

III Encontro de Estudantes de Doutorado em Ambiente e Agricultura

III PhD Students Meeting in Environmental and Agriculture

29th and 30th November 2018

Book of Abstracts

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Dear participants,

It is our great pleasure to welcome you to the **III Encontro de Estudantes de Doutorado em Ambiente e Agricultura / PhD Students Meeting in Environmental and Agriculture**, held in Évora on the 29th and 30th November 2018 and organised by ICAAM and IIFA from the Universidade de Évora. We have put together a two-day program combining communications by both well-established and junior scientists, organised in a way to encourage discussion and exchange of ideas and to explore new challenges in research regarding Environmental and Agricultural Sciences.

The meeting program comprises five sessions, namely **Different Approaches Applied to Environment and to Agriculture, Olive Grove, Vineyard and Other Cultures, Mediterranean Agro-Silvo-Pastoral Systems, Biodiversity and Ecosystems Functioning and Animal Production and Health**. Each session begins with two plenary lectures followed by several short talks selected from the abstracts submitted by the PhD students. Furthermore, several poster presentations will be displayed throughout the meeting. The program also includes a field trip entitled “As aves do montado e ribeira da Herdade da Mitra”.

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 authors who have contributed to the program of this meeting and 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!

The Organising Committee,

Marta Laranjo, ICAAM

Ana Alexandre, ICAAM

Teresa Pinto Correia, ICAAM

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Program

Tuesday, 29th November 2018

09:00	Registration
09:45	Opening Session
	Teresa Pinto Correia Diretora do Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)
	António Candeias Diretor do Instituto de Investigação e Formação Avançada (IIFA)

Different Approaches Applied to Environment and to Agriculture

Chair: Manuel Mota
Universidade de Évora – ICAAM, IHC

10:15 **Alergia e Ambiente: uma abordagem multi-disciplinar no estudo da doença alérgica respiratória sazonal**

Célia Antunes
Universidade de Évora - ICAAM, ICT

10:55 **O potencial desconhecido da cultura do tremço. Blad e deflamina**

Ricardo Boavida Ferreira
Instituto Superior de Agronomia - LEAF

11:35 Coffee break and poster session

Presentations selected form the submitted abstracts

12:00 **Atmospheric pollutants NO₂ and O₃ contribute to augmented allergenic activity of grass pollen**

Ana Galveias

12:15 **Time series analysis as a tool to study the behavior of airborne *Platanus* pollen during a 11-year period**

Beatriz Lara

12:30 **Establishment and standardization of soil enzymatic activity assays at Soil Microbiology Laboratory**

Taiana Conceição

12:45 **Effect of biological enrichment of compost and its storage period on the biological control of *Sclerotium rolfsii* in turfgrass (*Agrotis stolonifera* L.)**

Luísa Coelho

13:00 Lunch

13:45 Field trip (organized by LabOr)

As aves do montado e ribeira da Herdade da Mitra

14:30	Microbiological evaluation and physico-chemical and sensory characterization of cheese produced in Cape Verde Elyane Borges Dias
14:45	Effect of species, seasonal variation, and addition of transglutaminase and glucomannan fiber on the physical properties and sensory evaluation of fish hams prepared with gilthead sea bream, sea bass, and meagre Ana Teresa Ribeiro

Olive Grove, Vineyard and Other Cultures

	Chair: Augusto Peixe Universidade de Évora - ICAAM
15:05	Vírus de plantas: de agentes causadores de doença a protetores de plantas Maria do Rosário Félix Universidade de Évora - ICAAM
15:45	Cromatografia de alta resolução: Métodos analíticos para análise vestigial Marco Richter Gomes da Silva Universidade Nova de Lisboa - LAQV-requimte
16:25	Coffee break and poster session
	Presentations selected form the submitted abstracts
16:45	Study of Portuguese extra virgin olive oils according to the producing agricultural method: A qualitative and ecological approach Miguel Ferro
17:00	Evaluation of anthocyanin profile during ripening in <i>Vitis vinifera</i> red wine grape varieties grown in Alentejo Maria Inês Rouxinol
17:15	Mycorrhization of <i>Cistus</i> spp. with <i>Terfezia arenaria</i> (Moris) Trappe and its application in desert truffle cultivation Rogério Louro
17:30	Sustentabilidade caminho sem volta André Ramalho Flores Consultor técnico na área da Sementeira Direta
20:30	Meeting dinner at the restaurant "Cozinha do Cardeal"

Friday, 30th November de 2018

Mediterranean Agro-Silvo-Pastoral Systems	
	Chair: João Rabaça Universidade de Évora, ICAAM
09:00	Tecnologias na monitorização do montado: uma abordagem global sobre o ECO-SPAA João Serrano Universidade de Évora - ICAAM
09:40	Agro-Silvo-Pastorícia: Inovação com Tradição José Mira Potes Instituto Politécnico de Santarém – Escola Superior Agrária
10:20	Coffee break and poster session
Presentations selected form the submitted abstracts	
10:50	Visions and strategies for water management in agriculture: a transdisciplinary experience in the Alentejo Taiana Homobono
11:05	Climate change in the development of a model of occurrence of forest fires: determination of trend series and critical periods Leonel Nunes
11:20	Evaluation of the interactions soil - pasture - trees and animals in Montado ecosystem Emanuel Carreira

Biodiversity and Ecosystems Functioning	
	Chair: Anabela Belo Universidade de Évora - ICAAM
11:40	Estradas e biodiversidade. Coexistir ou não coexistir, eis a questão António Mira Universidade de Évora – CIBIO-InBIO, ICAAM
12:20	Bem-me-quer, mal-me-quer: uma história sobre cegonhas e linhas eléctricas Francisco Moreira Universidade do Porto– CIBIO-InBIO
13:00	Lunch

Presentations selected form the submitted abstracts	
14:05	Floristic analysis of four communities of <i>Prunus lusitanica</i> L. from Europe and North Africa Mauro Raposo
14:20	Effect of Alqueva Dam on terrestrial invertebrates communities: threat or opportunity Rui Raimundo
14:35	Accounting for connectivity uncertainties in predicting roadkills: a comparative approach between path selection functions and habitat suitability models Francesco Valerio
14:50	Regime of fire in Portugal: assumptions and facts Nuno Guiomar

Animal Production and Health	
	Chair: Catarina Lavrador Universidade de Évora - ICAAM
15:10	O papel do útero na sobrevivência e desenvolvimento do concepto Rita Payan Carreira Universidade de Évora, CECAV
15:50	Coffee break and poster session
16:05	Doença hemorrágica viral em coelhos bravos Conceição Peleteiro Universidade de Lisboa-FMV, CIISA
Presentations selected form the submitted abstracts	
16:45	Characterization of the Mertolenga Breed phenotypes under the influence of the CAP in the period between 1986 and 2012 Luís Santa Maria
17:00	Evaluation of elastase and cathepsin G inhibition on collagen transcription and prostaglandin secretion in mare endometrium Ana Amaral
17:15	Assessing pubertal age of male Bísaro pigs through testicular and epididymal morphometric parameters - preliminary results Gustavo Paixão
17:30	Environmental conditions in pigs facilities located in Southern Europe Teresa Morgado
17:45	Closing Session Including Best Poster Prize

Different Approaches Applied to Environment and to Agriculture

Allergy and Environment: a multidisciplinary approach in the study of seasonal respiratory allergic diseases

Célia M. Antunes (cmma@uevora.pt)

Department of Chemistry, School of Sciences and Technology, University of Évora

Institute of Earth Sciences, University of Évora

The burden of pollen allergies is increasing worldwide. Due to its environmental etiologic nature, the disease management in a changing environment has proven to be challenging. The Laboratory of Applied Biochemistry and Aerobiology is devoted to a multidisciplinary approach for the study of environmental factors involved in pollen allergy elicitation and aggravation. Our main goal is to contribute to a better understanding of this complex problem in the pursuing of the development of mitigation strategies.

In this context, a wide range of methodologies for the study of airborne pollen and allergens, the effect of air pollutants on pollen physiology and allergenicity, as well as the consequent impacts for human health are undertaken.

So far, our results have contributed to: the clarification of airborne pollen and allergen loads, distribution and their transport in the atmosphere in our region; the identification of new allergenic species and cross-reactivity patterns among allergens and its relevance for symptomatology; and, finally, to the development of a PolenAlert system aiming to contribute to a better management of seasonal respiratory allergic disease.

O potencial desconhecido da cultura do tremço. Blad e deflamina

Ricardo Boavida Ferreira

Instituto Superior de Agronomia – LEAF, Universidade de Lisboa

No que diz respeito à sua utilização na nutrição humana, a cultura do tremço ‘atrasou-se’ relativamente à da soja porque se encontra naturalmente adaptada a condições Mediterrânicas, caracterizadas por um clima quase semi-desértico. Nestas condições, em que alimento e hospedeiros escasseiam, as plantas têm de investir muito potencial metabólico em defesa para conseguir sobreviver. A presença de alcaloides tóxicos foi durante muito tempo um grande entrave à utilização generalizada dos tremços na alimentação do homem e dos animais, embora estas sementes contenham também algumas proteínas com interesse terapêutico para o homem.

Utilizando técnicas de melhoramento clássicas, os investigadores alemães foram os primeiros a produzir, há já várias décadas, semente doces (*i.e.* com teores residuais de alcaloides) de tremço, o que possibilitou a introdução desta leguminosa em todos os setores da alimentação humana e animal. Há mesmo alguns países que utilizam o tremço numa ampla gama de produtos alimentares, desde comida para bebés até à piscicultura.

Fatores de natureza política e económica têm condicionado e mesmo restringido a cultura de tremço na bacia Mediterrânica, onde os tremçoceiros crescem espontaneamente e se encontram, por isso, muito bem adaptados. É, pois, estranho é que a proteína animal ingerida pelos Europeus seja essencialmente conseguida à custa da soja, na sua grande maioria transgénica e importada do continente Americano.

Ao reduzir as importações massivas de soja do continente Americano, o cultivo generalizado do tremçoceiro na bacia Mediterrânica contribuiria para

- Uma balança comercial mais favorável na UE;
- Uma fonte de rendimento para os agricultores das regiões mais pobres da UE;
- Uma agricultura sustentável na bacia Mediterrânica;
- Uma fonte de proteínas edíveis bioativas para a saúde do homem e das plantas.

Por outro lado, a grande euforia que existe a nível mundial relativamente aos compostos naturais bioativos, assenta basicamente em metabolitos secundários que, se ingeridos fora de uma alimentação equilibrada e variada, exibem efeitos muito tóxicos, de que são exemplos os óleos essenciais, polifenóis antioxidantes, alcaloides e muitos péptidos.

Contrariamente à maioria das outras espécies, o tremço é fonte de três proteínas edíveis bioativas, cuja ação não se torna tóxica para o homem se ingerido em grande quantidade. São todas relacionadas com as principais proteínas de reserva da semente, as α -, β -, γ - e δ -conglutinas. A própria γ -conglutina tem sido estudada por investigadores Italianos pela sua bioatividade a nível da diabetes. O BCO e a deflamina foram descobertas no nosso grupo.

O **BCO** (do Inglês *Blad-Containing Oligomer*) é um oligómero de polipéptidos derivados da β -conglutina, comestível, de 210 kDa, que se acumula nos cotilédones de plântulas de *Lupinus* entre os dias 4 e 14 após o início da germinação. Descobrimos, em 1991, o seu principal

polipéptido, a **Blad** (20,4 kDa), com a qual temos trabalhado desde então e continuamos a descobrir coisas novas.

A Blad tem uma atividade antifúngica muito potente contra todos os fungos testados nos últimos 20 anos (incluindo fungos patogénicos de plantas e do homem, *food spoiling* e *food poisoning*), atividade bactericida contra bactérias Gram- e Gram+ e atividade bioestimulante potente nas plantas. A sua atividade é igual ou superior à dos melhores fungicidas químicos atualmente disponíveis. A Blad está já à venda nos E.U.A. (sob o nome comercial Fracture™, encontra-se incluída na FRAC Code List® de 2018 com o código BM01 e foi recentemente aprovada para utilização em agricultura biológica pela OMRI) e Canadá e, durante 2019 e 2020, ficará comercialmente disponível num número de outros países, incluindo a Europa, Austrália, China, Coreia do Norte, México, Japão, etc. Temos presentemente mais de 40 patentes internacionais sobre a Blad.

A **deflamina** é um oligómero de polipéptidos derivados da β - e da δ -conglutinas, comestível, presente nas sementes secas de sementes de *Lupinus* (e, em menor grau, também nas sementes de algumas outras leguminosas e não só), que apresenta potentes atividades anti-inflamatórias e anticancerígenas. A sua bioatividade resiste à ação de proteases, a baixos valores de pH, a temperaturas elevadas (pelo menos 180 °C, o que significa que resiste à cozedura e ao fabrico de biscoitos) e ao processo digestivo. Foi testada em modelos de doença (ratos), bem como em coelhos. Estamos presentemente a realizar experiências com tumores humanos em peixes-zebra (como modelos de doença) e estudos de nutrição em leitões desmamados (como modelos animais de digestão). Temos em preparação testes clínicos. Foi recentemente submetido um pedido de patente internacional.

Atmospheric Pollutants NO₂ And O₃ Contribute To Augmented Allergenic Activity Of Grass Pollen

Ana Galveias¹, Sara Mendes¹, Rute Arriegas¹, Helena Ribeiro², Ana R Costa¹, Célia M Antunes¹, Ilda Noronha²

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Superoxide dismutase (SOD), a known enzyme in several species and recently described as a grass allergen, that catalyzes the dismutation of toxic superoxide radicals to molecular oxygen and hydrogen peroxide, working against oxidative stress in plants. Airborne pollen during its transport become in contact with a variety of atmospheric chemicals, including common air pollutants O₃ and NO₂. Besides, their direct deleterious action in respiratory air ways, exposure to these pollutants, both independently or in combination, may change the pollen biochemical properties thus its allergenicity. Nonetheless, species-specific action of these pollutants or their synergic effects in pollen are essentially unknown. The aim of this research was to study the effects of O₃, NO₂ and the mixture of both pollutants on SOD activity of *Dactylis glomerata* pollen, a proposed grass allergen. Pollen samples were in vitro exposed to O₃, NO₂ and O₃+NO₂ in an environmental chamber system during 6hrs and concentrations around the ambient levels. A blank pollen sample, subjected to the same procedure but not exposed to the gases was used as control. Pollen extracts from unexposed (Control) or exposed to pollutants were prepared in phosphate buffered saline (PBS) and frozen until analyses. Total SOD activity was measured following riboflavin-induced reduction of nitro blue tetrazolium (NBT) for 40 min, and the absorbance (A) was measured at 560 nm every 5 min, triggered with cool white fluorescent light and enzymatic activity (U/mg protein) was determined. Pollen exposure to O₃ alone did not significantly affect SOD activity while exposure to NO₂ induced a 50% SOD activity increase (150±25%). When mixed together (O₃+NO₂) the SOD activity was higher (200±40%) compared to control and slightly but not significantly higher when compared to NO₂ alone. These results show that exposure to NO₂ induced a significant increase on SOD activity and suggest that, although O₃ alone did not induce any change, it may exert a synergic action on the effect of NO₂, contributing to amplify the response. These results suggest that NO₂ stimulates pollen antioxidant defenses while O₃ does not, characterized at least by an augmented SOD activity, an allergen. Consequently, pollen exposure to NO₂ can induce an increase in the allergenicity and directly correlated with the enhancement of respiratory diseases.

The work is co-funded by the European Union through the European Regional Development Fund, included in the COMPETE 2020 (Operational Program Competitiveness and Internationalization) through the ICT project (UID/GEO/04683/2013) with the reference POCI-01-0145-FEDER-007690 and by National Funds through FCT for the project PTDC/ATP-EAM/0817/2014.

Time series analysis as a tool to study the behavior of airborne *Platanus* pollen during a 11-year period

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Platanus pollen is an important cause of hay fever in many Spanish cities where plane trees (*Platanus orientalis* L. var. *acerifolia* Dryand in Aiton) are widely used as an ornamental species in parks, gardens and urban green areas. The aim of this study is to analyze the *Platanus* pollen time series by a commonly used method for time series analysis as a seasonal-trend decomposition procedure based on LOESS smoothing which aids to isolate the three different components (seasonal, trend and residual) to be studied in an independent way. In this study airborne *Platanus* pollen was monitored throughout the period 2005-2015 using a Hirst volumetric spore trap located on the roof of a building belonging to the University of Castilla-La Mancha (Spain), on the Science & Technology campus in Toledo.

The *Platanus* pollen season in the city of Toledo started in the latter half of March and is characterized by an intense and explosive pollination, leading to high airborne pollen concentrations over a short period of time, exactly most of the pollen season occur during a 40-day period (on average 95% of the annual pollen is recorded over that period). The daily maximum concentration tended to be recorded in late March or early April and a trend toward rising the pollen concentration on the peak days has been observed in our data, thus increasing the number of days with allergy risk in central Spain.

Also, time series clearly showed a 3-year cyclical pattern, in which two years of high pollen intensity were followed by a third year of lower pollen intensity likely linked to management techniques such as periodical pruning of plane trees in urban green areas. This information is crucial to generate prediction models as a tool for evaluating allergy risk to *Platanus* pollen. Models for forecasting were constructed combining the cyclical behavior of the pollen (seasonality of the time series) with the modelling of short-term fluctuations in airborne pollen concentrations prompted by daily changes in meteorological variables and pollen concentrations over the previous days. A model based on the 3-year cyclical pattern and predictive variables over a prior period 7-9 days accounted for 70% of the variance of the daily pollen concentration. In this case, calibration models based on a 3-year period are more stable and enable the use of longer prior time periods than those obtained with a 1-year period.

Establishment and standardization of soil enzymatic activity assays at Soil Microbiology Laboratory.

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By catalyzing many vital reactions necessary for the life processes of soil microorganisms, soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system. Soil enzymes have been extensively studied and a variety of methods were developed to measure their activity over the years, as they provide important information about the ecological and functional status of the soil microorganisms acting as "biological fingerprints" of soil microorganisms. The adjustment of techniques for the quantification of dehydrogenase (EC 1.1.1.), arylsulfatase (EC 3.1.6.1), β -glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), L-glutaminase (EC 3.5.1.2) and L-asparaginase (EC 3.5.1.1) activities have been performed at Soil Microbiology Laboratory, to establish a routine with standard procedures that can be applied in several contexts. Different scale protocols are being tested, the traditional bench scale and the microplate scale. To test the bench scale protocols under the laboratory conditions a soil with intentionally induced different microbial activities was studied. One treatment consisted of soil supplemented with a solution of sucrose, ammonia sulphate and potassium phosphate (10:1:1) and incubated for 48h/37°C, to increase biological activity. A second treatment consisted of a three series of soil sterilization (24h each) to completely inactivate biological activity, and a third treatment consisted in the soil as it was after field collection. All treatments were moisture to 70% of water holding capacity either with the nutrient solution or with sterile water for the two last treatments. The enzymatic assays in doped soil showed a significantly higher values than fresh soil, and for sterilized soil the values were near zero. So far, the results have shown that the bench scale protocols used reflect the differences imposed in the tested soil treatments and can reliably be used in laboratory routine for dehydrogenase, arylsulfatase, β -glucosidase and acid phosphatase activity evaluation. Though amidohydrolases (L-glutaminase and L-asparaginase) need more tests to achieve a standard procedure, because the two different bench scale protocols used have shown discrepancies of pattern recovery. The microplate format will also be tested as it allows the simultaneous analysis of multiple enzymes in microliter volumes, with reduce reagent costs and assay time.

Effect of biological enrichment of compost and its storage period on the biological control of *Sclerotium rolfsii* in turfgrass (*Agrostis stolonifera* L.)

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The suppressive capacity of composts against soil diseases may be caused by their microbiota activity. Compost enrichment with antagonistic organisms is a strategy to increase its effectiveness, allowing a more effective biological control of soil diseases. However, compost storage may affect its suppressive capacity, depending on compost and the soil disease.

Turfgrass (*Agrostis stolonifera* L.) are susceptible to a variety of fungal diseases, being essential to find ecofriendly alternatives for their control. Organic composts can be an efficient alternative to control plant diseases, reducing pesticide use. In this study two composts from agroindustrial residues were tested, P1 and P2. From the end of the thermophilic phase until the end of the process, fungi from both composts were isolated and identified. In *in vitro* experiments, *Trichoderma atroviride* showed the best results on *Sclerotium rolfsii* control and, for this reason, it was used in compost enrichment. Samples of both composts and peat (control) were enriched with *T. atroviride* suspension with 1.33×10^6 conidia mL⁻¹, in order to obtain 9.0×10^7 conidia L⁻¹ of the substrates. Samples of peat and composts (enriched and not enriched) were stored, and their suppressive capacity was tested after two weeks and after six months of storage. Turfgrass was sown in 100 mL pots, with the substrates: peat (T), composts P1 and P2, and the same substrates submitted to a thermic treatment of 60 °C (t) during seven days: Tt, P1t and P2t, respectively. The same substrates were enriched (E) with *T. atroviride*: TE, P1E, P2E, TTE, P1TE and P2TE, making a total of 12 treatments, with five pots and four replicates each. When turfgrass covered the surface of the pots, each pot was inoculated with *S. rolfsii*, by placing a PDA disk with the pathogenic.

In substrates stored for two weeks, it was verified that *T. atroviride* increased composts suppressive capacity. After six months of storage, P1, P2, P1E and P2E maintained their suppressive capacity, but the severity of the disease increased in P1TE and P2TE.

Considering these previous results, it will be important to evaluate composts suppressive capacity, with and without antagonist enrichment, after 1 year of storage.

Microbiological evaluation and physico-chemical and sensory characterization of cheese produced in Cape Verde

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The objective of this study is to establish the microbiological, chemical and sensorial characterization of the cheese of Cape Verde in order to commercialize them and implement the protected designation of origin (PDO) system for this excellent food product. The genuineness and typicality of the product, ensures its protection, enable fraud detection (through possible forgery) and also contributes to increasing the competitiveness of small-scale artisanal cheese production units. In this way it is intended to associate this product with the development and recognition of the region. There are no studies related to the microbiological, chemical and sensorial characterization of these cheese, so they do not have certification of Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI), negatively affecting their economic value. Thus, the exponential growth of the annual production, the total lack of knowledge of its properties and characteristics and the chemical composition, justify that studies are directed towards contributing to a more in-depth knowledge that allows us to make the production of the cheese profitable and the PDO certification and IGP. The project aims to provide the Cape Verde Region with powerful tools to progressively achieve a unique position in the export market under competitive conditions and on the other hand to provide Cape Verde's cheese with structures to implement the protected designation of origin system (PDO). This competition requires product quality, supported by modern technologies and high sensitivity, evaluated according to adequate indexes and compatible with the practices and techniques used. On the other hand, to guarantee the genuineness of the cheese produced in Cape Verde, it is necessary to know the product scientifically through microbiological, chemical and sensorial evaluation, allowing a more efficient control and the detection of possible adulterations. To this end, the objectives of the proposed work are as follows: To evaluate the microbiological quality of cheese produced in Cape Verde; Determine the physical-chemical parameters that define the quality of the cheese; Proceed to the sensorial analysis as an efficient approach in the identification and establishment of sensory markers of cheese; Establish the genetic profile of cheese as a way to guarantee its authenticity, typicality and protection of the product.

Effect of species, seasonal variation, and addition of transglutaminase and glucomannan fiber on the physical properties and sensory evaluation of fish hams prepared with gilthead sea bream, sea bass, and meagre

Ribeiro, A.T.^{1,2,3}; Elias, M.^{3,4}; Teixeira, B.²; Vieira, H.²; Mendes, R.²

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The increasing consumer demand for healthier products is driving the development of novel fish-based products, due to the well-known benefits of fish consumption. Within the variety of traditional meat products in market places, cooked ham is particularly appropriated, on account of its broad public acceptance.

This work aimed to study the influence of fish species, capture season, and addition of microbial transglutaminase (MTGase) and glucomannan dietary fiber from konjac (*Amorphophallus konjac*; KGM) in the preparation of fish hams and its effects on the physical properties and sensory evaluation.

Sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), and meagre (*Argyrosomus regius*), captured in the summer and winter seasons, were used individually to prepare hams. Different formulations varying the amount of MTGase (0-0.5 % MTGase) and KGM (0-1% KGM) were done. Color, water holding capacity, pH, texture (puncture, compression, and compression relaxation tests), and sensory analysis were determined in the different fish hams.

Results showed that in terms of species used for the preparation of fish hams, hams prepared with meagre had higher water holding capacity and the textural properties were closer to those of traditional hams prepared with pork. The capture season also affected several properties of fish hams, with a minor importance, and it was dependent on the species. The addition of MTGase and KGM in the preparation of fish hams resulted in hams with textural properties closer to those of pork hams. However, the use of KGM compromised the visual appearance of fish hams. In general, fish hams were well accepted by the sensory panelists.

This study showed that the fish hams produced have potential as novel fish-based products. In particular for each species, fish hams prepared with gilthead sea bream captured during winter or with sea bass captured in the summer, both prepared with 0.5 % MTGase and 1% KGM, and those with meagre captured in the winter with 0.5 % MTGase (without KGM) exhibited the most comparable physical properties to a pork ham.

Characterization of compounds with biological activity of *Opuntia ficus indica*

I. Miller

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The species *Opuntia ficus indica* (L.) Miller is native to Mexico, found widely distributed throughout Central America, South America, Australia, South Africa and in Mediterranean countries. The taxonomic classification of the genus *Opuntia* is complex, since many of the classification systems present errors in the concepts of genus and species, which ends up contributing to the high synonymy. This is an active genus of evolution and characterization, contributing to erroneous classifications, variation among species induced by genetic differences resulting from the influence of environmental factors, is perceptible for example in differences in cladodes size, flower color and length of leaf thorns. The *Opuntia* species has high importance in the agricultural economy, since it is a valuable resource, especially in periods of drought. In Portugal it is sub spontaneous in hedges and weavings, being cultivated for the formation of artificial hedges. From the morphological point of view, this can be divided into root, vegetative part, fruit and flower. The vegetative part corresponds to the cladodes, which consist of modified stalks, which replace the leaves in their photosynthetic function. These have an ovoid shape about 18-25 cm in length, and in addition to their photosynthetic function, also serve as storage water. However, the fruit has gained notoriety due to its nutritional benefits and different pharmacological actions, being rich in bioactive antioxidant compounds such as betalainas, polyphenols and ascorbic acid. Its extracts have demonstrated different activities, such as ant ulcerative, antioxidant, anticancer, neuroprotective, hepatoprotective and antiproliferative activities, thus justifying the theme of the following dissertation, since its purpose is to characterize the chemical composition and evaluate some nutritional and biological properties of the fruits (orange, red and green), cladodes, seed oil and "pie" (seed residue after oil extraction) of fig, aiming to contribute to the transfer of knowledge about its nutraceutical properties and, consequent appreciation and proper use of figs and its derivatives, and also contributing to the establishment of new criteria for the certification of marketed products.

COMPARATIVE STUDY OF ATMOSPHERIC CONCENTRATIONS OF POLLEN GRAINS OF AMARANTACEAE AND SAL K1 PROTEIN IN ALCÁZAR DE SAN JUAN AREA (CIUDAD REAL, SPAIN)

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Introduction: Previous studies have shown discrepancies between the pollination period and the allergic symptoms of patients by inhalation of Amaranthaceae pollen. This is attributed to the presence of non-aerovant grains, which are not detectable with pollen traps. *Chenopodium Album* and *Salsola kali* are the most representative species of this family from the allergological point of view and Sal k1 is the most representative allergen from *Salsola kali* pollen. The objective of this work was to analyze the correlation between the atmospheric concentrations of Amaranthaceae pollen grains and the allergen Sal k1 during 2015, 2016 and 2017.

Material and Methods: Pollen samples were taken with a volumetric collector type Hirst (Burkard®) and bioaerosols with a Burkard type Cyclon® sensor. The levels of Sal k1 were determined from the samples of bioaerosols with the ELISA-sandwich immunoassay method. **Results:** The sum of the daily concentrations of pollen grains was 371 g/m³ in 2015, 291 g/m³ in 2016 and 369 g/m³ in 2017. The highest concentrations of pollen grains were in August-September, with a peak day on September 7 in 2015 (35 grains/m³), on August 20 in 2016 (30 g/m³) and on August 19 in 2017 (19 g/m³). The highest concentration of Sal k1 was on August 18 (17.5 ng/ml) in 2015, on August 14 (20.1 ng/ml) in 2016 and on August 12 (16.8 ng/ml) in 2017. The relationship between the daily pollen concentrations of Amaranthaceae and the concentration of the Sal k1 allergen (Spearman statistical test) has resulted significant and with a positive correlation, with a coefficient of determination of 0.581 for 2015, 0.691 for 2016 and 0.397 for 2017.

Conclusions: The main period of pollination of Amaranthaceae comprises from May to October, with the existence of two pollen peaks: one in May-June and another in August-September. However, the peak of pollen in atmospheric air detected in spring does not correspond to high levels of Sal k1. Probably, in this area, the flowering of the species of Amaranthaceae in these months correspond mostly to other species such as *Chenopodium album* and the full flowering of *Salsola kali* occurs at the end of summer.

The adherence of North Alentejo population to Mediterranean Patterns: focus on the consumption of polyphenol-rich products and its relationship with saliva composition

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Adherence to Mediterranean Diet (MD) is being lost in most of the countries of the Mediterranean, namely Spain, Italy or Greece. Although Portugal being geographically not in the Mediterranean basin, an ancestral influence from the neighbor countries resulted in MD as culturally settled. Similar to other countries, also in Portugal, a shift in dietary habits has also been observed in the last decades. One of the characteristics of Mediterranean dietary patterns is the consumption of non-processed vegetable based products, and consequently to the consumption of high amounts of antioxidants, among which polyphenols. Despite of what has been referred above, in nowadays, a characterization of MD adherence in Portuguese population is missing. Moreover, even when dietary habits are characterized, the consumption of antioxidants is missing, what represents a limitation, since these compounds have particularly important health effects.

The major aim of the present study was to characterize the dietary patterns of the population of North Alentejo, searching for the level of adherence of this population to MD and for the levels of polyphenols intake. Moreover, and since the consumption of particular types of polyphenols are considerably linked to salivary proteome, an intervention study was performed, where the effect of polyphenols consumption, in saliva protein profile, was assessed.

In terms of MD adherence, it was possible to observe that only a small percentage of North Alentejo population (around 19%) presents a high score of adherence to MD. Even so, by comparing with reports from other populations, it is not a very low rate. Concerning the intervention study, it was possible to observe changes in salivary protein profile after the consumption of extra dose of polyphenol-rich foods. These changes were in the levels of salivary proteins that may also be related with oral food perception, suggesting that polyphenol consumption habits may influence further acceptance of foods.

These results will be detailed and discussed in the proposed presentation.

Unveiling food consumption patterns of rural dwellers in Europe – a critical approach to social innovation

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Abstract: The concept of social innovation has increased its popularity in current developmental research in rural studies in Europe, as it appears to respond to the various social and economic disparities faced by a fragile rural population and the confronting dynamics in the way we produce and consume food. Social innovation is a contested concept among policymakers, advocates and critics; however, it is a useful approach to the set of new ideas (products, services and models) aimed at meeting social needs and creating new relationships and collaborations to tackle rural issues. The objective of this thesis paper is to develop a suitable theoretical framework to sketch a methodology for mixed methods data collection to unveil the food consumption patterns by rural dwellers in selected regions across Europe. Assuming new forms of reconnecting producers and consumers are inspired from innovative food system models, the paper elaborates on the existing literature about social innovation and alternative food networks, as well as on debates on food security. This PhD chapter paper carries out a literature review on the topic from academic research and empirical work and identifies eight variables to be included in the following stage of the PhD work in the data collection phase. Main results from the research highlight the importance of alternative local food systems in shortening the linkages between producers and consumers, as strategies to address the environmental, structural and food quality issues derived from the current global food regime. However, a holistic understanding of complex local food systems must move beyond the 'productivist' trap of alternative food systems and the focus on market differentiation, plus acknowledge innovations' multifunctional nature and reflect on the degree of embeddedness of actors in these new designed food systems.

Keywords: social innovation, alternative food networks, food relocalization, food access, food availability

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Texture of Cachena meat.

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The meat quality, mainly tenderness, was affected by complex interactions of multiple antemortem and postmortem factors such as animal genetics, feeding, handling, slaughter process. Genetics determines an animal's potential for producing tender meat, and its interaction with ante- and postmortem environment and also management will determine the ultimate tenderness of the meat from each animal.

Cachena is a cattle breeds, part of the Portuguese genetic heritage, with high interest for the south Alentejo region due to the high rusticity of these animals. Animals are small and the meat is known by its excellent characteristics of texture and flavour. The determination of the factors that affect meat tenderness of Cachena is of extreme importance for the producers and for the industry. The goal of GOCACHENA project is understanding the relation among genetics, feeding, age and weight of animals, with the quality, mainly tenderness, of this exquisite meat. This research work is part of the refereed project and obtained on preliminary results useful for the persecution of the main goals.

The modalities of this trial are different due to the preparation of the meat of *Long dorsal*: sliced (aging 2, 5, 8 days) and entire pieces (aging 2, 8, 10 days). The animals were males, with slaughter weight from 165 to 225 kg, and age between 6 and 13 months. The texture was analyzed trough Texture Profile Analyze (TPA) using a Texture Analyzer TA.HD.Plus (©Stable MycroSystem). From each animal two parts of the *Long dorsal* muscles were separated and packed in bags each one with two samples and the measures were done in triplicate in each slice of each sample. The samples were prepared according to Fabre et al (2018) and Veiseth-Kent et (2018).

The results shown that there aren't significant differences for Tenderness, Springiness and Chewiness for sliced meat, however a slight decrease was noticeable. Considering the entire pieces, the only significant difference was found in the values of Springiness. The values of tenderness unexpectedly increase slightly.

The dramatic heterogeneity in the meat of animals may be caused by genetic factors. This statement confirms the necessity of deep studies like that of GOCACHENA

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Starch based edible coatings effectiveness in preventing fresh fruit phenolics degradation

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Starch-based edible coatings can enhance food quality, safety and stability. Unique advantages of edible coatings can lead to the development of new products, such as individual packaging for foods, carriers for various food additives, and nutrient supplements (M.A. García *et al* 2009)¹.

The extension of post-harvest life on fruits should consider challenges like extending senescence periods, reducing dehydration, the onset and microbial growth. Traditional preservation methods are focused on some of these challenges, like creating synthetic film packaging or antimicrobial treatments (A. El Gaouth, *et al* 1991)².

Phenolic compounds are responsible by the fruit's organoleptic properties, however despite their numerous beneficial proprieties, they are extremely sensitive and susceptible to degradation. Edible coatings have a high potential to extend the shelf life of fresh fruits.

The use of coatings made from natural products can be beneficial to prevent the degradation of phenolics and increase the fruits shelf life.

A sample of strawberries was tested with a modality with edible coating and one without, the study went for 8 days and samples from all days were analysed by an improved Folin- Ciocalteau method (data to be published).

The extraction for analysis was made by means of an ethanolic solution. And the results were expressed in

The results shown that, the fruits without coating there is a degradation on the phenolic content after the 2nd day of conservation. The fruits with coatings kept the phenolic content concentration stable. The same behaviour can also be observed in weight loss, in which fruits lost more weight, respiration rate was higher, and pH variation over time. The berries tested with coatings lost less weight, maintained pH level's thru time and decreased the respiration rate.

In sum, the use of edible coatings derived from potato starch can improve and enlarge the post-harvest life of fruits.

Future studies should have in mind new formulations and ways of application.

¹ María A. García, Adriana Pinotti, Miriam N. Martino, and Noemí E. Zaritzky (2009) Characterization of Starch and Composite Edible Films and Coatings. In: M.E. Embuscado and K.C. Huber (eds.), *Edible Films and Coatings for Food Applications*, 169–209

² El Gaouth A, Arul J, Ponnampalam R, Boulet M (1991) Chitosan coating effect on storability and quality of fresh strawberry. *J. Food Sci.* 56, 6, 1618 – 1620

Olive Grove, Vineyard and Other Cultures

Plant viruses: from causal agents of disease to plant protectors

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Traditionally, plant viruses are viewed as harmful, undesirable pathogens and those that affect olive trees are not an exception. The symptoms associated to this kind of agents in olive trees are, among others, chlorotic lesions, defoliation, deformation in fruits and leaves, and death of stems and trees. Viral diseases are not treatable and as such it is necessary to increase the knowledge on their epidemiology to improve sanitary rules and prevent infections in the field. In Portugal, high levels of viruses were detected, reaching up to 100%, in dozens of olive orchards sampled, being the most prevalent the necroviruses, from Alpha- and Beta-necrovirus genera. The molecular characterization of viral genomes and their replication strategies have been studied, and this has allowed the design and the improvement of molecular tools for olive viruses diagnosis. Apart from the harmful effects, viruses can provide several useful 'designer functions' or 'sequence modules' with which future vectors for general biotechnology may be developed. The study of viral genomes, particularly in the context of their pathogenic interactions with host plants, led us to the design of two types of vectors for plant protection use. One of those is based on virus-induced gene silencing (VIGS), using the natural plant gene silencing strategy to protect plants against viruses; and the other is based on the expression of antimicrobial peptides (AMPs), using the ability of virus genome replication to produce antibiotics and antifungal molecules to prevent diseases caused by bacteria and/or fungi. In both, a partial or complete gene is introduced in the vector and then the virus-related 'interfering' molecule is stably introduced into plants via the DNA-transfer mechanism of *Agrobacterium tumefaciens* or mechanical viral inoculation. The presence of these viral-vectors in plants confers them protection against specific diseases. The possibility to manipulate the plant-virus relationship is a powerful tool to increase and improve strategies for unconventional crop protection techniques.

High Resolution Chromatography. Method for target analysis

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Gas chromatography (GC) is a powerful analytical tool that can be applied to a variety of matrices in order to separate, detect and identify their constituents. Capillary gas chromatography was in the six decades used for complex matrices characterization as well as target analysis. New stationary phases, with broader application range, more robust and thus more versatile, together with developed column manufacturing procedures and instrumentation allow to enhance the resolution of the chromatographic systems, defining new productivity and throughput standards. Presently GC/MS is the most frequently used hyphenate technique for the characterization and identification of many volatile and semivolatile compounds. However, in spite of the continuous development of equipment, techniques and analytical methodologies, a total separation of all the sample components is still unachievable, due to the complexity of the samples and of the analysis (high number of components, structure similarities, isomers, and wide range of concentrations). High similarities are thus expectable between the retention times of several analytes, independently of the stationary phase used, that will result in coelutions. These coelutions are often impossible to detect and identify with some GC/MS instruments, in spite of the use of selective single ion monitoring or in tandem (SIM and MS/MS) mode or of complex deconvolution processes. An additional problem results from the wide range of analyte concentrations in their matrices. Consequently, the trace level analytes, that sometimes are the biologically active components in the matrix under study, may never be detected, if they are coeluting with high concentration compounds. Future developments are thus considered in order to mitigate these constraints.

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

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During the past decade, Portugal has significantly increased its olive production, competing for its place as a leading producer at international markets. Alentejo region, with a total exploited agricultural area of 2.100.762 ha (58% of the total national explored area), is by far the most dedicated Portuguese province for olive oil production. A total of 180.607 ha are exclusive devoted to olive production, with an input of 74% of the whole national olive oil production.

The replacement of traditional orchards by intensive and super-intensive ones represents a notorious developments 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 ecological EVOO's footprint is quite distinct according to the applied agricultural practices. Soil and water contaminations are among the most concerning factors when considering these new approaches, and they are mainly due to excessive application of agrochemicals.

Regarding the EVOO quality, to the best of our knowledge, no study has been reported associating agricultural practices to EVOO quality, either by fatty acid, phenolic or volatile characterization.

With this work, we intend to cross several information-related sources, such as the EVOO chemical characterization (fatty acid, hydrophilic phenolic and volatile profiles), the presence of chemical pollutants and pesticides in both EVOO and soil/water and its ecological impact, with distinct agricultural systems (organic, traditional, intensive and super-intensive). With this information, chemometric studies will be applied in order to create a tool to access the nutritional value and environmental impact of EVOOs production systems.

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Evaluation of anthocyanin profile during ripening in *Vitis vinifera* red wine grape varieties grown in Alentejo

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Anthocyanins are the natural compounds responsible for the red color in wines. They are the final products of a specific branch on the flavonoid pathway, meaning that they belong to the phenolics class. Their distinct color makes them extremely important for the fruit and its subproducts quality (namely red wine), enhancing the sensorial qualities. Also, it has been reported that anthocyanins (along with other phenolic compounds) have high antioxidant activity. Wine is widely consumed around the globe, and there is an increasing interest in its consumption due to its reported health benefits when consumed in moderation. In this study, four varieties (Syrah, Tempranillo, Touriga Nacional and Trincadeira) produced in Herdade da Mitra were monitored during version to understand the development of some phytonutrients and their respective antioxidant potential.

To allow these quantifications, phenolic compounds were extracted and evaluated. Total phenolic compounds content¹ and total flavonoids¹ were evaluated by UV/Vis spectroscopy. Anthocyanins separation, identification and quantification was determined by HPLC-DAD². Antioxidant activity of grape extracts was quantified using three different methods³ (DPPH method, total reducing power and β -carotene/linoleic acid system).

During ripening, Syrah, Touriga Nacional and Trincadeira presented an increasing of phenolic content, flavonoids and anthocyanins, on the other hand, Tempranillo presented a decrease in these compounds during verásion. Monoglucoside-anthocyanins have a higher content in grapes during all maturation process and Malvidin-3-O-glucoside is the most prevalent anthocyanin. Grape extracts have showed high antioxidant activity by all methods tested, highlighting the high ability of scavenging free radicals. Results showed a high correlation between total phenolic content and the antioxidant ability to scavenge free radicals.

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Mycorrhization of *Cistus* spp. with *Terfezia arenaria* (Moris) Trappe and its application in desert truffle cultivation

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Desert truffles are of considerable interest for ecological, agroforestry and commercial purposes. They represent a key component of the mycological flora in arid and semi-arid regions due to their important role as symbiotic partners of diverse host plants. Plus, their fruitbodies constitutes a potentially important food source, rich in proteins and poor in carbohydrates and lipids. Desert truffle increasing demand has recently boosted more research, aiming to achieve new strategies to enable their medium-large-scale cultivation, but so far only *Terfezia boudieri* and *Terfezia clavayi* were successfully cultivated together with *Helianthemum* spp. in basic soils.

T. arenaria is the most commercially valued desert truffle specie in central and southern Portugal. Usually, it is found in acid soils, establishing mycorrhizal associations with several *Cistaceae* plants. Since, *Cistus* species are the most important elements of Mediterranean scrublands in Alentejo (southern Portugal), the objectives of the proposed research were to expand the current knowledge about the mycorrhizal associations of *Cistus* spp. with *Terfezia arenaria* and develop strategies to allow its application towards desert truffle cultivation and the production of *Cistus* mycorrhized plants.

While working toward the proposed goals, we have repeatedly sampled numerous specimens of *Terfezia* throughout the Alentejo region and through molecular characterization of the collected specimens we were able to describe a new taxa, *Terfezia lusitanica* sp. nov. We also successfully developed novel micropropagation protocols for *Cistus salviifolius* and *C. ladanifer*, the two most common *Cistus* species in the region, which enabled us to effectively infect, *ex-vitro*, the root system of the abovementioned *Cistus* species and to obtain high mycorrhization rates in both cases. Recently, we were able to surpass the major challenge of the proposed research, the isolation of *Terfezia* spp in pure culture, and now we have collections of *T. arenaria* and *T. fanfani* mycelia growing in agar plates. In the next steps, we aim to develop an efficient *in-vitro* mycorrhization protocol using *Terfezia* mycelium, in order to, characterize the mycorrhizae formed between these two desert truffles and the abovementioned *Cistus* species and to evaluate which are the best plant-symbiont to enable *Terfezia* medium-large-scale cultivation in acidic situations.

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Olive flowering phenology variation of three traditional Portuguese olive varieties in Alentejo region

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In *Olea europaea* L. the beginning, the duration and the end of flowering are determined by genetic characteristics inherent to each variety and by the physiological response to meteorological factors of the year, mainly temperature.

During two consecutive campaigns (2017 and 2018), a study was carried out to collect information on the flowering phenology of the varieties 'Galega vulgar', 'Azeiteira' and 'Cobrançosa', in intensive olive grove on a farm located in a Portuguese town of Monforte.

For the campaign of 2018, the beginning of flowering (marked by stage BBCH 60) took place in a range of 3 days, between May 21th ('Galega', 'Azeiteira') and May 24th ('Cobrançosa'). The duration of the flowering period varied, depending on the variety, between 11 days ('Cobrançosa'), 14 days ('Galega') and 17 days ('Azeiteira').

Compared to 2017, in this campaign all varieties were in bloom much later (approximately 3 weeks for 'Cobrançosa' and 'Galega' and 4 weeks for 'Azeiteira'), and the flowering period was shorter (between 16 and 21 days in 2017).

The obtained results clearly demonstrated an inter-annual variability in the varieties under study, which is very important to cope with climate changing, presenting highly significant differences for all of the parameters when comparing the results of both campaigns. These results are crucial to the phenological characterization of these Portuguese olive varieties under study, however, to confirm its differentiated behaviour, it is necessary to repeat this phenological evaluation in further campaigns.

The experimental trial was financed by the EAFRD and the National Funds through the Regional Operational Program ALENTEJO 2020, Operation ALT20-03- 0145-FEDER- 000014 - "Valuation of Portuguese Olive Varieties (Oleavalor)".

Endophytic fungal communities of grapevine roots in Italian and Portuguese commercial vineyards afflicted by esca

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Amongst several pathogens reported in grapevine, the agents of esca represent one of the most destructive, causing serious economic problems in worldwide vineyards. The aim of this study was to detect difference in fungal endophytic population (species and quantity) with and without presence of esca pathogens. The survey was carried out from roots of 4 grapevine varieties: Verdicchio, Chardonnay, Trincaderia and Alicante Bouchet. The sampling was performed during summer 2017 from 6 commercial vineyards located in Alentejo (Portugal) and Marche region (Italy), selecting a total of 25 plants with and 25 plants with no symptoms of esca. A suitable method for external disinfection of vine roots not unsettling endophytes was developed and used successfully. Portions of disinfected roots were putted in growth in Petri dishes with Potato Dextrose Agar, and each single grown fungus was isolated. Isolates were grouped according with morphological characteristics, and species were identified by morphological characterization. The work allowed to isolate *in vitro* more than 160 fungal endophytes. The first results showed a sensitive difference (higher than 50%) of isolates between Verdicchio-Chardonnay and Trincaderia-Alicante Bouchet cvs. In Verdicchio and Chardonnay cvs, 9 species mainly identified as belonging to *Fusarium* spp. and *Trichoderma* spp. have been found as occurring in more than 26% of plants, and 5 species have been identified in more than 38% of plants. In Trincaderia and Alicante Bouchet cvs, 3 species (*Epicoccum* sp. and 2 unidentified spp.) have been detected only in asymptomatic plants. Amongst all the not found isolates in plants with symptoms, 13 of them occur as common in no symptomatic plants of same cv (eg.: *Phoma* sp., *Fusarium* sp., *Epicoccum* sp., *Chaetomium* sp., *Trichoderma* sp.). A specie, not identified as a common agent of esca, occurred only in plants that showed symptoms, deserving attention. A molecular characterization by ITS primers is currently underway. This first result allowed already to give a first contribute to further studies for biological control of esca.

Mediterranean Agro-Silvo-Pastoral Systems

Technologies for monitoring *montado* ecosystem (ECO-SPAA): a global approach

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Abstract

The main objective of this work was to present several proximal and remote sensors with potential to monitor relevant variables in the complex Mediterranean silvo-pastoral *montado* ecosystem (ECO-SPAA). The interest in using these two techniques in a complementary way can make an important contribution to:

- (i) Understanding the interaction between various factors and components that interrelate in this ecosystem (soil, pasture, trees and animals);
- (ii) The determination of key components of pasture productivity and quality;
- (iii) Support grazing management decisions; and
- (iv) Implementation of site-specific pasture management.

Agro-silvo-pastoral Systems: Inovation with Tradition

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A full characterisation of the Mediterranean environment is fundamental, in order to adequately frame agro-silvo-pastoral systems. Through the disaggregation of the agricultural, forestry and animal components, the respective productive subsystems are analysed. The integration needed for the good management of the overall system is performed through the *Montado* rotation and extensive livestock feeding scheme, finally focusing on the importance of the available environmental services. The Dynamics of the *Montado* will also be discussed.

Visions and strategies for water management in agriculture: a transdisciplinary experience in the Alentejo

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Agriculture faces the challenge of adapting to climate change and the context of natural resource crises while continuing to respond to global food needs. Thus, high ecological value agriculture, agro-ecological approaches and agro-silvo-pastoral systems can help to avoid damages to the ecosystems goods and services where they are developed. The complexity of these challenges requires the development of scientific approaches that promote the analysis of the agricultural sector in a holistic and inclusive way. The project proposes the implementation of a reflective and adaptive scientific production process, based on the principles of transdisciplinarity. The participatory approaches adopted put local actors as an active part in exploring the diversity of perspectives in understanding the water management role in agricultural sector decision making. This project is based on a case study in the municipality of Serpa, in the region of Alentejo (Portugal) and is supported by the international project DIVERCROP (<https://divercropblog.wordpress.com/>), which aims to analyze and understand productive systems in the western Mediterranean basin, at different scales. Through the transdisciplinary premises the projects aim to contribute to the construction of a systemic view of the relationship between water resources management and territorial development. The case study contextualization will be carried out through the socio-ecological systems framework proposed by Elinor Ostrom, which promotes a holistic understanding of complex issues such as the development of agricultural production in the current context. This characterization is based on the results of semi-structured interviews with key actors of the studied region, and will be complemented by a literature review. The results obtained in this stage will allow the design of the participatory approach stage, in which the Territory Game. This technique will be applied to identify the main perceptions about water changes and their effects on agricultural and land use activities, as well as, to the creation of a vision for the territory and the pathways needed to achieve the desirable future. To evaluate the transdisciplinary approach used, questionnaires will be applied to detect factors that can influence the actor's participation in the knowledge sharing. Two main results are expected at the end of the project: identification of measures adopted and challenges encountered regarding water management in the municipality studied, and to contribute to the promotion of transdisciplinarity in the management of natural resources.

KEY-WORDS: *water management; agriculture; transdisciplinarity; SES framework; Alentejo.*

Climate change in the development of a model of occurrence of forest fires: determination of trend series and critical periods

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Climate change is a phenomenon that could significantly affect the day-to-day activities of the rural communities, with particular emphasis on those directly dependent on the agricultural and forestry sector. In this way, the present work intends to base the impact that climate change has on forest risk assessment, namely on how the occurrence of rural fires is affecting the management of the forest areas and how the occurrence of these fires has evolved in the near past. Thus, a comparative analysis of the data provided by IPMA - Portuguese Institute of the Sea and the Atmosphere was carried out for the period from 2001 to 2017, with the climatic normal for the period between 1971 and 2000, for the variables of Average Air Temperature and for Precipitation. In this comparative study, the mean monthly values were considered and the months in which anomalies occurred were determined. Anomalies were considered in the months in which the Average Air Temperature varied more or less 1 degree than the value corresponding to the climatic normal, in at least 50% of the national territory. The same procedure was repeated for the variable Precipitation, counting as anomaly the occurrence of a variation in precipitation greater or less than 50%, also in 50% of the national territory. Then the calculation of the moving averages for cycles of 3, 5 and 7 periods was applied and the trend lines were projected. Subsequently, the relationship between the results obtained and the occurrence of rural fires was carried out, the spatial distribution of forest area, species and stand structure, was not analyzed in the present work. From the obtained results it was possible to confirm the existence of a tendency for the occurrence of climatic anomalies, highlighting the occurrence of an increasing number of months with temperatures higher by at least 1 degree. Even without it was also possible to foresee the co-relation between the occurrence of rural fires and the periods of anomaly and absence of precipitation. From the results obtained it is also possible to infer that, and analyzing the tendency for these phenomena to occur, it is necessary to change the "Critical Period of Rural Fires", since it is statistically verified that what is currently in force does not covers the entire period where anomalies occur and where large-scale rural fires can potentially happen.

Evaluation of the interactions soil - pasture - trees and animals in Montado ecosystem

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The work associated with ECO-SPAA, a project to be held at Herdade da Mitra (Évora University), have as main objective the monitoring of the Montado ecosystem (ECO) through monitoring and study of interactions between soil, pasture, trees and animals (SPAA). After the third year, and after soil correction and fertilization (2ha), the component related to the dynamic management of grazing will be implemented between November 2018 and June 2020. Thus, in two plots with about 2ha each, 12 trees (holm oaks) 6 were selected in a low zone and 6 in a high zone, with 4 exclusion boxes, 2 under the canopy and 2 outside the canopy. The following measurements are taken every month (between September and June): 1) NDVI, which is correlated with the amount of chlorophyll and with the vegetative vigor of the pasture; 2) Capacitance, which is correlated with pasture production (Kg MS / ha); 3) Soil moisture; 4) Thermographic photographs, which measure the temperature of the pasture; 5) Pasture height; 6) Pasture crops, for percentage of dry matter, crude protein and NDF.

Following this, a doctoral student will be held, with the following questions: 1) why are there differences in pasture growth under and outside the tree canopy?; 2) After correction of soil pH are there differences in productivity under and outside the tree canopy?; 3) What is the productivity of animals in this system?; 4) What is the effect of grazing on pasture productivity?. We will have 4 plots, in 2 we will have a fixed header, and in the other 2 there will be variable biotic loads depending on the availability of

pasture. To study the subject and try to answer the questions the methodology is as follows: 1) monthly performance of the measurements described above; 2) weighing and body condition of the sheep; 3) laboratory analysis of pasture. On the one hand, the soil-pasture interaction will be evaluated, on the other the pasture-animal interaction.

Biodiversity and Ecosystems Functioning

Roads and biodiversity. To coexist or not to coexist, that is the question.

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Roads are the most extended infrastructure on Earth and are present in most modern landscapes. They are critical for connecting human communities, but at the same time they pose a threat to biodiversity. There are several negative effects of the roads. However, the most relevant are habitat fragmentation and the associated barrier effect on the movement of animals; and road kill mortality, which at present is already higher at world level than kills from hunting. There are currently 64 million km of roads on Earth and an additional 25 million km are expected to be built by 2050, 90% of which in developing countries in areas where biodiversity is high. Thus, it is urgent to act for the promotion of more sustainable roads reducing their impact on wildlife. However, there is still a lack of information and prior to any action it is important to clarify several issues in order to promote the success of the interventions. This presentation focuses on an assessment of the main effects of roads on biodiversity and the main issues still open for debate, for which a research agenda will be needed to find efficient solutions that will reverse the global decline in biodiversity worldwide.

Love me, love me not: a story of storks and power lines

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After a strong decline since the 1950-60's, the breeding white stork (*Ciconia ciconia*) population has been increasing steeply since the 1980's. Along this recovery period, a curious behavioural change has occurred: storks have been “abandoning” their traditional nest sites in trees and buildings to be increasingly use electricity pylons to build nests. The result is that white storks are “wired”, so that no less than 25% (!) of the nests in the country are built on pylons, mainly in very high-tension power lines. These nests can cause management problems to electricity companies, as they are the cause of power outages. On the other hand, this preference by storks has a drawback: it increases the risk of mortality for birds, through collision with power line cables and electrocution. This presentation will report this “love and hate” story, trying to address the pros and cons for storks nesting on power lines, as well as the drivers of pylon occupation.

Floristic analysis of four communities of *Prunus lusitanica* L. from Europe and North Africa

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In the scope of a PhD thesis in Agrarian and Environmental Sciences, we aimed to understand the conservation state of *Prunus lusitanica* L. communities in its native territory, Europe and North Africa. To achieve this, a set of field trips to Portugal, Spain, France and Morocco were performed for data collection. These communities are true paleotropical relics of the Ibero-Maghreb territories, being recognized as a priority habitat for conservation in Europe (5230pt2), present in Annex I of the Habitats Directive (92/43/CEE). Also, this *taxon* is part of the IUCN Red List, presenting a Vulnerable status at a global level, according to the latest evaluation carried out using the IUCN criteria. Thus, this work presents a comparative analysis between four distinct *Prunus lusitanica* communities, located mainly in the Rife Mountains (Morocco), the Serra da Estrela (Portugal), the Leon Mountains (Spain) and the Western Pyrenees (France). In this analysis we compared the floristic originalities of each territory, as well as their similarities and differences in plant communities, highlighting its biogeographical and bioclimatic aspects. As a result, some management measures are proposed in order to improve its conservation status. Through fieldwork, it was also possible to identify new occurrence sites for this species, which are published for the first time in this work.

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Effect of Alqueva Dam on terrestrial invertebrates communities: threat or opportunity

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The implementation of a dam has serious impacts in the biodiversity on terrestrial invertebrates. As consequence, some islands that did not exist before are created. In the Alqueva (South of Portugal), the effect of the implementation of a large dam raises the main question: is it a threat or an opportunity for the biological communities? Thus, we focus our objectives to answer this question and others related with terrestrial invertebrate communities. The main goal of this Thesis is to measure the long-term influence of the Alqueva dam on the ecological communities of the terrestrial invertebrates in 30 permanent islands of the Alqueva reservoir archipelago generated by this dam, and understand the gains and losses of these communities in this enormous transformation of the landscape. The terrestrial invertebrates target species are spiders, carabid beetles and butterflies. The data will be analyzing to quantify the effect of the construction of the dam, comparing two scenarios: before and after the flooding. To achieve this goal, we proposed four specific objectives: 1. Determine which spiders and carabid beetles communities presented major differences between the islands in the different time periods; 2. Explore the relationships between spiders, carabid beetles and butterflies communities and environmental variables; 3. Explore relationships between parameters of biological diversity and environmental variables, using multiple regression models; correlations and predicting distribution of these species in the future; 4. Evaluate the composition and evolution of the analysed terrestrial invertebrate communities under the theory of island biogeography. To accomplish the specific objectives, multivariate methods will be used to explore the data to explain the distribution and changes of terrestrial invertebrate species and communities present in Alqueva islands.

Accounting for connectivity uncertainties in predicting roadkills: a comparative approach between path selection functions and habitat suitability models

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Functional connectivity modelling is increasingly being used to predict the best spatial location for animal road-crossing structures and to mitigate wildlife roadkills. This tool requires estimation of resistance surfaces, ideally modelled with movement data which are costly to obtain. An alternative is to use occurrence data within species distribution models to infer movement resistance, although this remains a controversial issue.

The aim of this study was: (i) to compare the performance of resistance surfaces derived from path versus occurrence data in identifying road crossing locations of a forest carnivore; (ii) to assess the influence of movement type (daily vs. dispersal) on this performance.

Resistance surfaces were built for genet (*Genetta genetta*) in South Portugal using 1) path selection functions with telemetry data, and 2) species distribution models with occurrence data. An independent roadkill dataset was used to evaluate the performance of each connectivity model in predicting roadkill locations.

The results show that resistance surfaces derived from occurrence data are as suitable in predicting roadkills as are path data for daily movements. When dispersal was simulated, the performance of both resistance surfaces was equally good at predicting roadkills. Moreover,

contrary to our expectations, we found no significant differences in locations of roadkill predictions between models based on daily movements and models based on dispersal. Our results suggest that species distribution models are a cost-effective tool to build functional connectivity models for road mitigation plans when movement data are not available.

Keywords: movement models; species distribution models; ecological process; mitigation; roadkill; dispersal period.

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Regime of fire in Portugal: assumptions and facts

Nuno Guiomar (ICAAM-Universidade de Évora)

Abstract

The definition of effective strategies for wildfire prevention, pre-suppression and suppression (both initial and extended attack) requires the strict knowledge of the fire regime. Due to the impacts that large fires have on landscape, economy and society in general, they foster reactions aimed at finding quick and easy solutions to a problem that is extremely complex. However, many of these options are supported by wrong assumptions about fire regime that, when evolving into axiomatic approaches and from those to dogmatism, not only limit the implementation of the necessary measures towards fire management, as they tend to increase the complexity of the problem over time. The claim of a "Portugal without Fires" results from the widespread incomprehension of the fire regime, while it is believed that strengthening the heavy aerial support, controlling the ignitions, or increasing the criminal framework for arsonists will result in a significant decrease of the annual burned area.

The number of fires and the burned area are the basic data used in the majority of approaches to the fire regime, but few studies have attempted to describe, simultaneously, large sets of characteristics that may derive therefrom. Since these two components of the fire regime are driven by different factors, and given the asymmetry in the distribution of the number of fires by burned area classes, it is critical that the analysis on the indicators derived from these two basic variables take into account, not only these evidences, but the whole spectrum of relations between them. Moreover, and since fire regime results from an accumulation of events that occur in time and overlap in space, the temporal and spatial aggregation of the indicators must also be conducted. In fact, the analysis of several fire regime indicators at different temporal and spatial scales allows, not only to detect hardly discernible patterns in studies focused on individual fires or developed in specific locations, but also to identify deviations or disruptions in their distribution and, consequently, particular aspects of each fire regime.

It will be presented the fundamental characteristics of the fire regime in Portugal mainland, considering not only different spatial and temporal scales, but also the previously described context that supported the choice of indicators and analytical processes.

Naturally-produced compounds for the control of two major plant parasitic nematodes

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The pine wood nematode, *Bursaphelenchus xylophilus*, and the root lesion nematode, *Pratylenchus penetrans* are two of the main plant parasitic nematodes responsible for productivity losses in a significant number of plant species. Despite their significant differences in terms of life cycle, dissemination, plant host range or feeding strategies, both nematodes are particularly difficult to control. The presently existing control methods are costly and hazardous to the environment and to humans, and in today's context of sustainable development, the selection of environmentally safe and effective ways to control these nematodes, become imperative. There is thus a need to search for sustainable alternatives, and plant natural products may play an important role in multi-pest pathogen control. Oxygen-containing- and monoterpene hydrocarbons were bioassayed separately in the two nematodes following standard methodologies. Preliminary results indicate that at 2 mg/mL after 24h-exposure, oxygen-containing terpene molecules (citral, carvacrol, geraniol, etc.) showed a higher mortality than the monoterpene hydrocarbon molecules (limonene, α -pinene, γ -terpinene, etc.). For the first group of molecules, corrected mortality was higher in *B. xylophilus* (75-100%) when compared with *P. penetrans* (60-100%). For the second group, corrected mortality values were slightly higher in *P. penetrans* (5-10%) in contrast to *B. xylophilus* (<5%). Compounds with a nematotoxic activity higher than 80% are being further tested at 1, 0.5, 0.25 and 0.125 mg/mL.

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Animal Production and Health

Uterine contributions for conceptus survival and development

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Contrary to most common assumptions, the uterus is not a friendly place for the embryo, unless adequately primed by the local interplay of sex steroid receptors. In fact, the interaction between the mother and embryonic structures is only possible for a short time frame that in humans is named as the window of implantation. Then, a multitude of structural and molecular changes occurs in the superficial endometrium that allows the embryo to attach to maternal structures to form the placenta. In dogs and cats, species presenting decidual placenta, invasion of maternal endometrium starts at the window of pregnancy. Even in non-decidual species, the endometrium must be sensitised to allow the attachment of the trophoblast to the epithelial lining of the maternal uterus.

Implantation and the start of pregnancy approaches a finely tuned inflammatory reaction, where the maternal uterine defence system tightly controls the trophoblast invading competence, in a species-specific pattern, that simultaneously ensures the connection between the two different structures (uterine lining and the trophoblast) while guarantees the embryo not to be attacked as foreign tissue. In all eutherian (placental) mammals, establishment of successful pregnancy requires synchronous interactions of the conceptus with the endometrium of the uterus.

From its role in accepting implantation, as well as for controlling the conceptus development in early implantation, the uterus is an essential driver of female fertility. Based on studies developed in dogs, we will discuss some of the changes occurring in normal and pregnant canine endometrium around the window of implantation.

Rabbit haemorrhagic disease virus infection in wild rabbits in Portugal. How to protect the species? Recent research and future developments

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The European wild rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758) is a key species of Iberia ecosystems. Subspecies *Oryctolagus cuniculus algirus* is only found in the Iberia, in all Portugal territory and central and southern (?) Spain¹. After 2005, the wild rabbit populations declined abruptly in the Iberian Peninsula, strongly due to Viral Haemorrhagic Disease which emerged in Europe in 1989 (with the etiological agent rabbit haemorrhagic disease virus). In Portugal, rabbit haemorrhagic disease virus 2 (RHDV2 or GI.2) was detected for the first time in 2012 and is presently disseminated both in the continent and in the islands^{2,3,4}.

Many studies have established the lesional patterns of the disease in non-vaccinated domestic rabbits to better understand its pathogenesis⁵. The main goal of this study was to evaluate the impact of commercial vaccine administration in the wild rabbits' response to infection at the tissue level, and to understand how protective the commercial RHDV2 inactivated vaccines developed for the rabbit industry are in wild rabbits.

Non-vaccinated and vaccinated naturally dead wild rabbits were collected from hunting reserves and reproduction centres, respectively. Viral loads⁶ were evaluated and the lesional patterns established. The fact that lesions were systematically more severe in the non-vaccinated rabbits suggests that the disease progressed more rapidly in this group that also showed the higher viral loads. However, vaccinated rabbits were also very susceptible to infection, with equal high mortalities, showing that the vaccine was not as effective in the wild rabbit as in the domestic rabbit industry.

An effective vaccine for wild rabbits should be delivered easily, not requiring handling of the animals since the stress involved often causes death and affects immune response.

Recently, the project FIGHT-TWO was approved for financial support by FCT aiming to produce an oral vaccine. This multi-institution research project is presently at its first steps, and intends to develop and provide a safe oral vaccine to be distributed in the field as bait aiming the immunological full protection of wild rabbits against RHDV2 infection. Also, this non-invasive immunization technology will be used to protect a broad proportion of the populations in affected

areas, crucial to reduce virus transmission and control the infection without capture and manipulation.

At the same time, because of a strategic partnership established between FMV-UL and FF-UL, an innovative study is already on-going to produce an automatic applicator of a non-toxic, natural-repellent of ticks, fleas and flying insects. The repellent will be applied to wild rabbits in field conditions reducing the interaction with arthropods. By decreasing indirect contact with the virus, this temporary shield will simultaneously tackle down the main wild-rabbit diseases - RHD and Myxomatosis.

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Characterization of the Mertolenga Breed phenotypes under the influence of the CAP in the period between 1986 and 2012

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Natural conditions in most of the Portuguese territory (marginal soils and severe water deficit in the summer) do not favor intensive meat production, which in turn determines a low stocking rate unless the pastures are well managed and the animals adequately supplemented whenever necessary. These improvements are costly and pointless in most cases explaining why breeders do not adopt this strategy. As grassland and forage production are in most cases not suitable for efficient fattening, the conversion of land use has led to an increase in the number of suckler cows in the period studied (1986 to 2012), since these animals are less dependent on forage quality and have lower feed costs under these conditions than in other farming systems. It is under this complexity of factors, strongly conditioned by the difficult conditions to which they are generally subjected, that the three phenotypes of the mertolenga breed stand out for their characteristics of rusticity, ease of delivery and low food requirements. Political conditions are added to the natural conditions and therefore the question arises as to the extent to which the productive efficiency of this breed and its phenotypes has been altered throughout the stages of the CAP, in what parameters and how can it be in the future ensure the economic viability of its staff regardless of Community support.

Evaluation of elastase and cathepsin G inhibition on collagen transcription and prostaglandin secretion in mare endometrium

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Mare endometrium fibrosis – endometrosis - is a major cause of subfertility/infertility in mares. Neutrophil extracellular traps (NETs), besides killing pathogens in uterus, may also contribute to endometrial fibrosis. We have shown that elastase (ELA) and cathepsin G (CAT) are NETs components that may stimulate *in vitro* endometrial collagen deposition. Thus, the aim of this study was to evaluate the effect of sivelestat (SIV) – an ELA inhibitor - and cathepsin G inhibitor (INH) – a CAT inhibitor - on collagen transcription and prostaglandin secretion, in different estrous cycle phases. Follicular-FP (n=8) and mid luteal-MLP (n=7) phase explants were cultured for 24 or 48h with ELA (0.5, 1µg/mL), SIV (10µg/mL), ELA (0.5, 1µg/mL) + SIV (10µg/mL), CAT (0.1, 1µg/mL); INH (1µg/mL) or CAT (0.1, 1µg/mL) + INH (1µg/mL). Collagen 1 (COL1) gene transcription was determined by qPCR and prostaglandins (PGE₂; PGF_{2α}) secretion in culture medium was assessed by EIA. In FP, at 24h, ELA0.5 and CAT1 increased COL1 transcription (P<0.005; P<0.05 respectively) and PGF_{2α} production (P<0.05; P<0.01), but its inhibition - ELA0.5+SIV (P<0.05) and CAT1+INH (P<0.05) - decreased them. Also, ELA0.5+SIV or ELA1+SIV raised PGE₂ production (P<0.01). At 48h, ELA1 increased COL1 transcription (P<0.01) and PGF_{2α} production (P<0.001), but its inhibition (ELA1+SIV) decreased them (P<0.01; P<0.05, respectively). CAT0.1+INH incubation was also able to reduce COL1 transcripts (P<0.05). PGF_{2α} augmented with ELA0.5 (P<0.001) but lowered with ELA0.5+SIV (P<0.01) and also PGF_{2α} secretion increased with CAT0.1 and CAT1 (P<0.05). Besides, ELA1+SIV incubation increased PGE₂ (P<0.05). In MLP, ELA0.5 (24h, P<0.01; 48h, P<0.001) and CAT1 (24h, P<0.001; 48h, P<0.05) up-regulated COL1 transcription, but ELA0.5+SIV (24h, P<0.05; 48h, P<0.001) and CAT1+INH (24h, P<0.001; 48h, P<0.05) decreased it. At 48h, incubation with ELA1 also up-regulated COL1 transcription and PGF_{2α} production (P<0.05), but PGF_{2α} production lowered with ELA1+SIV and also with CAT0.1+INH incubation (P<0.05). PGE₂ production was higher in ELA1+SIV incubation (48h, P<0.05) and CAT1+INH (24h, P<0.001). Inhibition of ELA and CAT pro-fibrotic action by SIV and INH, may reduce the establishment of mare endometrial fibrosis by stimulating the production of anti-fibrotic PGE₂ and inhibiting pro-fibrotic PGF_{2α}.

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Assessing pubertal age of male Bísaro pigs through testicular and epididymal morphometric parameters - preliminary results

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Bísaro pig (BP) had grown in numbers in the last decade, representing one of the most important Portuguese livestock breeds. As per farmer's observations, BP are sexually precocious, reaching puberty at around five-month-old. On average, boars are bred for the first time just after completing one year of age, but in the herdbook, breeding records start as early as four-month-old. Despite the farmers' random observations and breeding records, little is known about the pubertal age of BP. This study aims to estimate the age of puberty in male Bísaro pigs through testicular and epididymal morphometry. Fifty-six pairs of testis and epididymis were collected from male Bísaro pigs ranging in age from 1 to 8 months of age. Samples were collected post-mortem (n=26) or from surgical castration (n=30), from May 2017 to April 2018, sourced from six different farms. After collection, testis and epididymis were trimmed, weighed and measured. Testicles were then cut longitudinally to obtain three tissue samples of <1 cm deep and fixed in 10% buffered formalin solution. Tissue samples were processed for routine haematoxylin–eosin staining. Microscopic parameters used for the morphometric studies included the spermatogenesis scoring, the number of Sertoli and Leydig cells, the diameter of seminiferous tubule, and the presence of spermatozoa in the epididymis and vas deferens. Measurements were evaluated using an image processing software (ImageJ®). Correlations between testicular and epididymal length, width, depth, weight and volume, were highly positive (r: 0.866-0.997; p<0.001; n= 56). Positive correlation was also found between Leydig cell and nucleus diameter (r: 0.732; p<0.001; n=52), but no significant correlations were found between the diameter of seminiferous tubule and the Leydig cell and nucleus. Differently, tubular diameter increased proportionally to the age (R^2 : 0.69; p<0.001; n=52). A GLM model was used to predict BP testis mean±SEM length, width and depth and animal's age when spermatozoa were found in the epididymis (6.98 ± 0.87 , 4.50 ± 0.51 and 3.77 ± 0.13 cm, at 117.50 ± 6.36 days), and in the vas deferens (7.34 ± 0.79 , 4.75 ± 0.49 and 3.98 ± 0.47 cm, at 145.71 ± 3.79 days). The farm had significant effect on these parameters (p<0.001) and thus included in the model.

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Environmental conditions in pigs facilities located in Southern Europe

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In a world with increasing demanding for quality food, produced taking in consideration the animal welfare and by principles of ethics and by social and environmental responsibility, requires a special care with costs rationalization and increased efficiency. The constant need for change and growth, resize and reorganize makes pig farming an excellent case study for challenges in animal welfare. Pig production is an important sector in Portuguese economy, and almost half of the national pig herd (45%) is produced in Alentejo region. This region is where the climate variables are most extreme; demanding high monitoring of environmental and physiological variables to ensure sustainable and safe production. Nowadays the environmental control systems are limited to analysing temperatures and humidity, and actuate to opening windows and cooling or heating systems. These control systems forget the real impact in animal welfare. Therefore, there is a need to incorporate new variables to be monitored, in real-time, that are related to animal welfare, like physiological indicators of welfare.

The first activity of this project is the study of which variable/indicator are more indicated to evaluate the welfare in growing pigs. In a small lab 2 trials were performed, in each one with 7 growing gilts (± 45 kg starting weight) submitted to different environmental conditions (summer, winter and comfort). In each situation animal adaptation/stress was evaluated trough several indicators like physiological (e.g. cortisol levels), behavioural (e.g. video records) and productive (e.g. growth rate). In addition, environmental conditions were monitored (exterior and interior) and tested the interconnection between all the collected data in order to adjust environment according to animal responses in an automatic and real time basis. The second phase of the project is in a commercial farm, where the animals are submitted to real conditions. The aim of this phase is to analyze the influence of environmental parameters on the welfare of growing and fattening pigs and to test the equipment and methodology for collecting and monitoring information on indicators of animal welfare, provided by the animal itself, in real time.

Bioprotective solution against pathogens: selection of protective starter cultures for meat products

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The potential presence of pathogens on meat products needs to be controlled. The main control measures are the implementation of HACCP method coupled with technological strategies namely bioprotective solutions by the use of protective starter cultures. The aim of this work was to select presumptively safe *Lactobacillus* strains isolated from traditional dry fermented sausages regarding their potential production of bacteriocins. *Lactobacillus sakei* (n= 32) and *Lactobacillus plantarum* (n=82) were isolated from meat products, equipment surfaces and ingredients on different meat industries. All isolates were assessed regarding antibiotic susceptibility. Disc susceptibility testing was performed according to CLSI for nine antibiotics belonging to different classes. The PCR detection for *tet*(L), *tet*(K) and *tet*(M) genes was performed. Genomic typing was done by PCR-fingerprinting using the primers (GTG)₅ and M₁₃. *L. plantarum* and *L. sakei* isolates genetic background to express phenotypic bacteriocinogenic ability was achieved by applying PCR for known sakacin (*sakA*, P and Q) and plantaricin genes. Bacteriocinogenic potential was also assessed by Skalka qualitative test. The majority of isolates *L. plantarum* and *L. sakei* were susceptible to tetracycline (75% and 81%) and erythromycin (71% and 97%). The presence of *tetM* and *tetL* genes linked to mobile elements, were not frequent. However, among *L. plantarum* the *tetK* gene was detected on 44% of the isolates. The potential for producing Sakacin was detected only in three *L. sakei* isolates (L1B8, L3B8 and P2B3) with *sakA* and *sakQ* genes while the majority of the *L. plantarum* presented genes *plnC*, N, R, K, L for Plantaricin. *L. plantarum* P3B8 was selected to further studies as a safe strain with plantaricin genes and high bacteriocinogenic activity against *L. monocytogenes* and *S. aureus*.

Keyword: Bacteriocins, Protective starter, lactobacilli, Safety, Meat products

This study was supported by Fundação para a Ciência e a Tecnologia (FCT, Lisbon, Portugal) through CIISA project (UID/CVT/00276/2013) and project PTDC/AGR-ALI/119075/2010.

Combined effect of nisin loaded pectin nanoparticles and high hydrostatic pressure on the extension of *alheira*'s shelf life

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Alheira is a traditional Portuguese smoked and fermented meat 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 manufacture, storage and cooking, exposing consumers to microbial hazards. Considering these facts and with the aim of promoting its sustainable production, it is important to apply alternative technologies such as high hydrostatic pressure (HHP) and nisin loaded pectin nanoparticles (NLPN) to assure *alheira*'s safety and increase its shelf life while maintaining their organoleptic authenticity. Three batches of vacuum-packaged *alheira* were produced according to 4 conditions: without treatment, NLPN treatment, HHP processing (600MPa for 390 seconds at 10°C) and a combination of both treatments. The addition of NLPN was done during the mixing step of the process and the HHP processing was done after packaging. Microbiological analysis was carried out 1 day after treatments and then every month during a storage time of 5 months under 4°C. *C. perfringens* and *Listeria* spp. were not detected in any of the samples analysed. The total aerobic microorganisms at 30°C and lactic acid bacteria counts were over 7-8 log cfu/g before the application of technologies in study. The application of HHP significantly ($P < 0.05$) reduced the levels of all microorganisms analysed and this effect was maintained over the time. The benefit of the NLPN addition was only verified in the coagulase-negative *Staphylococci* counts, maintaining this effect during the storage time. Moreover, the application of HHP on *alheira* produced with NLPN showed a synergistic effect, with better results when compared with those obtained when the technologies were applied alone ($P < 0.05$). Overall, HHP combined with NLPN had a synergistic effect, and was considered the most suitable process for preserving and extending *alheira*'s shelf life. This study emphasizes the usefulness of using the synergism of two technologies to extend the shelf life of a fermented sausage and assure safety.

Keywords: Emergent Technologies, *Alheira*, Fermented Meat Product, Shelf life, Synergism.

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Chemical hazards screening in dry fermented sausages from Mediterranean influence countries

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Chemical compounds such as polycyclic aromatic hydrocarbons (PAHs), biogenic amines (BAs) and heavy metals are hazards identified and related with meat products. The introduction of these different compounds in dry fermented sausages (DFS) could be linked to raw materials and auxiliary ingredients used, to a specific step of their technological process, or by environmental contamination. This work aims to evaluate the presence of chemical hazards (PAHs, BAs and heavy elements) on commercially-available dry fermented sausages from different origins and provide preventive recommendations to assure products safety. Samples ($n=20$) were purchased at retail level in different markets of Lisbon (Portugal) and Novi Sad (Serbia). The levels of PAHs were determined by gas-chromatography coupled to mass spectrometry (GC–MS) while BAs and heavy elements were analyzed using reversed-phase high performance liquid chromatography (RP-HPLC) and atomic absorption/emission spectrometry, respectively. The most abundant PAHs in DFS were those with low molecular weight, comprising more than 90% of total PAHs. Serbian and most of the Portuguese DFS well meet the EU legislation for the PAHs content. The levels of BAs in DFS from distinct origin (Portugal and Serbia) show a wide variation, however, the major BAs found were tyramine (32.6–206 mg/kg and 5.0–246 mg/kg, respectively) and putrescine (3.5–401 mg/kg and 1.4–291 mg/kg, respectively) followed by cadaverine (0.6–202 mg/kg and 1.6–291 mg/kg, respectively). Histamine never reached considerable levels in both DFS. Contents of heavy elements in DFS from Portuguese and Serbian markets indicated that these potential hazards were present but at low levels. The most prevalent heavy element in DFS from both origins was lead (0.01–0.34 mg/kg). In summary, the data demonstrated that the health risks derived from the intakes of these chemical compounds present in both Portuguese and Serbian DFS is not a major concern for consumers. Nonetheless, additional research still has to be done regarding the selection of autochthonous starters and smoking step optimization to minimize the contents of potential toxic PAHs and BAs in dry meat sausages.

Keywords: Dry fermented sausage; polycyclic aromatic hydrocarbons; biogenic amines; minerals; safety.

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Optimization of the smoking process to control polycyclic aromatic hydrocarbons in a dry-cured meat sausage

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Polycyclic aromatic hydrocarbons (PAH) are potential chemical hazards related with smoked meat products. The composition and concentration of PAH in meat sausages depends on multiple factors throughout smoking process. This study aims to optimize smoking conditions for a high calibre dry-cured meat sausage (DCMS) and assess the presence of PAH. Three batches of DCMS were prepared from commercial crossbred pigs, seasoned, stuffed and smoked with natural smoke generated by burning wood from *Ilex aquifolium*, followed by a curing period of 45 days. Conditions for smoking process until 35% weight loss; were: a) no smoking; b) 20h of smoking; c) 60h of smoking, d) continuous smoking until cured. PAH from DCMS were extracted according to Alves et al. (2017) and determined by gas-chromatography mass-spectrometry (GC-MS) using a mixture of deuterated labelled PAH as internal standard. No heavy PAH, including the benzo[a]pyrene, were detected in sausages without a smoking period. However, benzo[a]pyrene was detected in meat sausages submitted to continuous smoking or in sausages with 60h of smoking, although its concentration did not reach the maximum legal limit of 5 µg/kg established by the European Union. The maximum level of 30 mg PAH4 (sum of Benzo[a]pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene, and Chrysene) per kg of smoked meat and smoked meat products (EC 835/2011) was not reached in any of the DCMS. However, DCMS with extended smoking times (i.e. 60h of smoking) showed a trend (P=0.061) towards higher levels of PAH4 compared with sausages submitted to 20h of smoking. Thus, to maintain typical organoleptic characteristics of DCMS, smoking periods as long as 60h are acceptable to avoid the accumulation of toxic levels of PAH in high calibre DCMS.

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Meat Product, Safety, Polycyclic aromatic hydrocarbons.

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