



II PhD Students Meeting in Environment and Agriculture

Book of Abstracts

16th and 17th November 2017
Universidade de Évora, Évora

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Dear participants,

It is our great pleasure to welcome each of you to the **II PhD Students Meeting in Environmental and Agriculture**, held in Évora on the 16th and 17th November 2017 and organised by ICAAM and IIFA from the Universidade de Évora. We have put together a two-day program combining presentations by both well-established and junior scientists, organised in a way to encourage discussion and exchange of ideas.

The meeting program, devoted to both environmental and agriculture subjects, comprises a total of four sessions, namely *Ecology and Environmental Sciences*, *Biological Sciences*, *Agricultural Sciences*, and *Animal and Veterinary Sciences*, each starting with two plenary lectures followed by several short talks selected from the abstracts submitted by PhD students. Furthermore, there are also several poster presentations, which can be appreciated during the coffee-breaks/poster sessions. A field trip will take place at Pólo da Mitra to highlight the interface between Environment and Agriculture on 17th November morning. Ultimately, 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 hope you will enjoy the Meeting and enjoy the beautiful city of Évora, an UNESCO World Heritage. You should find all detailed information in the meeting book, including the scientific program, abstracts and a list of participants.

Welcome to Évora!

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Clarisse Brígido, ICAAM

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Thursday, 16th November 2017

09:00	Registration
10:00	Opening session
	Teresa Pinto Correia, Universidade de Évora – ICAAM Diretor of the Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)
	Manuel Collares Pereira, Universidade de Évora – IIFA Diretor of the Instituto de Investigação e Formação Avançada (IIFA)
Ecology and Environmental Sciences	
Chairs	António Mira, Universidade de Évora – INBIO/CIBIO.UE, ICAAM Madalena Moreira, Universidade de Évora – ICAAM
10:20	Conservation and recovery of Mediterranean temporary ponds Carla Cruz, Universidade de Évora – ICAAM Invited speaker 1
11:00	Farming systems as tools to support policies for effective management of High Nature Value farmland – The FARSYD research project Paulo Flores Ribeiro, Universidade de Lisboa – ISA-CEF, CEABN Invited speaker 2
11:40	Coffee break
12:00	Agricultural Sciences
	System of indicators to assess the efficiency of water use and energy in State Irrigation Projects Fernanda Firmino Fiúza, Universidade de Évora – ICAAM OC 1
	Irrigation Precision planning in sugarcane cultivation Jannaylton Santos, Universidade Federal de Viçosa OC 2
	Antioxidant potential of <i>Vitis vinifera</i> bioactive extracts Maria Inês Rouxinol, Universidade de Évora – ICAAM OC 3
	Detection of Olive Viruses in Tunisia: an integrated approach Mohamed Salem Zellama, Université de Monastir OC 4
13:00	Lunch

Biological Sciences		
Chairs	Rosário Félix, Universidade de Évora – ICAAM Ana Cristina Costa, Universidade de Évora – ICT	
14:30	Biodeterioration in heritage- Know to act, act to preserve Ana Teresa Caldeira, Universidade de Évora – HERCULES	Invited speaker 3
15:10	Contribution of paleontology and paleobiology in the knowledge of evolution Octávio Mateus, Universidade Nova de Lisboa – GeoBioTec	Invited speaker 4
15:50	Coffee break and poster session	
16:30	Overexpression of either symbiotic or stress response genes can improve the symbiotic effectiveness of chickpea rhizobia José Rodrigo Silva, Universidade de Évora – ICAAM	OC 5
	A transcriptomic approach reveals new parasitism genes in the pinewood nematode, <i>Bursaphelenchus xylophilus</i> Margarida Espada, Universidade de Évora – ICAAM	OC 6
	New methods in air quality monitoring: is total protein a feasible marker of bioaerosol load in ambient air? Rute Arriegas, Universidade de Évora – ICT	OC 7
	Enzymatic mechanisms of extracts of four flavouring herbs from Alentejo: antioxidant and anti-inflammatory potential Andreia Piçarra, Universidade de Évora – HERCULES	OC 8
17:30	Alentejo de honra	
20:30	Dinner	

Friday, 17th November 2017

09:00	Field trip “The secret world of mushrooms” by Celeste Santos Silva, Universidade de Évora – ICAAM		
Agricultural Sciences			
Chairs	Gottlieb Basch, Universidade de Évora – ICAAM Miguel Elias, Universidade de Évora – ICAAM		
10:00	Arbuscular mycorrhiza and crop bioprotection -The role of cover crops and intact extraradical mycelium		Invited speaker 5
	Isabel Brito, Universidade de Évora – ICAAM		
10:40	Hazards and issues related to fermented meat products		Invited speaker 6
	Maria João Fraqueza, Universidade de Lisboa – FMV, CIISA		
11:20	Coffee break and poster session		
12:00	Portuguese forest biomass as a source of value-added compounds		OC 9
	Cláudia Santos Tavares, Universidade de Lisboa – FCUL		
	Morphological mechanisms of drought avoidance and phenotypic flexibility of taro (<i>Colocasia esculenta</i> L.) in drought conditions		OC 10
	Carla Silva Gouveia, Universidade da Madeira – ISOPlexis		
	Biological control of <i>Sclerotinia rolfsii</i> in turfgrass (<i>Agrotis stolonifera</i>) by organic composts		OC 11
	Luísa Coelho, Universidade do Algarve – MeditBio, ICAAM		
	Comparison of three optical geometries for the prediction of soluble solids content in ‘Rocha’ pear through short wave near infrared spectroscopy		OC 12
	António Brazio, Universidade do Algarve – CEOT		

	Bad company or better alone? Interactions between entomopathogenic soil organisms and the implications on biological control	
	Francisco Bueno-Pallero, Universidade do Algarve – MeditBio, CIMA	OC 13
13:00	Lunch	
Animal and Veterinary Sciences		
Chairs	Rui Charneca, Universidade de Évora – ICAAM	
	Elsa Duarte, Universidade de Évora – ICAAM	
14:30	One Health perspective for antimicrobial resistance Cristina Queiroga, Universidade de Évora – ICAAM	Invited speaker 7
15:10	Translational Research Starts with Pathology Tânia Carvalho, Universidade de Lisboa – IMM	Invited speaker 8
15:50	Coffee break	
16:10	Staphylococci that carry the <i>nuc</i> gene Nara Cavalcanti, Universidade de Évora – ICAAM	OC 14
	Establishment of a primary culture of an oral neoplastic tissue of dog Tarcísio Guimarães – Universidade de Évora	OC 15
	Photodynamic Therapy (PDT) as an approach for dog mammary tumors: from <i>in vitro</i> studies to clinical practice Karla Cardoso – Universidade de Évora	OC 16
17:00	Closing session	
	Best Poster Prize Tribute to Professor Solange Oliveira	

Oral Communications

Ecology and Environmental Sciences

Conservation and recovery of Mediterranean temporary ponds

C. Pinto-Cruz¹, A. Belo¹, A. Lumbreras², J.T. Marques³, C. Meireles², A. Mira³, P. Sá-Sousa¹

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Mediterranean Temporary Pond (MTP) have important conservation value due to their high contribution to local and regional diversity. However, most ponds are nowadays subjected to strong anthropogenic pressures and their conservation is facing many challenges.

In this context, the LIFE Nature-Project 'Conservation of Temporary Ponds in the Southwest Coast of Portugal aims to support and promote their long-term protection.

The most important challenges are the temporary ponds ecological restoration, as well as the increase of public awareness. Since MTP area is mostly privately owned, agreements with landowners are needed in order to establish management and conservation practices.

The first step was to do the cartography and biodiversity assessment of the several MTP in the southwest Portugal coastal plain. We classified the ponds according to their conservation status to identify associated biological indicators and create an efficient evaluation tool for non-experts. This tool represents an alternative to other common, but time-consuming, methods and can be readily used by trained practitioners. In order to recover, the natural topography of the ponds was re-established mainly by deepening the sediment bed. We expected to recover the natural hydroperiod, restore vegetation typical zonation and enhance local biodiversity. To assess the impact of the restoration actions we surveyed and monitored changes of several biophysical components (flora, fauna, water and sediment) before and after the interventions.

This work was co-funded by European Commission LIFE+ program – Project LIFE12NAT/PT/000997

Farming systems as tools to support policies for effective management of High Nature Value farmland – The FARSYD research project

Paulo Flores Ribeiro

Centro de Estudos Florestais e Centro de Ecologia Aplicada Prof. Baeta Neves. Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1340-017 Lisboa

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High Nature Value farmland (HNVf) have recently become a priority for European environmental policy due to growing evidence of its relevance for biodiversity conservation and ecosystem services provision. Because of the well-known impact of policy and socioeconomic drivers, HNVf are prone to changes resulting from intensification and the abandonment of farmlands, pressing need to understand how policy drivers of change may impact the natural asset, so that legal instruments within the Common Agricultural Policy (CAP) may converge to more effective environmental conservation.

The FARSYD research project aims at evaluating the resilience of HNVf under policy change based on a farming systems approach, seeking to contribute to improve the design of agri-environment policies. Its ultimate goal is to develop and test an integrative system-based framework that evaluates the potential of a farming system approach as a tool to assess HNVf support for the delivery of multiple ecosystem services under future scenarios of agricultural policy changes.

Three major research questions are to be addressed to accomplish these goals: 1) Can a farming system approach constitute a tool to assess biodiversity levels and ecosystem services provision in HNVf? 2) What determines the choice of a farmer towards a given farming system? and, 3) Is it possible to use the farming system approach to define optimal thresholds on farming practices that foster the delivery of biodiversity and ecosystem changes in HNVf under scenarios of policy change? To answer these questions, a spatially-explicit and multi-scale analysis of farming systems, from the field to the landscape level, will be implemented and tested in different study areas across Europe, including Portugal (Castro Verde e Peneda-Gerês), Spain (Galicia), Germany (Lower Saxony) and Scotland, UK (Uplands). Clarifying these research questions may show the potential of our farming systems approach as a promising tool to improve the cost-effectiveness EU agri-environment policy, suggesting the feasibility and benefits of a CAP direct payment scheme aimed at HNV farming systems.

FARSYD – FARming Systems as tool to support policies for effective conservation and management of high nature value farmlands (PTDC/AAG-REC/5007/2014 - POCI-01-01-0145- FEDER-016664) granted by national funds by FCT/MCTES (PIDDAC) and cofunded by FEDER funds through the Operational Programme for Competiveness Factors (POFC)-COMPETE.

Biological Sciences

Biodeterioration in heritage- Know to act, act to preserve

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Biodeterioration is an undesirable process, triggered by living organisms, which can affect cultural and built heritage and economically important materials.

Although the involvement of microorganisms in the deterioration process is well acknowledged, the specific role of the different groups and species that compose the communities is not yet well understood. The development of new methodologies to easily detect and identify the microorganisms colonizing cultural heritage and to determine the microbial community involved in its deterioration is crucial to design appropriate effective treatment or preventive strategies.

Culture-independent techniques, enable the differentiation of microorganisms in microbial communities and allow assessing the dynamics of communities. At the same time, the *in situ* analysis of assets by non-invasive techniques, namely x-ray fluorescence spectrometry (elemental composition of the support), spectocolorimetry (color parameters) and infrared spectrometry (molecular information) or the analysis of micro-fragments removed from visually biodegraded areas using a variety of techniques including Raman microanalysis (μ -Raman), infrared micro-spectrometry (μ -FTIR), and scanning electron microscopy coupled to x-ray energy dispersive spectrometry (SEM-EDX), allow material characterization and confirm the presence of microbial contamination or the resulting change of metabolic activity of microorganisms [1]. Indeed the complementarity of these approaches allows a greater understanding of the processes of degradation, paving the way for addressed mitigation/remediation strategies, with a higher level of effectiveness.

The present communication describes combined methodological approaches for the detailed characterisation of microorganisms thriving in heritage context as well as novel materials and tools for the safeguard of Cultural Heritage.

[1] Rosado T, Mirão J, Candeias A and Caldeira AT (2014) Characterizing Microbial Diversity and Damage in Mural Paintings. Microscopy and Microanalysis doi:10.1017/S1431927614013439.

This work was funded by "HIT3CH - HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage", Ref: ALT20-03-0246-FEDER-000004, and "MEDUSA-Microorganisms Monitoring and Mitigation-Developing and Unlocking novel Sustainable Approaches" ref. ALT20-03-0145-FEDER-000015, co-financed by the European Union through the European Regional Development Fund ALENTEJO 2020 (Regional Operational Programme of the Alentejo).

Contribution of paleontology and paleobiology in the knowledge of evolution

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Overexpression of either symbiotic or stress response genes can improve the symbiotic effectiveness of chickpea rhizobia

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Rhizobia can form endosymbiotic associations with legume plants. In this process, rhizobia reduce atmospheric dinitrogen into ammonium that becomes available to host plants. In exchange, legumes release photosynthesis products that are used as energy source by the microsymbionts. Biological nitrogen fixation is a very important process because it allows reduction of the use of nitrogen fertilizers, which represent a high financial cost and may have negative effects to the environment. Besides the known symbiosis genes, such as *nod* and *nif*, which are involved in nodule development and nitrogen fixation, other genes involved in molecular mechanisms, namely stress response may influence the symbiotic interaction plant-rhizobia. The objective of this study was to evaluate the effects of overexpressing symbiotic and stress response genes in the symbiotic performance of chickpea *Mesorhizobium*. *Mesorhizobium* strains were transformed with pRKnifA, pRKnodD, pRKenvZ and pRKgroEL (expression vector pRK415 with *nifA*, *nodD*, *envZ* and *groEL* genes from *M. mediterraneum* UPMCa36^T, respectively). From the four strains transformed with extra *nifA* copies, only V15-b was able to increase plant biomass, when compared to wild-type and empty vector strains. Among the four strains transformed with pRKnodD, ST-2 and PMI-6 showed a higher symbiotic effectiveness compared to wild type and control strains. Additional copies of *envZ* led to a higher symbiotic effectiveness when introduced in PMI-6 and EE-7. Evaluation of the symbiotic effectiveness of the four strains overexpressing *groEL* showed that only ST-2 improved, compared to wild-type and empty vector strains. For all these strains the rate of nodule formation was seen to be higher and further analysis of the infection process and nodule histological analysis were performed. Overall, these results show that extra copies of a given gene may have different effects in the symbiotic effectiveness, depending on the modified strain. This study contributes to a better understanding of the nodulation and nitrogen fixation processes, namely regarding the contribution of non-symbiotic genes, especially *envZ*, which to our knowledge was for the first time seen to be involved in the rhizobia-legume symbiosis.

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A transcriptomic approach reveals new parasitism genes in the pinewood nematode, *Bursaphelenchus xylophilus*

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Plant-parasitic nematodes cause huge economic and ecological losses in agriculture and forestry ecosystems worldwide. The migratory plant-parasitic nematode *Bursaphelenchus xylophilus* is the causal agent of the pine wilt disease, that affects conifer trees (genus *Pinus*) in Asia (Japan, Korea, China and Taiwan) and in Europe (Portugal). This nematode is unique in having both phytophagous and mycetophagous phases throughout its life cycle. During its phytoparasitic stage, the nematode migrates within pine trees feeding on the contents of cortex and xylem parenchymal cells. Nematode-plant interactions are mediated by parasitism proteins called effectors, which are secreted proteins originating from the pharyngeal gland cells. In this work, next-generation sequencing and bioinformatics analysis have been used as a neutral approach to identify novel effectors from this nematode. We have sequenced the transcriptome of *B. xylophilus* and compared gene expression in the mycetophagous and plant-parasitic stages to identify genes likely to be involved in plant parasitism, allowing us to identify a panel of new candidate effectors. We have used *in situ* hybridisation to demonstrate that some of these effectors are expressed specifically in the pharyngeal gland cells and then secreted into the host. In parallel, the sequencing of RNA extracted directly from the pharyngeal gland cells retrieved new data from the effectorome of the nematode. Functional analysis obtained suggests that effectors are one key part of a multi-layered detoxification strategy deployed by *B. xylophilus* in order to protect itself from host defence responses. It appears that *B. xylophilus* secretes detoxification enzymes into the host, while simultaneously upregulating other detoxification enzymes within its digestive system. This comprehensive analysis has given potential insights into molecular strategies used by *B. xylophilus* against the pine host.

This work was funded by National Funds through FCT under the project UID/AGR/00115/2013 and the PhD grant (SFRH/BD/84541/2012). This work was supported by the REPHRAME project (KBBE.2010.1.4-09). The James Hutton Institute receives funding from the Scottish Government Rural and Environmental Science and Analytical Services division (RESAS).

New methods in air quality monitoring: is total protein a feasible marker of bioaerosol load in ambient air?

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The burden of pollen allergies is increasing worldwide. Due to its environmental etiologic nature, disease management in a changing environment has proven to be challenging. Pollen counts are widely used by health professionals as markers for allergenic loads. Other strategies like direct allergen monitoring have also been attempted with reliable results. While the first strategy requires highly trained professionals and is tedious, the second is still expensive. Inexpensive and uncomplicated ways to follow allergenic loads are still missing. The aim of this work was to evaluate the validity of the measurement of total protein in ambient air samples as a marker for bioaerosol loads and therefore as a risk predictor for allergen exposure. The air samples were collected from 2009 and 2011 with a high-volume cascade impactor (ChemVol, Butraco Inc., Son, Netherlands), with two stages to capture two fractions of particulate matter (PM): M-stage for small particles $2.5\mu\text{m} < \text{PM} < 10\mu\text{m}$; XL-stage for $\text{PM} > 10\mu\text{m}$. The filters were extracted using phosphate buffer (10mM, pH=7,4) supplemented with 0.01% Triton X-100 for four hours protected from light. Supernatants were collected after centrifugation (10000g; 10min) and protein content was measured by a Bradford method. Quantification of total protein in the extracts was possible within the range 2-20 $\mu\text{g/mL}$. Protein content in ambient air showed a daily variation and varied between 10-500 $\mu\text{g/day}$. Protein content was 2-3 fold higher in the $\text{PM} > 10\mu\text{m}$ compared to $2.5\mu\text{m} < \text{PM} < 10\mu\text{m}$ fraction. Since, it is expected that pollen will constitute the major component of the bioaerosol during the spring time. Preliminary results indicate a correlation of protein content and grass and olive major allergens. This method, inexpensive, quick and easy to perform, proved to be effective in determine the protein load present in the atmosphere, a potential marker for bioaerosol, including allergen loads and account for air quality control thus for improving risk management strategies and reducing impacts on populations.

This work was supported by the Institute of Earth Sciences (ICT), under contracts UID/GEO/04683/2013 with FCT (the Portuguese Science and Technology Foundation), and COMPETE POCI-01-0145-FEDER-007690 and by project POLLENSORB - PTDC/ATPEAM/0817/2014

Enzymatic mechanisms of extracts of four flavouring herbs from Alentejo: antioxidant and anti-inflammatory potential

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Medicinal aromatic plants (MAPs) are used in folk medicine as treatment or prevention of some diseases, because they are a source of biological products. About 80% of worldwide population use MAPs as preventives or curative purposes, but some of them are also included in Mediterranean diet. Extracts of MAPs are recognized as having antioxidant and anti-inflammatory proprieties, with positive effects on human health against some neurodegenerative, cardiovascular and tumoural diseases. However, some of these properties have not been sustained by scientific/clinical studies, or they are not conclusive.

The aim of this study is to evaluate the antioxidant and anti-inflammatory potential of some flavouring herbs, often used in Mediterranean food, and contribute to MAPs valorisation. Aqueous extracts of *Calamintha nepeta* subs *nepeta* (L.) savi, *Coriadrum sativum* L., *Mentha spicata* L. and *Mentha pulegium* L. were obtained from decoction waters of plants and their chemical composition was accessed by total phenols and flavonoids quantification. Screening of antioxidant activity was performed by quantification of some enzyme activities related with antioxidant response, such as catalases (CAT), glutathione-S-transferases (GST) and glutathione reductases (GR) in hepatic mice homogenates. *In vitro* anti-inflammatory potential of extracts was evaluated by the ability of extracts to inhibit the albumin heating denaturation and lipoxygenases activity (LOX).

Aqueous extracts presented high contents of phenolic and flavonoid compounds and showed antioxidant capacity by inhibiting CAT and GST activities. Moreover, extracts showed high anti-inflammatory potential to inhibit the albumin denaturation and LOX activity. These results highlight the nutraceutical value of selected extracts and suggest their potential use as additives in food industry.

Keywords: Aqueous extracts, antioxidant, anti-inflammatory, *Calamintha nepeta*, *Coriadrum sativum*, *Mentha spicata*, *Mentha pulegium*

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Agricultural Sciences

Arbuscular mycorrhiza and crop bioprotection - The role of cover crops and intact extraradical mycelium

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Mycorrhizal colonisation (MC) of the second plant in a succession starts earlier and develops faster if the extraradical mycelium of the arbuscular mycorrhiza fungi (AMF) associated with the first plant is kept intact and is the preferential propagule type. When abiotic or biotic stressors are present in the soil, the benefits conferred by the AMF are greatly enhanced. Under manganese (Mn) toxicity, compared with any other type of AMF propagule, the presence of an intact ERM enhanced MC by 1.82 and 3.3 times, in wheat and subterranean clover, respectively. In consequence, the growth of both crops was more than doubled and was significantly correlated with a reduction in the Mn concentration of shoots of wheat and in the roots of subterranean clover. However, when the first plant in the succession was a member of the Fabaceae (*Ornithopus compressus* L.) shoot growth of both crops increased by 1.6 times relative to that determined if the first plant was from the Poaceae (*Lolium rigidum* L.), even though no differences were observed in MC or Mn concentration. These results seem to be associated with the AMF assemblage in the roots of the first plant that was passed to the second plant when the ERM was kept intact; whatever was the combination of plant families. The presence in the soil of an intact ERM also induced better growth of tomato plants (3.2 times at 21 DAP) after infection with *Fusarium oxysporum* (10^9 conidia/plant).

Hazards and Issues related with fermented meat products

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Worldwide there is a huge diversity of fermented meat products as a result of human needs and creativity. For centuries, traditional fermented meat products have been produced and consumed due to their delightful sensory characteristics. In fact, there is a significant and well-known history of fermented meat products consumption over several generations and in a large and genetically diverse human population. This gives a reasonable certainty that no harm will result from fermented meat products consumption. In spite of these facts that give us confidence, several biological and chemical hazards have been associated with fermented meat products production, which highlights the crucial and effective implementation of preventive measures to assure their safety. The World Health Organization (WHO) released a report by the International Agency for Research on Cancer (IARC), stating that meat products may increase the risk of colon cancer due to the presence of nitrite and polycyclic aromatic hydrocarbons. Consumers already have a negative picture of fermented meat products due to health issues associated with excessive salt and saturated fatty acids intake in these products, but now it has been deeply damaged by the press with the massive and often incorrect disclosure of the abovementioned report. Nowadays, consumers are demanding convenient, safer and healthier meat products with minimal content of chemical additives, but preserving their sensory characteristics. To meet these challenges, producers will need to innovate processes and products using alternative technologies. This talk will refer to the major hazards and issues related with fermented meat products and different technological strategies will be presented in order to control them.

System of indicators to assess the efficiency of water use and energy in State Irrigation Projects

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Improving the efficiency of the State Irrigation Projects (SIP) through the efficient use of water and energy is a priority of the PDR2020, not only due to increasing regulatory pressure from the EU Farm Policy, but also due to the promotion of economic and environmental efficiency of the PNUEA, ENAAC or the PNAEE. In SIP, the water losses can rise above 50% of the water inflows of the canal irrigation systems and 30% in pipeline systems. In recent years, there has been a gradual transformation of the secondary network, traditionally in open channels, by pressure pipes, which allows increasing the flexibility of the control systems and the efficiency of the water use. However, this change translates a significant increase in energy consumption which accounts for about 30 to 40% of operating costs. For this reason, any improvement in efficiency has a significant economic impact. The objective of this study is to develop a system of indicators to assess the efficiency of water and energy use in the primary and secondary networks of SIP, namely in three study-cases. The methodology used in this study-cases will include Management Entities (EG) of diverse dimension, complexity and availability of information, namely Sorraia Valley (predominantly an open channels system, with a benefited area of 15,897 ha), Odivelas (mixed system, 12,281 ha) and Vigia (predominantly pipeline system, 1,505 ha) to ensure that the results are applicable to the national universe (more than 50 EG). As a result, this study intends to enable Irrigation Associations to evaluate the efficiency of water and energy use in their primary and secondary networks through the use of a system of indicators common to all management entities. This evaluation system will take into account completed and validated water and energy balances. This study will contribute to a better understanding of the inputs and outputs of water and energy at the various levels of analysis. Furthermore, the Farmers are a key to this assessment once who are the end-users of water transported in the primary and secondary networks of SIP and who will make an essential contribution to the definition of consumption patterns.

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Irrigation Precision planning in sugarcane cultivation

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Since the 16th century, sugarcane has been one of the main agricultural crops in Brazil. The country has been leading the world production of this culture for decades. The sugar and alcohol industry accounts for at least 1.76% of the Gross Domestic Product (GDP). The exploitation of this agricultural culture has not yet reached its potential, with this it is expected to increase productivity with private and public investments in technology. The identification and quantification of the need for inputs, such as water, is fundamental to maximize the productivity of sugarcane, however, there is a need to use efficient techniques and low cost. The monitoring of large areas of sugarcane production, efficiently and with technical feasibility, can be obtained by Simple Algorithm for Evapotranspiration Retrieving - SAFER. The algorithm contains good accuracy in the mapping of evapotranspiration to water management. This methodology is important to be applied in irrigated agriculture, specifically in the design and management of irrigation. The objective of this study was to evaluate the behavior of evapotranspiration and biomass production of sugarcane before harvesting by means of remote sensing, in order to justify the use of irrigation. The study area was Fazenda São Pedro located in the municipality of Presidente Prudente, located in the western region of the state of São Paulo, Brazil. The crop evapotranspiration and sugarcane biomass were quantified using the SAFER algorithm. For this, images of the TM-Landsat 8 satellite were used, with spatial resolution of 30 meters, temporal resolution of 16 days and radiometric resolution of 16 bits. The results obtained point out the necessity of irrigation for a better management of the crop, due to the diagnosis of evapotranspiration variability. The variability of evapotranspiration resulted in different biomass stains in the same area. The verified unevenness is a result of the physiological response of sugarcane to the different contents of water in the soil, and can be overcome with precision irrigation from a variable rate of water depth. Irrigation is an important factor to be considered for agricultural planning in the coming cycles, aiming at a better uniformity of production.

Antioxidant potential of *Vitis vinifera* bioactive extracts

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Phenolic compounds are determinant for wine quality. They are also known for having antioxidant properties, protecting cells against oxidative processes and have a key role in the prevention of cardiovascular, neurodegenerative and carcinogenic diseases. Thus, it is important the quantification and characterization of phenolic compounds in grapes during ripening and at harvest. There are some difficulties in creating models to predict the phenolic compounds in grapes during maturation since it is difficult to reproduce the wine production process in laboratory. However, solvent extractions that simulate the winemaking process associated with extraction periods that approach the maceration process may allow the prediction of phenolic compounds content in grapes as well as their respective antioxidant properties. The characterization of phenolic compounds and the evaluation of antioxidant activity of grapes extracts with different extraction periods, will allow the optimization of laboratory methodologies even more similar to the wine production process. These studies can be used in the future in order to predict the phenolic compounds in wine through rapid analytical techniques in laboratory.

In this study two grape varieties commonly produced in Alentejo (Syrah and Aragonês) were harvested and the phenolic compounds were extracted within different periods using water:etanol (50:50 v/v), also different methods were tested to evaluate the antioxidant activity. Results showed that total phenolic content increased in both varieties with longer extraction periods, but Syrah presented higher values throughout all the extraction periods. The extraction period with better results in all tested parameters was the 6 hours extraction, indicating that this extraction period may be the most adequate for extracting a higher amount of polyphenols. Extracts showed antioxidant activity in all tested methods, this activity was higher using the DPPH radical method indicating that grape extracts have high potential for free radical scavenging.

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Detection of Olive Viruses in Tunisia: an integrated approach

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A survey was conducted to determine the virus incidence in olive trees from 43 different cultivars, native or introduced, produced under three different modes of management, in regions covering whole Tunisia. All olive-infecting viruses with a known genetic sequence were tested. TNV-A was also included due to its high similarity with the olive-infecting viruses OMMV, OLV-1 and TNV-D. TNAs and dsRNAs were extracted from the phloem tissues of 213 symptomatic or asymptomatic olive trees and used as viral targets. Prior to RT-PCR, dsRNAs were visualized in 180 sampled trees, suggesting the presence of viruses. RT-PCR tests using dsRNA as template, followed by sequencing of the amplicons, allowed the identification of viruses in 163 samples. When TNA was used as template, several non-specific RT-PCR products were obtained in each of the 213 samples and sequencing of all these RT-PCR products did not reveal the presence of any viruses. From the 13 viruses tested, only OMMV, OLV-1, OLYaV and TNV-D were detected. OLYaV was the most prevalent (61.5%), followed by OLV-1 (40.8%), TNV-D (28.63%) and OMMV (11.26%). The highest infection levels were observed in the northwest and center east regions, in olive trees under intensive modes of management and in native cultivars, mainly in the most widespread in Tunisia: Chemlali (87%), Chetoui (94%), Meski (94%). The introduced cultivars Picholine, Arbequina and Koroneiki also showed high infection levels (88%, 79% and 64%, respectively).

To our knowledge, this was the first time that OMMV and TNV-D were detected and that such a complete survey was performed, in olive trees in Tunisia.

Portuguese forest biomass as a source of value-added compounds

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Biomass represents one of the renewable sources with the greatest potential in energy production, biofuels and chemical products, being efficient as a raw material for industrial processes, including biorefinery. Despite the many advantages associated with the use of biomass in relation to renewable resources, there is a serious problem of the large amounts of biomass that are wasted, namely industrial and agroindustrial residues, which increasingly constitute an environmental problem. Forest biomass can be an example of such residues, due to the large quantities available and poorly used, which can also boost forest fires. Thus, the use of these wastes for the production of new value-added products is a growing objective. The main objective of the present study was the valorization of some Portuguese forest species residues, namely *Cupressus lusitanica* Mill biomass. In Portugal, different Cypress plants (Cupressaceae) are used primarily for reforestation and as ornamental plants, but their chemical constituents are also described to have several biological activities. In the present work, the biomass residues of *C. lusitanica* aerial parts, were steam distilled, providing four fractions: essential oil, hydrolate, extraction water and extracted solid residue. The essential oils composition was investigated by GC and GC-MS. The hydrolate and extraction water were evaluated for phenolic content by the Folin-Ciocalteu method and their profile was characterized by capillary electrophoresis (CZE). The antioxidant and anti-inflammatory activities of these fractions was also evaluated using both the free radical DPPH[•] capture and the ABTS^{•+} methods. Results obtained revealed that the major essential oil constituents were α -pinene, sabinene, δ -3-carene and limonene. The extraction water showed a greater amount of phenolic compounds, mainly tannins, and greater antioxidant activity than the hydrolate. Also for the phenolic composition, CZE revealed the presence of a complex mixture of phenolic compounds, mainly rich in catechin derivatives. Hydrolate and extraction waters also revealed antioxidant and anti-inflammatory activities that are worth exploring. Thus, these results indicate that *C. lusitanica* biomass is a source of natural bioactive compounds with potential applications in the pharmaceutical, cosmetic and food industries.

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Morphological mechanisms of drought avoidance and phenotypic flexibility of taro (*Colocasia esculenta* L.) in drought conditions

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Taro, *Colocasia esculenta* (L.) Schott is an important staple food for several tropical regions in the developing world. This crop has been cultivated on irrigated terraces in tropical Asia for more than 10,000 years. Among tropical root crops (cassava, sweet potato and yam), taro has the world-wide lowest yield production, registering a slight increase during the last decade.

Water scarcity is the most devastating abiotic stress, with great impact on crop productivity, food security and subsistence, which is expected to aggravate with ongoing climatic changes. Drought-tolerant crop genotypes with improved adaptive strategies that allows to maintain the plant's development and productivity under stress, will play an important role in water-stressed agriculture. Drought-tolerant taro genotypes can have improved skills in water use efficiency and nutrients allocation when compared to drought-sensitive ones.

The purpose of this study was to assess the drought avoidance and phenotypic flexibility as the main mechanisms of taro response under drought conditions, to better understand the drought tolerance mechanisms of this crop beyond the physiological traits of plant productivity.

Seven taro accessions originating from Madeira, Canaries and SPC (Secretariat of the Pacific Community) collections were grown in greenhouse conditions. Control and experimental lines were submitted to different watering regimes, using a drop irrigation system, during a one-year cycle. Taro roots and shoots were harvested and evaluated for total plant biomass, root:shoot ratio, whole-plant stress index (SI), nitrogen, starch, chlorophyll content index (CCI) and water use efficiency (WUE). SI showed positive correlations with biomass, WUE and starch content; CCI showed positive correlation with nitrogen content and negative with starch content; WUE showed negative correlations with nitrogen and starch content. All taro accessions revealed drought avoidance mechanism by decreasing the total plant biomass, and phenotypic flexibility with the corm as the main organ affected by drought response, and thus limiting foliar area and total plant growth under prolonged drought stress. The root:shoot ratio and the SI explained the whole-plant stress level. Accordingly, the tolerance response to drought stress increased the WUE for vital activities, increased CCI and nitrogen content by incrementing rate of photorespiration, and reduced starch content by hydrolysis for growth function.

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Biological control of *Sclerotinia rolfii* in turfgrass (*Agrostis stolonifera*) by organic composts

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Turfgrass are susceptible to a variety of fungal induced diseases, being *Sclerotinia rolfii* amongst the more frequent in the Algarve region. The resource to organic compost might be a good alternative in the control of plant diseases, leading to a reduction in the use of pesticides. In the present study two composts were obtained, P1 and P2, by composting agro-industrial residues in a pile with aeration by turning. During the end of the thermophilic phase and by the end of the composting process, fungi from both composts were isolated and identified. Twenty six species that might have antagonistic potential, were isolated, being 12 from P1 and 14 from P2. The antagonistic capacity was evaluated according to the method of direct confrontation. All the assays were run in triplicate. Both composts were tested, by sowing turfgrass (*Agrostis stolonifera*) in 100 mL pots, with six different substrates: peat (T), compost P1 and P2, and the same substrates submitted to a thermic treatment of 60 °C during seven days, TT, P1T and P2T respectively. When the surface of the pots was covered by turfgrass these were inoculated with *S. rolfii*, by placing a PDA disk with the pathogenic agent with a diameter of 6.5 mm. The control was not inoculated. Each modality had five pots with four repetitions.

Isolated fungi were identified by means of morphologic and molecular analysis. In the *in vitro* tests *Trichoderma atroviride* showed higher growth rates when compared with the pathogenic agent. The 26 fungus species isolated from the composts were tested by direct confrontation technique. Ten reduced pathogenic growth (*Trichoderma* spp. and *Fusarium* spp.), with disease inhibition rates between 51 and 64% derived according to Nikolajeva *et al.* (2012). From these 10 fungi, 7 were isolated from P2 compost and 3 from P1. The *in vivo* tests showed a reduction in the diseased area in the pots with the composts, particularly P2. With peat, with and without thermic treatment, the disease occupied the total surface of the pot, demonstrating the effectiveness of the compost in the biological control.

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Comparison of three optical geometries for the prediction of soluble solids content in 'Rocha' pear through short wave near infrared spectroscopy

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We are currently developing a prototype for automated grading of fruit by internal quality through non destructive optical measurements. The system is based on diffuse reflectance spectroscopy in the near infrared. As a first step, we have compared three different optical implementations of the diffuse reflectance measurements: direct reflectance, partial transmittance and transmittance spectroscopy.

In all cases the system comprises a spectrometer, a light source, optical fibers and collimation optics. In the direct reflectance geometry the illuminated and observed areas coincide; in partial transmittance there is a small spatial gap between illuminated and observed areas; and in transmittance mode the illumination and observation areas are placed 90° apart along the fruit surface.

For this test we have used 100 'Rocha' pears, for which we have collected the reflectance spectra in the three configurations and measured their soluble solids content (SSC). Finally, we have built prediction models for SSC from the reflectance spectra. In order to develop the models, the spectra were pre-processed (derivation by either the Savitzky-Golay filter or the Norris derivative, in a first step, and standardization in a second step). The transformed spectra were then fed into a PLS (Partial Least Squares) algorithm in order to build the regression model.

Special attention was paid to the validation procedure. Although the number of samples was low, their origin was heterogeneous, allowing us to perform stringent external validations, with results more realistic than those obtained through the classical (internal) validation method, where the validation set is uniformly sampled from the whole set (for example, the validation set obtained through random sampling of 20% of the whole set). The validations showed us the prediction accuracy of the different optical configurations and we were able to conclude that the partial reflectance configuration is more successful in predicting the SSC in 'Rocha' pear than the other two configuration tested in the research.

The PLS model also allowed us to pinpoint the wavelengths of interest and to check their spectroscopic meaning.

Bad company or better alone? Interactions between entomopathogenic soil organisms and the implications on biological control

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The basis of insect pests bio-control claim for better understanding of multitrophic interactions due to the complex nature of the soil and limited access to agrochemicals. Entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) are good biological control agents of insect pests. Nowadays, they are commercialized and used in crops. However, little is known about its compatibility in simultaneous application and how the presence of antagonists affects its bio-control efficiency. We speculate that the simultaneous application of two entomopathogenic species (nematode or fungus) might support greater bio-control, while the addition of natural antagonists (nematophagous fungi, NF) would unbalance the relationship. We studied the interactions between two NF: *Arthrobotrys musiformis* and *Purpureocillium lilacinus*; and two entomopathogens: one EPF, *Beauveria bassiana* and one EPN, *Steinernema feltiae*, both natives from Algarve. We analyzed the antagonistic interaction between EPF and NF *in vitro* assays in two media: Potato-Dextrose-Agar (rich medium) and Corn-Meal-Agar ¼ strength (limiting medium). In multitrophic interactions assays, we evaluated the effects of individual and combined applications of each type of organisms (EPN, EPF, NF) (two or three simultaneous application) on mortality of *Galleria mellonella* larvae, by employing three methods of exposure to fungi: mycelium, immersion and injection into insect larvae. The larval mortality was measured up to 5 days after exposure. Antagonisms assays showed growth inhibition of NF against EPF in both culture media. Growth rates were significantly slower when NF were alone than in the presence of EPF. In multitrophic interactions assays, both NF species were able to kill *G. mellonella* larvae mainly when injected. Fungal injection and direct exposure to mycelium increased larval mortality when combined with nematodes. EPN was the main driver of insect mortality when the three types of organisms were combined by immersion. Only slight additive effects were observed in most of the combined treatments. This study illustrates the need for a comprehensive study of the various multitrophic interactions naturally occurring in the soil to achieve successful insect pests control in agroecosystems.

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Animal and Veterinary Sciences

One Health perspective for antimicrobial resistance

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Infectious diseases and other infections treatment is absolutely dependent on the use of antimicrobial agents. Nevertheless, in recent decades antimicrobial resistance (AMR) has become a growing threat for both human and animals. In 2014, a report of the Review on Antimicrobial Resistance estimated that antimicrobial resistance could cause 10 million deaths a year by 2050. Recently the emergence of colistin-resistant enterobacteria rendered infections caused by these bacteria as untreatable. These bacteria were designated Superbugs as they are resistant to all known antimicrobials.

Commensal microbes that live on and in us are critical for our health, as this microbiome is like a filter between us and the world. Microbiome studies have clarified that microorganism exchange happens between people who cohabit certain spaces and even between animals and people living in the same environment. It is also well known that the consumption of food products of animal origin may provide access of microorganisms from the animal's microbiome to the consumer body.

Bacteria may transmit resistance genes to each other through the conjugation process, one of the DNA transfer processes. Therefore, when acquiring resistant bacteria, both humans and animals, there may be transmission of resistance genes to other microorganisms that inhabit the own organism and, thus, the individual may develop antimicrobials resistant populations in his particular microbiome. Therefore, food products of animal origin may constitute a route of transmitting resistant bacteria and resistance genes from animals to people. Direct contact with animals or the animal environment may also be responsible for transmission. Fruits and vegetables contaminated by animal waste or contaminated water may similarly constitute a transmission route. Several multi drug resistance (MDR) pathogenic bacteria are shared between companion animals and humans. These organisms spread between animals and humans, although the direction of transfer is often difficult to prove.

To control AMR a rational antimicrobial use must be followed. Antimicrobial stewardship refers to coordinated interventions designed to improve and measure the appropriate use of antimicrobials. But to face this serious problem, Antimicrobial Stewardship must have One Health approach, and collaborative efforts between Medical and Veterinary doctors must be implemented to achieve global health.

Translational Research Starts with Pathology

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Bent over a computer screen, scrolling over a scanned glass slide of a mouse lung, Drs. Cláudia Faria and Tânia Carvalho stare intently at a small group of cells. It is a lung metastasis, derived from a tumor that was surgically removed from the brain of a lung cancer patient, and immediately implanted subcutaneously in the dorsum of a mouse, weeks earlier. Claudia, a neurosurgeon at Hospital de Santa Maria and researcher at IMM, has a question: what drives tumor cells from the lung, breast, colon, skin, to invade the central nervous system, the core of our self, our sense, our commander? And how can we stop them? As they inspect a section of the mouse lung, Tânia, veterinary pathologist and head of Histology and Comparative Pathology Laboratory at the IMM, directs Cláudia's gaze to the tumor cells that appear completely adapted to that lung microenvironment, highly proliferative, suggesting that they probably know where they came from in the patient, what their place of origin is; it is the true meaning of tumor *homing*, and this case in particular was a home run! "We need to understand what drives these cells to disseminate, to invade the brain, so that we know how to block that process," Cláudia said. "This requires us to examine how the patients' tumor behaves *in vivo*, in a model organism that we can manipulate, challenge." Anatomical Pathology is a medical specialty concerned with the diagnosis of disease based on the macroscopic, microscopic and molecular examination of organs and tissues; it is the basis of medical and veterinary diagnosis and should play/plays a key role in bridging human and experimental disease.

Staphylococci that carry the *nuc* gene

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Inflammation of the mammary gland, known as mastitis, is a serious problem, because it is responsible for the decrease in milk yield and quality. Bacteria of the species *Staphylococcus aureus*, causing intramammary infections in small ruminants, are often isolated from milk samples and frequently resistant to antimicrobials. The thermostable nuclease encoded by the *nuc* gene is a virulence factor, as it promotes evasion of neutrophil extracellular traps (NETs), which are networks of DNA strands with antimicrobial proteins. The *nuc* gene is present in most *S. aureus*, however some isolates not carrying this gene have been described. Moreover, the *nuc* gene has also been detected in other *Staphylococcus* species, notably in *S. intermedius*, *S. hyicus* and *S. simulans*. The aim of this study was to assess the presence of the *nuc* gene in staphylococci isolated from goat and sheep milk samples. Three hundred and sixty-eight samples of milk were collected, from 187 animals, belonging to six flocks in Alentejo. Ninety-one isolates of the genus *Staphylococcus* were identified by classical biochemical methods and through the VITEK2 microbial identification system (BioMérieux, France). The presence of the *nuc* gene was assessed by PCR. The reference strain *S. aureus* ATCC 25923 was used as positive control. According to VITEK2, 24 *S. caprae*, 13 *S. epidermidis*, 13 *S. chromogenes*, 11 *S. aureus*, nine *S. simulans*, five *S. warneri*, five *S. lentus*, four *S. capitis*, four *S. haemolyticus*, one *S. auricularis*, one *S. hominis* spp. *hominis* and one *Staphylococcus* spp. (not identified to species level) were identified. Twenty-three out of the 91 isolates (25.3%) carry the *nuc* gene. With the pair of primers used, this gene was detected in seven different *Staphylococcus* species: 10 *S. aureus* (43.5%), three *S. warneri* (13%), three *S. lentus* (13%), two *S. caprae* (8.7%), two *S. epidermidis* (8.7%), two *S. chromogenes* (8.7%) and one *S. capitis* (4.3%). Furthermore, the *nuc* gene was not detected in one *S. aureus* isolate. We suggest that the presence/absence of the *nuc* gene is not a reliable method for the identification of *S. aureus* and that this virulence factor may be involved in small ruminant mastitis pathophysiology.

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Establishment of a primary culture of an oral neoplastic tissue of dog

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In vitro cell culture allows to assess intrinsic physiological functions of the cell and of the tissue from which they were originated. Culture of animal and human cells are widely employed in biomedical research, as experimental models in living organisms. The objective of this investigation was to develop a method to obtain a primary cell culture of an oral cancer of a dog. The tumor sample was obtained in the Veterinary Hospital of the University of Évora, from a canine patient, with a history of oral neoformation with rapid evolution. After excision, the neoplastic tissue was fragmented, one-third was submerged in 10% formaldehyde and sent for histopathological analysis, another third was frozen at -80 ° C and the remainder was collected in PBS with 2% antibiotic (penicillin and streptomycin - PenStrep), for further processing of cell culture. Two culture protocols were tested: explant culture and cell culture after chemical/mechanical dispersion of the tissue. The fraction intended for the explants was dissected into 1 to 2 mm pieces, which were placed in rosette form in a petri dish, followed by the addition of DMEM culture medium with 1% PenStrep and 10% FBS. The tissue fraction intended for dispersion was incubated in Versenio buffer (0.6 mM EDTA in PBS) for 30 min at 37 ° C, followed by mechanical homogenization of the tissue, sedimentation of the cell fraction and culture of the latter in culture flasks, in the same medium used for explants. Biologic material from both techniques were maintained in an incubator at 37° C, 5% CO and 90% humidity. The results showed that the mechanical dispersion was the most suitable technique for this type of tissue, since contamination of the explant cultures occurred. The cell culture was maintained for at least one week, and it was possible to identify several cell types, some of them with replicative capacity in culture, which suggests the possibility of maintenance in culture of the neoplastic tissue.

**Photodynamic Therapy (PDT) as an approach for dog mammary tumors:
from *in vitro* studies to clinical practice.**

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The evolution of veterinary medicine has contributed to the longevity of animals. Cancer has been extensively studied as well as different strategies for its treatment. The deepening of the studies shows that the most incidental neoplasms in bitches occur in the mammary gland and these tumors are equivalent to breast tumors in women, being appropriate models for the study of tumor biology. Photodynamic therapy (PDT) is a therapy based on the use of a photosensitizing molecule, which is activated by irradiation with light with a specific wavelength. The ensuing formation of singlet oxygen and other reactive species determines the activation of cell death pathways such as apoptosis and necrosis. The aim of the study is to test a group of new chlorins by an innovative method, as these photosensitizers, developed at the University of Coimbra, have been shown to be effective and potent against human melanoma cells. In addition, a platinum element has been incorporated into its structure which gives it thermostatic properties, i.e. the possibility of associating PDT with luminescence detection. A canine mammary carcinoma cell line REM 134 was purchased by the University of Évora, which will be cultured in Dulbecco's Modified Eagle's Medium (DMEM, at 37 ° C, 5% CO₂ / 95%), subsequently submitted to treatment with the photosensitizers and light of appropriate wavelength. Uptake, subcellular localization, metabolic activity, cell viability and types of cell death will be evaluated using techniques such as spectrophotometry, flow cytometry, and western blot. Anticipating valuable results in vitro, *in vivo* outcome will be accessed. For this, REM 134 cells will be implanted in Balb/c nu/nu mice to obtain xenografts. After PDT, tumor growth rate will be evaluated, and pathology examination will be performed. Results of this project may contribute for PDT indication in bitches with breast tumors. There is the possibility to perform a small trial in patients of the Veterinary Hospital of the University of Évora, which are indicated for mastectomy. This project stands out for its translational potential and for the innovation of the photosensitizers to be investigated.

Poster Communications

The communities of *Prunus lusitanica* L. in Portugal: Knowing how to conserve

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Integrated in the project LIFE - Relict (LIFE16 NAT / PT / 000754) it is intended to deepen the knowledge on the conservation status of the priority habitat 5230 – ‘Matagais arborescentes’ (Arbor Bushes) of *Laurus nobilis* * on the Natura 2000 sites. This habitat contains several subtypes, of which the communities of *Prunus lusitanica* L. (5230pt2) stand out as a priority. These plant clusters constitute true paleotropical relics, which are located in fragmented areas of small size on the European continent, practically exclusive to the Iberian Peninsula and punctually registered in the South of France. To achieve this objective, several management practices are presented with the aim of improving the quality of the *Prunus lusitanica* communities in the most representative areas of this habitat, within the RN2000 Sites, namely Serra da Estrela, with one of the most known areas of these species (Casal do Rei), which burned in a forest fire a few years ago; and the Complexo do Açor, that presents the largest known area of *Prunus lusitanica* in the Iberian Peninsula. It presents in the national territory the global assessment of the "unfavorable" state of conservation, which needs to be reversed. Thus, this project envisages the improvement in more than 50% of the areas occupied by these communities in the Portuguese RN2000, contributing to the objectives of the 2020 Strategy and the implementation of the Habitats Directive. One of the main threats faced by this habitat is invasive alien species, especially those belonging to the genera *Acacia* and *Hakea*, as well as the risk of forest fires. On the other hand, the high number of floristic species associated with the habitat of *Prunus lusitanica*, consisting of innumerable bindweeds (e.g. *Hedera hibernica*, *Lonicera periclymenum*, *Rubia peregrina*), several fetuses (eg *Asplenium onopteris*, *Asplenium trichomanes*, *Polypodium vulgare*, *Blechnum spicant*), bulbous (e.g. *Narcissus triandrus*, *Hyacinthoides hispanica*, *Orchis mascula*), among many others. In this sense, the actions implemented with this project aim to improve the conservation status of the priority habitat 5230pt2 and the dissemination of scientific knowledge about these communities to society in general, with a view to enhancing and restoring the long-term ecological functions of communities of *Prunus lusitanica* in Portugal.

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Impact of soil treatments on early root hair infection and nodule occupancy by rhizobia

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In the coming 50 years, with escalating global food demand, the major challenge will be to ensure the sustainability in both food production and ecosystems management. Sustainable agriculture aims to achieve high crop production, concurrently reducing the use of chemical fertilizers and herbicides, which have both economical and environmental costs. Rhizobia can play a significant role in improving the fertility and productivity of low-N soils, due to its ability of fixing atmospheric nitrogen in symbiosis with compatible legume species. Rhizobia inoculants can be applied to seeds of legume crops to provide efficient nodulation and nitrogen fixation thus reducing the use of chemical fertilizers. Broad-spectrum systemic herbicides such as glyphosate, are commonly used to destroy weeds. The application of both chemical fertilizers and herbicides affect the soil microbial community and consequently later on the productivity of crops. The aim of this work was to evaluate the diversity of rhizobia from nodules of chickpea plants grown in soil treated with glyphosate (Roundup), nitrate source (KNO₃) and *Mesorhizobium* LMS-1 inoculation. Another aim was to evaluate the impact of different levels of glyphosate and nitrate sources on chickpea mesorhizobia free-living growth and on the early stages of the infection of the host plant. To evaluate the diversity of rhizobia within the chickpea nodules, PCR amplification and sequence analysis of the 16S rRNA gene was used. The phylogenetic analysis showed that all the rhizobial 16S rRNA sequences obtained group with *Mesorhizobium muleiense* CCBAU 83963, a species previously known to be able to nodulate chickpea. This was also the case for sequences from the treatment where a *M. ciceri* LMS-1 strain was inoculated. The detection of *M. muleiense* as the predominant species, instead of higher diversity of *Mesorhizobium* species may be related to the high competitiveness of this species and preceding cultivation of chickpea plants. The early root hair infection process analysis demonstrated smaller root hairs and less curling for the highest concentrations of glyphosate and nitrate tested than in control. This work has contributed to have better understanding of the impact of chemical fertilizers and herbicide application on the legume-rhizobia symbiosis.

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Loss of airborne pollen integrity during transport: atmospheric conditions affecting *Cupressaceae* pollen

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Cupressaceae family include several species that are widely used as ornamental plants. These plants pollinate in late winter-early spring, depending on the temperature, and despite being considered moderately allergenic, are a great concern in some countries (Shahali et al, 2013). The most representative species in Portugal are *Cupressus lusitanica* and *Cupressus sempervirens*; as ornamental trees, are found scattered through the territory but there are a couple of geographical sites where species of this family are abundant, usually in mountains: Serra do Buçaco, Serra do Gerês e Serra de Sintra (www.flora-on.pt). *Cupressaceae* pollen is spheroid in shape, with 25- 30µm in diameter, thin exine and inaperturate (Gaussorgues and Demoly, 2013). Despite being traditionally captured in considerable amounts in Evora region, aerobiological features are poorly characterized. The aim of this work is to characterize the aerobiology of *Cupressaceae* pollen in Evora, during 2017 season and relate this with the atmospheric circulations and the meteorological conditions obtained from local observations and numerical weather prediction models. Pollen were collected using the standard Hirst methodology and identified by microscopy. During the last week of February, when large concentrations were detected, many of the *Cupressaceae* pollen grains were disrupted, ranging between 18.2% in February 22nd and 45.6% in February 28th. During this period desert dust and pollen grain were both identified in the sampling filters. Aerosol data will be analyzed to explore possible interactions between desert dust aerosols transported from North Africa and *Cupressaceae* pollen. To our knowledge, this is the first report of a considerable fraction of disrupted *Cupressaceae* pollen grains reaching the sampler, releasing cell contents, which may significantly increase ambient free allergen and contribute to enhance allergenic activity of this pollen type. A better understanding of this phenomenon may contribute to improve allergy risk management.

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Resistance of coagulase-negative staphylococci isolated from sausages to liquid smoke

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The industry of meat products produces an extensive diversity of traditional dry-cured sausages in Portugal and other Mediterranean countries. Currently, consumers are increasingly informed and demanding, preferring homemade regional products of high sensory quality. With the growing consumption of sausages, there is a need to improve their quality. One possible approach is the use of starter cultures, such as Coagulase-Negative Staphylococci (CNS) and/or Lactic Acid Bacteria (LAB). The use of starter cultures intends to ensure and improve the hygienic, sanitary, nutritional and sensory quality. CNS, such as *S. xylosus* or *S. equorum*, are naturally present at high levels, in sausages and on industrial environments. Moreover, microorganisms must be selected according to the conditions used in meat products industry, namely the use of a smoking step. However, little is known about how CNS tolerate smoking. In this study, CNS were used, because they promote the red colour of sausages, as well as other organoleptic characteristics desired by consumers, and are Generally Considered As Safe (GRAS) bacteria. This work consisted in determining the Minimum Inhibitory Concentration (MIC) for several CNS strains, in order to study their resistance to smoking. The strains used in this study were isolated from sausages and pure cultures were obtained in order to evaluate their resistance to liquid smoke. The MIC was determined for most of the strains used, however some of them continued to grow at the highest concentration, and further studies are required. It is possible to observe that strains have distinct growth patterns: some grow well in the culture medium used, but have a low MIC; in contrast, others grow poorly in the used culture medium, but have a high MIC. Based on these results, and taking some important technological features present in these CNS isolates into account, a selection of strains to be potentially used as starter cultures in the production of sausages will be done.

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Improving *Alheira* microbiological quality and safety using high hydrostatic pressure treatment and nisin loaded pectin nanoparticles

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Alheira is a traditional smoked fermented sausage produced in *Trás-os-Montes*, a region in the north of Portugal. Its industry represents an important economic resource for the region. Bad practices can occur during *alheira*'s processing, storage and cooking, exposing consumers to microbial hazards. Considering this, it is important to apply technologies to assure *alheira*'s safety and increase its shelf life, in order to promote the sustainability of this traditional product. This study determined the effect of nisin loaded pectin nanoparticles (NLPN) and high hydrostatic pressure (HHP) treatment alone or combined on controlling *Alheira*'s microbiota. The work was conducted with vacuum-packaged *alheiras* produced under 4 different conditions: 1- traditional *alheira*; 2- *alheira* with NLNP; 3- *alheira* processed with HHP; 4- *alheira* with NLNP and processed with HHP. The addition of NLNP was done during the mixing step. The vacuum packaged sausages were HHP treated at 600 MPa for 390s at 10°C. Assays were done in triplicate. Microbiological analysis were performed 1 day after treatments for total aerobic microorganisms at 30°C, total anaerobic microorganisms at 10°C, lactic acid bacteria, coagulase-negative *Staphylococci*, *Enterobacteriaceae*, *Clostridium perfringens*, *Listeria* spp., moulds and yeasts according to methods proposed by ISO. The application of HHP significantly reduced the levels of all microorganisms analyzed. Due to the low level of sausages contamination with *Listeria* spp., the observed reduction under the effect of HHP was not conclusive. *Clostridium perfringens* was not detected in the samples analyzed. No significant difference was observed between the application of HHP alone or combined with NLPN. The effect of NLPN addition alone was only verified in the coagulase-negative *Staphylococci* count, with a reduction of 1.26 log cfu/g. *Alheiras* with NLPN presented *Enterobacteriaceae* counts higher than the control group. Despite low efficacy of NLPN in reducing gram-negative bacteria counts, this result alerts to the importance of carrying out strict quality control in the NLPN production for incorporation in food. In further studies, we are interested in characterize the performance of NLPN over the shelf-life of *alheira* since nanoparticles are supposed to protect nisin from enzyme's substrate and slowly release it at a constant rate.

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Clinical and cytological characteristics of ocular lymphoma in dogT. Guimarães¹, K. Cardoso¹, S. Branco^{2,3}, N. Alexandre^{2,3}

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Lymphoma is the main hematopoietic neoplasia in the dog, originating from lymphoid or extranodal tissues. It is characterized by high aggressiveness, and the ocular involvement is associated with the multicentric form, manifesting clinically in a several forms. A female dog of undefined breed, 11 years old, with uncomfortable red eyes, was attended at the specialized veterinary ophthalmology service in the Rio Grande do Sul, Brazil. At the ophthalmological examination with a slit-lamp biomicroscope, there was generalized thickening of the conjunctiva and third eyelid, protrusion of the third eyelid gland, deep and superficial corneal vascularization, intra-stromal haemorrhage, uveitis with a presence of hyphal, keratitis, ocular hypertension (right eye 28 mmHg and left eye 26 mmHg) at the examination with tonometry of applanation. Physical examination revealed generalized lymphadenomegaly. The diagnosis of the disease was made by hematological, biochemical and cytological exams through fine needle aspiration of submandibular lymph nodes. The hematological results revealed mild anemia, thrombocytopenia, lymphocytosis with the presence of reactive lymphocytes. The cytological examination showed a high amount of neoplastic lymphocytes, high nucleus-cytoplasm ratio, highly basophilic cytoplasm, high eccentric nuclei and granular chromatin, related to large cell lymphoma or high grade lymphoma. The treatment was directed to uveitis secondary to lymphoma, and the initial ocular treatment consisted on prednisolone acetate, ceterolac tromethamine, timolol and dorzolamide. In the evaluation with the veterinary oncologist, the COP chemotherapy protocol was instituted with intravenous vincristine supplemented orally with prednisone and cyclophosphamide. The patient responded positively to the treatment instituted, showing a significant improvement of the ophthalmologic and systemic clinical picture, after 21 days. Lymphoma is a malignant neoplasm, which can affect and manifest in different organs, such as the eyes. Clinical, laboratory, and cytological classification are extremely important in determining the prognosis and diagnosis of multicenter lymphoma. Directing accurate diagnosis and appropriate therapy.

Ophthalmological evaluation, culture and antimicrobial sensitivity of Merino sheep from the University of Évora

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In Portugal, sheep production plays an important economic role, mainly in the region of the Alentejo, where it traditionally gathers a large part of the national herd, predominating the Merina breed and its crosses. It is of great importance to investigate ophthalmological diseases in production animals since they have an important impact on economic losses, as observed in the disease of infectious keratoconjunctivitis, requires drug administration and consequently withdrawal of the animal from the production during the treatment period. Ophthalmic exams are used to routinely evaluate and identify ocular changes in pets. The objective of this investigation will be to evaluate oculomotor reflexes, intraocular pressure (IOP), Schirmer's lacrimal test (TLS), lacrimal pH, fluorescein test, changes in funduscopy and culture, and conjunctival antibiogram. Also correlate the data with possible diseases, and correlation between Merina Branca and Merina Preta race, age of the animals and influence of the hour with the exams performed. The examinations and tests of this investigation will be carry out in an outpatient and routine manner in the veterinary ophthalmology. Before and after the ophthalmological examinations and tests, the animals will be able to return to the stable and their usual activities.

Volatile chemical characterization and biological activity assessment of Portuguese honey types – importance of botanical origin

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Honey is the sweet substance obtained from plants nectar, from secretions of living plant parts, or located on them, which honeybees collect, transform and combine with specific substances, storing it in honeycombs. Different methodologies have been conducted around the world to identify the volatile organic compounds from unifloral honey, responsible for its organoleptic and nutraceutical properties, to identify and prevent fraud and to guarantee its authenticity. Chemical volatile composition has great importance in characterizing the honey floral origin, which could be associated with its geographical source. Detailed characterization of different honey types occurring in Portugal is important but remains scarce. The traditional approach to identifying the botanical origin of honey relies on laborious pollen examination, thus other markers, namely volatiles markers can be explored. For these reasons, the objectives of this study are: 1. obtaining knowledge on the volatile compounds of selected honeys from Portugal, 2. identifying plants volatile marker compounds that allow assessing honey botanical and geographical origin, 3. determining the biological properties of the different honey types and honey derived products and 4. assessing the lack of toxicity effects of different honey types and honey derived products.

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Naturally-produced compounds for the control of the plant parasitic nematode *Pratylenchus penetrans* – the plant response

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The root lesion nematode, *Pratylenchus penetrans*, is one of the main plant parasitic nematode species responsible for productivity losses in a significant number of crops, among which potato crop. *P. penetrans* is the most common *Pratylenchus* species in Portuguese soils, although species distribution and abundance is still unknown. This organism is extremely difficult to eradicate, and control procedures are expensive and hazardous to the environment and human health. In the last few years, natural compounds have gained an increasingly importance as substitutes of the most effective chemicals used for controlling nematodes; however, their effect towards plants is largely unknown. Our main goal is to identify naturally-produced compounds able to control *P. penetrans* and simultaneously non-harmful to plant host. The research plan includes: (1) *in vitro* bioassays of plant (i.e. essential oils) and bacterial (i.e. toxins) compounds for biological control of *P. penetrans*; and (2) greenhouse trials to test the selected compounds in potato cultures under nematode presence with evaluation of plant physiological response, volatile profile and defence pathways. The compound(s) of interest should: be able to decrease significantly the nematode populations; be easily applicable; and allow minimal disturbance of plant host. Future results shall complement an ongoing project on biotechnological approaches towards the control of *P. penetrans*, and will be an add-value to the continuous investigation on environmental-friendly substances for the suppression of other plant parasitic nematodes.

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Evaluation of postharvest behavior under MA conditions of 'Crimson' seedless table grape from two different vineyards

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In recent decades in order to allow maintenance of good organoleptic and nutritional quality of fruits over an extended period of time modified atmosphere (MA) have been developed and implemented. 'Crimson' seedless table-grape (*Vitis vinifera* L.), is a non-climacteric fruit with low physiological activity at maturity stage. Being a highly perishable crop, it undergoes severe problems during postharvest phase as weight loss, color deterioration, accelerated softening, berry shatter, rachis browning and fungal infection. These new table grapes cultivars with commercial high value have stimulated market interest because of seedlessness, precocity and flavor. This type of table grapes is a commodity that allows good profits to producers and so they intent to storage them for a long period of time using easy and practical technologies. So the aim of this study was to evaluate the postharvest behavior of 'Crimson' seedless table grapes, harvested from two different vineyards locations, and stored in cold conditions at 1°C and RH 95%, with application of a plastic film (©PPlus), often used for fruit preservation. Samples were analyzed during a period of 60 days, considering 3 repetitions for each sample, and evaluating the following parameters: berry weight, weight loss during storage time, external color, fruit texture, soluble solids content, titratable acidity and total polyphenols. An analysis of variance was performed in order to fundament the results discussion and can be concluded that table grapes obtained in the different vineyards are significantly different being noticeable these differences not only at harvest time but also during the storage time. The consumers during a sensorial query found differences between grapes harvest in different vineyards. Considering the above referred quality parameters and also the observed fungus development the period of sixty days of storage can be pointed out as time storage the limit for these table grapes under these conditions. This study allows concluding that grapes from different vineyards have very different postharvest quality characteristics, and it is even possible to state that the grapes from one of the vineyard are better for conservation on MA condition, than those from the other vineyard being this an interesting information for producers.

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Evaluation of starch based edible coatings with two different surfactants in strawberry

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Edible coatings made from potato starch present high biocompatibility, can create a barrier between the fruit and the exterior environment, are well accepted by consumers mainly because they have no toxicity, and don't change the organoleptic food properties. (Han & Gennadios, 2005; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Nowadays starch based edible coatings are used in food industry due to their characteristics of transparency, lack of odour, insipidity and working as a good barrier for oxygen and carbon dioxide (Neetoo & Chen, 2011; Neetoo, Ye, & Chen, 2010). Starch is a low cost raw material and recently potato has been a surplus product in Portugal.

This work focused on the study of the effect of edible coatings made from starch using two different surfactants in postharvest strawberry behaviour mainly in respiration rate in order to extend fruits' shelf life.

Fruits were acquired at MARE in Évora, they were selected by colour uniformity and defects absence in the postharvest laboratory of University of Evora/ICAAM. Two solutions of starch were made as described in Maria Rodriguez et al. (2006), being used in one of them the Tween 20® and in the other the Span 80® as surfactants. The fruits were dipped in the solutions for 5min and dried for 30min. After that they were maintained in a cold camera at 3 °C and 75% RH. The texture was analysed with a texture analyser from Stable Micro Systems PA-HDplus, using a 3mm cylindrical probe; the weight loss values was calculated and the respiration was measured using a O2 CO2 gas analyser from Abiss Legend.

For results discussion, a multivariate variance analysis and a post-hoc LSD test ($p < 0.05$) with the SPSS statistical analysis software have been made.

From the collected data and discussion can be conclude that the starch coating with Span 80® allowed the solution to behave as a real coating after the drying process, and this one revealed statistical significant difference from the control, in preventing weight loss and to slowing the respiration rate.

Posterior studies should be made having in consideration the interaction between the starch and the fruit epidermis.

Spatial distribution of Esca disease in Italian and Portuguese commercial vineyards

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Esca is a multiple fungal syndrome which can cause serious economic damages in different cultivation areas. The aim of this study was to describe the epidemiology of Esca in three commercial vineyards located in Alentejo (Portugal) and in Marche region (Italy). The visual inspections were carried out in late summer 2017. The incidence of Esca disease was estimated about 10–30% in Alentejo, whereas 9.6% and 40% has been assessed in the vineyards located in Marche region. In these last ones, maps describing severity and spread of the disease within the vineyards were elaborated.

The severity of symptoms varied significantly from sporadic leaves that showed "tiger-stripes" up to the apoplectic stroke, represented by the entire dried plant. Longitudinal cracks of trunk and presence of typical basidiocarps of *Fomitiporia mediterranea*, an agent of Esca, have also been found.

Data of severity of symptoms (z) were defined with respect to number of rows (x) and position within the rows (y), allowing to reproduce a spatial distribution of infected plants in the vineyard using SYSTAT v.13 software (Systat Software Inc.). Contours were followed using the method of Lodwick & Whittle (1970) combined with linear interpolation. PASSaGE software v.2 allowed to examine the aggregation of adjacent vines and more complex spatial relationships over longer distances.

The data processing made possible to verify that 1) in no vineyard the symptomatic plants occurred in aggregated form; 2) none of the vineyards showed a gradient of infection among the rows; 3) there was a west-east gradient of infection in the vineyard OS1, and an east-west gradient in the vineyard CAST. Furthermore, in the vineyard OS2 a greater concentration of disease in the centre than the edges was observed.

The obtained result is a valuable information that will contribute to better understanding the epidemiology of Esca, still particularly damaging and difficult to control.

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