-regulated by HS in the Fm treatment, in Ri others were affected, such as miR3632, miR397a, miR3627-3p and miR398. Importantly, several target genes were predicted to be regulated by the differentially expressed miRNAs. Gene Ontology (GO) analysis revealed differences between targets of miRNAs up- or down-regulated by AMF/HS. Targets of up-regulated miRNAs presented GO terms such as response to endogenous stimulus, cell communication, response to stress, transmembrane transport, or signal transduction, whereas targets of down-regulated miRNAs included only metabolic processes' terms. Furthermore, 46 putative novel miRNAs were *in silico* predicted, of which 13 were found to be differentially regulated by AMF/HS. Taken together, our findings provide new knowledge on miRNA regulation in mycorrhizal grapevines under HS, which serve as a useful resource for further analysis of the interactions between miRNAs and target genes in crops responding to abiotic stresses due to climate change.

This work was funded by National Funds through FCT under the Project through the project MYCOVITIS (PTDC/AGR-PRO/0676/2014), the project Linking Landscape, Environment, Agriculture and Food Research Centre Ref. UIDB/04129/2020 and the contract CEECIND/01769/2017.

Poster 25

Antimicrobial Photodynamic Therapy as a controlling approach of *Pseudomonas syringae* pv. *actinidiae* transmission by kiwifruit pollen

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Pseudomonas syringae pv. *actinidiae* (Psa) is a relevant phytopathogenic bacterium responsible for the bacterial canker in kiwifruit plants. Pollen is proved to be responsible for the dissemination of Psa among orchards. This phytopathology is highly affecting kiwifruit production worldwide and the available treatments for this problem consist in spraying the orchards with copper derivatives and antibiotics. However, these approaches are highly toxic to the environment and can lead to the development of bacterial resistance.

Antimicrobial Photodynamic Therapy (aPDT), consisting in the use of a photosensitizer (PS) molecule that absorbs light in the presence of oxygen and promotes the formation of highly reactive oxygen species (ROS) causing irreversible damages on various cellular components, may be an effective alternative in the inactivation of Psa.

The aim of this study was to evaluate the effectiveness of aPDT in the inactivation of Psa in kiwifruit pollen, using new methylene blue (NMB) and methylene blue (MB) as PSs. The Psa inactivation was assessed *in vitro* using NMB at 1.0, 2.5, and 5.0 µM, in the presence/absence of the coadjutant potassium iodide (KI) (100 mM), under a light irradiance of 50 mW/cm^2. The best *in vitro* inactivation conditions were also tested with MB (5.0 µM) and KI. Subsequently, *ex vivo* experiments using artificially contaminated kiwifruit pollen were carried out using MB at 50 µM under light irradiance of 50 mW/cm^2 for 180 min. Also, pollen germination assays were performed to evaluate if pollen ability to germinate was affected by aPDT.

A reduction in Psa concentration of ca. 8 log CFU/mL in the *in vitro* assays after 45 min of irradiation was observed. In the *ex vivo* assays a decrease of ca. 3 log CFU/mL after 180 min in Psa concentration was achieved, and no negative effects were observed in the kiwifruit pollen germination capacity after aPDT treatment. The results obtained in this study showed that aPDT is an effective and safe method to inactivate the Psa in kiwifruit pollen, indicating that aPDT with MB, a PS already approved and with low cost, can be a promising alternative in the control of Psa transmission by kiwifruit pollen.

The authors are grateful to the University of Aveiro and the Departments of Biology and Chemistry where the experiments were carried out, and to the Portuguese Association of Kiwifruit Growers for the kiwifruit pollen that was kindly provided for the tests. The authors are also grateful for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020) and LAQV-REQUIMTE (UIDB/50006/2020), through national funds and, when applicable, co-financed by the FEDER, through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. Victor M. Balcão is grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil) (FAPESP Ref. No. 2018/05522-9, Project PsaPhageKill) for the BPE fellowship granted, and to CNPq, National Council for Scientific and Technological

Development Brazil, for the Research Productivity (PQ) fellowships granted (Refs. No. 306113/2014-7 and 308208/2017-0). Maria Bartolomeu and Cátia Vieira thank to the Fundação para a Ciência e a Tecnologia (FCT) for their doctoral grants (SFRH/BD/121645/2016, and SFRH/BD/150358/2019, respectively).

Poster 27

Polyphenol dissolution and extraction from agriculture and forestry residues

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Polyphenols have historically been used as dyes and tanning agents, being nowadays mainly known for their applications in the food and wine industries. However, these compounds can be used in many different fields[1].Polyphenols present many desirable characteristics and potentialto the pharmaceutical and material engineering fields. But, before being available for such applications, they need to be extracted from the plant matrix and usually common extraction solvents, such as acetone and methanol are used[2]. Our work aims to extract polyphenols from different biomass matrixes (e.g., pine needles and bark, peanut shells and carob kibbles) using greener and sustainable alternative solvents[3,4].For a better understanding on the interactions between the solvent and the extractable components,an initial systematic study using different solvent systems was performed involving acidic and basic systems. In respect to acidic systems, levulinic and acetic acids (4 M) showed to be promising solvents as the extracts obtained contain almost double the amount of polyphenols when comparing with 80% acetone or methanol, standard solvents systems used por polyphenol extraction. Alkali-based systems also showed good extraction efficiency but further analysis needs to be performed. Moreover, novel deep eutectic solvents (DES) were developed, and their extraction performance evaluated. Upon dilution, all the DES tested were found to remain remarkably efficient even for water contents as high as 65%. In all cases, through ultrasound assisted extractions, the extracts obtained from these mixtures were much richer in polyphenols andpresented a higher antioxidant (DPPH method) activity compared to traditional solvents. The extracted polyphenols are now being evaluated for biomaterials development, such as phenolic resins in which the petrol-based phenol is replaced by the natural sourced polyphenol. Overall, our work focuses on developing novel sustainable solvents regarding green chemistry principles to be used for the dissolution and extraction of natural polyphenols, targeting novelpolyphenol-based biomaterials with added value.

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Acknowledgments

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

Poster 28

Adjusting cultural practices to promote tomato mycorrhization and bioprotection against Fusarium diseases

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The production of industrial tomato is facing the great challenge of finding cultural practices that allow lower environmental impacts and more efficient use of resources, guaranteeing its long-term sustainability. Early mycorrhization of tomato plants by native soil arbuscular mycorrhizal fungi (AMF) provides several benefits, including bio-protection against biotic and abiotic stresses. This is especially important to attempt to reduce the incidence of Fusarium diseases, that severely affect tomato crop and can be attained by introducing cover crops during winter, to promote the native inoculum of AMF, combined with changing the schedule of cultural practices. Test fields were set in two locations – Salvaterra and Pancas –, soil loosening and plant-beds preparation were performed after harvest. Cover crops were used during winter (oat and a consociation of oat and rapeseed) and killed by systemic herbicide in spring. Plant-beds were rebuilt with minimum soil disturbance, keeping the AMF extraradical mycorrhizal mycelium intact and granting an early colonization of tomato. Mycorrhiza colonization rates, cover crop dry matter production, Fusarium spp. incidence and nematode counting were performed. Preliminary results showed low rates of mycorrhization for oat in cover crop and tomato roots. However, the same pattern of colonization was found in cover crop and tomato, highlighting its importance in the development of native inoculum. Higher mycorrhization was found for tomato roots of the consociation treatment in Pancas and for the oat treatment in Salvaterra, but showed no significant differences between treatments. Fusarium spp. was detected in most samples, but also no significant differences were found between treatments. Nematode incidence proved the nematicide effect of rapeseed, showing a tendency to be lower in the consociation treatment for both locations. The intensive use of these fields for tomato over the last 15 years contributed to a low AMF native inoculum and excess of nutrients in soil (high cover crop dry matter production). Under these circumstances, the results were not entirely surprising knowing that the mycorrhization level was low and therefore little protection was given to tomato plants. Persisting in the application of the strategy is fundamental for more tangible results, not showed yet because of soil degradation.

This work is funded by National Funds through Regional Operational Program Alentejo 2020 and co-founded by European Regional Development Fund (ERDF) under the Project ALT20-03-0246-FEDER-000056.

Poster 29

Tomato transcription factors regulate defence response against biotic stresses

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Tomato is one of the most economically important vegetable crops throughout the world. However, it is affected by a panoply of different pathogens that reduce yield and affect product quality, causing symptoms including wilts, leaf spots/blights, fruit spots and rots. Tomato diseases are mainly caused by fungi, but also by oomycetes, bacteria, viruses, viroids and nematodes. The study of plant-pathogen system in tomato arises as an ideal system for better understanding the molecular mechanisms underlying disease resistance, offering an opportunity of improving yield and quality of the products. Among several genes that have been identified in tomato response to pathogens, we highlight those encoding the transcription factors (TFs). TFs are considered central components of plant innate immune system and basal defence in diverse biological processes. They act through sequence-specific interactions with cis-regulatory DNA elements in the promoters of genes and are key regulators of tomato defence response against a wide array of pathogens linked to important diseases, together with a complex cross-talk between different signal transduction pathways.

Here we discuss recent studies of tomato TFs regarding defence responses to biotic stresses. Hence, we focus on the identification and role of different families of TFs selected for their abundance, importance, and the availability of functionally well-characterized members in response to pathogen attack. Genes that encode TFs as master regulators of stress-related genes offer extended possibilities related to their use for engineering pathogen resistance in tomato plants, arising as candidates for tomato breeding, taking advantage of the newly emerging molecular techniques applied to plant breeding in the genomics and genome editing era.

This work is supported by the project “Development of a new virus-based vector to control TSWV in tomato plants” with the references ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017, and the project “Control of olive anthracnose through gene silencing and gene expression using a plant virus vector” with the references ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017, both projects co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo), ALGARVE 2020 (Regional Operational Program of the Algarve) and through the Foundation for Science and Technology (FCT), in its national component. M. P. is supported by Portuguese National Funds through FCT/MCTES, under the PhD scholarship SFRH/BD/145321/2019, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo.

Poster 30

Microbial and chemical hazards in dry-cured sausages

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Dry-cured sausages have been part of the diet in Mediterranean countries for centuries. They enable the preservation of meat, through the fermentation with microbial cultures, while manufacturing a distinctive meat product with interesting organoleptic properties. However, in recent times consumers have become more aware and several health concerns regarding the consumption of cured meat products have arisen. In fact, health and safety issues, particularly chemical and microbial potential hazards, have been related to cured meat products. Microbial hazards include the presence of foodborne pathogens, such as *Salmonella* spp or *Listeria monocytogenes*, among others. Potential chemical hazards are due to their content in nitroso-compounds, associated with the use of nitrate/nitrite. Moreover, consumers demand safe low-salt and low-fat meat products without chemical additives, and the World Health Organization (WHO) recommends the reduction of salt content in foods to prevent cardiovascular diseases. To face all these challenges, the meat industry needs to work together with the academy to reformulate recipes and develop new strategies to meet the consumer demands.

In the present study, dry-cured sausages manufactured in different productions units both in the North and Alentejo regions of Portugal have been analyzed. Physicochemical parameters, namely pH and water activity (a_W), microbiological parameters, such as safety and hygiene indicators, and biogenic amines were assessed.

Generally, low pH values were registered for most sausages, which contributes to their stability and safety, associated also with low a_W values. The microbiota of the different manufacturing units differs regarding total mesophilic counts, enterobacteria and lactic acid bacteria, while significant differences were noticed throughout the manufacturing process for enterobacteria, staphylococci and lactic acid bacteria. Significant differences were observed in the content of biogenic amines throughout the process in the different manufacturing units.

Moreover, sensory analysis studies have revealed a good sensory acceptability of these dry-cured sausages.

We thank project GO77 PDR2020-1.0.1-FEADER-031373 funded by National Funds and co-funded by the European Union. This work was also supported by National Funds through FCT - Foundation for Science and Technology under Project UIDB/05183/2020.

Poster 31

Study of Portuguese extra virgin olive oils according to the producing agricultural method: A qualitative and ecological approach

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The replacement of traditional orchards by intensive and super-intensive ones represents a notorious development in the olive growing sector, focused on increasing productivity to its maximum. Opposing to the traditional distribution, these intensive and super-intensive orchards are characterized by making the most out of the cultivated area, in general with densities as high as 450 trees/ha and 1500 trees/ha, respectively. From the productivity perspective, super-intensive systems represent a breakthrough in extra virgin olive oil (EVOO) production, with extremely premature and productive trees aligned in high density systems. Nevertheless, not only productivity should be considered; the quality of produced EVOO must also be taken into consideration as well as the ecological impact of different intensification systems. Therefore, this work intends to qualitatively compare EVOO produced in different production systems, from traditional to super-intensive, also focusing on agricultural practices employed in each of the studied olive groves, mainly diverging from integrated and organic production systems.

For the development of the experimental work, three different cultivars were considered, two Portuguese traditional ones, 'Galega vulgar' and 'Cobrançosa', and an exotic cultivar with great expression currently in the Alentejo region, 'Arbequina'. With the results generated in this work, it is intended to evaluate the impact that the productive mode and respective agricultural practices may have on the nutritional quality of the produced EVOO.

Authors acknowledge FCT for the PhD grant of M. Ferro (SFRH/BD/140083/2018), and also for the financial support to MED research unit (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) (UIDB/05183/ 2020).

Poster 32

Tomato spotted wilt virus genes expressed in antisense orientation and their ability to control virus progression in *Nicotiana benthamiana*

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Tomato spotted wilt virus (TSWV) is a member of the *Tospoviridae* family and is ranked among the top ten economically important viruses in the world. The genome of TSWV consists of three linear negative-sense or ambisense RNA segments, denoted as segments L, M and S. Segment S RNA encodes the silencing suppressor NSs, and the nucleocapsid protein N. Segment M RNA encodes the cell-to-cell movement protein NSm and two glycoproteins (Gn and Gc). The TSWV is mainly transmitted by thrips and can infect a wide range of hosts, including tomatoes, an economically important crop. Thus, control measures need to be implemented to reduce the damage caused by this virus. In the present work, a TSWV isolate from *Nicotiana rustica* was acquired through Leibniz DSMZ Institute. The expression of antisense transcripts of the N, NSs and M viral genes in leaves of *Nicotiana benthamiana* was assayed for its ability to silence virus progression. For this, each construct in the binary vector pK7WG2 was co-agroinfiltrated with pK7WG2-GFP into *N. benthamiana* leaves, followed by inoculation with TSWV after 48h. Inoculated leaves were harvested 5 days after agroinfiltration for RNA extraction. The ability of antisense transcripts, expressed throughout the plant to control TSWV progression was also assayed using the *Tobacco rattle virus* viral vector (pTRV). In this case, partial sequences of the above-mentioned genes cloned into pTRV2 were expressed as antisense transcripts. New leaves were harvested 10 days after agroinfiltration of the pTRV viral vector. TSWV detection and absolute quantification was performed by a TaqMan real-time RT-PCR assay. Inoculated leaves with TSWV alone and new leaves showed a low viral titer, a result that indicates host plant resistance to TSWV infection. In both assays, TSWV accumulation was higher for constructs carrying the N or the NSs sequences than with M sequences. These studies allowed us to conclude that M gene transcripts in the antisense orientation greatly limit virus progression in *N. benthamiana* plants.

This research was financially supported by National Funds through FCT under the projects PTDC/ASP-PLA/28266/2017 and PTDC/ASP-PLA/28263/2017, and from the Investment and Structural European Funds (FEEI), by Portugal 2020 – Alentejo Regional Operational Programme (CRESC 2020) through projects ALT20-03-0145-FEDER-028266 and ALT20-03-0145-FEDER-028263. This study was also supported by FCT through Project UIDB/04326/2020 CEOT BASE.

Poster 33

Comparative proteome analyses of phloem sap of two citrus rootstocks that differ in their susceptibility to *Citrus tristeza virus*

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Citrus plants can be seriously affected by the *Citrus tristeza virus* (CTV), a positive single-stranded RNA of the *Closteroviridae* family. The disease severity caused by CTV depends on the virus isolate and the infected citrus plant. *Citrus macrophylla* (CM) and *C. aurantium* (CA) have differing susceptibility to CTV, with the latter species being less susceptible. High viral titers in the phloem tissue, where the virus circulates, has been correlated with the susceptibility to CTV and *CA* is a genotype that tends to have a very low viral titer compared to *CM*. To investigate the molecular response of *CA* and *CM* to CTV, the phloem sap proteome of plants of each species infected with the severe isolate T36 and the uninfected controls were analysed by nanoLC-MS/MS. A total of 5413 proteins were quantified in *CM* and 55 were differentially expressed (DE) ($p<0.05$, fold change ≥ 2 or ≤ 0.5) between infected and control plants. In CA plants 5681 proteins were quantified and 98 were DE in infected versus control CA plants. Significant differences were found between the infected CM and CA proteome. Proteins involved in thiamine, nitrogen metabolism and flavonoid biosynthesis were significantly modified in CM, while proteins involved in lipid metabolism, the citrate cycle and pyruvate metabolism were significantly modified in CA. In summary, understanding the divergent response of each rootstock to CTV infection may be the key to explaining their different susceptibility.

This research was financially supported by Fundação para a Ciência e a Tecnologia, FCT, Portugal through Project PTDC/BAA-AGR/30957/2017 and UIDB/04326/2020 and from the Investment and Structural European Funds (FEEI), by Portugal 2020 – Algarve's Regional Operational Programme (CRESC 2020) through project ALG-01-0145-FEDER-30957. Additional funds come from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121. LA was funded by FCT, under the "Norma Transitória" - DL57/2016/CP1361/ project CT0011.

Poster 34

Effects of nematicidal phytochemicals on potato germination

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Plant parasitic nematodes are responsible for significant productivity losses in the potato crop. Among them, the root lesion nematodes (RLN), ranks 3rd in terms of induced damage. Despite being mobile during the majority of their life cycle, RLN are root dependent in terms of feeding site, and moving in and out of the roots of host plants. Disease development can be exacerbated by the presence of soilborne pathogens such as fungi and bacteria. Integrated in a wider study that aims to replace the common application of synthetic compounds for the control of potato nematodes by environment safe plant secondary metabolites, this study intended to evaluate potato seed germination under the effect of compounds previously evaluated as nematotoxic. Preliminary *in vitro* experiments assessed the nematicidal activity of 35 standards of naturally occurring phytochemicals at 2 mg/mL for 24 h. Only 4 tested compounds achieved >99% mortality: benzaldehyde, carvacrol, octanol and thymol. For each of the mentioned compounds, 450 mL of a 2 mg/mL compound solution in 10% DMSO were hand mixed in 2.6 kg of pine forest sand and composted pine bark. Six ´Agria´ variety potato seeds were placed in pots filled with the previous substrate and grown in an Aralab© D1200PLH chamber. The same methodology was employed replacing DMSO by TRITON-X 5 mg/mL. The previous steps were redone, but each compound solution instead of being mixed was poured into 2.6 kg of substrate containing a potato seed. Control treatments (without any compound, with 10% DMSO and with TRITON-X 5 mg/mL) were performed with six potato seeds. The experiments were repeated twice. In the 10% DMSO control, only 4 out of 12 seeds germinated, but were shorter and pale green when compared with the control with no compounds. All tested compounds and TRITON-X inhibited potato development. Using a multiomic approach, the effect of these active compounds in the RLN will be further evaluated to understand their mode of action and the mechanisms responsible for causing nematode death.

Funded by National Funds through FCT under the PhD grant SFRH/BD/134201/2017, PTDC/ASP-PLA/0197/2020, UID/AGR/00115/2013, CESAM UIDB/50017/2020 + UIDP/50017/2020, FEDER PT2020-Compete 2020.

Poster 35

Evaluation of the improvement in the effectiveness of slow sand filtration with *Trichoderma atroviride*

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Soilless cultivation has increasingly been used in the cultivation of vegetables and berries, and can be applied in two ways: with hydroponic cultivation or on substrates. When cultivating on substrates, the nutrient solution that drains from the substrate is often discarded. However, the drained solution contains important amounts of nutrients. Drainage can be reused, if free of phytopathogenic agents and, at least, until the concentration of some elements, mainly Cl and Na, reaches incompatible levels with to the crop. Therefore, to reuse the drained solution it is advisable to use a disinfection system, such as slow sand filtration (SSF), which can be a sustainable biological control solution that promotes the best use of water and nutrients and reduces the use of synthetic pesticides.

The objective of this work was to evaluate the efficiency of SSF on a soilless cultivation system, by introducing beneficial microorganisms in the filter. To evaluate the effectiveness of the SSF (F) inoculated with *Trichoderma atroviride* (T) in controlling *Rhizoctonia solani* (R), 8 treatments were tested: $F^+T^+R^+$; $F^+T^+R^-$; $F^+T^-R^+$; $F^+T^-R^-$; $F^-T^+R^+$; $F^-T^+R^-$; $F^-T^-R^+$; $F^-T^-R^-$, on eight cultivation channels, with five pots per treatment. The filtration system consisted of eight PVC tubes, half of them filled with fine sand. Cucumber (*Cucumis sativus* L.) seeds were placed on pots filled with white peat. The nutrient solution, in constant recirculation, was supplied by drippers. *T. atroviride* was applied as a spore suspension in the filters. *R. solani* was grown in Petri dishes, on sterilized white peat, and later transferred to pots placed at the end of the plastic channels (R^+ treatments), before each filter. Two weeks after germination, the plants were evaluated regarding disease incidence, according to a visual scale of symptoms.

Both filtration systems (with and without *T. atroviride*), were effective in controlling *R. solani*. No increase in filter efficiency was observed due to *T. atroviride* inoculation. When no filtration was applied, *R. solani* did not always infected the plants. This can be related to the fact that *R. solani* does not produce spores, being dissemination promoted by hyphae fragmentation, which is less effective in disseminating the disease.

Poster 36

The effect of the recovery pruning in the productivity and fruit quality of two late orange cultivars

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Citriculture is important in agriculture at national level and mainly in the Algarve region. However, there are some limitations in citrus production. The way citrus trees grow can be a limitation. Growth is irregular, branching occurs in a chaotic way and trees reach, sometimes, large sizes. This aspect restricts the air circulation through the canopy, as well as the entry of solar light, and phytopharmaceuticals to the inner parts of the canopy. Flowering and fruiting occur only outside the canopy. The harvest is also more difficult and, therefore, more expensive. The lack of light inside the canopy leads to the death of the branches in that area. The dead branches can serve as inoculum, where some species of microorganisms can remain in resistance structures. In the favorable conditions for development, these microorganisms may cause fruit diseases. In addition, the fruits located at the tips of the branches and outside the canopy have, usually, inferior quality.

Pruning is a cultural practice that allows the control of these problems through the proper management of the canopy. However, for importing high costs and sometimes direct production losses, some citrus producers avoid, postpone or prune insufficiently. In the case of late orange varieties, the production cycle is over a year and the trees always have fruits. Pruning, in these cases, cause direct production losses.

In the PodaCitrus project two experiments were installed in two late orange cultivars: 'Valencia Late' and 'Dom João'. In both experiments was made, in November 2019, a severe recovery pruning, in large trees, in which was not made a maintenance pruning for several years. In the 2020 production, there were no differences in the fruit size and there was a decrease in production in the pruned trees, as expected. In the 2021 production, although the pruned trees had a smaller canopy than the unpruned trees, the production was equal. In 'Dom João' orange, the fruits were bigger. In 'Valencia Late' orange, several quality parameters were superior in fruits from pruned trees.

The PodaCitrus Operational Group is co-financed by the Rural Development Program PDR2020. Grant number PDR2020-101-031881: PodaCitrus: Optimização da poda em citrinos.

Poster 37

Potential of phage ϕ6 in the inactivation of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit plants

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In the last decade, the worldwide production of kiwi fruit has been highly affected by *Pseudomonas syringae* pv. *actinidiae* (Psa), a phytopathogenic bacterium responsible for the bacterial canker of kiwifruit. The available treatments for this disease are still scarce, with the most common involving frequently spraying the orchards with disinfectants, copper-based bactericides and/or antibiotics. Moreover, these treatments should be avoided due to their high toxicity to the environment and promotion of bacterial resistance. Phage therapy may be an alternative approach to inactivate Psa. The present study investigated the potential application of the already commercially available bacteriophage (or phage) ϕ6 to control Psa infections. The inactivation of Psa was assessed *in vitro*, using liquid culture medium, and *ex vivo*, using artificially contaminated kiwifruit leaves with two biovar 3 (a highly aggressive pathogen) strains (Psa CRA-FRU 12.54 and Psa CRA-FRU 14.10). In *In vitro* experiments, the phage ϕ6 was effective against both strains (maximum reduction of 2.2 and 1.9 CFU/mL for Psa CRA-FRU 12.54 and Psa CRA-FRU 14.10, respectively). In the *ex vivo* tests, the decrease was lower (maximum reduction 1.1 log and 1.8 CFU/mL for Psa CRA-FRU 12.54 and Psa CRA-FRU 14.10, respectively). The results of this study suggest that the commercially available phage ϕ6 can be an effective alternative to control Psa infections in kiwifruit orchards.

Poster 38

Sustainable production of processing tomato through the application of the principles of Conservation Agriculture

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The current production system of processing tomato is based on monoculture and intensive soil tillage practices, which lead to issues in soil quality and control of weeds, pests and diseases, thus compromising the crop's sustainability.

The principles of Conservation Agriculture consisting of reduce soil disturbance, permanent soil cover with plants or their residues and diversified crop rotation, will be compared to the conventional production system to solve the issues identified by the producers. Two alternative production systems will be tested against the conventional: i) reduced soil disturbance and cover crops between two tomato crops; and ii) reduced soil disturbance, cover crops, and a different main crop between two tomato seasons.

Traditionally, the raised bed preparation process occurs in spring, immediately before the tomato transplanting. Applying the principle of reduced soil disturbance the raised bed preparation process will take place after tomato harvest in autumn instead of in spring. This process will be followed by the establishment of a cover crop based on a mixture of grasses and legumes that will grow during the main crop-free period. Tomato transplanting will be performed by a narrow strip tillage, leaving the space between tomato rows covered by the cover crop residues. With these practices both an increase of soil structure stability and retention of nutrients are expected, offering better growth conditions for the tomato crop from the beginning of cycle. The diversified crop rotation principle will be applied by the introduction of sunflower in a biennial rotation with tomato. The crop rotation is expected to reduce the consumption of water and fertilizers as well as to provide better weed and pests control.

This study aims to identify practices capable to promote an improvement in the agronomic, environmental and economic sustainability of the processing tomato production system. Considering the soil as a key element of the system a special focus will be given on the effect of the introduced practices on its physical, chemical and biological properties. The impact of these practices in the mitigation of climate change will be analyzed through the carbon balance, and also the tomatoes' quality and its economic performance.

This work is being funded by Syngenta Crop Protection - Soluções para a Agricultura, Lda under the Project TomAC - Conservation Agriculture approaches in tomato growing. The authors acknowledge the support of Universidade de Évora, APOSOLO – Associação Portuguesa de Mobilização de Conservação do Solo and Ag-Innov – Centro de Excelência Agrícola do Grupo Ortigão Costa.

Poster 39

Characterizing the distribution of *Trioza erytreae* in southern Portugal

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The African citrus psyllid, *Trioza erytreae*, is the vector of the bacterium *Candidatus* Liberibacter spp. which causes the disease HuangLongBing (HLB). HLB has a very harmful effect on citrus, blocking the phloem and so the transport of nutrients throughout the plant, thus leading to tree decline. Losses of up to 70% in citrus production were reported in infected citrus orchards.

In 2014, this vector of HLB was detected for the first time in mainland Portugal, in Porto, but not the bacteria. Since then, it has spread along the entire Portuguese coast, having recently arrived in the western part of Algarve, very close to the most important Portuguese citrus area which is also connected to the Spanish production area.

As HLB disease is very devastating and there is no effective way to fight it, vector control is essential in order to block the spread of the bacteria should it arise. There are control measures implemented by national authorities, such as the biological control program that consists in the release of *Tamarixia dryi*, a parasitoid of *T. erytreae*. *T. dryi* parasitizes *T. erytreae* only in its nymph stages, so it is crucial that *T. dryi* releases are made in places where these stages of the insect exist.

The characterization of the distribution of *T. erytreae* populations was carried out south of Lisbon, with special emphasis on the Algarve region, in order to better understand this threat to citriculture and contribute to the fight against it. The presence of symptoms in leaves, the presence/density of adults, eggs and nymphs, the presence and level of parasitism, among other parameters, were evaluated.

It was found that although the insect is disseminated along the entire coast, south of the Tagus River, populations are low, and the level of parasitism is high throughout the area occupied by *T. erytreae*. Sites with the presence of nymphs have been reported to official authorities to release the parasitoid *T. dryi* in these sites and minimize the advancement of the psyllid.

Rita Poeira is a research fellow in the project GA 817526: PRE-HLB and Beatriz Duarte is a research fellow in the project Life Vida for citrus LIFE18 CCA-ES-001109.

Poster 40

"Farm-to-fork" transmission of antimicrobial resistance – A Review

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Over the years antimicrobials have changed modern medicine by improving both human and animal health. Unfortunately, the emergence of resistant bacterial isolates, namely among the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) bacteria, has been reported in recent years. Antimicrobial resistance (AMR) is considered a complex problem since resistant bacteria are emerging in food, as well as in food-producing animals, such as pigs. With the increase in antimicrobial-resistant bacteria, the World Health Organization (WHO) considered AMR to be a global health problem from a One Health perspective.

The surveillance of AMR falls under the One Health umbrella and can be considered as one of the main objectives. Particularly, it is important to monitor the use of critically important antimicrobials for human medicine, and whether resistance to these is arising. Moreover, this monitorization is important towards the Sustainable Development Goal 12-Responsible Consumption and Production. Antimicrobials considered by WHO as the highest priority among the critically important antimicrobials were quinolones, third- and fourth generation cephalosporins, macrolides and ketolides, glycopeptides, but also carbapenems.

The role of pigs and pork meat in the "farm-to-fork" transmission chain of AMR is reviewed, with particular emphasis on the most hazardous food pathogens that exist in their gut microbiota.

Finally, innovative co-constructed communications strategies are needed to warn farmers of the excessive use of antimicrobials, which are often used to promote animal growth, and in the treatment of bacterial infections.

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

Poster 41

New and sustainable trends in food packaging: edible packaging

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The agri-food sector has focused on the development of improved preservation techniques that effectively slow down food spoilage and maintain food quality throughout the shelf-life.

Packaging is one of the main processes for preserving and maintaining the quality of food products for distribution, storage, and final consumption. Apart from its usual function of containing the food, it is also responsible for ensuring freshness, flavour, and nutritional value. The use of packaging based on polymers derived from fossil fuels, such as polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polystyrene (PS), polyamide (PA), among others), has increased in recent years, generating serious environmental problems. These materials are environmentally unfriendly, and come from non-renewable and non-biodegradable sources, ending up in landfills or in the oceans.

Therefore, and considering the changes in the consumer's lifestyle and the legislation in force, there has been an increase in the demand for safe and high-quality products, fresh, minimally processed, and ready-to-eat, with an extended shelf-life, which creates the need for modernised packaging technologies. The use of edible packaging developed from different biopolymers has proven to be a good alternative. It is possible to add other natural compounds, such as antioxidants, probiotics, and antifungals, among others, to the formulations of these packages. These can be obtained by exploiting the valorisation potential of some forestry and agri-food sector by-products, contributing to the bioeconomy, that promotes the replacement of fossil resources and the implementation of new waste management strategies. The use of these edible packaging will add value to food products, extend their shelf-life, and maintain their safety and quality.

The growing awareness and concern of both consumers and food industry with health and environment, and the increasing plastic waste associated with foods, has led to the need to consider the use of eco-friendly, natural, and sustainable technologies. As defined by the European Commission, it is expected that by 2030 all plastic packaging considered for food products should be recycled, because plastics and plastic packaging are an integral and important part of the global economy. Thus, the need arises for the use of alternatives to the use of ultralight plastic bags and plastic cuvettes for fresh food products. This topic is of extreme importance considering the recently published legislation and the urgent need to come up with improved food preservation technologies, decrease food loss and of waste, and reduce the use of petroleum-based plastics.

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

Poster 42

Influence of soil management practices on the vineyard: a multilevel approach

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The upcoming agricultural and viticultural challenges, related to the need of more sustainable agroecosystems, have raised the awareness of the importance of soil microorganisms. Particular groups of soil microbes can benefit plant growth and/or plant resilience to stressful conditions, so understanding the influence of different soil management practices on the profile and function of grapevine associated microbiome represents a starting point to a more detailed analysis on the effects on plant health and quality of wine grape production. The vineyard microbiome has been extensively investigated to decipher the associated microbial populations in soil, as well as the dynamics of microorganisms in grapes and phyllosphere (Canfora *et al.*, 2018). Differences in soil bacterial community composition are significantly associated with differences in fruit and wine composition and the environmental factors affecting wine terroir may also be mediated by changes in microbial structure (Zhou *et al.*, 2021). Previous studies have shown that the microbial diversity is also affected by land management practices (Canfora *et al.*, 2018). However, little is known to which extent and how soil and vineyard-associated microbiomes can be manipulated through different soil management practices. The hypothesis of this study is that the management of the inter-row, using cover crops, represents a tool to influence soil microbiome and impact the vineyard and the terroir definition. Hence, two different management systems of the inter row will be compared: bare soils and the presence of a cover crop, using as case study vineyards located in Alentejo. The influence of the presence or absence of inter-row cover crop will be evaluated at several levels: soil microbial activity and soil microbiome composition, grape-associated microbiome, grapevine nutritional status and grape and wine quality.

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