



UNIVERSIDADE
DE ÉVORA

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

11 e 12 de novembro | 11th and 12th November 2019

IV Encontro de Estudantes de Doutoramento em Ambiente e Agricultura

11 e 12 de novembro 2019

IV PhD Students Meeting in Environmental and Agriculture

11th and 12th November 2019

Pólo da Mitra, Universidade de Évora

Book of abstracts

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Dear participants,

It is our great pleasure to welcome you to the **IV Encontro de Estudantes de Doutoramento em Ambiente e Agricultura / PhD Students Meeting in Environmental and Agriculture**, held in Évora on the 11th and 12th November 2019. 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.

This event is organized by ICAAM – Institute of Mediterranean Agricultural and Environmental Sciences and IIFA – Institute for Advanced Studies and Research, University of Évora and is supported by UNIMED – Mediterranean Universities Union, which gives this EEDAA an international context. On the other hand, we will have in this edition an active participation of researchers from the current MeditBio of the University of Algarve, who together with ICAAM will in 2020 be part of the new research centre MED – Mediterranean Institute for Agriculture, Environment and Development.

This year edition of EEDAA is organized in four sessions, namely Biology and Biochemistry, Veterinary Sciences and Animal Production, Agricultural Sciences and Food Sciences as well as Ecology, Environment and Landscape. The meeting will be structured in four thematic sessions, with conferences by guest researchers and PhD students, and will include two invited plenary lectures. Furthermore, several poster presentations will be displayed throughout the meeting. The program also includes a workshop on “Comunicar Ciência”.

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

Cláudia Marques, IIFA

COMMITTEES

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Teresa Pinto Correia, Universidade de Évora – ICAAM

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Program

Monday, 11th November 2019

09:00	Registration
09:30	Opening Session
	António Candeias Vice-Rector of Universidade de Évora Diretor of Instituto de Investigação e Formação Avançada (IIFA)
	Teresa Pinto Correia Diretor of Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)

10:00	Plenary Lecture <i>Risk of companion animal to human transmission of antimicrobial resistance</i> Constança Pomba Universidade de Lisboa – Faculdade de Medicina Veterinária / CIISA
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Biology and Biochemistry

10:45	Chair: Manuel Ramiro Pastorinho Universidade de Évora - ICAAM
	Invited talk <i>Olive Fruit Fly: Can the bacterial microbiome be source of highly specific pest control agents?</i> Tânia Nobre Universidade de Évora-ICAAM
	Invited talk <i>Plant microbiome at work: how beneficial bacteria help to achieve a more sustainable agriculture</i> Esther Menéndez Universidade de Évora-ICAAM
11:30-11:50	Coffee break and poster session
	Invited talk <i>Development of a viral vector to control TSWV in tomato plants</i> Carla Varanda Universidade de Évora-ICAAM
Presentations selected from the submitted abstracts	
	<i>Ripening of the yellow-fleshed kiwifruit 'Jintao' in orchards located in Braga region</i> Andreia Afonso Universidade do Algarve - CEOT
	<i>Molecular Markers associated to the cynaropicrin trait in Portuguese cardoon population</i> Ana Paulino Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo - ICAAM

12:45 – 14:30	Lunch Workshop “Comunicar Ciência” (lunchbag included for the participants) Maria Fátima Nunes e Mariana Soler Universidade de Évora, Instituto de História Contemporânea IHC-CEHFCi-UÉvora
	<i>Effect of meteorological parameters on Cupressaceae pollen in southwestern Iberia</i> Ana Galveias Universidade de Évora - ICT
	<i>Antimicrobial and antitumoral potential use of essential oils from Calamintha nepeta, Origanum virens and Thymus mastichina (Alentejo)</i> Sílvia Arantes Universidade de Évora - HERCULES
	<i>The effect of plant type and disturbance on soil enzymatic profile</i> Taiana Conceição Universidade de Évora - ICAAM
	<i>Anti-inflammatory potential and toxicological properties of Lavandula spp. aqueous extracts</i> Andreia Piçarra Universidade de Évora - HERCULES

Veterinary Sciences and Animal Production

15:20	Chair: Sandra Branco Universidade de Évora - ICAAM
	Invited talk <i>Whole genome sequencing analysis as a powerful tool to dissect the genomic architecture of indigenous Portuguese pig and sheep breeds</i> Marcos Ramos Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo - ICAAM
	Invited talk <i>Human and animal saliva proteome analysis for the study of ingestive behaviour</i> Elsa Lamy Universidade de Évora - ICAAM
16:10- 16:30	Coffee break and poster session

Presentations selected from the submitted abstracts

	<i>Comparative RNAseq analysis of backfat tissue from local pig breeds</i> André Albuquerque Universidade de Évora - ICAAM
	<i>Dietary condensed tannins to improve the ruminant antioxidant status and quality of their edible products – Elucidation of action mechanisms</i> David Soldado Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo - ICAAM
	<i>Biological Control of Gastrointestinal Parasites in Free-Range Poultry Production: A New Trend?</i> João Lozano

	Universidade de Lisboa – Faculdade de Medicina Veterinária / CIISA
	<i>How human diet shifts will influence global climate change and nitrogen use</i> Tiago Morais Instituto Superior Técnico - MARETEC
	<i>Lifecycle-based economic and environmental assessment of meat production as a driver of ecosystem services</i> Manuel dos Santos Instituto Superior Técnico - MARETEC
17:50	Closing of the first day of the IV EEDAA
20:30	Dinner at the restaurant “Cozinha do Cardeal”

Tuesday, 12th November 2019

10:00	Plenary Lecture <i>Sustainable Chemistry in the 21st Century: Impact on Agriculture, Energy and the Environment.</i> Anthony Burke Universidade de Évora - CQE
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Agricultural Sciences and Food Sciences

10:45	Chair: Maria Eduarda Potes Universidade de Évora - ICAAM
	Invited talk <i>Advances in cellulose dissolution and regeneration: From structural features to novel biomaterials</i> Bruno Medronho Universidade do Algarve - MeditBIO
	Invited talk <i>The key role of sample pre-treatment in analytical workflow: challenges and highlighted applications</i> Raquel Garcia Universidade de Évora - ICAAM
11:30- 11:50	Coffee break and poster session
	Presentations selected from the submitted abstracts
	<i>Phthalates a danger in food: a new analytical approach for risk assessment</i> Flávia Freitas Universidade Nova de Lisboa – REQUIMTE-LAQV
	<i>Molecular basis of resistance/susceptibility of Vitis vinifera L. to wood infection by fungal trunk pathogens in Alentejo region</i> Mariana Patanita Universidade de Évora - ICAAM
	<i>Growing under Alentejo's sun: a two-year comparative study between Syrah and Touriga Franca</i> Maria Inês Rouxinol Universidade de Évora - ICAAM
12:45- 14:30	Lunch
	<i>Response Surface Methodology as an Optimization Tool in the Analysis of Seaweeds' Volatile Organic Compounds</i> Bruno Leite Universidade Nova de Lisboa – REQUIMTE-LAQV
	<i>Phenolic profile characterization of olive fruit along the ripening stages</i> Miguel Ferro Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo - ICAAM

Plant secondary metabolites for the control of two major plant parasitic nematodes

Pedro Barbosa

Universidade de Évora - ICAAM

Ecology, Environment and Landscape

15:15 Chair: **Ana Cristina Gonçalves**
Universidade de Évora - ICAAM

Invited talk

From simple diet studies of owls to understanding complex interspecific interactions

Rui Lourenço

Universidade de Évora - ICAAM

Invited talk

Threatened voles in fragmented Mediterranean farmland: Insights on habitat use, movement, occupancy and demography

Ricardo Pita

Universidade de Évora – ICAAM

16:00-16:20 Coffee break and poster session

Presentations selected from the submitted abstracts

Chemical, biochemical and ecotoxicological evaluation of the use of materials from the pulp and paper industry to remediate soils affected by mining activities

Clarisse Mourinha

Escola Superior Agrária de Beja

Estimating Cabrera vole abundance based on presence sign counts: An evaluation using genetic non-invasive sampling

Dinora Peralta

Universidade de Évora – InBIO/CIBIO-UE

Remotely sensed indicators and open-access biodiversity data to assess bird diversity patterns in Mediterranean rural landscapes

Inês Ribeiro

Instituto Superior Técnico - MARETEC

The propagation of invasive species as a consequence of rural Fires: a case study in Casal do Rei (Seia)

Leonel Nunes

Universidade de Évora - ICAAM

17:20 Closing Session
Best Poster Prize

Note: The posters will be displayed throughout the Meeting.

ABSTRACTS

Risk of companion animal to human transmission of antimicrobial resistance

Constança Pomba¹

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Abstract

Antimicrobials are important tools for the therapy of infectious bacterial diseases in companion animals. Loss of efficacy of antimicrobial substances can seriously compromise animal health and welfare. A need for the development of new antimicrobials for the therapy of multiresistant infections, particularly those caused by Gram-negative bacteria, has been acknowledged in human medicine and a future corresponding need in veterinary medicine is expected. During the last fifty years, the number of companion animals has substantially increased, and companion animals are often considered as “family members” enjoying close contact to their owners. Thus, humans may acquire antimicrobial resistance via direct contact from their pets. Problems of resistance development and of infection control in companion animal hospitals are mimicking those in human hospitals (microbiological hazards are identified in Table 1)¹. Several studies have reported the colonization and sharing of *Escherichia coli* strains between companion animals and humans and very recently a first report of the fecal colonization and sharing of *K. pneumoniae* clonal lineages between healthy humans and dogs living in close contact has been reported². In China, the detection of mcr-1 in colistin-resistant CTX-M-15-producing *E. coli* strains isolated from companion animals and the possible transmission of mcr-1-harboring *E. coli* between companion animals and a person was reported¹. Also, the transmission of NDM-5 ST167 and CTX-M-9 ST69 *E. coli* between dogs and humans in a family was described in Finland³.

Table 1. Selected microbiological hazards identified¹

Antimicrobial-resistant bacteria in companion animals	Type of hazard	Sources
MRSA	direct hazard	dogs, cats and horses
MRSP	direct hazard ^a	dogs, cats and horses
VRE	indirect hazard ^b	dogs and horses
ESBL-producing Enterobacteriaceae	indirect hazard	dogs, cats and horses
Carbapenem-resistant Gram-negative bacteria	indirect hazard ^b	dogs and cats
Colistin-resistant <i>E. coli</i>	indirect hazard	dogs and cats

^aLow number of cases of human infections originating from companion animals.

^bNo human infections originating from companion animals have been reported.

References

- [1] C. Pomba, M. Rantala *et al.* J Antimicrob Chem (2017); 72(4):957-968.
- [2] C. Marques, A. Belas *et al.* J Clin Microbiol. (2019); 57(6); e01537-18.
- [3] T. Grönthal, M. Österblad *et al.* Euro Surveill (2018); 23(27); pii=1700497.

Acknowledgements

With financial support of CIISA and FCT through Project UID/CVT/00276/2019 and PET-Risk Consortium Project JPIAMR/0002/2016.

Sustainable Chemistry in the 21st Century: Impact on Agriculture, Energy and the Environment

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In a nutshell, Sustainable Chemistry is the term we use to describe the realization of (bio) chemical processes that either use renewable materials, or have low energy consumption, or have a weak environmental impact or include recycling of one or more components. So one way or the other, they are environmentally friendly. (Bio)Catalysis is a very important topic within the context of sustainable chemistry. (Bio)catalytic methods, both natural and unnatural, have been known for centuries and still make several important products, such as; medicines, food, personal care products, clothing and textiles, fuels and other economic products. The current impact that catalysis has on agriculture, energy production, transportation (including space exploration) and medicine will be discussed.

Workshop Comunicar Ciência

Maria de Fátima Nunes ,¹ Mariana Galera Soler²

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²Bolseira NEI. ¹IHC-CEHFCi-U.E. (Instituto de História Contemporânea – Grupo Investigação Ciência – Universidade de Évora. Palácio Vimioso – Largo Marquês Marialva, 8, 7002-554 Evora. Portugal)

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This workshop is part of the European Researchers Night Project (2018-2019), whose theme and practice are guided by “Science in the City”. Overcoming the barriers of laboratories and research centers and bringing science into the public space brings challenges for researchers and public. The squares, streets, libraries and museums are open to the entire population, from kids to seniors. However, is science for everyone? What to disclose? How to spread? What science and what ideas to disseminate? Does science have no color or ideology or owner? Is it possible to talk correctly about Science on Facebook, Twitter or Instagram? We can ask if science has to be disseminated as a social role, as scientists with a commitment with society. We are not the professionals of dissemination - *e.g.* the media, science centers, educational services of Museums. We are scientists who have an ethical and social obligation to bring to society what we are doing, to be open to listening to questions from people and flourishing a dialogue that provides critical tools for society to consume encrypted scientific dissemination packages. But this requires understanding who is listening to us and how to raise understanding. Starting from the initial point that there is no “general public”, which means listening to beyond speaking and understanding which reality we are ourselves scientifically involved. Make clear that a more accessible language does not mean less science, incomplete or wrong. In this workshop we propose to organize the public into interactive teams; each of them provide an instrument, a theme for analyzing and reflecting on target audiences and the merit and appropriateness of the way science is disseminated. We live in a society surrounded by products of scientific research and technological solutions, while groups question global warming or the effectiveness of vaccines. Therefore, it’s urgent that we leave our academic circles and talk to other people in different places and cultural and social contexts.

1.

Biology and Biochemistry

Olive Fruit Fly: Can the bacterial microbiome be source of highly specific pest control agents?

T. Nobre

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The notion of biological-individual is crucial to all fields of life sciences. Each individual is better seen in the frame of interactive relationships among species, and the individual boundaries are shifting to accommodate a symbiotic view of the organism and of life itself. The applied importance of symbiosis has also been gaining recognition. The relevance of symbiosis has been increasing in agriculture, in developing sustainable practices, including pest management.

Insect symbiotic microorganisms' taxonomical and functional diversity is high, and so is the potential of manipulation of these microbial partners in suppressing pest populations. These strategies, which rely on functional organisms inhabiting the insect, are intrinsically less susceptible to external environmental variations and hence likely to overcome some of the challenges posed by climate change. Rates of climate change in the Mediterranean Basin are expected to exceed global trends for most variables, and this warming will also affect olive production and impact the interactions of olives and their pests. The olive fruit fly (*Bactrocera oleae*), specialized to become monophagous, remains the most important olive tree pest. Production losses are estimated on an average of more than 15% yearly, and this fly has been responsible for losses of up to 80% of oil value and 100% of some table cultivars. Conventional olive fruit fly management strategies include the use of baits, attracting the olive fruit fly by colours and/or pheromones, but mainly the use of insecticides, and particularly over the last decades through the use of organophosphates (OPs). Resistance to the most commonly used OP, dimethoate, had evolved but also to other types of insecticides (e.g. pyrethroids, spinosad).

I will summarize the current knowledge on olive fly symbiotic bacteria towards the potential development of symbiosis-based strategies for olive fruit fly control. Particular emphasis will be given to *Candidatus* *Erwinia dacicola*, an obligate, vertically transmitted endosymbiont that allows the insect to cope with the olive-plant produced defensive compound oleuropein - the most promising target for a symbiosis disruption approach.

This work is supported by the "Fundação para a Ciência e Tecnologia" (FCT – Portugal), through the research project PTDC/ASP-PLA/30650/2017.

Plant microbiome at work: how beneficial bacteria help to achieve a more sustainable agriculture

Esther Menéndez¹

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Plants harbor complex networks of microbes, interacting among them and with the plant, inside and outside of its tissues. These microbes' networks act like a second genome of the plant, which is known as the plant microbiome. Amongst the microbes forming part of the microbiome, there is a subset which is commonly called plant probiotic bacteria. This group involves rhizobial and non-rhizobial bacterial endophytes which benefit the plant performance and protect it against biotic and abiotic stresses through several mechanisms.

The composition and functionality of this microbiome can shift under different conditions, with a major influence of the plant and the soil type. Changes in the environment and different agricultural practices might also have influence on microbiomes. This talk will be mainly focus on our case of study, the Montado, an agro-silvo pastoral system adapted to Mediterranean climate conditions occupying acidic soils with low fertility, mainly due to the presence of toxic levels of manganese (Mn) under low soil pH. In order to improve plant productivity, we followed an appropriate management, involving the establishment of permanent pastures in pH-corrected soils, by the application of dolomitic limestone-based amendments. Nevertheless, the bacterial communities present and the effects of these amendments in these conditions are poorly studied from a microbiological point of view. In this work, we hypothesize that the application of dolomitic limestone-amendment plus the establishment of legume plant hosts will influence the soil microbial community structure.

In this microbiological perspective, this talk will summarize some of the works on plant microbiome research that are currently running in our research group as well as a glimpse of the techniques that are being applied to achieve our objectives. Finally, we will discuss future research directions and some ideas for the translation of basic science to a field application of the plant (and soils) microbiomes to reach a more sustainable agriculture.

This work was funded by FEDER Portugal2020/Alentejo2020 project ALT20-03-0145-FEDER-000039, the COMPETE 2020 - Operacional Programme for Competitiveness and Internationalization (POCI) in the framework of the project POCI-01-0145-FEDER-016810 (PTDC/AGR-PRO/2978/2014) and the Strategic Project UID/AGR/00115/2019 from Fundação para a Ciência e a Tecnologia (FCT). EM acknowledges a Junior Researcher contract from the Individual Call to Scientific Employment Stimulus 2017 (CEECIND/00270/2017).

Development of a viral vector to control TSWV in tomato plants

C. M. R. Varanda¹, M. R. Félix², P. Materatski¹, M. D. Campos¹, N. Marques³, M.I. Clara², G. Nolasco⁴

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Tomato spotted wilt virus (TSWV) is a virus that causes a disease of major concern in tomato plants all over the world. TSWV is responsible for extremely significant economic losses in this crop, which constitutes 72% of the value of fresh vegetables produced worldwide. So far, plant viral control strategies rely only on preventive sanitary measures or genetic resistance as antiviral products are not available for field use which turns essential the development of innovative and efficient means of control.

Viruses are responsible for several important plant diseases, however, they have also been used in biotechnology with different purposes. Virus induced gene silencing (VIGS) allows specific silencing of foreign genes that can be inserted in an optimized virus vector and then inoculated in plants. When a sequence of a viral gene is introduced in a VIGS vector, the plant infected with this vector will be signaled to target that foreign viral RNA. This will give to the plant a significant advantage in its protection against a possible infection of that virus. The VIGS approach provides the generation of rapid phenotype, no need for plant transformation and can be used for plant protection purposes, at a relatively low cost. Several plant viruses have been used as VIGS vectors however, their large genomes, their difficult manipulation and the reduced number of hosts they infect restrain their use as vectors. The Alphanecrovirus *Olive mild mosaic virus* (OMMV) has characteristics that place it as a very promising vector tool. Its small genome makes it easy to manipulate, in addition, it causes only mild systemic symptoms in a wide range of crops, which will facilitate their manipulation into symptomless constructs and allow its application to a high number of plants. To our knowledge no studies have been done so far concerning this matter using necroviruses.

This new virus-based vector for protection of tomato plants against TSWV through silencing, with no need of plant genome transformation, is being developed under the project TOMVIRPROTECT (ongoing, from October 2018 to October 2021) here presented, and where it is also intended to turn the vector available for the control of other important plant diseases.

This work is funded by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through FCT, in its national component under the Project with references ALT20-03-0145FEDER-028266 and PTDC/ASP-PLA/28266/2017. P.M. acknowledges project with references ALT20-03-0145-FEDER028263 and PTDC/ASP-PLA/28263/2017.

Molecular Markers associated to the cynaropicrin trait in Portuguese cardoon population

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Cardoon has gained a growing interest, as a multipurpose crop representing a natural source of sesquiterpene lactones, namely cynaropicrin. Portugal has a tremendous natural variability of *Cynara cardunculus* (Cc) at a morphological and biochemical levels, conducting to the necessity of genetic diversity studies, for further plant selection/breeding within certain desired agronomic traits. The present work intends to study the development of molecular markers to select the best cardoon genotypes for cynaropicrin production, leading to the basis of a future breeding program. With great biological activity, cynaropicrin can be valorized within different industries, being the pharmaceutical and biotechnological options those with greater added-value. Chemical Cc profile, genetic variability, transcriptomic studies, and identification of potential molecular markers associated to cynaropicrin traits, will be assessed. Furthermore, this work intends to assess the potential of Genome-Wide-Association-Studies in the genetic improvement of cardoon, exploring its natural variability, as a source of genetic variability (novel alleles), for further identification of plants, with specific desired profiles. This research is a necessary step for a better conservation of the wild cardoon gene pool and for a more efficient use for future breeding programs of Cc.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495). Authors also acknowledge FCT for UID/AGR/00115/2019 to ICAAM, Contrato – Programa to L. Marum (CEECINST/00131/2018) and PhD grant to A. Paulino (SFRH/BD/145383/2019), D. Rosa (SFRH/BD/143845/2019) and T. Brás (SFRH/BD/110969/2015).

Effect of meteorological parameters on *Cupressaceae* pollen in southwestern Iberia

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Atmospheric pollen is a form of biogenic contamination recognized as a major cause of respiratory diseases. The severity of the allergic reactions depends on both the pollen concentration and the potency (allergen release per pollen). The incidence of pollen on public health may become increasingly worrying in the coming years as the effects of climate change become more intense, especially in Mediterranean regions where the type of cultures are rapidly changing due to water scarcity issues, with modifications in the biogenic aerosols emitted and intensification of their allergenic potential in certain periods of the year. The pollen allergy of *Cupressaceae* is reported worldwide. It is considered a significant source of allergens in the air due to great variability of species belonging to this family (Bunderson and Levetin, 2014). The period of pollination of this species is end of winter and beginning of spring, depending on the temperature (Shalali et al, 2013).

The objective of this study is the analysis of the influence of meteorological conditions in pollination of *Cupressaceae* in two different sites, Évora and Granada in the last three years: 2017, 2018 and 2019. The *Cupressaceae* pollen was harvested using standard Hirst-type traps and identified with optical microscopy, according to the traditional methodology. The meteorological parameters were obtained from ICT/CGE platform and Agencia Estatal de Meteorología (AEMET). Back-trajectories of air masses arriving at Évora and Granada have also been used in the analysis. The 12-hour back-trajectories were calculated using the HYSPLIT model (Hybrid Single-Particle Lagrangian Integrated Trajectory), at 12:00 and 15:00 UTC for different height levels.

Large concentrations of pollen were detected, with pollen index at Évora in 2017, 2018 and 2019 reaching 5887 pollen/m³, 2258 pollen/m³ and 7841 pollen/m³, respectively. In Granada the samplings yield 25584 pollen/m³, 16557 pollen/m³ and 58478 pollen/m³ for 2017, 2018 and 2019, respectively.

The temperature and relative humidity correlate well with pollen index of *Cupressaceae*. The precipitation and solar radiation present inversely proportional relationships with pollen concentrations. The back trajectories showed that pollen was mostly from local origin. These results suggest that meteorological parameters are important for pollination of all species, possibly affecting pollen concentrations.

This 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 also through ALOP (ALT20-03-0145FEDER- 000004) project.

Antimicrobial and antitumoral potential use of essential oils from *Calamintha nepeta*, *Origanum virens* and *Thymus mastichina* (Alentejo)

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Aromatic plants are appreciated as food flavors, being preserved and consumed by the local population in daily diets, and in Mediterranean Diet. Essential oils (EOs), complex mixtures rich in terpene compounds, have shown several biological properties, mainly as antimicrobials and antioxidants. Alentejo, S.W. of Portugal, is very rich in flavoring autochthonous aromatic plants. Therefore, it is important to evaluate their phytotherapeutic potential for their application as food preservatives, nutraceuticals and/or therapeutically products.

For this study were selected autochthonous Lamiaceae plants: *Calamintha nepeta*, *Origanum virens* and *Thymus mastichina* in order to evaluate the chemical composition and antioxidant, antimicrobial and antiproliferative activities of their essential oils.

EOs were extracted by hydrodistillation from aerial parts of flowering plants, and their chemical composition was evaluated by GC-FID. EOs antioxidant potential of was evaluated *in vitro* by radical DPPH, total reducing power and β -carotene linoleic acid methods. Antimicrobial activity was assessed by solid diffusion disk assays and minimal inhibitory concentration. Cell viability was performed by methylthiazol tetrazolium (MTT) assay using MDA-MB-231 breast cancer cells.

Results showed EOs very rich in oxygenated monoterpenes but *O. vulgare* EO showed similar content in oxygenated monoterpenes and monoterpene hydrocarbons. EOs are important antioxidants, with ability to scavenge free radicals, inhibit Fe^{2+} oxidation and protect the lipid substrate oxidation. EOs presented a large antimicrobial spectrum, with high inhibitory activity against Gram-positive and Gram-negative strains. Additionally, EOs showed high antiproliferative activity for breast cancer cell line.

Results point out the antioxidant, antimicrobial and antiproliferative potential of EOs of *C. nepeta*, *O. virens* and *T. mastichina* of Alentejo and suggest their potential use as health promoting agents in food and/or pharmaceutical industries.

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The effect of plant type and disturbance on soil enzymatic profile

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Soil microbiota is essential to biogeochemical process maintenance and has a decisive role in nutrient cycling. The soil enzymatic activity can work as an indicator of biological activity levels and therefore the functional status of the soil. The biological activity quantified by soil enzymes to interpret the effects of soil management practices can give a reliable description of the soil state under different uses. The objective of this work was to study the effect of agricultural practices (cultivated plant and soil tillage) on enzymatic activity profile in the soil. Four different plants were chosen according to its levels of mycotrophy, (legumes and non-legumes). The following enzymes involved in soil organic matter mineralization were measured: Dehydrogenase (DH) that indicates biological activity, Arylsulphatase (AS) related to sulphur mobilization, B-glycosidase (BG) involved in carbon metabolism and Phosphatase (PH) involved in soil phosphorus mobilization. A pot experiment was carried out and 3 sampling times were considered: (1) before plant seeding, (2) after plant harvest and (3) 10 days after soil disturbance. The growth of the plants significantly interferes in the soil enzymatic profile by increasing the activity of all the enzymes, although the mycotrophic plants lead to greater enzyme activities and in the case of PH this was particularly evident. Except for PH, soil disturbance negatively interferes with soil enzyme activity leading to similar levels to the ones observed before any plant growth. That same variation pattern was observed for respiration and microbial carbon, which were significantly greater after the mycotrophic plants growth and decreased after soil disturbance. Soil disturbance promotes a rapid mineralization of the organic matter and reduce the substrate availability for soil microbiota. The low values of enzymatic activity found 10 days after soil disturbance illustrate this situation. The differences of soil enzymatic profile found among the cultivated plants are certainly associated to differences in their rhizosphere, since the root architecture, the exudates release and their level of mycotrophy also differently contribute to the establishment and influence the structure, diversity and activity of the populations that make up the communities of microorganisms it encloses. The results presented showed that type of plants and the soil disturbance, along with its interaction, significantly interfere in the soil enzymatic activity profile which can be used as a tool to illustrate the effect of soil management practices on soil microbiota.

Anti-inflammatory potential and toxicological properties of *Lavandula* spp. aqueous extracts

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Polyphenols are products of the secondary metabolism present in some extracts of aromatic plants. These compounds have important antioxidant and anti-inflammatory properties and the intake of these natural products has been related with the decrease of some chronic disorders. Nevertheless, to a secure use is mandatory to develop toxicological approaches that allow to define a safe dose.

The aim of this study was to evaluate the anti-inflammatory and toxicological proprieties of aqueous extracts of *Lavandula* spp. and correlate that with their phenolic content. For this purpose, three wild growth *Lavandula* species, *L. stoechas* subsp. *luisieri*, *L. pedunculata* and *L. viridis* were selected and their aqueous extracts were prepared from decoction waters resulting of hydrodistillation of fresh plants. Chemical composition was evaluated based on total phenols, flavonoids and tannins content. *In-vitro* anti-inflammatory potential was carried out by three mechanisms: inhibition of 5-lipoxygenases activity, inhibition of trypsin activity and reduction of albumin denaturation. Toxicity approach was performed by the estimation of *Artemia salina* lethality, with determination of lethal concentration (LC₅₀), and *in vivo* oral toxicity was determined with lethal dose (LD₅₀) in *Swiss* mice. Moreover, oral toxicity assays were achieved based on Hippocratic screening to despite any signs of toxicity and quantification of biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkanine phosphatase (ALP) and bilirubin in hepatic and creatinine in renal homogenates for monitoring liver and kidney functions, at the end of the assay.

The aqueous extracts of *Lavandula* spp. presented high amount of total phenols, flavonoids and tannins compounds. Results revealed their anti-inflammatory properties with high ability to inhibit 5-LOX (32 <IC₅₀< 56 mg/L), trypsin activity (120 <IC₅₀< 200 mg/L) and albumin denaturation (152 <IC₅₀< 450 mg/L). Extracts showed very low toxicity against *A. salina* (4700 <LC₅₀< 6300 mg/L) and low toxicity in *Swiss* mice (LD₅₀>>>>5000 mg/kg). Moreover, Hippocratic screening and quantification of hepatic and renal biomarkers indicated very low toxicity.

Results point out the anti-inflammatory potential and low toxicity of aqueous extracts of these autochthonous flavouring herbs and suggest their potential use in nutraceutical or pharmaceutical products.

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Can food smell induce changes in salivary proteome? - the particular case of bread odor

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Food consumption induces changes in saliva, which may influence oral food perception. Changes in saliva protein composition are known as a consequence of chewing and food intake. Some of these changes occur at the level of proteins involved in the oral perception of food, namely the perception of basic tastes and astringency. Although it is known odor is an extremely important stimulus for food evaluation, acceptance and preference, and even though it is known to participate in triggering pre-ingestive processes, its effect on salivary protein composition is unknown.

This paper aims to identify the changes induced by olfactory stimulation in salivary proteome, relating these changes with the emotions triggered by that type of stimulation, and to try to understand if these changes are similar to those caused by chewing the same food.

Odor induced increases in salivary secretion rate were observed for proteins such as α amylase, cystatins, immunoglobulins and cytoplasmic actin II. Increases in secretion rate were augmented with the increase of desire triggered by the odor of bread. Parameters such as salivary cortisol and amylase have higher increases in individuals for which bread smelling provoked higher desire.

Concerning protein spots corresponding to cystatins and amylase, augmented in response to smell, they also increased with bread chewing, but not after chewing a different food. This suggests that a food odour may cause changes similar to those resulting from chewing that food.

This study concludes that odour induced pre-ingestive effects on salivary proteome and the level of these effects is dependent on the emotions that food triggers. Since protein changes are associated with oral perception, it is possible to hypothesize that olfactory stimulation, by changing the salivary proteome, may change the sensory evaluation of the food ingested following the stimulus. This work opens the way for a better understanding about oral dynamics, during food intake and consequent perception and acceptance.

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PineChem – Pine host chemistry and environmental factors driving the epidemiology of the pinewood nematode

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causative agent of pine wilt disease (PWD), is a major threat to pine forests worldwide. Native from North America, its dispersal goes as far as Eurasia, and for the last 20 years, it can be found in the Portuguese pine forests. However, different pine species have different levels of susceptibility to *B. xylophilus*. In its native forests, in North America, there are no apparent damages to pine trees, while in Portugal, *Pinus pinea* and *Pinus pinaster* coexist in the pine forests, but their level of susceptibility is distinct; the latter is more affected by the PWN. Such differences seem to be related with the host constitutive and inducible defenses which determines their resistance or susceptibility to the nematode. The PineChem project aims to identify the pine host phytochemical profiles and tissue morphology that most likely confer susceptibility or resistance to the PWN, and to isolate specific bioactive compounds against the PWN, by testing the following hypothesis 1) co-evolution of pine species with the PWN lead to higher resistance in pine species; 2) environmental and geographic clines determine phenotypical variation of pine species defenses over large geographic scales, and thus the outcome of invasion by the PWN; 3) pine species life history strategies leads to different strategies in relation with the production of chemical defenses, determining the susceptibility to the PWN; and 4) seasonal variation on the constitutive composition of the pine host tissues, is determinant for the seasonal patterns and epidemiology of PWD. As a long-term goal, we expect to gather enough knowledge to build predictive models for the PWD that includes different pine species and geographical areas and climates.

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Synthesis of new fluorescent labels for biomolecules and photosensitizers for DSSCs using coumarin derivatives.

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Coumarins, whether natural products or synthetic ones, have also aroused a growing interest of the scientific community in last decades due to their very significant pharmacological activity. Coumarins constitute also the major class of fluorescent dyes, used as fluorescent labels and probes for physiological measurement, fluorescent whiteners, optical brighteners, nonlinear optical chromophores, emission layers in organic light-emitting diodes, and more recently, in caging and labelling. The application of coumarin derivatives as organic dyes has been hindered due to their colour spectra falling in the UV range and the relatively low intensity of their absorption bands. One solution to this problem arises from increasing the delocalization of the conjugated π -electron system. A recurring strategy that became essential for the development of new organic dyes and that can be applied for coumarins, involves Donor– π bridge–Acceptor molecules. Applying our knowledge on the conjugation extension of coumarins at position 3, we observed that the presence of electron-donating substituents in position 7 and electron-withdrawing moieties in position 3, contribute to coumarin derivatives with improved photophysical and spectroscopic properties, with high quantum yields. Additionally these kind of substitutions allows a further decrease in the energy gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital. In this work, using 7-(diethylamino)-4-methyl-3-vinylcoumarin as intermediate, we developed a simple, low cost and effective synthetic strategy to produce new promising fluorescent labels for biomolecules and photosensitizers for DSSCs.

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2.

Veterinary Sciences and Animal Production

Whole genome sequencing analysis as a powerful tool to dissect the genomic architecture of indigenous Portuguese pig and sheep breeds

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Modern agricultural practices emphasize using highly selected livestock breeds, leading to great biodiversity loss of local genetic resources. These resources contain vital genetic variation for traits like adaptation to extreme environmental conditions and disease resistance. The advances observed in next-generation sequencing technologies over the last years now allow the development of powerful strategies that can accelerate genetic characterization of local animal resources, a key requirement for their preservation, improvement and valorization.

Portugal has a significant number of local animal resources, which represent an extremely valuable national heritage. The present availability of high-throughput sequencing and genotyping technologies opens new and exciting opportunities for research performed in local livestock breeds. Together with the advances also observed in fields like animal breeding and genetics, bioinformatics and biostatistics, these technologies allow that advanced research may be targeted, including whole genome (re)sequencing, marker development and the identification of genetic variants associated with improved performances.

In this study whole genome sequencing was applied to characterize the genomic architecture of several Portuguese breeds of livestock, which included Porco Alentejano individuals with contrasting meat quality characteristics, and several sheep breeds.

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Human and animal saliva proteome analysis for the study of ingestive behaviour

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Interest in salivary proteomics emerged in the last years, mainly due to the potential of saliva as a non-invasive source of biomarkers for different diseases and physiological conditions. But, besides a potential way of diagnosis, saliva is the fluid that bathes the structures of the oral cavity and that interacts with food. As such, it has interest for understanding individual's food oral perception and consequent preferences. Animals and humans share many mechanisms and understanding saliva-food interaction in one of the species can benefit the understanding of this process in the others. In this presentation, the actual knowledge about the relationship between salivary proteome and oral food perception will be presented, highlighting differences and similarities between humans and animal species and how this contributes to their food acceptance and choices.

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Comparative RNAseq analysis of backfat tissue from local pig breeds

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Alentejano (AL) and Bísaro (BI) are the main local pig breeds in Portugal, but have no information comparing their transcriptomic activity. AL belongs to the Iberian branch, presenting lower growth rates, precociously high adipogenic activity and higher levels of unsaturated fatty acids (FAs) while BI pig is from the Celtic group, sharing ancestors with higher growth rate and leaner commercial breeds.

This work intended to explore the genome function of AL and BI to better understand the underlying physiological mechanisms associated with body fat accretion, lipid composition and meat quality. Dorsal subcutaneous fat (DSF) samples were collected from AL and BI fattening pigs, with ~150kg BW at slaughter. Total RNA was obtained and sequenced for transcriptome analysis. Bioinformatic analyses using three different tools (Cufflinks, EdgeR and DESeq2) were performed.

A total of 367, 137 and 155 differentially expressed genes (DEGs) (q -value<0.05, $|\log_2 FC|>0.8$) were found using the Cufflinks, EdgeR and DESeq2 pipelines, respectively, between AL and BI DSF samples. EdgeR and DESeq2 shared a total 121 DEGs (~71% overlap) while Cufflinks showed divergent results (2.7% overlap with EdgeR and 5.5% with DESeq2).

A functional enrichment analysis of the candidate DEGs was performed using Ingenuity Pathway Analysis. Synthesis of lipid, depletion of glycogen, mass of organism and accumulation of oleic acid were revealed as main involved functions (p -value<0.05) though no directional activation state was observed ($-2<Zscore<2$). Potential upstream regulators that explain the obtained results such as *TCF7L2* and *RIPK2* were predicted to be activated and inhibited in AL, respectively. Moreover, 4 causal networks with *RIT2*, *KL*, *FLCN* and *RIPK2* as master regulators were inhibited in AL while another with *PPARGC1B* was activated.

These results present the first high-throughput transcriptomic data involving these local breeds and can help explain the metabolic differences that occur in the adipose tissue and shed light into specific meat quality traits.

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Dietary condensed tannins to improve the ruminant antioxidant status and quality of their edible products – Elucidation of action mechanisms

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Livestock animals are often exposed to oxidative stress, resulting in the deterioration of many physiological functions that compromise their health and productivity. Utilization of dietary antioxidants is recommended to limit the oxidative stress, and preserve the animal health and their products quality. Condensed tannins (CT) are phenolic compounds derived from secondary metabolism of plants, being the most common type of tannins present in forage legumes, shrubs and tree leaves, and in several agro-industrial by-products. Plants and byproducts rich in CT are widely available in Mediterranean countries, however they are almost not used. So, development of new applications for these resources will contribute to their valorisation. Overall, CT are known to have antioxidant properties and inclusion of CT-rich plants and plant extracts in ruminant diets have been shown to be an effective approach to improve animal antioxidant status and oxidative stability of their products. However, the mechanisms by which dietary CT are able to induce such effects remain to be established. Direct mechanism, by transfer of CT to animal tissues might be limited by polymeric nature and high molecular weight of CT. Moreover, CT degradation in small metabolites along gastrointestinal tract (GIT) and absorption are still unclear. Indirect mechanisms, by CT antioxidant action into GIT and/or by interaction with other antioxidant systems, have also been proposed. In order to explore the possible mechanisms by which dietary CT induce antioxidant effect will be performed several *in vitro* and *in vivo* experiments. Two distinct CT sources will be used – grape seed (GS) and *Cistus ladanifer* (rockrose, a very abundant shrub in Mediterranean area). Possible degradation of CT by rumen microorganisms will be tested through *in vitro* experiments. An *in vivo* experiment with lambs will be performed to elucidate the questions related to eventual degradation of CT in the gastrointestinal tract (GIT), absorption and deposition of CT in tissues, contribution of CT to antioxidant activity, antioxidant activity of CT in GIT, vitamin E metabolism and interaction with antioxidant enzymes.

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Biological Control of Gastrointestinal Parasites in Free-Range Poultry Production: A New Trend?

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In extensive poultry production systems, chickens are exposed to a high risk of parasitism and sometimes heavy burden of parasites, with direct and indirect life cycles, in most of their lifetime (3-4 months). In chicken production, the most common gastrointestinal parasites belong to the genera *Eimeria*, *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria*, and their control have been made mainly through drug treatment and house cleaning and disinfection. However, the growing prevalence of poultry parasites resistant to the most common sanitary control programs, led to the search for novel solutions, from which can be highlighted the use of nematophagous fungi. The current review aims to highlight the recent progress and the main research studies focusing the use of nematophagous fungi for the potential control of gastrointestinal parasites of poultry, namely developed by Brazilian, Danish, Portuguese and Spanish research teams. The main nematophagous fungi referenced by these authors are *Duddingtonia flagrans* and *Monacrosporium thaumasium* (larvicidal fungi), as well as *Pochonia chlamydosporia* and *Mucor circinelloides* (ovicidal fungi). These fungi can be administrated to animals via feed or disseminated on soil/grass, they are resistant to the gastrointestinal passage in most of the domestic animals, as well as, their respective activities are developed either inside the fecal environment or on soil/grass. *In vitro* assays performed in water-agar medium (2 %) and outdoor trails developed inside plastic boxes, revealed that *D. flagrans* and *M. thaumasium* attack free-living nematodes detected in poultry fecal samples, results that can be transferred to the parasitic forms, as well as demonstrated that *P. chlamydosporia* and *M. circinelloides* develop their lytic activity against parasitic nematode eggs. Also, as revealed by Portuguese and Spanish authors, *M. circinelloides* also has tropism to oocysts of *Eimeria* spp. from poultry, being the concentration of spores positively correlated with the speed of lytic activity against coccidia. The results found by this review study highlight the potential use of nematophagous fungi in poultry parasite control programs, as well as conclude that this biological strategy can be an accurate and sustainable tool to worldwide aviculture.

Keywords: Free-Range Poultry, Biological Control, Nematophagous Fungi, *Duddingtonia flagrans*, *Mucor circinelloides*, Portugal.

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How human diet shifts will influence global climate change and nitrogen use

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The goal of Ph.D. project “Studies in environmental sustainability assessment of land use systems” is to use models of carbon (C) and nitrogen (N) cycles in agro-ecosystems to assess the sustainability of agricultural innovations and their response to local and global environmental change. The project uses a multi-scale framework, in order to assess and optimize global diets and in particular the role of animal production. Here, we performed an integrated feasibility assessment of the main promising sustainable dietary shifts (reduced meat, vegetarian and vegan diet) using conventional and organic production. We compared each diet with the continuation of a ‘Business-As-Usual’ (BAU) diet. After taking into account scenarios of food demand, we assessed for each diet in 2050 if there was sufficient area for production, enough N availability, and if it was possible to reduce GHG emissions. We used the ‘BioBam’ model to calculate the balance between biophysical supply and biomass demand. We modified BioBam in order to compute C and N mass balances, which enabled the assessment of the N budget of each diet as well as the calculation of GHG emissions. Results show that the largest decreases of GHG emissions are obtained by reducing or eliminating animal products from diets. However, eliminating animal products requires large yield increases that are only potentially achievable through conventional farming. It is still possible to reduce GHG emissions in meat-based diets by replacing ruminants with monogastric animals, which decreases emissions due to enteric fermentation. For example, full conversion to monogastric products can lead to higher GHG reductions than changing to a vegetarian diet (40% and 30% less than the BAU emissions, respectively). Organic diets avoid the environmental burden of synthetic fertilizer and are potentially feasible. However, they require animal sources of N. Without animal fertilization, organic diets are unable to provide the N required to feed the world population. Consequently, N scarcity is the highest in complete plant-based organic diets, which lack manure as source of N. We also show that alternative sources of N, such as municipal solid waste, are insufficient for solving N scarcity.

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Lifecycle-based economic and environmental assessment of meat production as a driver of ecosystem services

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As livestock production is one of the main sources of global greenhouse gas emissions, the Portuguese government recently announced the intention of strongly decreasing the number of cows in the country until 2050. However, pasture-based beef production can also drive positive effects on the environment as it provides ecosystem services (ESS) such as climate change mitigation and adaptation through carbon sequestration in soils. Those positive effects, particularly relevant in Mediterranean areas where livestock are key for managing ecosystems, are rarely assessed. The goal of the PhD project “LEAn-on-Meat” is to assess the economic and environmental effects of pasture-based beef production as a driver of change in ESS, in order to assess the true costs and benefits of reductions in beef cattle herds. The PhD project will develop an innovative method to perform economic and environmental assessments of the effects of pasture-based beef production as a driver of change in ESS. The approach involves the evaluation of ESS at farm-level as well as throughout supply chains and is based on a layered evaluation and monetization of ESS: at farm level, using modelling, and at supply chain level, using environmentally extended input-output analysis. This approach can be applied in multiple regions but will be implemented for the Alentejo region as a first step. The foreground will be characterized using data from 40 case study beef farms in Alentejo that is currently being collected by the Animal Future (SusAn/0001/2016) project team. Detailed farm-level economic and agronomic questionnaires are being filled out. The economic data involves the entire farm accounting, while the agronomic data has all information regarding materials and energy consumption and all depicts all management practices (feeding, soil operations, etc.). At the end, the project will combine these features into a multi-level hybrid LCA-based tool specially tailored to assess and monetize ESS in grazing land, as well as to identify and test performance options for sustainable pasture management.

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Evaluation of the pasture productivity and quality in *Montado* ecosystem-prospects for introducing the concept of dynamic grazing

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Abstract

This study has the objective to quantify the effect of the soil pH and manganese toxicity corrections and tree canopy on 4 ha of natural pasture productivity and quality in *Montado* ecosystem located in Mitra farm.

In the autumn of 2017, dolomitic limestone was applied in half of the pasture area (2 ha) (COR) with the other two ha remaining uncorrected (UCOR). From December 2018 to May 2019, twenty-four pasture samples were collected in both areas (COR and UCOR), half of the samples (12) under tree canopy (UTC) and another half (12) outside tree canopy (OTC). The previous results collected presented: (i) higher productivity in COR and UTC areas; (ii) better pasture quality in COR and OTC.

Based on these previous results, the second phase of this study aims the effect of dynamic grazing effect on the quality and productivity of those pastures. A split-plot design was planned, with both areas (COR and UCOR) subject to two treatments: i) continuous grazing (7 sheep); deferred grazing, based on the amount of pasture. The expected results are a better acknowledgement about the effects of the grazing pressure and animal selectivity over productivity parameters as well the quality of pasture and the extent of phenological cycles.

Biological Control Trials with *Mucor circinelloides* against *Eimeria* spp. from Free-Range Poultry

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The rise of multi-drug resistance in the most pathogenic parasites of domestic animals stimulated the search for novel control strategies, such as the use of nematophagous fungi as a biological control tool. The current pioneer study aimed to test the use of the nematophagous fungi *Mucor circinelloides* against coccidia from free-range poultry, stimulating its ovicidal activity under *in vitro* and outdoor environments. Suspensions of *M. circinelloides* were used to perform two types of assay: first, three Petri dishes with water-agar medium (2 %) were used, two with oocysts previously isolated from fecal samples of freerange chickens, in which were inoculated spores of *M. circinelloides* (3,3 x 10⁵ spores/ml) and one as control. Plates were incubated at 26-27 °C, for 2 weeks; the second trial was made inside plastic boxes and under outdoor environment, for 2 weeks and in each season of the year, mixing different concentrations of spores with fecal samples positive for coccidia. The type of ovicidal activity was analyzed periodically. In both assays was identified with success ovicidal activity of *M. circinelloides* against coccidia, having been detected oocysts with morphological changes and walls destroyed (activities type II and III, respectively). Winter was the season in which the action of *M. circinelloides* against oocysts was faster, possibly due to the higher concentration of spores inoculated. The results obtained with this study suggest the use of predatory fungi in the Biological Control of poultry coccidia and allowed to conclude that temperature, moisture and concentration of spores affect the intensity and speed of action of these fungi.

Keywords: Biological Control, Nematophagous Fungi, Coccidia, Poultry, Portugal.

This work was funded by FCT under the Project UID/CVT/00276/2019 (CIISA) and aims to set up a basis for the future PhD Project to be developed by the first author.

Economic viability of the Mertolenga breed - Study of exploitation models -

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The reality of extensive or even semi-intensive beef cattle exploitation must have a technological level that allows it with existing resources (natural, financial, economic, labor / social and political) to achieve good economic and financial results. At the same time it must conserve or improve the genetic heritage of its herd and ensure the conservation of environmental public goods - notably the rural landscape, the quality of soil and ground and surface water, the maintenance of habitats needed by threatened or vulnerable species among others - by adopting greener production measures as part of a system to support farm multifunctionality. Over 3 years a survey of the costs and revenues was carried out and from the technical and economic parameters per cow throughout the CAP stages we developed four types of theoretical holdings, namely: the average holding of the whole information within Step 4 of the CAP (2008 to 2012); experimental exploration, with the average values of the total information that exists for it; a model exploration with parameters that ACBM considers ideal; lastly, a Mertolenga industrial cross-breeding as proposed by ACBM technicians to Associates in the future. In the end we decided to use two theorizing lines to analyze the evolution of economic results per cow, within each group: Line A - Traditional process involving animal and premium costs; Line B - Experimental process involving results per day of intercalving interval and excluding animal premiums (animal efficiency itself). The Mertolenga cow is found to pay daily labor, food and other costs, except in Stage 4 (from 2008 to 2012), however, it is completely dependent on Community aid for positive economic results.

3.

Agricultural Sciences and Food Sciences

Advances in cellulose dissolution and regeneration: From structural features to novel biomaterials

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As the major carbohydrate produced by plant biosynthesis, cellulose occupies a prominent place as a 'green' polymer for the production of innovative and sustainable materials. Unlike other polymers, cellulose is not meltable and therefore most of its applications rely on an efficient dissolution step followed by shaping processes where the properties of the regenerated material are strongly dependent on how well cellulose is dissolved and organized in solution. In this lecture the basic cellulose fundamentals will be reviewed together with controversial thoughts and current perspectives. Some of the projects being developed at our group will be briefly presented and discussed.

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The key role of sample pre-treatment in analytical workflow: challenges and highlighted applications

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Sample pre-treatment, also called sample preparation, is a preliminary and common step encompassed in most of the analytical workflow for the analyses of different kind of samples- e.g. biological, pharmaceutical, environmental and food matrices. Often combining physical and chemical procedures, it will enable to isolate or pre-concentrate the target analytes, minimizing the presence of interferents in the final extract. Particularly, for trace or ultra-trace analytes in complex matrices, this step is mandatory being one of the most time-consuming in the overall analytical workflow, representing almost 80% of the total analysis time. Although, some advanced instruments have been developed recently to perform the automation of sample preparation, analysis and detection, it is necessary to pretreat samples appropriately before proceeding with the analysis. Indeed, the separation and/or preconcentration phenomenon promoted by pre-treatment step is crucial, enabling to achieve lower limits of detection and improvements in the accuracy of the analysis method. The choice of a sample preparation procedure is dependent of several parameters, namely: i) the chemical properties of the target compounds and the interferents; and ii) the analytical technique used to quantify the compounds that will determine the need to convert them into a more suitable form for detection. The relevance of the sample preparation step cannot be underestimated as all errors that occur cannot be corrected, even by using the more powerful separation or detection methods.

This communication will be focused on the key role of sample pre-treatment in the chemical analysis methods. Thus, it will be discussed the path for selecting a specific sample preparation technique, their role in the analytical workflow and the strategy to assemble a new analytical method. Nowadays, the requirements of sample preparation procedures that are more selective, cheap, quick and environmentally friend brings new challenges into the field of sample preparation. Therefore, improved pre-treatment methodologies are emerging with applications covering different scientific fields, namely food contaminants. Some highlighted applications will be revised and the future trends in the field will be also discussed.

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Phthalates a danger in food: a new analytical approach for risk assessment

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Phthalate esters (PE's), better known as phthalates, are a group of chemical compounds widely used since 1960 as plasticizing agents in order to impart flexibility, durability and longevity to plastics.^[1] Given their unique physicochemical properties, some phthalates and their metabolites have a severe toxic effect on human health, primarily in the reproductive, endocrine and respiratory systems.^[2,3] Several studies have led the EU and the USA, among other countries, to intervene and regulate exposure to phthalates.^[4] Exposure to PE's is daily, causing an accumulation in the body, leading to long-term harmful effects. The control must be rigorous with very low levels of detection (ppb or lower), so it is important to define methodologies that respond to this need. Traditionally, the analysis of PEs is performed using 1D gas chromatography techniques. In the future, this project will apply classical and alternative 2D analytical methodologies (GCxGC and/or MD-GC) in order to obtain better separation, detection and sensitivity for PEs in complex food matrices, wine and olive oil. Up to this moment, nine phthalates have been quantified in Portuguese olive oil and different materials used in it's production, such as hoses. Liquid extraction with hexane/Methanol was performed, and chromatographic analysis was carried on a LECO GC/TOFMS with an apolar capillary column. The limit of detection ranged from 0.2 to 1 ppm (mg/kg) for all analytes. Further matrices are under study, namely wine.

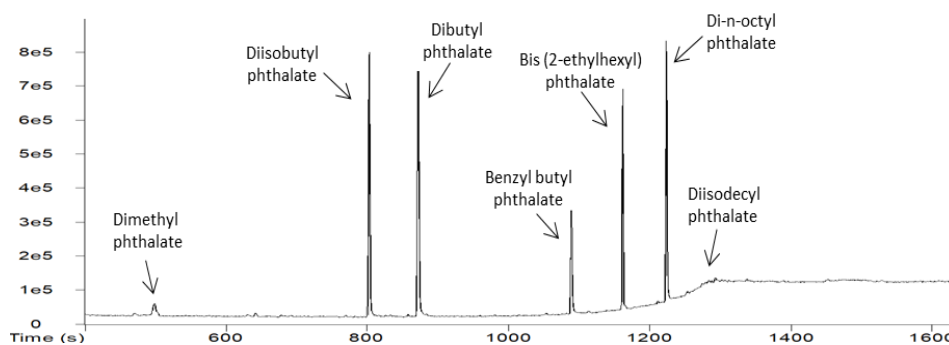


Figure 1: Extracted ion chromatogram showing m/z 149, displaying seven phthalates at a concentration of 60 ng/mL

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Molecular basis of resistance/susceptibility of *Vitis vinifera* L. to wood infection by fungal trunk pathogens in Alentejo region

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Grapevine (*Vitis vinifera* L.) is one of the most cultivated crops around the globe and has a high commercial value for fresh table grape, dried fruit and wine production. Grapevine is affected by several diseases among which grapevine trunk diseases (GTDs) are the most spread. These diseases are caused by several species of fungi that infect and damage the wood, causing chronic infections, affecting the longevity and productivity of grapevines in all major growing regions worldwide. Until now, no effective treatments are known. The recent increase of GTDs incidence is believed to be the consequence of several factors such as the grapevine planting 'boom' as occurred in Alentejo region, which increased the circulation of potentially contaminated propagating material, the drastic changes in production methods that greatly favor fungal infection, and finally because of the European laws concerning pesticide restrictions which have banned the use of effective chemical products available against GTDs fungi.

The recently accepted PhD project that will be here presented intends to exploit the natural phenotypic expression of plants' resistance/susceptibility to GTDs and to identify the gene(s) involved in these processes. Firstly, a preliminary study on the fungal community present will be performed in plants from selected cultivars from different vineyards in Alentejo region, with and without trunk diseases symptoms, using a strategy based on next generation sequence analysis (NGS). This study will give a better knowledge of the endophytic and pathogenic fungal communities and update the information on the diversity and abundance of the fungi responsible for trunk diseases. The antagonistic capacity of the identified endophytic fungi against the most frequently GTDs will also be tested. The information obtained on fungal communities, together with natural phenotypic expression of resistance/susceptibility to these diseases, will be the basis for the selection of plants for all transcriptome analyses. The comprehensive transcriptome data set will provide molecular insights into gene network responsible for activation of antifungal responses to trunk diseases, in resistant and susceptible grapevine cultivars, indicating novel candidates to be latter tested in strategies involving gene knockout and overexpression and contribute to the development of effective protective methods against these diseases.

This work will be funded by Portuguese National Funds through FCT under a PhD Scholarship (SFRH/BD/145321/2019) attributed to Mariana Patanita and by the project UID/AGR/00115/2019.

Growing under Alentejo's sun: a two-year comparative study between Syrah and Touriga Franca

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Grape varieties constitution is extremely affected by the *terroir* (the set of all environmental factors that affect a crop's phenotype). Grapes are so variable that in the same vineyard we can find varieties with different the maturation status, development, phenolic compounds and cell wall structure. The differences found between varieties are important for wine producing since they allow the production of a distinct wine with higher quality. The significative differences found between varieties lead us to develop this work, by selecting two distinct grape varieties.

The varieties Syrah and Touriga Nacional were collected in a vineyard installed in Herdade da Mitra, Valverde, Évora. For two years (20017 and 2018) samples were collected from verásion to harvest and key parameters were analyzed. Berries were heighted and the skins were taken off manually. The skins were used to produce extracts that allowed the quantification of phenolic compounds, flavonoids, tannins, anthocyanins and antioxidant activity of the extracts using DPPH, reducing power method and β -carotene/linoleic acid system.

Berries were bigger and heavier in 2018 in both varieties with statistical significative differences ($p < 0,05$), probably due to different weather conditions, namely temperatures and rain. Touriga Nacional had bigger berries in both years, with statistical significative differences ($p < 0,05$). Total phenolic compounds were higher in Syrah variety, although, between years, there are no statistical significative differences at harvest ($p > 0,05$). Total flavonoids were higher in Touriga Nacional in both years with statistical significative differences between varieties and years ($p < 0,05$). Tannin content was lower in 2018 (with statistical significative differences, $p < 0,05$) and Syrah was the variety with less content. Anthocyanin content was higher in Touriga Nacional variety, with no statistical significative differences between years. There was a high antioxidant activity in both years studied, with no statistical significative differences between dates studied ($p < 0,05$), although with differences between varieties.

These results are clear about the influence of the weather conditions on vineyard, with statistical significative differences between years in both varieties.

Response Surface Methodology as an Optimization Tool in the Analysis of Seaweeds' Volatile Organic Compounds

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Seaweeds are an important source of biological compounds exploited in various research fields. Most of the work published deals with the economic or nutritional benefits that seaweeds can promote in food formulations, rather than valuating them as an element of flavor. They are also a very vulnerable ingredient, undergoing changes after harvesting and processing. One of the objectives of the work under development is to study the impact of certain conservation techniques on the organoleptic profile of selected seaweeds from the Portuguese coast. The volatile organic compounds (VOCs) of these seaweeds should be analyzed by gas chromatography coupled with mass spectrometry (GC-MS)¹. The method used for sample extraction and concentration is headspace solid-phase microextraction (HS-SPME), using a DVB/CAR/PDMS fiber. Analytical chemistry has been using “design of experiments” (DoE) in order to obtain the best experimental conditions with the least use of resources. Response surface methodology (RSM) used in this work was the three-level factorial Central Composite Design (CCD)². A total of 18 experiments were realized and the response variable selected was the total area of the chromatogram. Results showed a lack of fit for the model: the independent variable “weight of sample” wasn’t statistically significant, and there wasn’t an optimum solution for the surfaces generated – because both other explanatory variables had a maximum outside the experimental region. Nevertheless, it was possible to infer that the extraction was more efficient the longer the extraction time and temperature. The next step is to redesign the model, studying the impact of other variables, with the aim of achieving the optimized HS-SPME parameters.

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¹ GC-MS Program: splitless desorption for 5min. in a DB-WAX Column (60m X 0.25mm X 0.25um) with helium as carrier gas (flow of 1mL/min); total run time of 63min [50°C (hold 2.5min), 3°C/min until 90°C, 6°C/min until 140°C, 2°C/min until 180°C, 20°C/min until 250°C (hold 15.33min)]; the detector temperature was set to 280°C and collision energy to 70eV.

² Time ranging from 10 to 90min., temperature from 20°C to 80°C, sample weight from 1g to 5g, and 4 replicas in the central point.

Phenolic profile characterization of olive fruit along the ripening stages

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The phenolic composition of olive fruits, represent a vast and unique source of health beneficial compounds due to the presence of specific hydrophilic phenolic compounds, such as hydroxytyrosol, tyrosol, oleuropein and its derivative complex molecules. Despite of being some of the most critical compounds regarding olive oil quality, these phenolic compounds are mostly abundant on olive fruit and leaves due to their hydrophilic nature. The phenolic fraction of olive fruits is well known for their antioxidant properties, mainly conferred by the presence of high hydroxytyrosol concentrations, but also by the significant presence of other phenolic molecules, such as tyrosol, verbascoside and oleuropein. In olives, the phenolic profile suffers a deep change along the fruit maturation process, being the simple phenolic compounds, such as tyrosol and hydroxytyrosol, mainly formed by the degradation of oleuropein and/or oleuropein aglycone molecules. The present work aims to study the maturation evolution of olive fruits from two different traditional Portuguese cultivars, 'Galega Vulgar' and 'Cobrançosa', in regard to their specific phenolic profile, as well as other physical parameters. Samples were collected from two to two weeks within a total period of ten weeks, starting in an early stage of ripening process, until harvesting time. A total of five different blocks located in different areas of the same olive orchard were considered for samples collection. Each block was constituted by five sequential trees. Hydrophilic phenolic extracts were produced and their profiles analyzed by HPLC-UV, as well as physical characteristics, including maturation index, of olive fruits and basic chemical composition. The present work was developed within OLEAVALOR project, led by University of Évora, aiming the valorization of traditional Portuguese olive cultivars.

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Plant secondary metabolites 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, worldwide, in a significant number of plant species. Chemically based nematicides offer systemic protection but all are powerful acetylcholinesterase inhibitors. Due to their short-term effects on human health and in the environment, they are extremely hazardous. Most of these compounds are forbidden or in the process of being phased out. In the search for more sustainable control measures, natural products gained increasing importance. Among them, compounds from plant secondary metabolism have long been recognized for their nematicidal effectiveness. The nematicidal activity of 25 terpene molecules (13 oxygen-containing- and 12 monoterpene hydrocarbons molecules) was assessed separately in the two species following standard methodologies. Preliminary results indicate that at 2 mg/mL after 24h-exposure, oxygen-containing terpene molecules (e.g. citral, carvacrol and geraniol) showed a higher mortality than the monoterpene hydrocarbon molecules (e.g. limonene, α -pinene, γ -terpinene). For the first group of molecules, the corrected mortality was higher in *B. xylophilus* (75-100%) when compared with *P. penetrans* (60-100%). For the second group, the corrected mortality values were slightly higher in *P. penetrans* (5-10%) compared to *B. xylophilus* (<5%). Ongoing research is evaluating if the differences between the two nematode species' response is due to each individual defensive pathways or if it is related with the bio-assayed molecules structure.

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Ripening of the yellow-fleshed kiwifruit '*Jintao*' in orchards located in Braga region

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The kiwifruit, unlike other fruits, shows minor external changes as it approaches ripening and as a climacteric fruit, an appropriated harvest date gains increased importance to insure that, after months in cold storage, the quality of these fruits would be approved by the consumers. This work followed the ripening of the yellow-fleshed kiwifruit *Actinidia chinensis* Planch cv '*Jintao*' at two different orchards (different edapho-climatic conditions, plough, pesticides, machinery, etc.), in the north of Portugal, in order to determine their optimal harvest date. To achieve this goal, in each orchard, twenty five trees were tagged and followed two months before the respective harvest season, throughout it and at least one week after it. The major quality/ripening attributes for kiwifruit, which are the soluble solids content, firmness and dry matter, were evaluated according to standard procedures, since August 2018 until November 2018. For this yellow-fleshed kiwifruit, since it retains the green flesh colour if harvested too soon, it was also important to include the flesh hue as parameter, once its unique golden colour has a major impact on the consumer acceptance [1].

The results showed, as expected along time, not only a clear decrease of the firmness and flesh hue but also a clear increase of dry matter and °Brix. However, although the orchards were located at the same region, the kiwifruits from one of them became yellow-fleshed and with a higher °Brix sooner than the other one, leading to different harvest dates for each orchard. Overall, this work clearly shows the importance of assess the major ripening attributes of '*Jintao*' kiwifruit along time and in each orchard, to establish more precisely the optimal harvest date at each site, and therefore provide the best quality fruits to consumers.

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Amino Acid and Volatile content of Aragonez Wine: The Influence of Soil Fertilization

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Vineyard fertilization is an important practice as it provides soil nutrients to the levels required for optimum grapevine growth and yield. Most soils contain adequate amounts of micronutrients, however Nitrogen (N), Phosphorus (P) and Potassium (K) (principal macro-nutrients) as well as Magnesium (Mg), Calcium (Ca) and Sulphur (S) (secondary macro-nutrients) are the ones that usually can limit grape production. Mg is required as a component of chlorophyll molecules and for metabolic processes and influences fruit formation and berry ripening. In Alentejo region soils usually tend to have low pH, which translates in deficiency of Magnesium.

The aim of this work was to understand the influence of several nutrients applications to soil vineyards on the amino acid and volatile content of the wines from Aragonez grapes. The experiment was conducted in a randomized block design, with three replications, in a split-plot arrangement. Two different doses of Mg were applied (D1 and D2). For each one there was six different treatments: 1) with N, P, Ca, S, K; 2) with P, Ca, S, K; 3) with N, Ca, S, K; 4) with N, P, S, K; 5) with N, P, Ca, K; 6) with N, P, Ca, S. A control plot with N, P, Ca, S and K without Mg addition was also considered, in a total of 13 plots. The amino acid content of the wines was quantified using a HPLC-DAD system from Waters, USA. The column used was an ACE HPLC column (5 C18-HL) particle size 5 μm (250 mm x 4.6 mm). Prior to injection, samples were derivatized. The volatile content of the wines was also analyzed using a GC/MS system from Bruker, USA. Chromatographic separation was achieved on a ZB-WAX PLUS capillary column (60 m x 0.32 mm i.d., 1.0 μm df). Prior to injection, an HS-SPME extraction was performed on the samples. Results shown that some differences can be observed among the amino acid and volatile content of the wine samples.

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The effect of temperature on NIR spectra measurements in pears.

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Quality control and waste management is a growing concern in today's enterprises, in particular in agricultural and food industry. The main quality criteria used in the fresh fruit sector are the total soluble solids content (TSS) and the pulp firmness. The most usual method to measure them is destructive, which is time consuming and leads to waste. The use of reliable nondestructive methods to obtain this information would speed up the measurements and decrease the waste being produced in quality control, while still ensuring a good measurement of the quality criteria through a more extensive screening of the fruit. The use of NIR spectroscopy as a nondestructive way to predict the value of TSS and firmness has been shown to be a valuable solution, but there is still room to improve its reliability and robustness. One of the ways to improve it is to understand the external factors that influence the results. In real world measurements, outside the controlled laboratory conditions, one of the more important external factors is the fruit temperature, which may vary within a significant range. The main goal of this study is to improve the knowledge on the influence of temperature on pear spectra and eventually its influence on prediction models. To achieve this goal various pears were refrigerated and/or heated, then placed in a fixed position to measure the interactance spectra and temperature of the same spot every 3 minutes until room temperature was reached. We observed that the fruit temperature affects the spectra, with some wavelengths showing more responsive patterns than others, mostly linear with the temperature. Some of these effects seem to be possible to override with adequate transformations on the spectra. Preliminary results suggest that the temperature effects on the spectra are not TSS or firmness dependent. Prediction models being developed in our laboratory will be used, in a near future, for testing the temperature effects on the model's accuracy.

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Comparison of Vis/NIR and NIR spectroscopy for monitoring of internal quality attributes of 'Rocha' pear (*Pyrus communis* L.) and 'Valencia Late' oranges (*Citrus sinensis* (L.) Osbeck)

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Fruit consumers demand healthy and high-quality produce with good taste, acidity and adequate soluble solids content (SSC). The nutritional value of fruit depends on various internal quality attributes (IQA). These are usually measured by standard methods that involve the destruction of the fruit, are very time-consuming, and require sample preparation. In addition, these standard methods rely on a limited number of samples that seldom account for all the variability of fruit found in an orchard and/or in a packinghouse. Spectroscopic methods allow to overcome these problems since they carry out fast and cost-effective non-destructive measurement of IQA. Near-infrared (NIR) is one of the most advanced methods in the field of food science and technology. NIR range covers from 900 to 2500 nm and provides information about the vibrations of molecules and their functional groups. Otherwise, Vis/NIR range covers from 400 to 1100 nm and yields information on electronic transitions and higher order overtones of the vibrational modes. The objective of this research was to compare the Vis/NIR (interactance mode) and NIR (direct reflectance mode) ranges for the evaluation of the internal quality attributes of 'Rocha' pear (*Pyrus communis* L.) and 'Valencia Late' oranges (*Citrus sinensis* (L.) Osbeck). The fruits studied were purchased in several local supermarkets. A batch of 255 oranges and 239 pears were collected and evaluated. Reflectance measurements were performed with a Vis/NIR spectrometer (USB2000, Ocean Optics, USA) and a NIR spectrometer (AvaSpec-NIR256, Avantes, Holland). Firmness and SSC were determined in both fruit groups, and the titrable acidity (TA) in the oranges, in order to calculate the respective maturity index (MI) [SSC/ TA]. The preliminary results showed that for both spectrometers, the best correlations were obtained for SSC. On the other hand, the NIR spectra provided the best results for oranges, while for the pears there were no significant differences between the correlations obtained in the NIR and Vis/NIR ranges. Preliminary results suggest that it is possible to build calibration models based on the Vis/NIR and NIR spectra for the nondestructive prediction of the internal quality of both oranges and pears.

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Prediction of 'Ortanique' internal quality by Vis/NIR spectroscopy-based calibration models

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Visible-Near Infrared (Vis-NIR) spectroscopy coupled to Partial Least Squares (PLS) have been shown to provide reliable calibration models to predict fruit's internal quality attributes (IQA). Assessing the ripening of "Algarve Citrus" orchards in real-time and accounting for representative samplings is mandatory in a precision agriculture approach concerning these crops. The aim of this study was the non-invasive determination of 'Ortanique' (*Citrus reticulata* Blanco x *Citrus sinensis* (L) Osbeck) critical IQAs by Vis-NIR spectroscopy throughout ripening. Spectra from fruits of 25 georeferenced trees, located in two different orchards, were acquired on-tree using a portable Vis-NIR system (Jaz, Ocean Optics, USA), in the range of 680-1100 nm. Fruit temperature was also measured in loco. After spectra acquisition, each fruit was harvested and their IQA determined by the standard procedures. PLS was used to build the calibration models of the various IQA, by correlating those determined through the standard methods, with the spectra collected on-tree. A low bias and Root Mean Square Error of Prediction (RMSEP) and high determination coefficient (R^2) models were achieved when calibration and validation data sets belonged to the same orchard (internal validation), revealing a good predictive performance. On the contrary, models worsened their predictive behaviour, when challenged by samples from another orchard (external validation). Additionally, in the internal validation approach, PLS scores showed that both calibration and validation subsets were more homogenous. On the contrary, calibration and external validation subsets showed a heterogenous behaviour, revealing fewer common spectral characteristics between them, leading to deviations between the IQA measured and the ones predicted by the model. Overall, our results show that model's robustness must be improved by collecting more data from other production years and orchards in order to cover most variability found among them. Besides the challenges presented by the need for improving model's robustness, this work has shown a strong potential for the use of Vis-NIR as a smart tool to provide an extensive and real-time assessment of 'Ortanique' orchards ripening stage.

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Using LED light in plant growth chambers: Evaluation of the equipment efficiency and plant development parameters

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Urbanization of human societies and increasing environmental concerns led to an increased demand for local food production in the proximity of the city centres, aiming to reduce its carbon footprint. This contributed to the development of plant production units using artificially controlled environment, mainly in Asia. The basic technical knowledge for the design of these units comes from plant growth chambers equipment, that has long been used by researchers to produce plants all over the year in controlled conditions. In production, as well as in research, homogeneous responses from the plants are fundamental, so, uniformity on light, air temperature and humidity distribution, are important issues for those equipments. The main problem with these systems is the high energy consumption, that needs to be mitigated in order to allow an expansion in the agrobusiness sector. In the last decade, several measures have been taken to reduce energy consumption, the most significant being the replacement of traditional fluorescent light tubes with LEDs. However, the data that quantify this reduction are still scarce and even less information is available on the crop physiological parameters that affect the production, or on the uniformity of the different inside environmental parameters (air temperature, air humidity, etc.). In this work a comparison between energy consumption and air temperature conditions and uniformity is analysed based on data recorded in a plant growth chamber Model Fitoclima 1200, build by ARALAB (Portugal), equipped either with Philips Master PL-L 55W/840/4P fluorescent tubes and Green Power LED research modules, for the same light intensity ($270 \mu\text{mol m}^{-2} \text{s}^{-1}$). Physiological and production parameters are also presented for two crops: *Lactuca sativa* 'Maravilha de Inverno' and *Spinacea oleracea* 'Gigante de Inverno', such as the fresh and dry matter, photosynthesis rates, photosynthetic efficiency f_v/f_m , stomatal conductance, chlorophyll levels) and growing rate for both light conditions.

The use of Black Soldier Fly larvae frass as organic fertilizer for lettuce – a study

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There has been a lot of research regarding the use of insects, such as dipterans, to biodegrade and digest organic wastes, since they are natural decomposers of organic matter. They can grow on different types of organic wastes, due to their larvae digestion process, they also have high reproductive rates and short lifecycles, making mass-rearing relatively easy. *Hermetia illucens* L., known as Black Soldier Fly (BSF), is one of the best candidates. BSF larvae, while feeding, incorporate fat and protein into their body and create a value-added compost (frass), rich in organic matter, simultaneously reducing waste volume. Dehydrated larvae can be used as livestock feed and larvae frass (BSFF) is a potential organic fertilizer, because of its composition in plant nutrients. This is a very interesting approach, that allows the reintroduction of lost nutrients back into the value-chain thus closing the loop to a sustainable circular economy. The aim of this study was to evaluate the agronomic value of the BSFF, using lettuce as test plant. The experiment was conducted in a glasshouse in INIAV (Oeiras, Portugal), for five 5 weeks. The experimental design consisted of 6 treatments (control, mineral fertilization, 1 part mineral and 2 parts BSFF, and three different BSFF rates). Both plants and BSFF were obtained from specialized producers and transferred into pots composed by 4 parts soil, collected from Ribatejo, and 1 part perlite. Pots were watered daily, to ensure 80% of field capacity. The results showed significantly higher yields in the mineral treatments, that may be explained by the slow mineralisation rate of organic composts along with the choice of using a short life-cycle plant such as lettuce, that requires high nutrient availability. Leaf nitrogen content, using N-Kjeldahl method, was also higher in mineral treatments. However, when evaluating soil microbial activity in each treatment, through the measurement of the enzymatic activity dehydrogenase, higher values were obtained with higher amounts of BSFF applied to the soil. These results indicated that BSFF increases soil microbial activity, which is linked to soil quality enhancement. Nonetheless, further chemical analysis concerning macronutrients content and organic matter determinations will be performed.

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Effect of the storage period of organic compost enriched with *Trichoderma atroviride*, on its biological control capacity of *Sclerotium rolfsii* in *Agrostis stolonifera* L.

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Composts resulting from agro-industrial residues may be rich in microorganisms with suppressive capacity against plant diseases. The enrichment of composts with antagonistic organisms is a strategy used to increase its effectiveness, allowing for a more effective biological control of soil diseases. However, compost storage may affect its suppressive capacity depending on the type of compost and the soil disease. Finding environmentally friendly alternatives to control plant diseases is crucial, and organic composts can be an efficient alternative to reduce pesticide use, contributing also to the circular economy.

In this study, two composts of agro-industrial residues, P1 and P2, were tested. From the end of the thermophilic phase to the end of the composting processes, fungi from both compost piles were isolated and identified. *In vitro* assays with the isolated fungi indicated *Trichoderma atroviride* has the most effective in the control of *Sclerotium rolfsii* and was therefore used in P1 and P2 composts enrichment. Compost (P1 and P2) and peat (T) samples (control) were used as substrates, being previously enriched with a suspension of *T. atroviride* (1.33×10^6 conidia mL⁻¹) to obtain 9.0×10^7 conidia L⁻¹ of the substrates. T, P1 and P2 were subjected to heat treatment of 60 °C (t) for 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. Peat and compost samples (enriched and not enriched) were stored at room temperature and their suppressive capacity was tested after two weeks, after six months and after one year of storage. *Agrostis stolonifera* was sown in 100 mL pots, on the 12 substrates, with five pots and four repetitions each. When the plants covered the surface of the pots, each pot was inoculated with *S. rolfsii*, by placing a 6 mm PDA disc with the pathogen mycelia.

In the substrates with a two weeks storage period, *T. atroviride* increased the compost suppressive capacity. In the substrates with a six months storage period, P1, P2, P1E and P2E maintained their suppressive capacity, but the severity of the disease increased in P1TE and P2TE. After one year of storage, the lowest severity was achieved in the enriched substrates (P1E and P2E), followed by P1 and P2.

These results indicate that stored composts are more suppressive if previously enriched with *T. atroviride*, maintaining their suppressive capacity after one year.

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Characterization of chicken houses in Mozambique

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In Mozambique the chicken meat is the largest source of protein for the population diet. However, the production systems are inefficient and have limitations in terms of the adequacy of buildings to climate characteristics. This study was performed in order to characterize and discuss the different types of chicken houses, having as reference the most productive regions in the country. Three categories of producers from Nampula (northern region), Manica (central region) and Maputo provinces (southern region), respectively small, medium and large, were identified, classified and grouped according to the range of production. For data collection, producers operating in integration systems (feed production, incubation, rearing and slaughterhouse) were selected. In this context, visits and semi-structured surveys were carried out with the producers and those responsible for all production systems, in order to obtain a detailed description of the installations (chicken houses). The results showed that there are no standard chicken houses adopted to all regions of the country, probably due to the fact that each region has specific limitations regarding the availability and accessibility of building materials. It was possible to observe the need for technical qualification not only of the producers but also for all actors in the production system, including the improvement of the constructions, to comply with the necessities throughout the production cycle. In general, the most common facilities are open, with natural ventilation, sheet metal covering (100% and 53.3% for Maputo and Nampula) with limitations in terms of technological level. Many of the installation models visited are the result of innovations introduced by producers, having as building materials the existing and abundant resources in the region, such as palm straw for the construction of roofs and clay mixed with natural fibers for the construction of the walls, also known as ``taipa`` or ``adobe``. As future work, we intend to develop small models of small and medium size of chicken houses, using available resources and innovative techniques that best contributes to condition the production.

Keyword: chicken production, chicken houses, animal environmental conditioning

Mucilage extraction from crops - a source of feedstock for biopolymers production

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As the world is facing several problems with the increase of the desertification processes as well as the decline of water resources, and considering that approximately 50% of the world area is considered arid, semi-arid it is mandatory to find alternative crops able to grow in those conditions. Crops like *Opuntia* spp grows essentially in the tropical and subtropical regions, under arid climate conditions, preventing soil degradation and being helpful in the control of desertification processes. The fruits are widely used for human consumption and the cladodes are essentially used as feed. The cladodes have in its composition mucilage, a complex polymeric carbohydrate structure with the ability to retard water loss and being able to form viscous or gelatinous colloids. Its gelling, thickening and emulsifying properties turn cactus mucilage useful to be applied in different areas such as cosmetics, pharmaceutical, and some other industries. In the food industry, it could find application in food packaging as edible films and coatings because of its elastic flow characteristic and its ability to form a molecular network. Therefore, the aim of this work is to develop an efficient and sustainable method to extract the mucilage from the cladodes with characteristics suitable to be used as a biopolymer. The methodologies tested comprised the same general steps: 1) Removal of the outer layers from cladodes; 2) Washing and cutting step; 3) Mix with a solvent (water or ethanol); 4) Pressing or centrifugation; 5) Precipitation with ethanol; 6) Drying step to obtain mucilage powder (drying and lyophilization). Based on preliminary results, a yield (in dry basis) of 0.72 and 15.29%, was obtained for pulp and peel, respectively. A critical assessment of the methods tested indicate that there are still options for improvement (e.g. use of different solvents and solvents mixtures) and that higher yield can be obtained.

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Establishment of a somatic embryogenesis protocol for *in vitro* olive plant propagation – a focus on the valorization of the Portuguese cv. 'Galega vulgar'

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Due to its capacity to regenerate plants from a single cell, *in vitro* somatic embryogenesis, apart from being an efficient plant propagation system is also fundamental to assist plant breeding by genetic transformation, and fundamental research for validation of data coming from different omics. The establishment of efficient protocols for plant regeneration through somatic embryogenesis must be taken as the first achievement on the successful regeneration of a transgenic plant. Focused on the validation of transcriptomic data, achieved in the frame of a running project (OLEAVALOR) and in the potential use of genetic engineering methodologies to further assist olive breeding programs, a research work has been carried out to establish an efficient somatic embryogenesis protocol for the Portuguese olive cv. 'Galega vulgar'. This cultivar is characterized by high quality olive oils and is still today the most representative of the traditional Portuguese olive orchards. However, it also presents several agronomic problems that highly limit its use in modern high density olive orchards, being mandatory the establishment of a breeding line for its valorisation. This study aimed on taking a first step in this direction, seeking to obtain an efficient somatic embryogenesis protocol that could later be used in breeding programs using genetic engineering approaches (e.g. cisgenesis, CRISPR/Cas9). Leaf petioles, taken from *in vitro* growing plantlets, as well as radicles and cotyledons, taken from mature seeds, were used as initial explants. The culture media composition and culture conditions were adapted from available reports (Rugini and Caricato 1995; Rugini and Silvestri 2016; Cerezo *et al.* 2011). A protocol for plant regeneration via somatic embryogenesis was achieved when mature zygotic embryos were used as initial explants. From the same initial explants, repetitive somatic embryogenesis was also successfully established, making available plant material for genetic transformation experiments. Regarding the use of leaf petioles from mature tissues, despite the formation of *calli* and the neoformation of leaf structures, it was not possible to obtain somatic embryos. Nevertheless, the results allow to foresee encouraging perspectives for further development of a protocol based on the use of adult plant material as initial explant.

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Bioherbicide *Cynara*: weed control through natural resources for sustainable agriculture

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Weeds represent a negative impact to crop plants productivity, on average, weeds can lower crop productivity by 34%. The intensive use of synthetic herbicides in the last decades not only has led to herbicide-resistant weeds development, but also the bioaccumulation of synthetic compounds in the environment resulting in health and ecological concerns. Sesquiterpene lactones (SL), abundantly present in *Cynara cardunculus* leaves (Ccl), have recently been described for its allelopathic effect on common target species showing strong evidences of their phytotoxicity and potential use as bioherbicides. The main objective of this PhD proposal is to develop a green bioherbicide based on Ccl extract, rich in SL to be used in olive orchards, grapevines and cereals production. Ccl extract will be prepared by ultrasound assisted extraction methodology, followed by membrane nanofiltration technology, to obtain Ccl- derived SL rich fractions. SL identification and quantification will be monitored by HPLC-DAD in fractions obtained, with special attention to cynaropicrin, grosheimin and deacylcynaropicrin. Simultaneously, phytotoxicity activity, using wheat coleoptile bioassay, will be performed to select SL fractions with higher activity. Then, selected fractions based on the phytotoxicity activity will be further assessed against a panel of weed species commonly found on agriculture crops mentioned. Formulations will be developed considering type, extract stability in solution and ecological excipients. The formulations produced will be first tested in vitro against weeds, in which case phytotoxicity will also be evaluated at different stages of the plant development in order to observe physiological effects. Furthermore, the formulations will be also tested on crop fields to verify phytotoxicity potential.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495). Authors also acknowledge FCT for PhD grant to Daniela Rosa (SFRH/BD/143845/2019), Teresa Brás (SFRH/BD/110969/2015) e Ana Paulino (SFRH/BD/145383/2019).

***Cynara Cardunculus* as a source of cynaropicrin – extraction and purification**T.BRAS^{1,2}, A.F.C. PAULINO¹, D. ROSA¹, L.A. NEVES², J.P.G. CRESPO², M.F. DUARTE^{1,3}

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Cynara cardunculus (cardoon) is a perennial plant, from the Asteraceae family, exhibiting a vigorous growth, with great adaptation to Mediterranean climates. Within the different plant parts, leaves represent about 35.4% dry weight of the total plant, and are a great source of natural compounds, with known biological potential, and high applicability in food and pharmaceutical industries. Cynaropicrin (CYN), a guaianolide sesquiterpene lactone, was firstly found in *Cynara cardunculus* var. *altilis* (cardoon) leaves, by Ramos et. al. with a content of approximately 87.5% of dried weight (DW). Considered as the main compound responsible for the artichoke bitter taste, it is an interesting compound due to its large availability on cardoon leaves and its biological potential. In general, extraction and separation processes to recover bioactive compounds typically need high working times, involving exhausting procedures, high energy consumptions and use toxic organic solvents. Recent studies from our research group, underlined the contribution of cavitation effect, within ultrasound assisted extraction (UAE) with ethanol as extraction solvent, as the best methodology for CYN extraction. The extraction method achieved presented a reduction of 99% of extraction time, a 30% increase on CYN extraction yield and reduction of 97% of energy consumption, expressed in kWh/g CYN extracted, when compared to the conventional CYN extraction methodology described. Chemical characterization of the obtained extract showed that, sesquiterpene lactones may represent 45% of the extract, where CYN is the major compound. However, UAEderived cardoon leaves extracts may also present 31% of compounds with low biological potential, namely monosaccharides. Nanofiltration of ultrasound assisted ethanolic extract from *Cynara cardunculus* leaves showed to be an effective process for the removal of monosaccharides, and recovery of CYN from the extract. Two final streams were obtained within the purification and concentration process, a permeate rich in monosaccharides, and a concentrate stream with higher CYN content. Interaction between UAE and ethanol followed by fractionation by nanofiltration lead to an innovative optimized CYN extraction and purification process, with low environmental and economic impact, allowing a *Cynara cardunculus* valorization with potential application on agro food, pharmaceutical or cosmetics industries.

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Comparison of seedless table grape varieties

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Table grapes are one of the oldest and widely grown fruit crops in the world. Although for a long time they are widely appreciated fresh or in sub products (such as juices, jams and raisins), nowadays consumers are becoming more discerning and seeking products that suit their healthy lifestyles. The benefits of table grapes consumption are well known and due to their high nutrient and antioxidant contents. Besides the refereed nutrient aspects consumers asses table grapes for fresh consumption by evaluating several characteristics: the appearance, size of bunches and berries, the uniform color, flavor and texture typical of each cultivar. It should be noted that the demand for apyrenic table grapes, has increased in recent years, and so the number of varieties of seedless table grapes in production is increasing too. Grape breeders have been trying to respond to the consumer's preferences for seedless grapes, characterized by not having noticeable seeds in their berries, by development of numerous improved varieties. Apyrenic table grapes presents a great market potential. The main objective of this work is to characterize six varieties of seedless table grapes 'Timco', 'Melody', 'Scarlota', 'Alisson', 'Melissa' and 'Autumn Royal'. The grapes were produced and collected in their commercial ripe stage in Herdade do Vale da Rosa, Alentejo, located in Ferreira do Alentejo (38°05' 23,80" N; 8°04' 52,7 1" O). Several widely measured product quality attributes were analyzed, including caliber, berry weight, color (CieLab), texture using penetration test with a 2 mm diameter stainless steel cylindrical probe using a TA.HD.Plus, total soluble solids by refractometry, titratable acidity, Folin-Ciocalteau total phenolic compounds and antioxidant activity (DPPH method). The differences among the six varieties are very important considering the measured attributes, since they are essential for quality evaluation by consumers. However, it is noteworthy for their importance the high values of phenolic compounds and antioxidant activity found in variety Autumn Royal. The variety 'Melody' also presented also a high value of antioxidant activity. The presence of polyphenols has effect on many biological activities concerning health such as cardioprotective effect, anticancer, anti-inflammatory, anti-aging and antimicrobial properties (Seccia et al 2019). These results, that specifically characterize the varieties, one by one, can be interesting for giving detailed information to interested consumers and for marketing strategies, and even for decision of public diet and health like canteen menus.

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Post-Harvest behavior of SugraOne Seedless grape variety with different methods

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Grapes are consumed both as fresh and as processed products, such as wine, jam, juice, jelly grape, seed extract, dried grapes, vinegar and grape seed oil. (FAO, 2016). International trade of perishable fruits is increasing noticeably thanks to the progress made in the cultivation and storage techniques allowing long term conservation and transport of the commodities. Many different approaches are often used by producers and stake holders in order to reduce decay and improve shelf life. However, the unique response of each variety requires specific essays in order to understand the efficiency of the treatments.

A trial was carried out with seedless table grapes from the variety SugraOne that were kept in cold storage at 2°C (±1°C) and 90% RH. The grapes were subject to different treatments: modified atmosphere modalities of packaging, namely the Control (C), other one with sodium metabisulphite included in the package material (Sulfurous modality) and a pre-harvest treatment with CPPU [forchlorfenuron, N-(2-chloro-4-pyridinyl)-N¹- phenylurea], a synthetic cytokinin-like plant regulator that is supposed, among other effects, to promote grape berry set, increase berry size and to improve storage quality (Zutahy,2008) (CPPU modality).

The samples were removed from cold storage to be analysed after 7, 21, 35 and 49 days. The fruits to be tested were kept until reaching the room temperature (20^o C). The analyses performed, included each grape weight and caliber, color through CieLab system, texture using penetration test, total soluble solids by refractometry, titratable acidity, total phenolic compounds using Folin-Ciocalteau method, and antioxidant activity (DPPH). From the data collected we can affirm that there are no meaningful differences between the control and the different used treatments along the storage period. So, considering the objective of reduce decay and improve shelf life of seedless SugraOne variety, during this period of time and these conditions, this trial has no evidence of the efficiency of the tested treatments.

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Microbial hazards' evaluation in nitrite/nitrate-free dry-cured sausagesS. Conceição^{1*}, M. Elias^{2,3}, M. J. Fraqueza⁴ AND M. Laranjo²¹Mestrado em Bioquímica, Departamento de Química, Universidade de Évora, Ap. 94, 7006-554 Évora, Portugal.²ICAAM- Instituto de Ciências Agrárias e Ambientais Mediterrânicas), IIFA-Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.³Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Ap. 94, 7006-554 Évora, Portugal.⁴Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Av. da Universidade Técnica, Polo Universitário, Alto da Ajuda, 1300-477 Lisbon, Portugal.*mail: m39590@alunos.uevora.pt

Nitrates and nitrites are used as curing agents in the manufacturing of dry-cured sausages, among other reasons to assure food safety. The most recently published IARC-International Agency for Research on Cancer report, highlighted the risks of consuming dry-cured sausages. These are considered hazardous due to their high salt and fat contents, but also due to the presence of nitrosamines. These are formed when nitrite/nitrate derived nitrosating agents react with biogenic amines. The food industry would like to reduce or eliminate the addition of nitrite/nitrate curing salts. However, there is a risk regarding food safety, namely due to the presence of *Salmonella* spp., *Listeria monocytogenes*, and more important of the botulinum toxin produced by sulphite-reducing clostridia. The abovementioned are considered some of the main safety microbial hazards for dry-cured sausages, which may result from the contamination of raw materials or from a poor cleaning and disinfection of materials and equipment in the industrial environment. The aim of the present work was to evaluate the microbial safety of nitrite/nitrate-free dry-cured sausages. Three independent batches of large calibre dry-cured sausages ('Paio') were produced in a local manufacturing unit both for control as well as for nitrite/nitrate-free sausages. Two samples were collected throughout the manufacturing and curing process (raw meat, meat batter, half-cured sausage and end-product) for both conditions. Physicochemical analyses, namely pH and a_w , and microbiological analyses (*Salmonella* spp. detection, and sulphite-reducing clostridia, *L. monocytogenes*, enterobacteria, lactic acid bacteria (LAB), and staphylococci counts) were performed. Regarding physicochemical parameters, significant differences ($P < 0,05$) were observed throughout the manufacturing and curing process between control and nitrite/nitrate-free dry-cured sausages, with nitrite/nitratefree sausages showing higher a_w values. The presence of *Salmonella* spp. and sulphite-reducing clostridia was not detected in any of the analysed samples. No differences were observed in the counts of all other microbial groups between control and nitrite/nitrate-free sausages. In summary, physicochemical and microbiological analyses show no significant differences between control and nitrate/nitrite-free samples. Moreover, the addition of nitrites/nitrates seems unnecessary to assure microbial safety. Nevertheless, the sensory acceptability of these nitrate/nitrite-free sausages by consumers must be evaluated, before recommending the manufacturers to eliminate the use of these curing agents in the production of this type of drycured sausages.

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Edible coatings effect on postharvest fungal growth

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Food waste during the postharvest period is around 27% of total production, mainly from physiological, mechanical and microbial infection. Table grape loss can reach values over 50% in developing countries. One of the most important postharvest diseases in table grapes is the grey rot caused by the fungus *Botrytis cinerea* Pers. The grey rot may originate from latent infections that begin before harvesting from spores in fruits that have not been eliminated during cleaning and selection operations. This fungus is capable of growth and development at temperatures up to 0.5° C and symptoms of infection begin with changes in berry colour and easy detachment of the epidermis, 'Slipskin'. Another disease is the blue rot caused by the fungus *Penicillium* spp., which although less important than grey rot, also causes damage during the preservation period of table grapes. Symptoms of this disease are the development of a white, watery rot, in which the epidermis acquires a light brown colour that can quickly affect the whole berry. The aim of this work was to evaluate, *in vitro*, the action of edible coatings in the control of postharvest table grape fungi, such as *Botrytis* spp. and *Penicillium* spp.. The procedure consisted in using petri dish of 90 mm diameter with edible coatings, chitosan [0.8%] and *Aloe vera* [0.25%] where each fungus was inoculated. The fungi were isolated in petri dish from naturally infected berries from the same farm. The concentrations of each fungus were adjusted to 1.0×10^6 . In petri dishes with PDA was added after solidification of this 100 µl of each coating and spread onto the plate. Subsequently 10 µl of the spore suspension was placed in the centre of the petri dish. The results allowed to say that Chitosan and *A. vera* had retarding action on the appearance and development of fungi. After 24 hours of inoculation the presence of the fungal development was observed in the control plates, and in the *A. Vera* and chitosan plates the development of the fungi was inhibited. After 48 hours of inoculation the fungi could be observed in all the plates. It should be noted that *A. Vera* and chitosan have at these concentrations and this test conditions considered effective in controlling the development of both fungi, *Botrytis cinerea* and *Penicillium* spp., although *A. Vera* has a more marked inhibitory effect. So these two types of material for coatings, Chitosan and *A. vera* may be a valid alternative to the use of fungicides in post-harvest decay and losses control.

This work integrates the Project "Criação de um Núcleo de I&D para a geração de novo conhecimento relacionado com a uva de mesa sem grainha Vale da Rosa", Portugal 2020. This research was partially supported by National Funds through the FCT – Fundação para a Ciência e Tecnologia, project UID/AGR/00115/2013.

Tenderness analysis and consumer sensory evaluation of fresh meat

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During the purchase of the meat there are three attributes, among others, that are usually taken into account: the appearance, the colour and the presumed tenderness of the meat, assumed from the beef cuts that had been chosen. After that, while the meat is eaten the most determining attribute is tenderness. The present study aims to establish an index for the differentiation between hard and tender beef of commercial origin. Different beef cuts (“chã de fora^a”, “rabadilha^b”, “lombo^c” e “vazia^d”) were considered for this study being simultaneously by consumers using a hedonic scale and at the same time by instrumental tests, for tenderness evaluation through (Warner-Bratzler shear force and texture profile analysis-TPA). For about 250 consumers were asked about the frequency and mode of consumption of beef, and what their palatability for beef. Four samples were given, corresponding to the muscles described, in order to the consumer assess which tenderness category was most appropriate for each sample considering an affective acceptance test through a 5-category hedonic scale (Very Hard, Hard, Ideal Tenderness, Tender and Very Tender). It was found that “chã de fora”, “vazia” and “rabadilha” were rated mainly as Hard and Ideal Tender. The highest evaluation was found in “lombo”, which is always rated as tender. The “lombo” is found to be significantly tenderer than the remaining beef cuts considering instrumental evaluation, which is in agreement with the assessment made by the consumers. The compression force was higher when testing “chã de fora” beef cut, however the results of the shear force for this same cut beef are identical to those of the “rabadilha” and “vazia”. This observation can emphasize the importance of the probe used in the test and the fibers direction to understand the tenderness of the meat. The results showed that a tender meat should have shear force values between 15 and 32 N and compression force between 11 and 20N, while a hard meat should have shear force greater than 37 N and compression force greater than 30N.

^a“Chã de fora” is the muscular part that covers the outer thigh and extends through the posterior region to the level of the tendon of the muscles that are inserted in the calcaneus. ^b“Rabadilha” is the muscular part that surrounds the lateral and anterior faces of the femur, until the kneecap. ^c“Lombo” is the muscle part taken from the ventral face of the roast beef, and includes the portion inserted into the iliac bone. ^d“Vazia” is the muscular piece that fills the vertebral drip of the spinal portion.

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4.

Ecology, Environment and Landscape

From simple diet studies of owls to understanding complex interspecific interactions

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There is a long history of studies of the diet of top predators all across the world. Ecologists, naturalists, or just enthusiasts have accumulated basic knowledge that help understand the trophic ecology of top predators. These “simple” diet studies are the necessary building blocks to understanding more complex interspecific interactions, which in turn are intrinsic forces modelling the structure of biological communities. Owls is one of the groups among the vertebrate top predators whose diet has been more frequently studied. Therefore, owls are good case studies to understand how top predators may influence the structure of communities.

I started climbing this ladder in 1999 with the study of the diet of eagle owls, and have since then collaborated several colleagues. After observing some differences between the diet of eagle owl breeding pairs, we realized that apart from the usual prey, eagle owls frequently captured other predators. This raised our interest on lethal interactions among vertebrate top predators. We then worked on the formal designations of the lethal interactions, namely interspecific competitive killing, intraguild predation, and superpredation. Further on, we explored the frequency of predation of meso-predators (i.e. superpredation) by four European top predators, to conclude that this is a relatively common phenomenon. Departing from this, we studied the causes and consequences of lethal interactions among vertebrate top predators. As potential causes of lethal interactions we explored food-stress, opportunism, competitor removal, and predator removal. We observed an interactive effect of the different causes, namely food-stress and opportunism. The consequences for the prey/victim can be grouped as (1) lethal or (2) non-lethal, and within the latter, we have explored in particular the behavioural effects of predation risk on meso-predators. As consequences for the predator, we observed the increase in contamination levels associated with biomagnification as a result of a more frequent inclusion of prey from higher trophic levels.

Along the track, we have shown how diet studies are fundamental pieces to understand the complexity of biological communities.

Threatened voles in fragmented Mediterranean farmland: Insights on habitat use, movement, occupancy and demography

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Habitat fragmentation has been long recognised has a major threat to many species living in human-dominated landscapes. A main consequence of habitat fragmentation is that species may become restricted to remnant habitats, often exhibiting spatially structured distribution patterns. This may result in a decreasing of their persistence abilities, which in the long term may result in significant decreases in overall biological diversity. Understanding the effects of habitat fragmentation on spatially structured populations requires analysing processes operating at multiple spatial scales (within patches, among patches, across patch-networks). I will present empirical results of my current research focusing on threatened voles (namely Cabrera and water voles) in fragmented Mediterranean farmland, particularly regarding processes that influence species persistence within and beyond local habitat patches, including habitat selection, movement, occupancy, and demography. I will also draft on possible conservation guidelines favouring species persistence and coexistence in patchy, heterogeneous environments.

Chemical, biochemical and ecotoxicological evaluation of the use of materials from the pulp and paper industry to remediate soils affected by mining activities

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The objective of the study was to evaluate the sustainable use of materials from the pulp and paper industry to improve the quality of soils degraded by mining activities, taking the example of the Aljustrel mine soil. The materials were tested at a pilot scale, in a trial installed in São Domingos, including: (1) biomass ash granules (AG), stabilized for about six months outdoors; (2) AG and composted cellulosic sludge; and (3) a mixture of 1:3 (volume basis) of “fresh materials”, biomass ash and cellulosic sludge without stabilization. The original soil of the Aljustrel mine was used as a positive control. The effects of the amendments on soil characteristics were assessed one month after the amendments applications by measuring different chemical, biochemical and ecotoxicological properties. The chemical parameters were soil pH (1:2.5 w/v, in deionized water), EC (1:5 w/v, in deionized water), OM content, N_{Kjeldahl} content, and extractable P and K (Egner-Rhiem). The biochemical status of the soil was assessed by measuring the activity of dehydrogenase and of some soil exoenzymes (β -glucosidase, cellulase, acid phosphatase and protease). Improvement in soil phytotoxicity was evaluated using germination and growth tests with garden cress (*Lepidium sativum*), a dicotyledonous plant and with barley (*Hordeum vulgare*), a monocotyledonous plant, while the ecotoxicity of the soil-water extracts (1:10 w/v) was evaluated by three different bioassays with organisms representative of different trophic levels: (i) luminescence inhibition of *Vibrio fischeri*; (ii) mortality test with *Thamnocephalus platyurus*, and (iii) growth inhibition of the green microalgae *Pseudokirchneriella subcapitata*.

The results showed an improvement in the soil characteristics by application of materials, and better results were obtained by simultaneous application of stabilized materials. Significant improvements in soil fertility were observed: increase in pH, OM content, N_{Kjeldahl} content, and extractable P and K. Furthermore, the EC did not significantly increase by application of stabilized materials. Enzymatic activity of dehydrogenase increased, as did β -glucosidase, protease and cellulase; acid phosphatase showed a distinct behavior, decreasing with some treatments, which may be a consequence of the increase of soil pH. The phytotoxicity decreased, as demonstrated by the bioassays used. The results suggested that the waste-based additives tested are adequate to be used as mine-contaminated soil amendments, since their application allowed a clear improvement in soil properties, which are crucial to the recovery of its functions.

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Estimating Cabrera vole abundance based on presence sign counts: An evaluation using genetic non-invasive sampling

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Understanding the factors that affect the spatial and temporal variation of species population size provides baseline guidance for adequate conservation management, either targeting the protection of endangered species, or the control of pest outbreaks. Despite the great efforts and developments, knowledge of the size and abundance of wild populations remains a main challenge in conservation ecology studies, particularly for elusive species of conservation concern. Capture–recapture (CR) methods remain the ‘golden standard’ for estimating species population size, but these are often hard to implement in the case of rare and elusive species that are difficult to capture using standard methods (e.g. life-trapping, camera-trapping). In addition, CR methods are largely impractical and cost-prohibitive to conduct over large spatial scales, thus hampering proper evaluation of species population status and trends. Therefore, population size indexes (PSI) based on species presence sign surveys have been often used as alternative measures for population monitoring. However, before being used in wildlife management decisions, PSI need to be validated against known standards. Here we assess the utility of presence signs counts as PSI for the Cabrera vole, a nearthreatened, elusive small mammal, endemic to the Iberian Peninsula. We generated 18 PSI based on presence sign counts under varying sampling efforts and used GLMM to relate those PSI to the population size estimates based on CR data obtained from genetic non-invasive sampling. Overall, our results suggest that PSIs based on latrine counts conducted over relatively short-time sampling efforts may provide a useful, cost-effective alternative for monitoring Cabrera vole populations over large spatial scales, which should be crucial to inform conservation management of the species.

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Remotely sensed indicators and open-access biodiversity data to assess bird diversity patterns in Mediterranean rural landscapes

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In a time of fast environmental change, biodiversity monitoring at simultaneously fine spatial resolutions and large spatial extents is needed but limited by operational trade-offs and costs, thus calling for the development of cost-effective and expedite approaches. Open-access data may contribute towards that goal. We test the use of open-access satellite imagery (NDVI texture variables) and biodiversity data, assembled from GBIF, to investigate the relative importance of variables of habitat extent and structure as indicators of bird community richness and dissimilarity in Montado systems, in the Alentejo region (Portugal). After controlling for data quality, our dataset of bird occurrences included data from 78 species (7858 observations) in 40 landscape-sized cells. We analyzed two species groups (forest/farmland) at two spatial scales (landscape/main habitat) in two seasons (spring/summer satellite imagery). We used generalized linear models and generalized dissimilarity models to investigate the patterns of species richness and community dissimilarity. Results show that, at the landscape scale, forest bird richness is better indicated by the availability of tree cover in the overall landscape than by the extent or structure of the forest habitats. Open-land birds also respond to landscape structure, namely to the spectral homogeneity and size of open-land patches and to the presence of perennial vegetation amid herbaceous habitats. Moreover, structure variables were more important than climate variables or geographic distance to explain community dissimilarity patterns at the regional scale. Overall, summer imagery, when perennial vegetation is more discernible, is particularly suited to inform indicators of forest and open-land bird community richness and dissimilarity, while spring imagery appears to be also useful to inform indicators of open-land bird richness. Finally, this study illustrates the potential of open-access data to inform large scale biodiversity monitoring.

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The propagation of invasive species as a consequence of rural Fires: a case study in Casal do Rei (Seia)

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The Natura 2000 Network results from the application of Directive 79/409/EEC, of 2 April 1979 (Birds Directive), repealed by Directive 2009/147/EC, of 30 November, and Directive 92/43/EEC (Directive Habitats), has as its objective the conservation and preservation of European Union species and habitats, as defined in the Convention on Biological Diversity, adopted at the Rio de Janeiro Earth Summit in 1992. In mainland Portugal, the network is constituted by 42 Special Protection Zones and 62 Sites of Community Interest [1].

The Natural Park of Serra da Estrela was classified by Decree-Law No. 557/76, of 16 July, and by Regulatory Decree No. 50/97, of 20 November. Much of its extension has been included in the Natura 2000 Network by the Council of Ministers Resolution 76/00 of 5 July, as it includes a diverse set of habitats of particular interest for biodiversity conservation [2].

Despite this protection imposed by law, this natural space undergoes changes and pressures, mainly due to the effects of human presence, but also due to the increasingly present effects of climate change and its impacts on the natural space, as is the case of the recurrence of rural fires, and the dispersion of invasive forest species such as acacia species [3].

The present work deals with the evaluation of the invasive potential of forest species and its relationship with the occurrence of rural fires, as a consequence of the impacts caused by climate change on forest development. For this evaluation, an area within the Serra da Estrela Natural Park was selected, in the place of Casal do Rei, municipality of Seia, where the occurrence of rural fires and the development of forest cover from these occurrences was analyzed, with emphasis on *Acacia dealbata* Link., because it is the most common invasive species and is occupying the largest extent.

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Advances in knowledge of Portuguese-laurel (*Prunus lusitanica* L. subsp. *lusitanica*) communities in Portugal Continental

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As part of a doctoral dissertation on the management and conservation of Portuguese-laurel of *Prunus lusitanica* L. subsp. *lusitanica*, which is being carried out at the University of Évora, a new Portuguese-laurel community with high chorological and floristic originality was identified. This community represents the southern boundary of the Portuguese-laurel distribution area in Portugal, occurring disjunctly on the right bank of the Tagus River basin, namely in Ribeira do Aziral (Mação Municipality). Although two phytosociological associations are recognized on the national territory, namely *Frangulo alni-Prunetum lusitanicae* for the Estrela, Açor and Lousã mountain ranges and *Luzulo henriquesii-Prunetum lusitanicae* for the Gerês mountain range, this new community features a set of thermophilic plants, such as *Pistacia lentiscus*, *Smilax aspera* and *Myrtus communis*. In this sense, we propose a new plant association which was called *Smilaco asperae-Prunetum lusitanicae*. This community lives at the headwaters of permanent and torrential waterlines, where it represents the local climax. Further downstream, where sediment is deposited, it still appears on the edge of the alder and willow potential. It is, therefore, a wet, thermomediterranean, temporo-hygrophilic association of the Biogeographic South Beira District. In syntax terms, the affiliation of this new community to *Fraxino angustifoliae-Ulmenion minoris* is suggested due to the strong presence of plants characteristic of this alliance and higher units such as *Salix atrocinerea*, *Osmunda regalis*, *Brachypodium sylvaticum*, and *Frangula alnus*. Finally, the current state of conservation of these relic communities is evaluated, with a view to adapting a set of management practices and improving the resilience of the structure to possible future impacts.

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Payment for Environmental Services (PES) in Brazil and the case of the Mantiqueira Highlands

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The sustainable use of natural resources means the regard of their use under the economical, social and environmental aspects. To promote this use, environmental political instruments were created. Among those, there is the Payment for Environmental Services (PES), also known in Portuguese as Pagamento por Serviços Ambientais (PSA) or Pagamento por Serviços Ecosistêmicos (PSE), intended to compensate those who properly manage the environment and the natural resources, producing environmentally friendly goods and services that benefit the society. The compensation strategy for farmers, especially for family farmers, represents an additional income, and takes place as an environmental management tool, enabling the co-existence of environmental preservation and economic growth. It is considered a promising mechanism for solving problems related to ecosystem degradation through an environmental mechanism that determines values of these assets. Therefore, it is necessary to analyze the PES for small scale farming and the benefits generated by its application. For this purpose, a basis for the establishment of the existing PES in Brazil was studied, using an exploratory research, analyzing the history and evolution of the concepts on the subject, describing the Brazilian farming structure, with family farming and analysis of the Bolsa Verde Program in the State of Minas Gerais. Finally, through a case study, ways were proposed to evaluate natural resources, identifying eligibility criteria for the implementation of a PES program in the Mantiqueira's highlands – a demo unity in Itamonte, Minas Gerais. It was concluded that the effectiveness of PES projects depends crucially on the adaptation to social, economic, environmental and political contexts, where the program will be deployed, and, therefore, further discussion on the subject and dissemination of studies become very important in building a foundation to assist the creation of new programs in the country, especially a National Policy for Payment for Environmental Services.

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