



V Encontro de Estudantes de Doutoramento em Ambiente e Agricultura

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V PhD Students Meeting in Environmental and Agriculture

9th December 2019

Pólo da Mitra, Universidade de Évora

Book of abstracts

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Editors:

Marta Laranjo

Ana Alexandre

Cláudia Marques

Address:

Universidade de Évora,

Largo dos Colegiais, 2

7004-516 Évora

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Dear participants,

It is our great pleasure to welcome you to the **V Encontro de Estudantes de Doutoramento em Ambiente e Agricultura (EEDAA) / PhD Students Meeting in Environmental and Agriculture**, held in Évora on the 9th December 2019. We have put together a one-day program with the aim of encouraging scientific discussion. This Meeting represents an excellent opportunity for young researchers to exchange ideas and to explore new challenges in research regarding Environmental and Agricultural Sciences.

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

The EEDAA focuses on four main areas: Biology and Biochemistry, Veterinary Sciences and Animal Production, Agricultural Sciences and Food Sciences, and Ecology, Environment and Landscape. The meeting will include one invited plenary lecture and several presentations selected from the abstracts submitted by PhD students. In addition, all authors will have the opportunity to present the posters in the two poster sessions included in the program.

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 scientific program and hope you will enjoy the meeting and appreciate the beautiful city of Évora, an UNESCO World Heritage. You should find all detailed information in the meeting book, including the scientific program, abstracts and a list of participants.

Welcome to Évora!

The Organising Committee,

Marta Laranjo, MED

Ana Alexandre, MED

Cláudia Marques, IIFA

COMMITTEES

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Program

Wednesday, 9th December 2020

| | |
|---|---|
| 08:45 | Registration |
| 09:15 | Opening session |
| | António Candeias Vice-Rector of Universidade de Évora Director of Instituto de Investigação e Formação Avançada (IIFA) |
| | Teresa Pinto Correia Director of MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento |
| Presentations selected from the submitted abstracts (10 min for each presentation) | |
| | Chair: Bruno Medronho Universidade do Algarve - MED |
| 09:30 | <i>Disease-relevant interactions between Mycobacterium bovis wildlife hosts and cattle: a systematic review and a meta-analysis</i> Eduardo Ferreira Universidade de Évora - MED |
| 09:45 | <i>Relationship between the level of adhesion to Mediterranean Diet and saliva composition</i> Teresa Louro Universidade de Évora - MED |
| 10:00 | <i>Differentially expressed genes involved in lipid metabolism in the muscle tissue of Alentejano and Bísaro pig breeds</i> André Albuquerque Universidade de Évora - MED |
| 10:15 | <i>Effect of early feeding management on post-weaning rumen biohydrogenation pathways</i> Letícia Fialho Instituto Politécnico de Beja - CEBAL |
| 10:30 | Coffee break |

| Presentations selected from the submitted abstracts (10 min for each presentation) | |
|---|--|
| 11:15 | <i>An overview of the presence of potato cyst nematodes in Portugal: geographical distribution, phylogenetic relationships and integrated pest management outcomes</i> Maria João Camacho Universidade de Évora – MED e INIAV |
| 11:30 | <i>Phenolic compounds as a defensive mechanism against olive fruit infestations: a case study of 'Galega Vulgar' and 'Cobrançosa' cultivars</i> Miguel Ferro Instituto Politécnico de Beja – CEBAL-MED |
| 11:45 | <i>Cynara cardunculus leaves extract fractionation – an enriched source of allelochemicals</i> Daniela Rosa Instituto Politécnico de Beja – CEBAL-MED e Universidad de Cádiz - INBIO |
| 12:00 | <i>Metagenomic analysis of fungal microbiota associated to grapevine trunk diseases in Alentejo region</i> Mariana Patanita Universidade de Évora - MED |
| 12:15 | Lunch |
| 13:30 | Poster Session I (includes poster presentations – 2 min for each poster) |
| 14:30 | Plenary Lecture <i>Ciência, Ambiente e Agricultura: Algumas questões fundamentais</i> Mário Carvalho Universidade de Évora - MED |
| Presentations selected from the submitted abstracts (10 min for each presentation) | |
| 15:30 | <i>Agro-industrial waste valorization of <i>Salicornia ramosissima</i> to produce cellulose nanofibers (CNFs) by acid and enzymatic hydrolysis</i> Alexandre Lima Universidade do Algarve - MED |
| 15:45 | <i>Potato growth under environment chamber conditions</i> Pedro Barbosa Universidade de Évora - MED |
| 16:00 | <i>Integrated use of chemical, biochemical and ecotoxicological tests to assess the effects of remediation actions of soils degraded by mining activities</i> Clarisse Mourinha Instituto Politécnico de Beja |
| | <i>Assessment and Improvement of Climate Change Adaptation Capacity of Smallholder Farmers</i> Joana Rocha Instituto Politécnico de Coimbra - CERNAS |
| 16:30 | Poster Session II (includes poster presentations – 2 min for each poster) <i>(coffee break included)</i> |
| 17:30 | Closing Session Best poster Prize and Best Oral Communication Prize |



Miguel Silvério

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

“The applicability of molecular markers to assist a grapevine breeding program aiming to develop new varieties resistant to pathogenic fungi”



Mariana Patanita

was awarded with the **Best Oral Communication Prize** for the presentation

“Metagenomic analysis of fungal microbiota associated to grapevine trunk diseases in Alentejo region”



ABSTRACTS

Plenary Lecture

Ciência, Ambiente a Agricultura: Algumas questões fundamentais

Mário Carvalho¹

¹MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: mariogpcarvalho@gmail.com

Há frequentemente a tendência para separar a ciência entre fundamental e aplicada, como se fossem dois mundos à parte. No entanto, ciência há só uma ou, se quisermos, há boa e má ciência. Só existe boa ciência quando os seus pressupostos são cumpridos, ou seja, há questões identificadas que se traduzem em perguntas, são levantadas hipótese(s) de trabalho de forma coerente. Posteriormente é necessário montar um dispositivo experimental que permita testar a hipótese(s) colocada(s). Depois é necessária uma correta análise estatística dos dados, o seu confronto com o conhecimento actual e, finalmente, a retirada de conclusões. A única diferença entre a dita investigação fundamental e investigação aplicada é a natureza dos problemas que nos propomos estudar. Na investigação fundamental os problemas/questões levantados partem de necessidades da própria ciência, enquanto que na investigação aplicada os problemas/questões iniciais resultam de uma necessidade do mundo exterior à ciência. Estas duas faces da ciência não vivem uma sem a outra. A ciência não existe sem pesados investimentos que se justificam pelos benefícios que a sociedade dela retira. Por outro lado, sem um corpo teórico que responda às questões de base, quanto muito poder-se-á fazer experimentação e ter-se-á muita dificuldade em interpretar e generalizar os resultados obtidos. Considerando as limitações financeiras que o país atravessa é muito importante uma escolha criteriosa da ciência a desenvolver. Os sistemas de agricultura Mediterrânicos enfrentam desafios enormes à sua sustentabilidade económica e ambiental, desafios esses que são agravados pelos cenários de alteração climática previstos para a região. A economia e o ambiente estão intimamente ligados no ecossistema agrícola e não existe solução para a sua sustentabilidade desligando os dois aspectos. A interligação entre clima, solos, sistemas de culturas e práticas agronómicas tem que atender, simultaneamente, às dimensões económica e ambiental. Nesta apresentação serão analisados alguns aspectos fundamentais para a ciência poder responder a estes desafios.

1.

Biology and Biochemistry

Disease-relevant interactions between *Mycobacterium bovis* wildlife hosts and cattle: a systematic review and a meta-analysis

Eduardo M. Ferreira^{1*}, Elsa L. Duarte¹, Mónica V. Cunha², Sara M. Santos¹

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Departamento de Engenharia Rural, Escola Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²cE3c- Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Email of the corresponding author: ferreiraeduardo.mr@gmail.com

Animal tuberculosis (TB) affects livestock and wildlife, having economic and animal health implications. Current knowledge postulates that *Mycobacterium bovis* maintenance and spread to other species is largely driven by interactions between cattle and wildlife. Despite recent insights about the role of direct and indirect interactions on TB dynamics at the wildlife-livestock interface, standardization of experimental approaches and data interpretation is lacking, which hampers inferences on the underlying transmission processes. Here, we seeked to examine global patterns of interactions between *M. bovis* wildlife hosts and cattle worldwide. We began by systematically reviewing the different concepts underlying the study of interactions and revised the main methodological approaches currently used to shed light on the ecological processes behind *M. bovis* transmission. Then, a meta-analysis of studies that have quantified interspecific interactions between wildlife and cattle was performed through a literature search on ISI's Web of Science. Additionally, relevant literature cited there was also screened. We used generalized linear mixed models to investigate the relative importance of direct and indirect interactions, while accounting for the effect of potential ecological and methodological sources of variation. We found four definitions adopted by researchers to evaluate indirect interactions between wildlife hosts of *M. bovis* and cattle using camera trapping, proximity and gps collars; and two definitions used to assess direct interactions encompassing camera trapping and proximity collars. Global estimates of direct interactions were low and even a rare event. On the opposite, indirect interactions frequencies were higher, with the mean estimated frequency representing 78.4 times more than the mean of direct interactions. Although we found no evidence for a significant effect of method type, species-trait, and landscape context on the frequencies of interactions, our results suggest that certain species (wild boar and badgers) are more likely to interact with cattle, particularly during warmer periods and at sites near water sources. Indirect interactions in shared environments might be a concern for TB transmission. The little scientific evidence on this topic along with complex ecological and methodological factors still limits drawing conclusions on general impacts and the source of interactions patterns at the wildlife-cattle interface.

Eduardo M. Ferreira was supported by a PhD fellowship funded by Fundação para a Ciência e a Tecnologia (SFRH/BD/146037/2019).

Relationship between the level of adhesion to Mediterranean Diet and saliva composition

Teresa Louro¹, Maria João Penetra², Carla Simões¹, Laura Carreira², Paula Midori Castelo³, Henrique Luis⁵, Pedro Moreira⁶, Elsa Lamy^{1,*}

¹MED (Mediterranean Institute for Agriculture, Environment and Development), Institute for Advanced Studies and Research (IIFA)IIFA – Instituto de Investigação e Formação Avançada; University of Évora, Évora, Portugal.

²Student from the Department of Biology, University of Évora, Évora, Portugal

³Dept. Pharmaceutical Sciences, Universidade Federal de São Paulo (UNIFESP), Diadema, Brazil

⁴School of Dental Medicine, University of Lisbon, Lisbon, Portugal

⁵Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Porto 4200-465

*contacting author, email: ecsl@uevora.pt

Mediterranean Diet is actually considered as one of the most healthy and sustainable dietary patterns. However, the adherence to this dietary pattern is not elevated, neither in countries form the Mediterranean area. Different aspects may be related with this, among which sensory characteristics, such as bitterness and astringency, associated to the polyphenol rich foods that compose it. Saliva composition is associated to the intensity with which these sensations are perceived. Moreover, evidences from studies with animal models suggest a potential of these compounds to produce changes in saliva composition. The objective of this study was to access if saliva protein composition is related with the level of adherence to MD, and in particular with the polyphenol levels of the diet. It was confirmed a growing polyphenol intake with increasing adhesion to MD. One protein band, containing cystatins presented higher expression levels in individuals with medium adherence to MD, comparatively to high and low-adherence groups., although this protein was not related with the polyphenol intake of these individuals. Polyphenol intake, that was observed to be negatively associated with Body Mass Index (BMI), was positively associated with the expression level of one alpha-amylase band, but not with the total enzymatic activity of this salivary protein.

This study allows the conclusion about differences in saliva proteome among individuals with different dietary habits. As well as in animal species, it is observed that polyphenol intake is associated with saliva composition. Despite the meaning of this salivary variability needs to be elucidated, to understand if it is the cause or the consequence of different food choices, these results reinforce that saliva is a promising fluid to be studied in the context of Mediterranean Diet.

This paper was funded by the FEDER as part of the Operational Programme for Competitiveness Factors (COMPETE) and received funding from the Science and Technology Foundation (FCT) under the Project UID/AGR/00115/2019 (ICAAM, University of Évora). Funding was additionally provided by the FCT–Portuguese Science Foundation, research contract CEECIND/04397/2017 to Elsa Lamy, while the foundation was not involved in carrying out this study or submitting it for publication. We acknowledge the support received by the State of São Paulo Research Foundation (FAPESP), grant n. 2017/26400-6. The authors would like to thank all the subjects who took part in the study.

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

Ana Galveias¹, Maria João Costa², Juan Luís Guerrero-Rascado³, Paloma Cariñanos⁴, Ana Rodrigues Costa¹ & Célia M. Antunes¹

¹Institute of Earth Sciences - ICT & Department of Chemistry, School of Sciences and Technology, University of Évora, Évora, Portugal

²Institute of Earth Sciences - ICT & Department of Physics, School of Sciences and Technology, University of Évora, Évora, Portugal

³Applied Physics Department Sciences Faculty, University of Granada, Granada, Spain

⁴Andalusian Institute for Earth System Research (IISTA-CEAMA) and Department of Botany, University of Granada, Granada, Spain

Email: acgjorge@uevora.pt

The *Cupressaceae* is considered a significant source of airborne allergens and allergy to its pollen is reported worldwide. The period pollination for this species is the end of winter and beginning of spring, depending meteorological factors. The objective of this study was to compare the aerobiology of *Cupressaceae* pollen and to analyse the influence of meteorological factors in the *Cupressaceae* pollination in two different sites: Évora and Granada. Data were collected in two cities of southwestern Europe (Évora: 38.568542°N; 7.910526°W and Granada: 37.1041°N 3.3555°W). The *Cupressaceae* pollen was monitored using standard Hirst-type traps (2017-2019) and identified by optical microscopy, according to the standard methodology. The meteorological parameters were obtained from ICT/CGE platform and Agencia Estatal de Meteorología (AEMET). According to the Köppen climate classification Evora and Granada are Csa, characterized by a temperate climate with warm and dry summer. The annual mean air temperature is 15.8 °C and 15.7 °C, respectively. The rainfall period, occurring mostly between autumn and spring, presents an average annual precipitation of 608.5 and 352 mm, respectively. Large concentrations of pollen were detected in both cities, 5-6-fold higher in Granada (Annual Pollen Integral, API, ranging from 3137- 8201 pollen/m³ in Évora and 17851- 81798 pollen/m³ in Granada). The peak date occurred between 25th February and 14th March in both cities and the pollen season duration was similar, ranging from 128-212 days. Granada presented twice the days >100 pollen/m³. During the season, temperature and solar radiation positively correlated with API while precipitation and relative humidity were negatively correlated. Accumulated precipitation from September-November and accumulated temperature and global solar radiation from December-January correlated with higher seasonal API.

These results have shown higher prevalence of *Cupressaceae* pollen in Granada, probably due to the surrounding forests. Meteorological factors, particularly, the autumn rain and the temperature during winter months are relevant for *Cupressaceae* API and might constitute indicators for the prediction of pollen seasonal intensity.

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

***Cynara cardunculus* leaves - a natural source of cynaropicrin**

A. Paulino^{1,2}, J. Santos¹, T. Brás^{1,3}, D. Rosa^{1,4,5}, R. Pires¹, A.F. Belo^{5,6}, C. Pinto-Cruz^{5,6}, I. Guerreiro⁷, M. Regato⁷, O.S. Paulo², L. Marum^{1,5}, M.F. Duarte^{1,5}

¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801908 Beja, Portugal.

²Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal.

³LAVQ/REQUIMTE, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

⁴Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

⁵MED – Mediterranean Institute for Agriculture, Environment and Development, CEBAL, 7801-908, Beja, Portugal.

⁶Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, 7002-554 Évora, Portugal

⁷Escola Superior Agrária, Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

*Email: ana.paulino@cebal.pt

Cynara cardunculus as a multipurpose crop, represents a natural source of sesquiterpene lactones, namely cynaropicrin [1]. Portugal has an incredible natural variability of *Cynara cardunculus* conducting to the necessity of genetic diversity studies, for further plant selection/breeding within certain desired agronomic traits [2].

About 175 individuals (F0 generation) of wild cardoon from 25 different geographical locations in Alentejo region, were identified in the framework of ValBioTecCynara project. From each natural population, 7 different genotypes were collected and chemically and genetic characterized. The F1 generation from half-sib families (total of 1061 individuals) is currently installed in two experimental fields in the south of Portugal, Beja and Évora.

Currently, and following the previous studies, a genomic characterization is ongoing, aiming the identification and selection of high added value cardoon plants, according to cynaropicrin production profiles, and to evaluate the differential expression of relevant transcripts. 49 cardoon plants were evaluated and collected in the experimental fields installed. Over 4 months (March, April, May and June) fresh leaves were collected, extracts obtained and further chemically characterized, in terms of Cc leaves-lipophilic extracts cynaropicrin content (mg/g DW). Pulsed ultrasound extraction has performed as previously described [3], using ethanol as solvent, and high-performance liquid chromatography for cynaropicrin quantification. The results obtained in the different plants over the 4 months collection points show that there is a great variability in terms of cynaropicrin content, concerning the genotype and collection period. Different chemical profiles were identified, *Cynara cardunculus* leaves lipophilic extracts presented a remarkable range between 12.7 and 80.7 mg/g DW of cynaropicrin in March and April, respectively. The next step will be the analyse of transcriptome. For that total RNA will be extracted from ten biological samples from each group (H, L). RNA concentration and integrity will be assessed by Agilant BioAnalyzer. cDNA libraries will be prepared, and stranded pairedend sequencing will be performed on Illumina Sequencers, for transcriptomic analysis.

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 Contrato – Programa to L. Marum (CEECINST/00131/2018), PhD grant to A. Paulino (SFRH/BD/145383/2019), T. Brás (SFRH/BD/110969/2015) and D. Rosa (SFRH/BD/143845/2019), and Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED).

[1] Ramos, P. A. et al. J. Agric. Food Chem., 2013, 61(35), 8420-8429

[2] Castro M. M. et al. Jornadas MED, 2019, pp 135

[3] Brás, T et al. EPO nº PCT/IB2017/053152, PCT – The International Patent System, 2017

How different snacks produce a distinct effect in salivary protein composition

Carla Simões^{1†}, Inês Caeiro^{1†}, Andreia Colaço², Beatriz Sabino², Cristina Piteira², Inês Ferreira², Márcia Fontes², Laura Carreira^{1, 3}, Nádia Correia³, Elsa Lamy^{1*}

¹ Mediterranean Institute for Agricultural, Environmental and Development, University of Évora

² Department of Biology, School of Sciences and Technology, University of Évora

³ Department of Chemistry, School of Sciences and Technology, University of Évora

*Corresponding author: ecls@uevora.pt

† Authors contributed equally to this work

Saliva is the fluid that first contacts with the external environment and, besides participating in oral and general defense it interacts with food, participating in the perception of its sensory characteristics and digestion. It is known that dietary constituents may influence saliva composition. Most of this knowledge come from animal studies, reporting increases in the levels of cystatins induced by the consumption of capsaicin or polyphenols, or changes in salivary proline rich proteins (PRPs) levels in response to tannin-enriched diets. More recent studies start to relate salivary proteome with diet composition, in humans. Recently we observed that saliva is capable of quick responses to food stimuli, such as odor or taste/mastication. However, it remains to elucidate if the response is the same for different types of foods.

The objective of the present work was to access the saliva responses for different foods. Twelve healthy non-smoking women (20-30 years old) were tested for four different snacks (yoghurt, bread, apple and nuts), in a design where all individuals tested each snack (one in each day) and all snacks were tested in the same day. Tests occurred 2h after standardized breakfast. Nonstimulated saliva was collected before, and immediately, 5 min and 15 min after snack intake. At each time of saliva collection, subjects rated satiety using a 9-point scale Flow rate, total protein concentration, α -amylase enzymatic activity and proteome were analyzed. Two-way ANOVA repeated measures (factors: collection time and snack) showed different effects of the snacks for protein band containing albumin, increasing after apple and decreasing after yoghurt. Cystatins containing bands increased in response to bread and apple, but not for other snacks, whereas the bands containing polymeric-Ig receptor and Ig light-chains decreased significantly after the consumption of all snacks. Although the expression levels of amylase bands didn't change, its enzymatic activity (per volume) increased with the consumption of all snacks, except nuts.

These results show that saliva response to foods occur not only in the amount of flow produced, but also in proteome, being specific for each type of food.

This work was supported by the FCT–Portuguese Science Foundation, through UID/AGR/00115/2019 and research contract CEECIND/04397/2017 to Elsa Lamy.

Biological aspect of the catadromous thin-lipped grey mullet trophic migration

Pereira, E¹; Rato, A¹; Quintella, B^{1,2}; Quadrado, F³; Telhado, A³; Pinto, V³; Batista, C³ and Almeida, PR^{1,4}

¹MARE- Centro de Ciências do Mar e do Ambiente, Universidade de Évora, Évora, Portugal

² Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

³Departamentos dos Recursos Hídricos e do Litoral e Proteção Costeira, Agência Portuguesa do Ambiente, I.P., Portugal

⁴ Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal

Email: d39006@alunos.uevora.pt

Among the diadromous fish species, catadromous mullets have one of the most plastic life-cycles. Most of this species tolerate in some degree, different conditions of salinity, temperature and turbidity, being able to enter brackish water environments and in some few cases, perform extensive migrations to freshwater habitats, as occur with the thin-lipped grey mullet *Chelon ramada* (Risso, 1827). This species, is the most abundant diadromous fish in Portuguese rivers, yet biological aspects of their trophic migration and patterns of freshwater habitat use are still poorly understood. Thus, between 2013 – 2019, migratory movements of *C. ramada* through a vertical slot fishway, located 45 km from the Mondego river mouth, were studied using PIT telemetry and video-image processing. A total of 1270 animals were PIT tagged and individual movements though the fishway were analyzed to infer the individual movement patterns, their migration strategy (at an annual and interannual basis) and identify any differences in migratory behavior. Video-images collected at the fishway monitoring window were used to obtain, the total number of species movements upstream freshwater stretch, seasonal and circadian patterns (video counts), as well as species size-composition.

The present work shows that river fragmentation can have serious impact on this species life-cycle and fishway design should take into consideration multispecific requirements to safeguard fish passage in both directions.

Changes in salivary proteome and metabolome and their effects on oral food perception – the particular case of xerostomia

L. Carreira ^{1,2}, J. Zamora ³, J. Nunes ³, J. Coromina ³, C. Cordeiro ⁴, E. Lamy ²

¹ Biochemistry PhD student, Chemistry department, University of Évora, 7002-554 Portugal,

² MED- Mediterranean Institute for Agriculture, Environment and Development; University of Évora, 7002-554 Portugal

³ Dry mouth institute, Teknon Hospital, 08022 Spain;

⁴ FT-IRC and Structural Mass Spectrometry Laboratory, Faculty of Science, University of Lisbon, Campo Grande 1749016, Lisbon, Portugal

Email address: d47128@alunos.uevora.pt

Saliva is a fluid composed of water, electrolytes and organic components (e.g. proteins), which constitution will vary depending on factors such as age, sex, the use of drugs, eating habits, pathologies, among others. Its' contribution in oral food perception is now recognized, and changes in composition may result in modified perceptions of the sensory characteristics of foods.

Studies have indicated a relationship between food taste, trigeminal sensations and salivary proteins, namely higher amylase enzymatic activity and lower sweet taste sensitivity, higher carbonic anhydrase VI expression levels in individuals perceiving higher intensity of bitter taste, or salivary cystatins and proline rich protein peptides involved in bitterness perception. Moreover, astringency, an oral tactile sensation involved in food rejection, is recognized to be dependent on salivary protein composition. In addition, regarding retronasal aroma, saliva is starting to be recognized as an important factor modulating the access of volatile molecules to olfactory receptors.

Xerostomia is a pathology characterized by the sensation of dry mouth, which is likely to be of variable origin such as autoimmune diseases, polymedication, radiotherapy of the head and neck area, or it may be of idiopathic origin. The symptoms are oral discomfort, loss of taste and difficulties in chewing, among other things. As such, nutritional aspects may be particularly compromised. And, while it is known that saliva volume may be compromised in some patients, the exact changes in saliva proteome and metabolome are not well understood. Acquiring this knowledge is essential, both to better understand the disease, and to manage the nutritional aspects of the individuals' who suffer from it.

The aim of this work is to review existing literature about xerostomia and saliva biochemistry, and xerostomia and oral food perception/food choices. As an outcome of this review, a synthesis of the major salivary changes in these patients and what is known about their major difficulties in oral food perception and ingestion, will be presented. This work will serve as the basis for the development of experimental work, where saliva proteome and metabolome will be characterized, to identify the main salivary components responsible for altered food perception and nutritional contribution.

Anthocyanin fingerprinting of *Vitis vinifera* varieties produced in Alentejo: a two year comparative study

M. I. Rouxinol¹, J. M. Barroso², M. R. Martins³, A. E. Rato²

¹MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³Departamento de Química & Laboratório HERCULES (Herança Cultural, Estudos e Salvaguarda), Universidade de Évora, 7000-809 Évora

Email: mir@uevora.pt

Anthocyanins are one of the most important class of polyphenols and pigments in plants, namely in red wine grapes. In *Vitis vinifera* are mainly found delphinidin, cyanidin, petunidin, peonidin and malvidin. They occur in nature in the form of heterosides (linked to carbohydrates), acetylated forms of glucosides or p-coumarylated glucosides. In some varieties and in some specific cultural conditions, some grapes also might develop pelargonidin in the grape's skins.

The aim of this work was to evaluate the anthocyanin composition of four grape varieties (Syrah, Trincadeira, Aragonês and Touriga Nacional) during 2 different years in Alentejo. Samples were collected in a vineyard installed in Herdade da Mitra, Valverde, Évora from verásion to harvest. Berries were weighted and the skins were taken off manually. Extracts obtained from grape skins were used to determine the total phenolic content (TPC) using Folin Ciocalteau Method and anthocyanins profile by HPLC using a UPLC Dionex Ultimate 3000, equipped with a diode array detector (DAD) and Chromeleon 6.8. software. Also, a PCA analysis was performed to understand which anthocyanins are the main contributors to the total anthocyanin content.

TPC was generally higher in 2017. Syrah, Trincadeira and Touriga Nacional have an increase in the phenolic content during maturation. Among all varieties, only Aragonês variety had an increase in the first 15 days, followed by a decrease. In 2018 all varieties had an increase during the first 15 days, followed by a decrease. In both years, at harvest, Touriga Nacional had the highest phenolic content.

In all varieties were found 3 distinct anthocyanin groups: monoglucosides, acetyl-glucosides and coumaroyl glucosides during maturation. The main anthocyanin found in all varieties during verásion was Maldivin-3-O-glucoside. The total content of anthocyanins was higher in 2017 in all varieties, with statistical significative differences. In 2017 maturation the main anthocyanin group found in all varieties was anthocyanin-monoglucosides. In 2018 Syrah had a higher content of the coumaroyl anthocyanin group at harvest when compared with other groups, while the other varieties kept with anthocyanin-monoglucosides has the majority group.

In vitro establishment of almond (*Prunus dulcis* Mill.) portuguese varieties and rootstocks for micrografting assays

S. Caeiro^{1,2}, R. Pires², A. Faustino², A. Rosa⁴, A. Marreiros⁴, J. Canhoto¹, S. Correia¹, L. Marum^{2,3}

¹University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

²Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

³MED – Mediterranean Institute for Agriculture, Environment and Development, CEBAL, 7801-908, Beja, Portugal

⁴Direcção Regional de Agricultura e Pescas do Algarve, Apartado 282, Patação, 8001-904 Faro, Portugal

Email: caeirosandra@hotmail.com

Almond demand and production has increased in recent years, due to its high nutritional value and benefits for human health. In Portugal, almond culture is traditional from the Algarve, Trás-os-Montes and Alto Douro regions, but recently new orchards have emerged in other regions such as Beira Interior and Alentejo.

Cultivated almond varieties are usually grafted to increase production and improve fruit quality. Grafting, allows the movement of molecules (e.g., hormones, proteins, small RNAs) between the scion and rootstock thus controlling both stem and root growth. Such regulators are also essential for graft compatibility and agronomic performance. It has been reported that gene expression is affected by the genotype of both scions and rootstocks, and that specific miRNAs and transcription factors contribute not only to the success of the grafting but also control the growth and architecture of the grafted plants. The main objective of this work was to analyse physiological and genetic regulation aspects involved in almond micrografts with focus on Portuguese traditional varieties. To achieve this goal, protocols for *in vitro* establishment of scions and rootstocks, shoot proliferation and micrografting will be carried out and optimized. Micrografts will be performed using different combinations of scions (commercial and traditional varieties) and rootstocks (commercial rootstocks or seedlings of bitter almond) previously established, micropropagated and selected based on propagation and rooting rate; this feature being particularly relevant for selection of rootstocks. A germination assay was performed to evaluate the role of genotype, flowering time, cold treatment, growth regulators and explant types on seed germination and seedling growth. The results showed that the germination rate was >46.7% for all varieties tested. The *in vitro* establishment of traditional and commercial varieties and bitter almond seedlings was achieved and multiplication rates are now being evaluated. Following optimization of *in vitro* multiplication protocols micrografting will be carried out, and levels of endogenous IAA (índole-3-acetic-acid) and auxin-related transcription will be determined. This study aims to contribute for a better understanding of the molecular mechanisms associated with grafting, and the interaction between scion and rootstocks in almond.

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Sorption of okadaic acid lipophilic toxin onto plastics in seawater

Sara T. Costa^{1,2,3}, Alisa Rudnitskaya⁴, Carlos Vale², Lúcia Guilhermino^{2,3}, Maria João Botelho^{1,2}

¹IPMA, I.P. (Portuguese Institute for the Sea and Atmosphere), Av. Alfredo Magalhães Ramalho 6, 1495-165 Algés, Portugal.

²CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), University of Porto, Global Changes and Ecosystems Services Research Line, Rua dos Bragas 289, 4050-123 Porto, Portugal.

³ICBAS (Instituto de Ciências Biomédicas de Abel Salazar) Universidade do Porto, Departamento de Estudos de Populações, Laboratório de Ecotoxicologia e Ecologia, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

⁴CESAM (Centre for Environmental and Marine Studies), Campus Universitário de Santiago, University of Aveiro, 3810-193 Aveiro, Portugal.

Email: satcostaa@gmail.com

Plastic debris achieved increasing proportion in the marine litter found in various Ocean regions, which puts at risk the world's oceans health. Plastics present in closed bays, gulfs and seas with densely populated coastlines are available to marine organisms inhabiting the water column and sediments. Several studies have shown that persistent pollutants derived from anthropogenic activities can be adsorbed onto plastic debris and microplastics in marine systems due to their affinity to virgin and aged plastic surfaces, which increases the dangerousness of ingestion of those particles. Several marine phytoplankton species are known to sporadically overgrow causing harmful algal blooms with impacts on humans and marine life. Toxic metabolites released during these blooms – marine biotoxins – can be accumulated in shellfish's tissues and cause adverse effects in these resources and human consumers. The present study tested, under laboratorial conditions, the sorption of okadaic acid (OA), a regulated lipophilic biotoxin with respect to human health, onto 6-mm plastic circular fragments. Transparent water bottles, white plastic plates, yellow food packaging, and grey dossier tabs are commonly used objects made of polyethylene terephthalate (PET), nonexpanded polystyrene (PS), expanded polystyrene (EPS), and polypropylene (PP), respectively. These materials were used as sorbents in sorption experiments. Fragments (4 g L^{-1}) were exposed for 96 h to artificial saline water (ASW, NaCl, salinity 35) spiked with 10 ng mL^{-1} of OA. Results of the experiments revealed that dissolved OA sorbed onto random floating plastics in ASW. A broad percentage of OA removed from the water by plastics after 48 h of exposure followed the decreasing ranking order based on the mean ($\pm \text{ SD}$): $83 \pm 1.9\%$ (PS) $> 62 \pm 7.1\%$ (EPS) $>> 37 \pm 9.5\%$ (PP) $\approx 30 \pm 5.1\%$ (PET). Sorption appears to be highly influenced by polymer's characteristics, such as polarity and degree of crystallinity. These results point to the plausible interaction of OA and plastics in coastal waters and it may potentially increase bioavailability and accumulation of this toxin in shellfish. Further studies on the stability of OA plastic aggregates in seawater should elucidate its impact on bivalves.

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2.

Veterinary Sciences and Animal Production

Differentially expressed genes involved in lipid metabolism in the muscle tissue of Alentejano and Bísaro pig breeds

Albuquerque, A.^{1*}; Félix, M.R.^{1,2}; Charneca, R.^{1,4}, Laranjo, M.¹; Martins, J.M.^{1,3}

¹MED-Mediterranean Institute for Agriculture, Environment and Development, IIFA-Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*Email: andrealb@uevora.pt

The Alentejano (AL) and Bísaro (BI) pigs stand as the predominant local breeds raised in Portugal. AL pigs are characterized by lower growth rates and precocious and higher lipid deposition, particularly of monounsaturated fatty acids, when compared to BI pigs. Lipid and fatty acids composition of meat and meat products are important features for consumers health, and gene expression is a decisive process determining fatty acid composition and deposition rates in the muscle tissue of every mammal. This study investigates the transcriptomic fundamentals associated to the phenotypical differences through a real-time qPCR approach on a set of candidate genes responsible for regulating lipid metabolism processes. *Longissimus lumborum* samples were collected at slaughter from five animals of each breed raised under similar conditions until ~150kg BW. Total RNA was extracted, and target cDNA was amplified to estimate expression levels when compared to endogenous control genes. Several lipogenic related genes were found to be overexpressed in the AL breed including *ACLY* ($\log_{2}FC=0.50$, $p<0.05$), *ELOVL6* ($\log_{2}FC=0.67$, $p<0.01$), *ME1* ($\log_{2}FC=0.78$, $p<0.05$), while other major lipogenic markers, such as *FASN* ($\log_{2}FC=0.45$, $p=0.09$) and *ACACA* ($\log_{2}FC=0.28$, $p=0.17$) did not attain statistical significance, but indicate a tendency towards AL. The gene coding for the central appetite regulation hormone (LEP) was detected as significantly overexpressed in AL ($\log_{2}FC=1.60$, $p<0.05$), which may suggest the occurrence of leptin resistance in this breed, comparable to what happens with the genetically similar Iberian pig, failing to decrease feeding and increase stored energy expenditure. Similarly, adiponectin resistance is predicted to have developed in AL pigs since higher *ADIPOQ* levels ($\log_{2}FC=0.90$, $p<0.01$) are associated with leaner animals due to the activation of fatty acid oxidation pathways by this regulator. These results agree with the previously described high intramuscular fat content profile of the AL breed, essential in enhancing the overall sensory quality of its meat and meat products.

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Effect of early feeding management on post-weaning rumen biohydrogenation pathways

Letícia Fialho¹, Rui Bessa², Eliana Jerónimo³

¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

²Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

³MED - Mediterranean Institute for Agriculture, Environment and Development, CEBAL, 7801-908 Beja, Portugal

Email: leticia.fialho@cebal.pt

Ruminant fat is characterized by high levels of saturated fatty acids (SFA), low contents of polyunsaturated fatty acids (PUFA) and variable amounts of trans fatty acids (FA). This FA profile, which is generally considered to be harmful to human health, results from the intense metabolism by which dietary lipids are subject in the rumen. Ingested lipids are quickly hydrolyzed, and then the unsaturated FA released are extensively biohydrogenated by the action of microbial population, with the production of SFA and several unsaturated C18 FA isomers, including trans FA [1,2]. Several nutritional factors and dietary strategies have been explored to modulate the ruminal biohydrogenation (BH) in order to reduce the SFA content and increase the content of healthy FA in fat, such as vaccenic (t11-18:1) and rumenic (c9,t1118:2) acids. However, under specific dietary conditions, a considerable inter-animal variation in the BH pathways is observed, limiting the effectiveness of dietary ruminal BH modulation strategies to improve the FA composition of ruminant fat. Reasons for this inter-animal variation in the BH pathways are not known, but probably are due to rumen ecology and animal factors [2].

Rumen microbial colonization begins as early as the first day of life [3], and the early life feeding management affect the establishment of the rumen microbial community, but also its composition and/or activity in the post-weaning period [4]. Results show that dietary intervention during early life could potentially be used to manipulate rumen microbiome composition to achieve specific outcomes, which lead us to believe that the early life intervention might be an approach to modulate the ruminal BH. However, the impact preweaning dietary regime on ruminal BH pathways in the post-weaning is not known. So, the objective of this work was to explore the early life feeding management as a tool for modulate the ruminal BH and create favourable conditions to enrichment of ruminant fat with desirable FA.

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Dairy calves welfare indicators

F. Silva¹, J.L. Cerqueira^{1,2}, C. Conceição³, S. Silva¹

¹CECAV (Centro de Ciência Animal e Veterinária), Universidade de Trás-os-Montes e Alto-Douro (UTAD), Quinta de Prados, Ap. 1013, 5000-801 Vila Real, Portugal.

²Escola Superior Agrária do Instituto Politécnico de Viana do Castelo, Refóios do Lima, 4990-706, Ponte de Lima, Portugal

³MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Zootecnia, Universidade de Évora (UE), Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: fsilva@uevora.pt

Animal welfare, nowadays, is a meaningful worldwide topic in animal production, regarding ethical, legal and commercial concerns. Despite many advances in this area, recent literature suggests that there is still much work to be done. The morbidity and mortality numbers in dairy calves before weaning reveals either a lack of scientific knowledge and/or a deficiency in the communication between academic research and dairy farms. The morbidity and mortality in dairy calves is also a societal issue that translates into an ethical concern related to animal production. Therefore, reducing those problems in dairy calves allow better welfare, higher efficiency in productivity and ethical standards. A better comprehension of welfare indicators in dairy calves is essential to implement management strategies that all in all are efficient, allow proper growth and reduce discomfort and illness. From a protocol between UTAD and UE, a project has the main objective to improve the scientific knowledge of dairy calves welfare, using physiological, behavior, health and morphometric measures as welfare indicators. The specific objectives are: (1) To evaluate the impact of the farm infrastructures and physical features on the animal welfare; (2) To assess behaviors mechanisms associated with discomfort, pain, disease and fear; (3) To identify factors that affect the calf's passive and active immunity and how it is related to health and performance; (4) To identify and validate specific calf welfare indicators, using recent and non-invasive methodologies, such as infrared thermography to detect infectious diseases and saliva to measure stress. The data will be collected from female calves of Holstein-Friesian breed from dairy farms in the north and south of Portugal. The dairy farm hazards are going to be analyzed according to the European Commission indications regarding calves welfare. From 72 welfare indicators revised in the literature, the ones with more validity and feasibility are going to be analyzed. The results are going to be divulged in scientific journals, symposium and national farm meetings during the next four years.

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Use of almond hulls in ruminant nutrition

L. Cachucho¹, K. Paulos², C. Costa², M.T. Dentinho^{2,3}, S. P. Alves³, E. Jerónimo^{1,4}

¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801908 Beja, Portugal

²Instituto Nacional Investigação Agrária e Veterinária, Polo de Investigação da Fonte Boa (INIAV-Fonte Boa), 2005048 Vale Santarém, Portugal

³Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade

Lisboa, Avenida Universidade Técnica, 1300-477 Lisboa, Portugal

⁴MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, CEBAL, 7801-908 Beja, Portugal

Email: liliana.cachucho@cebal.pt

Ruminants are characterized by its ability to convert inedible feed inputs into high quality food. Several plant by-products, from agricultural and food industry, are safe and suitable for use in ruminant nutrition, providing nutrients and bioactive compounds with potential beneficial effects on animal health and well-being, feed efficiency and quality of products. Almond hulls (AH), which consist of the green outer covering of the almond, is available in several regions of the world. In Portugal, about 14,304 t of shelled almonds are produced annually [1], and it is estimated that AH represent 52% of the total fresh weight of fruit. The objective of this study was to explore the utilization of AH in ruminant nutrition, promoting the exploitation of this local feed resource and the sustainability of the meat production systems.

Almond hulls collected in Baixo Alentejo Region, southern Portugal, contain high sugar content (28% of dry matter (DM)), moderate amounts of fiber (25% and 18% DM of neutral detergent fiber and acid detergent fiber, respectively), low amounts of protein (6% DM) and fat content (< 1% DM) and high levels of ashes (10% DM). The main fatty acids (FA) are linolenic and oleic acids (40 and 35% of total FA, respectively), while linolenic acid represents 15% of total FA. The most abundant amino acids are lysine and aspartic and glutamic acids (9.16, 7.53 and 5.20 g/kg DM), respectively. Almond hulls present moderate organic matter digestibility (57%). Moreover, AH is a source of phenolic compounds (10–60 mg gallic acid equivalents/g DM), and has variable antioxidant activity (24–230 mg FE equivalents/g DM and 27–181 mg Trolox equivalents/g DM, in FRAP and ABTS assays, respectively). Despite the number of studies is still limited, various levels of AH were applied in diets for dairy cows [2,3], sheep [4] and goats [5] with beneficial effects of animal performance, quality of products and feed efficiency. However, additional research is needed in this area, particularly on use of AH in nutritional strategies to improve the FA composition and the oxidative stability of ruminant products.

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Contribution for the improvement of oral mycosis treatment in captive vultures

M. Couto¹, F. Lopes², M. Limede², D. Vieira², M. Grilo¹, E. Cunha¹, L. Tavares¹, R. Dias³, M. Oliveira¹

¹CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa

²CERAS - Centro de Estudos e Recuperação de Animais Selvagens, Castelo Branco

³BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa

Email: mpcouto@campus.ul.pt

Vultures, as scavenger birds, have developed the ability to feed on carcasses containing pathogenic microorganisms. In the wild, debilitated individuals, mostly at a young age, might develop oral mycosis, which lesions can impair animals' food intake, increasing morbidity. It can also occur in captivity, mostly due to prolonged antibiotic therapy and stress, affecting recuperation rates. Therapeutic protocols currently applied in Rehabilitation Centers often consist of removing the plaque-like areas with an antiseptic solution, or, in severe cases, the use of antifungal systemic protocols. By characterizing yeast species responsible for captive individuals' oral infections and understanding their degree of susceptibility to antifungals, we aim to optimize therapeutic protocols in use.

Eurasian Griffon Vulture (*Gyps fulvus*) and Cinereous Vulture (*Aegypius monachus*) oral swabs were collected at CERAS, Castelo Branco, by sampling five healthy vultures with no gross signs of disease, and 3 vultures with oral candidiasis. Samples were cultured in Sabouraud dextrose agar at 27°C for 48 hours, and yeast-like colonies were further isolated for morphological and biochemical identification using API 20C AUX. *Candida* isolates susceptibility to Caspofungin, Posaconazole, Itraconazole, Voriconazole, Amphotericin B and Fluconazole was determined by the disk diffusion method.

Besides *Candida*, other yeast genera were present in the oral cavity of these animals, including *Rhodotorula*, *Cryptococcus* and *Trichosporon*, being the latter an emerging fungal pathogen. Most animals were colonized by more than one species, with 50% of the individuals simultaneously presenting 3 different species in their oral cavity. Levels of resistance were worrisome, as of the three *Candida* isolates identified, none was susceptible to all antifungals tested, with one being only susceptible to Caspofungin.

Considering that in Portugal the Eurasian Griffon and Cinereous Vultures are classified as Near Threatened and Critically Endangered, respectively; and the relevance of oral mycosis in these animals' period of hospitalization, these results will contribute for improving the therapeutic protocols available at Rehabilitation Centers.

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3.

Agricultural Sciences and Food Sciences

**An overview of the presence of potato cyst nematodes in Portugal:
geographical distribution, phylogenetic relationships and integrated pest
management outcomes**

Maria João Camacho^{1,2}, Maria Lurdes Inácio², Eugénia de Andrade², Manuel Mota^{1,3}

¹ NemaLab, MED – Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² INIAV - National Institute for Agriculture and Veterinary Research, Quinta do Marquês, 2780-159 Oeiras, Portugal.

³ Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006554 Évora, Portugal.

Email: mjoaoc@uevora.pt

The potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, pose one of the greatest threats to potato crops worldwide and are subject to strict quarantine regulations. In Portugal, *G. rostochiensis* was first reported in 1956 and *G. pallida* in 1988, both in Northern Portugal. Since their first detection, both species have been found throughout the country. To ascertain the PCN status in Portugal, the national official control was established in 2010. In mainland Portugal, from 2013 until 2019, 748 soil samples were surveyed from potato fields, to detect and identify both species through morphological and molecular analyses. Duplex PCR with species-specific primers (ITS5/PITSp4+PITSr3) was used to identify PCN species, as recommended by EPPO protocols. Both species are present in all potato producing regions. In fact, during the survey period, an incidence of 22.5% was estimated for the tested samples, with a greater presence of *G. pallida* alone (49.4%), compared to *G. rostochiensis* alone (28.6%) and mixed populations (22%). According to the obtained statistical results, infestation patterns vary among regions, increasing from south to north, where PCN were first detected. To study phylogenetic relationships among Portuguese *Globodera* isolates, the ITS-rDNA region was amplified using the primers 18Lmod/ITS4 and sequenced. GeneBank-BLAST homology search using 14 obtained sequences of *G. pallida* and 20 sequences of *G. rostochiensis* shows that ITS-rDNA sequences display high homology with these species and no significant intraspecific variation was observed. For *G. pallida*, variation was slightly smaller (98–99%) than for *G. rostochiensis* (97–100%) which can be due to its later introduction in the country. The relationship among presence, infestation rate, spread and geographical distribution of PCN is discussed in terms of behavioural responses of the potato cultivars and the implications for developing new integrated crop protection measures. Statistics revealed that the use of potato cultivars resistant to *G. rostochiensis* led to a decrease of this species but had no influence on *G. pallida* detections, which continues to spread freely since there are no effective resistant cultivars for this species. Therefore, there is a need for a new approach to the management of PCN, mainly *G. pallida*.

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**Phenolic compounds as a defensive mechanism against olive fruit infestations:
a case study of 'Galega Vulgar' and 'Cobrançosa' cultivars**

M. Ferro^{1,2*}, E. Lopes^{3,4}, M. Afonso¹, A. Peixe⁵, F. Mondragão-Rodrigues^{3,6}, M. F. Duarte^{1,2}

¹CEBAL (Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo)/IPBeja (Instituto Politécnico de Beja), 7801-908 Beja, Portugal.

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), CEBAL, 7801-908 Beja, Portugal.

³Instituto Politécnico de Portalegre, 7300-110 Portalegre, Portugal.

⁴Departamento de Fisiología Vegetal, Universidad da Extremadura, Av. de Elvas, 06006 Badajoz, Spain.

⁵MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁶MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*Email: miguel.ferro@cebal.pt

The phenolic composition of olive fruits represents a vast and unique source of health beneficial molecules due to the presence of specific phenolic compounds (PCs), such as verbascoside (VERB), oleuropein (OLE) and its derivative molecules. Despite of being some of the most critical compounds regarding olive oil quality, these PCs are mostly abundant in olive fruits and leafs due to their hydrophilic nature. In olives, the phenolic profile suffers a deep and constant change along fruit ripening, being the phenolic alcohols, such as hydroxytyrosol (HT), mainly formed by OLE, and/or OLE aglycone molecules degradation. Also, OLE is associated with an endogenous defensive system against invasive species, such as *Bactrocera oleae*, therefore, high OLE values should confer it a relatively good natural resilience against infestations. As shown by the present work, if not accelerated by external factors, OLE hydrolysis will naturally occur along fruit ripening, leaving the olive fruits more susceptible to external agents infection. Within the scope of this work, the ripening process of olive fruits from the two major traditional Portuguese cultivars, 'Galega Vulgar' and 'Cobrançosa', was studied, in regard to their specific phenolic profile. Results show that both cultivars present distinct phenolic profiles along their ripening, with 'Galega Vulgar' reaching a much higher maturity index at a considerably earlier ripening stage when compared with 'Cobrançosa', in agreement with the moment when OLE accumulation was registered as maximum.

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***Cynara cardunculus* leaves extract fractionation – an enriched source of allelochemicals**

D. Rosa^{1,2,3}, T. Brás^{1,4}, A. Paulino^{1,5}, F. A. Macías², B. Maçãs⁶, MF. Duarte^{1,3}

¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), Beja, Portugal.

²Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz, Spain.

³Mediterranean Institute for Agriculture, Environment and Development MED-Universidade de Évora, 7002-554 Évora, Portugal

⁴LAQV/ REQUIMTE, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

⁵Center for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciéncia, Universidade de Lisboa, 1749-016, Lisboa, Portugal

⁶INIAV - National Institute for Agrarian and Veterinarian Research, Estrada Gil Vaz, Ap. 6, 7350-901 Elvas, Portugal

Email: daniela.rosa@cebal.pt

Allelopathy, a biological phenomenon naturally occurring in ecosystem, has proven to be a powerful tool for weed management as an alternative to the use of synthetic herbicides. [1] The influence between allelochemicals and weed target species have already been studied for a vast list of compounds. [1] The Sesquiterpene lactones (SL), abundantly present in *Cynara cardunculus* leaves (95 mg/ g DW) [2], reveal a strong phytotoxic activity and potential use as bioherbicide. [3], [4] The aim of the present study is to obtain enriched SL fractions, with enhanced phytotoxic activity, derived from *C. cardunculus* leaves extract (CCLE) by using membrane separation technology. CCLE was obtained by pulsed ultrasound extraction using ethanol as solvent. [5] CCLE fractionation was performed by using ultrafiltration GE membrane GK (high molecular weight removal) and DuraMem® 200 (low molecular weight removal). System was operated on diafiltration mode. Fractionated extract concentration was performed on a nanofiltration experiment, with the DuraMem® 200 membrane. SL (cynaropicrin, grosheimin and 11,13-dihydroxy-8-deoxygrosheimin) identification and quantification was monitored by HPLC-DAD. [6] A removal of 55% of chlorophylls and almost total depletion of sugars was obtained, as well as 2.5 times increase of SL concentration (mg/mL). After obtaining SL concentrated fractions, it is intended to evaluate its phytotoxic activity, using wheat coleoptile bioassay, and then against a more specific panel of weeds, commonly found in Mediterranean agricultural crops.

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Metagenomic analysis of fungal microbiota associated to grapevine trunk diseases in Alentejo region

Mariana Patanita¹, M. Doroteia Campos¹, André Albuquerque¹, Carla M. R. Varanda¹, Patrick Materatski¹, Maria do Rosário Félix²

¹ MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: mpatanita@uevora.pt

Grapevine trunk diseases (GTDs) are considered among the most important problems affecting the longevity and productivity of vineyards in all the major growing regions of the world, causing important economic losses. They are caused by wood inhabiting fungi, namely by 133 species belonging to 9 families and 34 genera, with similar life cycles and epidemiology. Until now, no effective treatments are known. Aiming to gain a better knowledge of these diseases and search alternatives to limit their development, the present work intended to molecularly identify GTDs-associated fungi, grapevine endophytic community and fungi with antagonist ability against GTDs. For this study, two important cultivars from the Alentejo region were selected, 'Alicante Bouschet' and 'Trincadeira', which demonstrate different levels of susceptibility to GTDs. Samples consisted of cuttings from plants with and without trunk diseases symptoms from both cultivars in a vineyard located in this region. Total DNA was extracted from cortical scrapings and sequenced using a metagenomic approach based on next generation sequence analysis.

Deep sequencing of fungal-directed ITS1 and ITS2 amplicons led to the detection of 215 taxa in grapevine fungal microbiota, with nine fungi previously described as responsible for GTDs. Unexpectedly, symptomatic plants showed a lower relative abundance of GTDs-associated fungi and a higher relative abundance of possible antagonist fungi, in opposition to what was obtained in asymptomatic plants in both cultivars. Nevertheless, symptomatic plants showed greater diversity of GTDs phytopathogenic fungi when compared to asymptomatic plants. These facts corroborate previous reports referring that trunk diseases symptoms are intensified by a set of several associated fungi on the same plant. Some fungal species with biological antagonist characteristics were also identified but their role in GTDs still need further investigation. This study allowed a deeper knowledge of grapevine fungal communities of the selected cultivars and updated the information on the abundance and diversity of GTDs associated fungi and their relationship with the symptomatology in plants. Additional studies are still required to better understand plant-pathogen interactions and contribute to the mitigation and control of GTDs in the Alentejo region.

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Agro-industrial waste valorization of *Salicornia ramosissima* to produce cellulose nanofibers (CNFs) by acid and enzymatic hydrolysis

A. R. Lima¹, N. Cristofoli¹, M. C. Vieira^{1,2}

¹MED (Instituto Mediterrâneo para o Ambiente, Agricultura e Desenvolvimento), Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal.

²ISE (Instituto Superior de Engenharia), Universidade do Algarve, Departamento de Engenharia Alimentar, Campus da Penha, 8005-139 Faro, Portugal.

Email: carlima@ualg.pt

Salicornia ramosissima is a halophyte plant commonly found in saltmarshes and coastal areas worldwide. In Portugal, there are some companies producing *Salicornia* for human consumption in greenhouses. After harvesting, the lower part of its stem is discarded as there is no commercial value to it. This study was developed to find solutions for the valorization and application of this by-product, by extracting and isolating its nanocellulose and investigating its potential for the development of biopolymer composites. To obtain the cellulose nano fibers (CNFs), *salicornia* waste samples were first subjected to alkaline treatment to eliminate noncellulosic components. Then, bleach (H_2O_2) broke down the phenolic compounds which were next easily removed, whitening the pulp. Finally, for the separation of cellulose fibrils from the cell wall and obtaining CNFs, the insoluble residue was hydrolyzed by either the acid treatment (AT) or the enzyme treatment (ET). The analysis of FTIR spectra revealed that both AT and ET effectively removed the amorphous components from the bran structure and allowed the isolation of CNFs. From the analysis of XRD patterns, it was possible to observe the elimination of partial hemicellulose and lignin, resulting in an increased crystallinity degree of CNFs. The CNFs from AT (CNFsAT) presented a lower mechanic resistance due to their smaller particle size compared to CNFs from ET (CNFsET). The zeta potential values for CNFsAT and CNFsET were respectively, -29.9 and -52.2 mV. The higher potential for CNFsET suggests more electrically stable nanofibers. SEM micrographs indicated fiber exposure caused by both the AT and ET, in addition to the irregular bran structure. TEM images confirmed the presence of nanofibers in both CNFs-ET and CNFsET samples. ET successfully isolated cellulose nanofibers from the by-product of *Salicornia*, encouraging the use of this agro-industrial residue as a renewable source of nanofibers as in contrast to the AT, it does not generate toxic residues, presented mild thermal conditions, and produces nanocellulose fibrils with high-value applications.

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Potato growth under environment chamber conditions

Pedro Barbosa¹, Rui Machado², Renato Coelho³, Cláudia Vicente^{1,4}, A. Cristina Figueiredo⁵ and Manuel Mota^{1,3,6}

¹NemaLab/MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁴Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal.

⁵Centro de Estudos do Ambiente e do Mar (CESAM Lisboa), Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal (CBV), DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal.

⁶Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal.

Email: pbarbosa@uevora.pt

Potato (*Solanum tuberosum* L.) is one of the world's most common vegetable, source of calories, essential nutrients and vitamin C. Most studies with potatoes are performed *in vitro*, under strict environmental conditions and high levels of asepsis. Integrated in a larger research study that aims at simulating *in vivo* conditions for application of nematicides for potato pest control, this study intended to evaluate the best growth chamber conditions for potato growth. To optimize the best conditions, type of substrate, potato variety, seed depth, day/ night period, environment conditions and watering/ fertilization routine were evaluated between September 2018 and February 2020 in 14 trials with 20 plants each. In March and in June 2020, 16 potato seeds were placed in 3 L plastic pots, filled with pine forest sand and composted pine bark (9:1 ratio). Plants were grown in an Aralab© D1200PLH chamber at MED/ U. Évora facilities. Day period was set to 14 h, 23°C and 50% relative humidity and night period to 10 h, 12°C and 70% relative humidity. Plants were routinely watered and fertilized. Destructive sampling was performed 7, 14, 21 and 28 days after plant emergence (DAE). Non-destructive sampling was measured every 2 days, starting on the 7th day after emergence. Biomass related parameters (height, fresh weight, leaf area, etc.) increased as plant develops, with some overlay between consecutive time-points. Stomatal conductance, chlorophyll content and chlorophyll fluorescence values are more variable as plants grow. A plant with 14 DAE has a maximum quantum efficiency (F_v/F_M) value of 0.82 and an average Performance Index (PI_{abs}) of 7.91; a plant with 28 DAE has an average F_v/F_M value of 0.83 and an average PI_{abs} of 8.10. Leaf chlorophyll content varied between 73.66 for a 14 DAE plant and 80.20 for the 28 DAE plant (SPAD index).

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The X-Ray microdensitometry technology in cork from *Quercus suber* L. analysis

Poeiras A. P¹, Surový P.² and Ribeiro N. A¹

(¹) MED (Mediterranean Institute for Agriculture, Environment and Development), University of Évora, Pólo da Mitra, Apart. 94, 7006-554 Évora, Portugal

(²) Department of Forest Management, Faculty of Forestry and Wood Science, Czech University of Life Science Prague (CULS), Kamýcká 129, 16500 Praha 6, Suchdol, Czech Republic

Email: apcp@uevora.pt

Cork extracted from *Quercus suber* L. presents unique characteristics and properties which made of it one of the most import non-wood forest product in the Mediterranean system. Cork cells comes from a traumatic phellogen, which lives all the life of the tree, in a continuous formation over cork debarkings. Several characteristics are responsible for cork quality. The most important are the caliber or radial growth, density and porosity. These three characteristics, related each other's, can determine the value of the cork and tells the usage of a cork plank. Cork growth happen specialty in spring and autumn, originated the cork rings with their different densities and lengths.

X-Ray microdensitometry is a tool specially used in wood science to determine wood density but it can also be used in cork tissue, allowing to determine cork density along the sample, density in each cork ring and at the same time measuring rings growth. Calculations were made automatically considering the sample thickness, density and X-Ray attenuation. The objective of this analysis was to show the importance of some cork characteristics and to confirm the microdensitometry technology as an important and effective tool for cork from *Quercus suber* L. analysis.

A part of this research was already submitted for publication.

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Comparison of organoleptic characteristics of different species of pitaya.

Ana Rita Trindade¹, Pedro Matias¹, Diamantino Trindade², Amílcar Duarte^{1*}

¹MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro

²Mil Plantas, Sítio do Pereiro, caixa postal 250 X, 8700-123 Moncarapacho

*artrindade@ualg.pt

Among the exotic fruits, the pitaya (*Hylocereus* spp.), with red skin and white or red pulp, depending on the species, is one of the crop cultures that has been gaining featured in European markets. Due to its unique appearance, high nutritional value and high adaptability to different conditions, pitaya, also known as dragon fruit, can be an interesting food crop to study.

The low water needs of pitaya make this crop more sustainable, especially in Algarve where water scarcity is a dominant problem. In addition, Algarve is a region whose thermal amplitudes are not significantly accentuated. Another important factor is the probability of the frost occurrence, which although dependent on the location of the orchard, is generally low. The pitaya will be a good option to make economically viable small areas with agricultural potential, that cannot be used for more demanding fruit crops in irrigation and soil fertility.

Initially, a survey was carried out to assess the presence of this culture in Portugal. A collection of 15 varieties was collected that were planted outdoors in Cacela Velha, and in a greenhouse at the Mil Plantas nursery in Estoi, to characterize growth, flowering and fruiting. In the outdoor experiments, several types of support structures, ground cover and compass are being tested. We also count on the collaboration of farmers who grow pitaya in greenhouses, in soil and in hydroponics.

This summer flowering and fruiting has already been seen in some of the varieties (*H. undatus*, *H. costaricensis*, 'JC01' and 'Hybrid'), thus allowing a preliminary characterization of them. At the University of the Algarve, organoleptic tests were carried out to determine consumer acceptance and to compare varieties. Several parameters were evaluated, such as the appearance of the fruit and pulp, aroma, flavor and texture. Respondents were also asked whether they had ever tasted dragon fruit, and if they plan to include this exotic fruit in a future diet.

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Psyllids and its parasitoids on South Portugal

Beatriz Duarte, T. Magalhães, A. Duarte, L. Neto

MED - Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro, Portugal

Email: bzduarte@ualg.pt

The invasion of exotic species poses important challenges concerning their natural control. One of the issues is the possibility that native parasites and predators can exercise effective control action against invasive species. For this, it is crucial to know which species have the possibility of exercising this action. These auxiliary insects are usually associated with similar pest species in the area of invasion. Another issue relates to the use of exotic enemies, are the potential side effects on the native species which must be prevented.

With the recent invasion of *Trioza erytreae* in Portuguese territory and the consequent release of *Tamarixia dryi* for biological control, besides studying the evolution of the populations and distribution the vector and its parasitoid, the need of knowledge about native psyllids bioecology, plant hosts and natural control agents grows even bigger.

With that intent, plant direct observations and sweep net sampling were carried out on citrus trees and on various other plant species (cultivated, ornamental and spontaneous) in some areas of South Portugal. The samples are taken and analysed in the laboratory to account for the presence of psyllids. When immature stages were recorded, plant samples are taken to rear potential parasitoids.

Our preliminary results revealed psyllids adults and/or immatures stages in some native and exotic plants. On two plant species (*Pistacia lentiscus* and *Jacaranda mimosifolia*) parasitoid emerged from plant samples.

In the future, these results must be pursued to determine which are the most suitable time of the year to collect immature stages and determine its possible parasitism.

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The dual role of Plant Viruses in CRISPR

C. Varanda^{1*}, M.R. Félix², M. D. Campos¹, M. Patanita¹, P. Materatski¹

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

*Email: carlavaranda@uevora.pt

Plant viruses cause devastating diseases in many agriculture systems, being a serious threat for the provision of adequate nourishment to a continuous growing population. At the present there are no chemical products that directly target the viruses, and their control rely mainly on preventive sanitary measures to reduce viral infections that, although important, have proved to be far from enough. The current most effective and sustainable solution is the use of virus-resistant varieties, which require too much work and time to obtain. In the recent years, the versatile gene editing technology known as CRISPR/Cas has simplified the engineering of crops and has successfully been used for the development of viral resistant plants. CRISPR stands for Clustered regularly interspaced short palindromic repeats and CRISPR-associated (Cas) proteins, and is based on a natural adaptive immune system that most archaeal and some bacterial species present to defend themselves against invading bacteriophages. Plant viral resistance using CRISPR/Cas technology has been achieved either through manipulation of plant genome (plant-mediated resistance), by mutating host factors required for viral infection, or through manipulation of virus genome (virus-mediated resistance), for which CRISPR/Cas systems must specifically target and cleave viral DNA or RNA. Viruses present an efficient machinery and comprehensive genome structure and, in a different perspective, they have been used as biotechnological tools in several areas such as medicine, materials industry and agriculture with several purposes. Due to all this potential, it is not surprising that viruses have also been used as vectors for CRISPR technology, namely to deliver CRISPR components into plants, a crucial step for the success of CRISPR technology. Here we discuss the basic principles of CRISPR/Cas technology, with a special focus on the advances of CRISPR/Cas to engineer plant resistance against DNA and RNA viruses. We also describe several strategies for the delivery of these systems into plant cells, focusing on the advantages and disadvantages of the use of plant viruses as vectors. We conclude by discussing the constraints faced by the application of CRISPR/Cas technology in agriculture and future prospects.

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More than meets the mouth: a systematic review and meta-analysis of auditory contributions to taste perception

D. Guedes^{1,2}, M. Prada¹, M. Garrido¹, E. Lamy²

¹Center for Research and Social Intervention, Iscte – University Institute of Lisbon, Av. das Forças Armadas, 1649-026, Lisboa, Portugal.

² Mediterranean Institute for Agriculture, Environment and Development – MED, University of Évora, Pólo da Mitra, 7002-554 Évora, Portugal.

Email: dhfgs@iscte-iul.pt

Flavor perception is a multisensory experience. What we see, ear, smell or touch can influence our expectations towards food and beverages and modulate how we subjectively perceive tastes and flavors. Among all the senses, the role of audition has been the least studied to date. Recent research has shown that people may reliably associate certain auditory parameters (e.g., pitch) with different basic tastes (e.g., low pitch and sweet taste). Based on those patterns of crossmodal association, researchers and practitioners have been interested in identifying and developing stimuli which modulate how we perceive basic tastes.

In the present work, we systematically review the existing literature on the crossmodal associations between audition and taste. For that purpose, four indexing services (EBSCOhost, SCOPUS, Web of Science and PubMed) were searched, in April 2020, using three sets of keywords on the crossmodal or multimodal interactions (e.g., cross-modal* OR multi-sensor*) between audition (e.g., audition OR sound* OR music*) and basic tastes (e.g., sweet* OR tast* OR flavor*). Empirical, quantitative studies with healthy subjects, either in field, lab or online settings, were considered for inclusion. Review articles, opinion/commentary and conference papers, as well as studies with clinical samples (e.g., synesthesia) were excluded. The initial search resulted in 1910 records ($n = 1193$ after removing duplicates) which were subject to two consecutive stages of abstract and title screening and full text screening ($n = 60$), which resulted in 47 articles for data extraction (*in progress*).

The primary outcome of the review will be a synthesis of existing evidence on the crossmodal associations between audition and taste. The results will allow for a comprehensive and integrative perspective on this topic of research, with the potential of informing future studies and interventions. The area of multisensory food perception may be a promising avenue for promoting healthier and sustainable eating habits, by supporting reduction of sugar or salt in foods and beverages with minimal compromises for taste perception and hedonic experience.

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Effect of dolomitic limestone application, grazing type and stoking rate in pasture productivity and quality in *Montado* ecosystem

E. Carreira¹, F. Costa², A. Pereira³, J. Serrano⁴, J. Castro⁵ & M. Carvalho⁶

¹Aluno de Doutoramento em Ciências Agrárias e Ambientais, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²Aluno de Mestrado em Engenharia Agronómica, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³Professor Associado com agregação do Departamento de Zootecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

⁴Professor Associado com agregação do Departamento de Engenharia Rural, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

⁵Professor Auxiliar do Departamento de zootecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

⁶Professor Catedrático do Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

e-mail: d41989@alunos.uevora.pt

Montado is an agro-forestry-pastoral ecosystem multifunctional. The Montado degradation is frequently associated with overgrazing. This trial aimed to study the combined effect of the correction of manganese toxicity (corrected – COR vs uncorrected – UCOR), with different sheep stocking rates and types of grazing (continuous vs dynamic), on the quantity and feeding values of Montado biodiverse pastures. The study was carried out at Mitra farm ($38^{\circ} 31' 52''N$; $8^{\circ} 00' 33''W$) - University of Évora - between November 18th, 2019 and May 31st, 2020. A 4 ha plot was divided into four sub-plots (P1UCOR, P2UCOR, P3COR, P4COR), with different stoking rates and two types of grazing. In P1UCOR and P4COR with 8 sheep/ha grazed continuously. In P2UCOR and P3COR there has been intermittent grazing 14 sheep/ha corresponding to different grazing pressure along the seasons. The criterion for removing the sheep from the P2UCOR and P3COR plots was the pasture has a height less than 2.5-3 cm. The grazing days in each sub-plot P2UCOR and P3COR counted. After the exit and before returned the sheep of the P2UCOR or P3COR, 12 points of height measurements were made, and the respective samples pasture were cut to estimated productivity (Kg GM and DM/ha) and CP and NDF. At the end of the trial, at the beginning of the summer, it is possible to show that the P3COR plot allowed a more significant number of grazing days, compared to the P2NCOR. From September to April, the P4COR pasture always has the highest hight, followed by P1UCOR. In May P3COR presented higher values than P1NCOR. However, P4COR keep higher values than P1COR and P3COR. P2UCOR has shown the lowest hight and productivity. At the end of May, P3COR presented a more considerable amount of GM and DM than the other sub-plots. In this month and in P3COR, the size and density of the legumes species was higher, which could indicate better conditions of competition to the grasses.

Proteomic study of proteolysis during ripening of cheese made with *Cynara cardunculus* L.

Garrido, A. L.¹, Freitas, S.¹, Silva, F.¹, Lamy, E.², Conceição, C.^{1,2}

¹Escola de Ciências e Tecnologias, Departamento de Zootecnia, Universidade de Évora (UE), Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Zootecnia, Universidade de Évora (UE), Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: cristinaconceicao@uevora.pt

The pistils of wild and cultivated cardoons are used as vegetal coagulant to produce several traditional ewe's and goat's cheeses, some of them benefit from protected designation of origin (PDO) status in which *Cynara cardunculus* flower extracts have been successfully employed and legally required. The type of coagulant agent is one of the main factors responsible for the variability in cheese characteristics, being therefore its effect on the proteolysis a subject of profound study. A precise analytical technique is essential to understand the impact of different factors on cheese proteolysis. In this study three electrophoretic techniques have been used to evaluate the cheese proteolysis namely (1) urea polyacrylamide gel electrophoresis (Urea-PAGE) and (2) sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for the insoluble fraction of cheese and (3) twodimensional gel electrophoresis (2-DE) for the soluble fraction. Results showed that urea-PAGE was the best method for cheese insoluble fraction analysis. Both urea PAGE and 2-DE methods are complementary and proteomic tools are helpful in understanding the proteolysis of ewe's cheese.

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Modelling the relationship between the Évora, Serpa and Nisa cheese casein fractions and their degradation products using linear regression analysis

Silva, F.¹, Garrido, A. L.¹, Conceição, C.^{1,2}, Duarte, M. F.^{2,3}

¹Escola de Ciências e Tecnologias, Departamento de Zootecnia, Universidade de Évora (UE), Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

²MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Zootecnia, Universidade de Évora (UE), Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³CEBAL - Centro de Biotecnologia Agrícola e Agro-alimentar do Alentejo, Rua Pedro Soares, s.n. - Campus IPBeja/ESAB Ap. 6158, 7801-908 Beja | Portugal

E-mail: ccp@uevora.pt

Some Portuguese and Spanish ewe's cheese are made with the aqueous extracts of *Cynara cardunculus* L. dried flowers. The renewed interest in vegetal coagulant prompted to the investigation of its proteolytic effect. Different *Cynara cardunculus* L. populations (*Cynara* 1, *Cynara* 2 and *Cynara* 3) were used in the cheesemaking of PDO Évora, Serpa and Nisa cheeses. Cheese samples were analysed by urea-PAGE to identify the casein fractions, enabling the evaluation of the extent of proteolysis. The casein fractions obtained were separated in four areas: β -caseins, α_s -caseins and their degradation products, γ -caseins and pre- α_s -caseins (CN), respectively. The aim of this work was to identify the relation of the caseins fractions and their degradation products through a simple linear regression method (LRM), regarding the effect of *Cynara cardunculus* L. coagulant, and the matrix of three PDO cheeses, during ripening process. Results showed significative correlations between the predictors variables (α_s - and β -CN) and the responses variables (pre- α_s -CN and γ -CN) in the three cheeses. Coefficients of determination (R^2) between α_s -CN and pre- α_s -CN in all cheeses was superior to 0.80, while the R^2 between β -CN and γ -CN was less than 0.80. These results suggest that α_s -CN can be a good predictor of pre- α_s -CN, providing viable information regarding the proteolysis process in DOP Évora, Serpa and Nisa cheeses.

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Physico-chemical characterization of fruits from three varieties of *Opuntia ficus indica* (L.) Miller

G. C. Murta¹, A.E. Rato², M.R. Martins³

¹MED (Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento), IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

²MED (Instituto Mediterrâneo para Agricultura, Ambiente e Desenvolvimento), Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

³Laboratório HERCULES & Departamento de Química, Escola de Ciências e Tecnologia, Universidade de Évora, Rua Romão Ramalho, 59, 7002-671 Évora, Portugal.

Email: gabriela.murta@gmail.com

The *Opuntia ficus indica* (L.) Miller is a specie native from Mexico, found throughout all over the world. In Portugal this specie became well adapted to natural conditions and currently it is a sub spontaneous one. The quality of the fruits depends on their physical-chemical characteristics of each cultivar. Fruit quality attributes can be altered by mechanical injuries during harvest. Also, organoleptic composition of the fruits are important characteristics both to industrialization and to fresh consumption. In order to improve the quality control during the postharvest period it has become necessary to evaluate physical and chemical characteristics. This study focus is in the characterization of 3 *Opuntia* varieties at ripening maturity. After harvest 10 fruits from each *Opuntia* variety were weighted, and several measures of size were taken and, pulp/seed ratio, texture, skin color, were also registered. Organoleptic composition of the fruits was characterized through the content of soluble solids, titratable acidity (TA) and SSC/TA which are important indicators of fruit quality since the perception of a tart flavour caused by organic acids is strongly influenced by the presence of sugars. The determination of SSC was made through refractometry. The fruits from 3 varieties were characterized with an acceptable level of soluble solids of 12.92 (%) for the variety Bianca with green color, 14.02 (%) for the variety Gialla yellow and 11.80 (%) for the variety Rossa. The physical parameters of the fruits were for Bianca variety 6- 8 cm long and 5-7 cm wide, for Gialla variety 7-9 cm long and 6-8 cm wide and for Rossa variety 6-8 cm long and 6-7 cm wide. The weight variation of the fruits was 112-131 g and DP 0.3, for the three ecotypes. The average TA found indicates a slightly acidic fruit and among varieties the Bianca variety was the most acidic one. The TA is one of the criteria used for fruit classification through the flavour, where the percentage found was in average 1,41% for Bianca variety, 1,51 % for Gialla and 1,41% for the Rossa cultivar. On physical and physicochemical parameters there was no statistical difference between varieties.

Genetic bases of almond quality

G. Almeida^{1,2}, A. Faustino², R. Pires², D. Soldado², L. Cachucho², A. Rosa⁴, A. Marreiros⁴, M.M. Oliveira¹, E. Jerónimo^{2,3}, L. Marum^{2,3}

¹Genomics of Plant Stress Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, New University of Lisbon, Av. da República, 2780-157 Oeiras, Portugal

²Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

³MED – Mediterranean Institute for Agriculture, Environment and Development, CEBAL, 7801-908, Beja, Portugal

⁴Direcção Regional de Agricultura e Pescas do Algarve, Apartado 282, Patacão, 8001-904 Faro, Portugal

Email: goncalo.almeida@itqb.unl.pt

Almond is one of the most important tree nut crops, due to its high nutritive value, namely in lipids and tocopherol richness. Its production in Portugal has been increasing during the last years, as traditional varieties have been exchanged to commercial varieties. Tocopherol (Vitamin E) is an antioxidant who prevents the peroxidation of unsaturated fatty acids, increasing storage-life of almond. As an antioxidant, it is highly effective preventing cardiovascular diseases by inhibiting platelet aggregation. Genes involved in its biosynthesis have been characterized in other species, but few research have been made in almond. This work aims to study the tocopherol profile of three traditional varieties (Gama, Joao Dias and Fura Sacos) and three commercial (Lauranne, Soletta and Antoneta) varieties produced in Alentejo at different kernel developmental stages. Moreover, the fruits collected at different developmental stages will be morphologically characterized. In addition, the differentially expression level of candidate genes involved in tocopherol synthesis (*vte1*, *vte3*, *vte4*, *vte5*, *HGGT*) will be characterized using RT-qPCR.

Until now, the last stage of almonds development was morphologically characterized and the analysis in terms of tocopherol profile and expression level of candidate genes involved in tocopherol synthesis are ongoing. The tocopherols were extracted using methanol:dichloromethane (1:1) and quantified by high-performance liquid chromatography (HPLC), using a fluorescence detection and a normal-phase silica column (Zorbax RX-Sil). Total RNA extractions were optimized using a Qiagen kit, according to the manual instructions, with minor modifications. The gDNA was removed using a DNase (Qiagen). The concentrations and quality of RNA samples were analysed by Nanovue, Qubit and electrophoresis techniques.

In the future, the differentially expression level of candidate genes will be evaluated by RT-qPCR, using specific primers. To understand the tocopherol profile and the molecular mechanism of its synthesis during kernel development, the experiment will be performed using immature kernel developmental stages collected from February to June.

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Calorespirometry – an efficient phenotyping tool to assist plant breeding focused on seed resilience

L. Rodrigues¹, A. Nogales², L. Hansen⁴, F. Santos¹, S. Groot³, A E. Rato⁵, H. Cardoso^{1*}

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Formação e Investigação Avançada, Universidade de Évora. Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²LEAF – Linking Landscape, Environment, Agriculture and Food. Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

³Wageningen Plant Research, Wageningen University & Research Centre, Wageningen, The Netherland.

⁴Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA.

⁵MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*hcardoso@uevora.pt

The continuous increase in human population and global as well as local changes in climate and soil conditions are factors that have been gaining special attention. While there is an increased demand for sustainability in agricultural systems, availability of agricultural land and natural resources such as water and fertilizers are limited. In order to support the needs imposed, several breeding programs have been implemented focused on the development of more resilient varieties/cultivars able to grow under unfavorable natural conditions. A strong focus has been given to seed sector, in order to develop elite genotypes able to produce more robust seeds with enhanced plasticity upon environmental constraints and high germination rates. The European Research project LIVESEED (<https://www.liveseed.eu/>) is an example that seed production companies and research/breeding Institutes are all engaged on the development of more resilient plant varieties contributing for more sustainable agricultural systems. As partner of the LIVESEED project, UEvora is responsible for the development of a phenotyping tool able to assist different breeding programs focused on the selection of genotypes with increased resilience upon environmental stresses. Calorespirometry, a technique that simultaneously measure metabolic heat rates and CO₂ emission rates of biological samples, allows to determine carbon use efficiencies (ϵ) and growth rates. This technique allows to monitor plant response upon different temperatures and does not require *in vivo* trials involving growing plants under different environmental temperatures. Calorespirometry has been used by the UEvora group and its applicability has been demonstrated in different biological systems. Considering that seed germination involves the activation of several metabolic pathways, including cellular respiration to provide the required energy for embryo development, calorespirometry appears to be a promising tool to assess seed plasticity upon environmental temperatures. In the current work, the applicability of this technique for phenotyping different pea cultivars already at seed level as well as the potential of calorespirometry for assisting on the selection of the most resilient genotypes upon temperature stress will be presented.

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Tomato - a model plant to study molecular resistance mechanisms to pathogens

M. D. Campos¹, M.R. Félix², M. Patanita¹, P. Materatski¹, C. Varanda¹

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

²MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: mdcc@uevora.pt

Tomato (*Solanum lycopersicum*) is one of the most economically important vegetable throughout the world. It is one of the best studied cultivated dicotyledonous plants, often used as model system for plant research into classical genetics, cytogenetics, molecular genetics and molecular biology. Tomato cultivation has been limited by an abundance of infectious diseases, that reduce yield and affect product quality, causing symptoms including wilts, leaf spots/blights, fruit spots and rots. The diseases are mainly caused by fungi, but also by oomycetes, bacteria, viruses, viroids and nematodes. So, it is crucial to establish effective and integrated control methods (i.e. cultural practices, disease resistant varieties, use of chemicals), and understand tomato-pathogen interactions. This diversity of pathogens that affect tomato, emphasizes the importance of the tomato-pathosystem as a favourable model for studying plant-pathogen interactions, contrarily to other ‘traditional’ model plants as *Arabidopsis*. The responses of plants to biotic stresses are very complex as the multitude of interactions involves two living organisms, the plant and the pathogen. The use of the tomato as model plant-pathogen system helps to accelerate the discovery and understanding of the molecular mechanisms underlying disease resistance. The identification of key functional genes in susceptible responses and the understanding of molecular basis of compatible interactions are possible with techniques that allow the study of differential gene expression in tomato plants affected by different pathogens. Although various resistance genes have been functionally identified in the resistance system, little is known about the complex molecular mechanisms involved in defense responses. Next-generation sequencing (NGS) technologies, which produce massive quantities of sequencing data, have greatly accelerated research in biological sciences and offer great opportunities to better understand the molecular networks of plant–pathogen interactions. Here we discuss on the use of functional genomics as a mean to study tomato plant immunity, with a special focus on emerging techniques that are allowing to acquire a deeper knowledge in view of plant breeding. The identification of plant regulatory components involved in protection against pathogens can therefore be of major importance for a sustainable plant-disease management, namely relying on the plant innate immune mechanisms.

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The applicability of molecular markers to assist a grapevine breeding program aiming to develop new varieties resistant to pathogenic fungi

M. Silvério¹, M. Marques¹, C. Mendes¹, D. Tavares^{1,2}, R. Martins¹, R. Félix³, J. Böhm², A. Peixe³, H. Cardoso¹

¹MED - Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²Viveiros PLANSEL Lda., Apartado 2, 7050-909 Montemor-o-Novo.

³MED - Mediterranean Institute for Agriculture, Environment and Development, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: mavisilveriowork@gmail.com

The European grapevine (*Vitis vinifera* L.) is a major crop worldwide with its fruits being used for wine production, fresh and dried consumption or juice production. This species exhibits high sensitivity to pathogenic fungi, which attack all plant green tissues and grape-clusters, representing a strong reduction on yield and grape quality. The downy mildew (*Plasmopara viticola*), the powdery mildew (*Erysiphe necator*) and the grey mould (*Botrytis cinerea*) represent the most nefarious pathogenic fungal diseases. The knowledge about grapevine resistance against powdery and downy mildew dramatically increased in the last decades with the identification of several resistance *loci*. Aiming the introgression of resistance into Portuguese varieties, a breeding program based on conventional hybridization has been recently established at Viveiros Plansel Lda., Montemor-o-Novo (Portugal) under the scope of a PDR2020 project. In 2019 more than 20.000 controlled crosses were made using as a pollen donor a multi-*locus* resistant variety provided by the JKI Institute – Germany. After a first selection of the progeny made by *in vitro* inoculation of leaf disks using a downy mildew spore suspension and simultaneously evaluation of plant growth under natural high-pressure of powdery mildew, 90 genotypes were selected. The resistant variety used on the crosses presents multi-*locus* resistance and a Marker-Assisted Selection (MAS) approach must now be followed to identify the resistance *loci* transmitted to the progeny and to confirm parentage. This approach is based on the use of Simple Sequence Repeats (SSRs) markers. In case of parentage confirmation, the SSRs work as Neutral Markers (not linked to a specific trait) and appear as codominant. Nowadays, a set of nine SSRs are well known for *V. vinifera* genotyping, which can be easily used for confirmation of progenitors. In the identification of the resistance *loci* transmitted to the progeny, SSRs work as Functional Markers directly linked to fungal resistance. The introgression of new *locus* in a Portuguese variety genome will represent the presence of a new allele, not detectable in the Portuguese progenitor (dominant markers). Both approaches have been applied to assist the Portuguese breeding program. The methodology followed and the results achieved will be presented.

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Differences in phloem metabolic profile between *Citrus macrophylla* and *Citrus aurantium* could explain the degree of susceptibility to *Citrus tristeza virus*

Susana Dandlen¹, JP Silva², L. Anjos², A. Duarte¹, D. Power², N. Marques³

¹MED - Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro, Portugal

²CCMAR – Center of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

³CEOT - Center for Electronics, Optoelectronics and Telecommunications (CEOT), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Email: sdandlen@ualg.pt

Citrus tristeza virus (CTV) from family *Closteroviridae* is the causal agent of devastating diseases of citrus that changed the course of the citrus industry worldwide, having caused the death of millions of plants and forced to change rootstocks in many countries. This virus infects phloem-associated cells that enables a long-distance transport inside the host. However, the systemic distribution of CTV in phloem tissue depends on the citrus host. CTV is restricted to citrus and citrus relatives and different species exhibit distinct degrees of susceptibility to infection that may result from a differential ability of the virus to move and infect surrounding sieve tube elements. Phloem sap is a mixture of organic compounds including proteins and enzymes that have the ability to reprogram the plant metabolism and thus serve as vehicles for the plant's responses to a virus infection. Therefore, the metabolite profiles of phloem sap should reflect the distinct susceptibility of citrus species to the virus. The objective of the present work was to analyze the metabolic profile of phloem sap in two citrus species, *Citrus macrophylla* and *Citrus aurantium*, that display different susceptibilities to CTV infection. Considering the virus spreading ability in the host, *C. macrophylla* is more susceptible than *C. aurantium* to CTV infection. Both citrus species are widely used as rootstocks for citrus trees. Four two-year-old plants of each *C. macrophylla* and *C. aurantium* were evaluated and the major metabolites determined by LC-MS following a metabolomics approach. LC-MS analysis of phloem sap taken from *C. macrophylla* and *C. aurantium* showed different metabolite profiles, namely of terpenes, flavonoids, phenylpropanoids and organic acids families. In *C. aurantium* some compounds showed contents about two orders of magnitude higher than in *C. macrophylla*. Differences in the relative contents of some compounds could reflect different defense antiviral mechanisms that would explain the reduction of spreading ability of CTV in *C. aurantium*.

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Recovery of natural polymers from an exhausted culture medium of microalga *Porphyridium cruentum* production by different methods

N. L. Cristofoli¹, A. R. Lima¹, J. S. Varela², M. C. Vieira^{1,3}

¹MED (Instituto Mediterrâneo para o Ambiente, Agricultura e Desenvolvimento), Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Ed. 8, 8005-139 Faro, Portugal.

²CCMAR (Centro de Ciências do Mar), Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Ed. 7, 8005-139 Faro, Portugal.

³ISE (Instituto Superior de Engenharia), Universidade do Algarve, Departamento de Engenharia Alimentar, Campus da Penha, 8005-139 Faro, Portugal.

Email: nlcristofoli@ualg.pt

Porphyridium cruentum microalga is a source of compounds with nutritional and biological value, rich in health-promoting lipids and sulfated polysaccharides. These compounds present interesting biological and rheological properties with potential use in the food, cosmetic and pharmaceutical industries. Europe has some companies that cultivate microalgae on an industrial scale, where a great part of the compounds is excreted into the culture medium and later discarded when biomass is separated. The main objective of this study is to add value to the wastewater of *P. cruentum* through the recovery of polysaccharides by different methods for future biotechnological applications. To obtain the exopolysaccharides (EPS) the exhausted culture media was subject to precipitation with cold ethanol and the mixture was kept overnight at -18 °C, followed by the recovery of the precipitate by centrifugation and then freeze-dried (EETO). Was performed a purification of the precipitate by dialyzes against distilled water (EETD) and another experiment using the trichloroacetic acid (ETCA) before ethanol precipitation. The yield of recovery was significantly higher to EETO (3.12 g L⁻¹) but the purified sample (EETD) presents more total carbohydrate (1.37 %). Fourier transform infrared (FTIR) analysis provided a similarity in the distribution of the peaks (C-O-C, C-OH e C-H) to EETO and ETCA samples. The particle size is related to the purification technique, where deproteinization and dialysis provide a decrease in size, as well as for zeta potential values. These values can be influenced by the concentration and type of polysaccharide present since the change in surface charges is associated with the aggregation of the compounds present. Microalgal polymeric by-products are a sustainable source to recovered valuable compounds, being important to choose the appropriate purification method to obtain different yields and monomeric composition.

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Effect of fruit thinning on fruit size of 'Setubalense' mandarin (*Citrus deliciosa*)

Pedro Matias^{1*} Tiago Guerreiro² Ana Trindade¹ & Amílcar Duarte¹

¹MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro.

²João Santana Unipessoal, Sítio dos Mortais 8700-120 Moncarapacho.

*pmmatias@ualg.pt

Some citrus cultivars tend to alternate bearing. This aspect consists of the variation between years of abundant production, followed by years with scarce production. In the years of high production, the fruits are generally smaller, compared to the years of scarce production. Fruit thinning can emerge as a technique that seeks to regularize production. In addition, fruit thinning favors, according to previously developed studies, an increase in fruit size.

The 'Setubalense' mandarin is a traditional Portuguese cultivar with excellent organoleptic characteristics, but some problems, such as the tendency to alternate bearing and the small size of the fruit, are leading to the rejection of this mandarin. Works that improve the production of this cultivar can help to keep it in production, which allows us to differentiate our citriculture from those of other countries.

The PodaCitrus project aims to optimize citrus pruning, to improve fruit quality and reducing the incidence of diseases that affect the appearance of the fruit. As part of this project, two fruit thinning experiments were performed on 'Setubalense' mandarin (*Citrus deliciosa* Tenore 'Setubalense'), a significantly alternate bearing cultivar. The experiments were installed in August 2019, in a year of abundant production. Manual thinning of 50% of the fruits in the entire tree was compared to identical fruit thinning in isolated branches. This work aims to determine which of the two types of fruit thinning is most effective in increasing the size of the fruit.

It was observed, in the 2020 yield, that the size of the fruit is higher in the trees where the fruit thinning was done. On the other hand, there were no significant differences in fruit size, between branches where the fruits were thinned and branches without thinning. In addition, the number of fruits harvested in 2020, a year of low production, suggests that the thinning of the fruits did not influence the cycle of alternate bearing and production is similar in all trees of the experiment. Fruit quality was also evaluated.

Keywords: Citrus, alternate bearing, tangerine

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Unraveling somatic embryogenesis signaling pathways – the role of extracellular molecules as efficiency modulators

R. Pires¹, A. Peixe², F. Santos³, H. Cardoso¹

¹MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

²MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³Unidad de Proteómica, Centro Nacional de Biotecnología, CSIC, Calle Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain.

Email: rnpires@uevora.pt

Somatic embryogenesis (SE) can be described as a process involving the formation of structures similar to the zygotic embryos, arising from dedifferentiation of somatic cells and not requiring the occurrence of fertilization. This process, widely used in clonal propagation and genetic transformation of several plant species, is not routinely used in olive (*Olea europaea* sp. *europaea* L.), due to the recalcitrant behaviour that characterizes olive adult tissues. In fact, most of the protocols established for olive cultivars and wild olive genotypes, are based on tissues taken from zygotic embryos, which are characterized by high genetic variability. If adult tissues can be used, *in vitro* SE may be an interesting alternative for vegetative propagation of high agronomical interesting olive cultivars that exhibit a recalcitrant behaviour when propagation procedures requiring the formation of adventitious roots are used. An important factor referred as affecting SE in plants, but not yet receiving the necessary attention, and to the best of our knowledge, never considered in any olive study, is the release of organic bioactive molecules by the explants into the culture medium. These molecules are involved in several biological processes and can play an important role in cellular communications. Based on this knowledge, a new research line focused on the role of extracellular molecules and its involvement in efficient signalling of SE was started in olive, and here it will be presented: i) the different molecules that could be secreted to the culture medium during SE and its potential role in determining SE efficiency, ii) the approach that was defined in frame of this research line to identify biomarkers associated to SE efficiency using different OMIC approaches (transcriptomics, proteomics and metabolomics), iii) the strategy to evaluate the potential applicability of extracellular molecules to overcome recalcitrant behaviour upon SE stimulus.

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Content in biogenic amines of nitrite/nitrate-free dry-cured sausages

S. Conceição^{1*}, P. Bernardo², M. J. Fernandes², M. J. Fraqueza², M. Elias^{1,3} & M. Laranjo¹

¹MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, IIFA-Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mira, Ap. 94, 7006-554 Évora, Portugal

²Centre 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

³Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Ap. 94, 7006-554 Évora, Portugal

*Email: d44723@alunos.uevora.pt

Biogenic amines are basic nitrogenous compounds, the main source of which is the decarboxylation of amino acids by microorganisms, such as enterobacteria and lactic acid bacteria. According to their origin, they can be classified as endogenous and exogenous; and according to their action they are classified as vasoactive and psychoactive. In dry-cured sausages, the most prevalent amines are histamine, tryptamine, β -phenylethylamine, tyramine, putrescine and cadaverine. The most problematic ones are histamine and tyramine, as their high intake can cause intolerance, intoxication, and poisoning reactions. Nitrites/nitrates are naturally occurring chemical compounds, which are used as food preservatives. They are added in the manufacturing of dry-cured sausages because they boost their characteristic colour and flavour. Furthermore, nitrite also exhibits an important antimicrobial activity. However, there is a need to reduce the use of nitrites/nitrates in food production as they promote the formation of nitrosamines when the nitrous agent reacts with secondary biogenic amines. The aim of the present work was to evaluate the profile of biogenic amines in nitrite/nitrate-free dry-cured sausages. Three independent batches of large-calibre dry-cured sausages ('Paio') were produced at a local manufacturing unit for both control and nitrite/nitrate-free dry-cured sausages. Two replicate samples were collected at different steps throughout the manufacturing and curing process (raw meat, meat batter, half-cured sausage, and end-product) for both conditions. Quantification of eight biogenic amines (tryptamine, β -phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) was performed. The content in vasoactive and total amines was calculated. The content in nitrites and nitrates was also determined. No significant differences were observed between control and nitrites/nitrates-free samples, regarding biogenic amines ($P < 0.05$), as well as nitrite and nitrate content ($P < 0.05$). Our results have shown that it may be possible to reduce or eliminate nitrites/nitrates from the formula of dry-cured sausages without compromising their safety.

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Establishing tenderness threshold of beef using instrumental and consumer sensory evaluation

S. Ricardo-Rodrigues^{1*}, A. C. Agulheiro-Santos^{1,2}, M. Laranjo¹, M. E. Potes^{1,3}, M. Elias^{1,2}

¹ MED (Mediterranean Institute for Agriculture, Environment and Development), IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

² MED (Mediterranean Institute for Agriculture, Environment and Development) & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

³ MED (Mediterranean Institute for Agriculture, Environment and Development) & Departamento de Medicina Veterinária, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

Email: sirr@uevora.pt

The consumers consider tenderness one of the most important qualitative characteristics of meat, so they are willing to pay a higher price for beef as long it is guaranteed tender. However, tenderness is also a highly variable property, depending on many intrinsic and extrinsic factors, such as animal breed, feed, and management, and on their interaction. Establishing a tenderness threshold could serve as a precise quality control system to guarantee tender meat and to assure consumer acceptability. A threshold is defined as a point on the sensory stimulus scale at which a transition occurs in a series of sensations or judgements. The present study aims to establish a tenderness threshold for beef steaks using a consumer sensory test. About 250 consumers were asked to rate consumption frequency, mode of preparation, and their palatability of beef. Consumers were further asked to assess which tenderness category was most appropriate for each one of four beef samples of commercial origin, considering an affective acceptance test through a 5-category hedonic scale (Very Hard, Hard, Ideal Tenderness, Tender and Very Tender). The same beef samples were evaluated through Warner-Bratzler shear force (WBSF) and texture profile analysis (TPA) to define their instrumental tenderness. The results obtained allowed us to conclude that there is a correlation between the sensory and instrumental evaluation of beef tenderness. However, a correct definition of the threshold must always consider both instrumental evaluation methods, WBSF and TPA. A tender meat should have shear force values between 15 and 32N and a compression force between 11 and 20N, while a hard meat should have a shear force greater than 37N and a compression force greater than 30N. Both instrumental tests give different information: WBSF test evaluates the meat fibres perpendicularly, as do incisor teeth, while TPA mimics the chewing with molar teeth. It is not possible to discard one of the instrumental tests, if the aim is to correlate beef measured tenderness with its sensory evaluation.

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Increasing shelf life of *Opuntia ficus-indica L.* fruits using different packaging materials

Sofia Trindade¹, Maria Inês Rouxinol², Ana Cristina Agulheiro-Santos³

¹Aluno de Mestrado de Engenharia Agronómica, Universidade de Évora

²Aluno de Doutoramento em Bioquímica, Universidade de Évora; MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

³MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: m41741@alunos.uevora.pt

The concern over climate change, the necessity of natural products and healthpromoting foods, gave relevance to the *Opuntia ficus indica*, prickly pear cactus, as an effective food product. This crop requires very low levels of water and is adaptable to poor soils so the economic viability of this culture could play an important role in depressed areas. The prickly pear is a nonclimacteric fruit and highly perishable; usually there is a loss of about 70% in the twenty days following the harvest, so it's important to extend their shelf life. Nowadays consumers look for environmentally friendly products and packaging; according to a survey done by a consumer association DECO, 46% of Portuguese consumers prefer biodegradable plastic. The objective of this study was to evaluate the evolution of the prickly pears for a large period of time, using different packaging materials and simultaneously assess their behaviour using a biodegradable packaging. The fruits used were of the regional "orange" variety, produced by "PepeAromas"® in Alentejo region, and they were stored at 2° C, 90% R.H. using three different types of packaging materials, the commercially used cardboard box with an opening on the top, a rigid PET (Polyethylene Terephthalate) box that is normally used in the storage and sale of small fruits and a biodegradable plastic flexible bag from SILVEX®. This trial had the duration of forty days, and every ten days, three packages of each type were taken out of the cold chamber and the fruit quality was evaluated trough several tests. The prickly pear juice was also frozen for later analyses on phenolic content and antioxidant activity. Overall, this study showed that the "orange" variety prickly pear fruits can be stored at 2°C for the maximum period of thirty days and the biodegradable plastic bag is the best, maintaining the fruit quality throughout that period of time. The fruits stored into the cardboard box exhibited the worst behaviour, showing great weight loss, more cases of fruit contamination by fungus such as *Botrytis spp.* and *Penicillium Spp.* and the fruits displayed worse appearance than those maintained in the biodegradable bag.

Innovative strategies to optimize the use of Fe in horticultural crops

Teresa Saavedra^a, José Paulo Da Silva^b, Pedro José Correia^a

^aMED – Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Faro, Portugal. ^bCCMAR- Centre of Marine Sciences, Universidade do Algarve, Faro, Portugal.

Email: tmr.saavedra@gmail.com

Higher plants develop physiological and morphological mechanisms as a response to Fe deficiency. Dicots and some monocots (gramineous) use, respectively, a reduction strategy (Strategy I) or a complexation strategy (Strategy II) for iron acquisition from the soil. Strategy II is based on the production of Phytosiderophores (PSs), non-proteinogenic amino acids able to complex the Fe present in soil solution. The role of these Fe-complexes in Fe-transport and Fe mobilization is not fully understood. In a previous research, it was found that the gramineous plants extract (GE) was able to promote a full recovery of Fe chlorosis in strawberry plants. Chlorotic plants were sprayed with the GE extract and newly formed leaves became green. The aim of this work was to evaluate the recovery of iron deficient strawberry plants by foliar spray, using the GE. Bare-root transplants of strawberry (*Fragaria x ananassa* Duch. cv "Diamond") were grown in a Hoagland's nutrient solution with iron (Fe10) or without (Fe0). Forty-two days after Fe deprivation, plants grown without iron became chlorotic and GE was applied. Three foliar applications were done and the regreening was monitored. After two applications, a rapid but transient regreening was detected in young leaves. The concentration of organic acids and ferric chelate – reductase (FC-R) activity was also significantly higher in GE treatment. It is possible that the high chelating capacity of the extract was responsible for the Fe internal transport and use. To study and characterize the production of PSs under Fe-stress conditions, seeds of *Poa* sp., *Lolium* sp. and *Festuca* will be germinated and subsequently transplanted to nutrient solution. After the appearance of chlorosis symptoms, plants will be collected and PSs will be identified in aboveground biomass, in roots, and also in root exudates by ESI- electrospray ionization mass spectrometry (ESI-MS).

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The use of remote sensing to develop a Citrus map, case study of the Algarve region

T. Magalhães, A. Duarte

MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento), UALG (Universidade do Algarve), Campus de Gambelas, 8005-139 Faro, Portugal.

Email: ttmagalhaes@ualg.pt

Algarve is Portugal's main Citrus producing region, this production is very important for the region. Land use maps are important resources for planning, management, and monitoring programs, and can be used for local, regional, and national scales. Citrus maps can be very useful for pest monitoring, planning control methods and subsequent management, with a current particular interest in *Trioza erytreae* and *Ceratitis capitata*. There are different ways to obtain these maps. In-field census is a very precise method, however it is very time consuming, expensive and cannot be repeated with high frequency. Another method is through remote sensing, using satellite imaging data. This method has the advantages of being less time consuming, more cost effective and having a high temporal resolution. Disadvantages of this method include a higher need in expertise, and less rigor. This study aims to develop a method of producing a citrus map for the Algarve region using open data resources from winter and summer seasons, analyzing its accuracy. The method used images from the Sentinel-2 satellite from ESA (European Space Agency). These images were then clipped using an open-source layer from governmental authorities where the general fruticulture areas are outlined. Within this fruticulture outline areas where the NDVI (Normalized Difference Vegetation Index) were below 0.5 were removed. Then orchards of different species were selected as training sets. The spectral signatures were analyzed, and two macro classes were outlined: the "Citrus" and the "Non-Citrus" classes. Three different classification methods were applied for each season: maximum likelihood, minimum distance and spectral angle mapping. Every classification was evaluated for its accuracy. Differences were found between classification methods and between seasons. In winter the overall accuracy of the classification methods ranged from 71.62 % to 54.94 %, in summer the classifications varied less (from 64.98 % to 68.59 %). These results are useful to identify the best season to use satellite data to better identify the citrus land use in citrus growing regions and the resulting maps can be starting point for a citrus production map for the Algarve region.

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4.

Ecology, Environment and Landscape

Integrated use of chemical, biochemical and ecotoxicological tests to assess the effects of remediation actions of soils degraded by mining activities

C. Mourinha¹, A. Catarino¹, I. Martins¹, P. Alvarenga², P. Palma^{1,3}, N. Cruz⁴, F. Silva⁵, A.P. Gomes⁵, L.A.C. Tarelho⁵ e S.M. Rodrigues⁴

¹DTCA, Departamento de Tecnologias e Ciências Aplicadas, Escola Superior Agrária de Beja, 7801-295, Beja, Portugal

²LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017, Lisboa, Portugal

³ICT, Instituto de Ciências da Terra, Universidade de Évora, Rua Romão Ramalho 59, 7000-671, Évora, Portugal

⁴CESAM & Departamento de Química, Universidade de Aveiro, 3810-193, Aveiro, Portugal

⁵CESAM & Departamento de Ambiente e Ordenamento, Universidade de Aveiro, 3810-193, Aveiro, Portugal

Email: clarissemourinha_17@hotmail.com

Aiming the rehabilitation of soils affected by mining activities, efforts have been made to reduce the risk of exposure to contaminants, namely metals, using *in situ* stabilization techniques, through the incorporation of amendments (organic or inorganic), that contribute to their immobilization. On the other hand, wastes produced in different activities must be integrated back into the production cycle, to reduce the consumption of natural resources and the need for their landfill disposal.

In this context, the objective of this study was to evaluate the use of wastes from the pulp and paper industry to improve the quality of soils degraded by mining activities. The materials were tested in a pilot plot, installed in São Domingos, with soils from different mines from the Iberian Pyrite Belt (Aljustrel, Lousal and São Domingos). The materials tested included: biomass ash granules (AG), stabilized; AG and composted cellulosic sludge; and a mixture of fresh materials, without stabilization.

Soil physicochemical characteristics (pH, electrical conductivity, organic matter content, macronutrients (NPK) concentrations), and enzymatic activities (e.g. dehydrogenase and hydrolases) were used to assess soil quality. The decrease in soil phytotoxicity was evaluated using germination and growth tests with watercress (*Lepidium sativum*), a dicotyledon, and with barley (*Hordeum vulgare*), a monocotyledon. The ecotoxicity of soil-water extracts comprised bioassays with organisms representative of different trophic levels: inhibition of luminescence of *Vibrio fischeri*; inhibition of the growth of green microalgae *Pseudokirchneriella subcapitata*; and mortality of the crustacean *Thamnocephalus platyurus*.

There were significant improvements in soil fertility, with increased pH, MO content, total N, extractable P and K. As a consequence, the dehydrogenase activity increased significantly and, although it decreased over time, it shows an improvement in soil microbial activity. The behavior of β-glucosidase, protease and cellulase was similar. Phytotoxicity decreased, as evidenced by the increased growth of *L. sativum* and *H. vulgare*, and the other bioassays also pointed to a decreased toxicity towards *P. subcapitata* and *T. platyurus*, with an increase in EC₅₀ values. The best results were obtained for the plots amended with AG and composted cellulosic sludge.

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Assessment and Improvement of Climate Change Adaptation Capacity of Smallholder Farmers

J. Rocha¹, C. Dias-Ferreira¹, I. Dinis¹, T. Pinto-Correira²

¹CERNAS (Research Center for Natural Resources, Environment and Society), College of Agriculture, Polytechnic Institute of Coimbra, Bencanta 3045-601 Coimbra, Portugal.

²MED (Mediterranean Institute for Agriculture, Environment and Development), Department of landscape, environment and planning, University of Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: joanarocha@esac.pt

According to the United Nations now is the moment to act upon climate change threat. While, globally, agriculture is the second This PhD aims at closing a knowledge gap concerning the types of smallholder farmers on the Portuguese Centro region, and their climate adaptation capacity. Since the development of the work plan is in its very early stage, presenting and discussing the proposed methodology allows for improvement. The work starts with a smallholder farmers' typology and development of a climate adaptability index (CAIx), followed by the development of a bioeconomic model for each farm type. A typology study classifies farms into defined groups by taking into consideration variables of the whole farming system as well as their relationship with the ecological, environmental and social contexts. For this study data will be collected through surveys and then will be statistical tested using multivariate analysis and cluster identification (Principal Component Analysis and Hierarchical Clustering). The index CAIx will be developed to measure farmers adaptability to climate change. Indexes are important tools to identify and manage fragilities and strengths of a sector, and to support further development of climate related policies. Over the last decades there has been an increasing interest concerning the relationship between agriculture and the environment. A joint ex-ante assessment of the economic and environmental effects of agricultural policies or innovation adoption (by farmers) presents clear advantages because it helps to better target policies towards their intended outcomes. Not only are farmer responses assessed, but the environmental consequences of farmer reactions are also accounted for. A common approach to integrate biophysical and economic models is bioeconomic modelling (BM). Bioeconomic farm models are based on the optimization of a utility function under multiple constraints. BM allows for the integration of the multiple dimensions of sustainability, it is the most widely used approach to assess the complex interrelations between climate change, agricultural production, resource and social sustainability. This work's main output is a novel multidisciplinary characterization of climate change adaptability of smallholder farmers and the assessment of potential socio-economic and environmental impacts arising from the adoption of tailored climate adaptation practices.

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Wool dyeing Wastewater treatment for water and natural dye recovery

A.I. Arroyo-Escoto^a, F. Carvalho^{b, c}, M. C. Fernandes^{a, d}

^aCentro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), Apartado 6158, 7801-908 Beja, Portugal.

^bDepartamento de Tecnologias e Ciências Aplicadas, Instituto Politécnico de Beja (IPBeja), Ap 158, 7801-902 Beja, Portugal

^cFiberEnTech, Fiber Materials and Environmental Technologies, Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal

^dMED - Mediterranean Institute for Agriculture, Environment and Development, CEBAL — Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo; Apartado 6158, 7801-908 Beja, Portugal.

e-mail: alonso.escoto@cebal.pt; maria.fernandes@cebal.pt

Textile industry is one the most polluting in terms of its water effluents. During the different processes involved, the dying stage is one of the most important. Since is here where colour is obtained and being this feature one of the most related to consumer, aside to texture and quality, requires from the industrial sector in charge to invest much care about this step. Nevertheless, the levels of water consumed by the dyeing industry is quietly alarming, not only for the volume required, but also for the wastes produced. Textile dyes are in general, aromatic molecules that confer to the fabric the desired colour through different processes. This stage, besides its complexity due to the characteristics of the selected fabric source has also another limiting step. The dying molecule has shown some capabilities to interact with people's skin producing allergic and toxic reactions for different reasons. Coupled to the polluting capacities related to the Oxygen Chemical Demand (OCD) value for the metals used as mordants, makes its treatment and care of great importance. This feature has provided to the natural dyeing process a new chance to be reintroduced in the industrial way due to it is more noble with human skin and its biodegradable capabilities. As said before, not only colour, but texture and quality are also important. Wool is one of the textiles with those desired characteristics for consumers. Its durability and weather facing capabilities makes it one of the best options to reinforce the industry with eco-friendlier and lesser toxic characteristics.

Based on the circular agriculture movement, "Grupo Operacional Tinturaria Natural" is dedicated to the improvement of dyes production coming from natural sources that are suited for wool dying. This project has a multi-academic participation in which CEBAL's role is focused on dying extraction and purification scaling and wastewater treatment of the effluents with aim on the recovery of these molecules to be reused in the process as much as possible using membrane filtration technology. To achieve these objectives a thoroughly review has been done to find the best reported reaction conditions for making the process as economically suitable as possible based on the UBI and INIAV laboratory scale reactions conditions already used.

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Ants associated with the vines of the Évora region

E. Venâncio¹, A. Oliveira², J. Tiago Marques S³

¹PhD Student in Biology – Universidade de Évora

²MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra

³Unidade de Biologia de Conservação, Polo da Mitra

Email: eunycassia@gmail.com

Agriculture represents the dominant form of land use worldwide. This practice affects the macroinvertebrates of the soil including ants. Few work has been carried out in Portugal on ants associated with agricultural crops, namely vineyards. In order to know the diversity and abundance of ant species in vineyards in the Évora region and to understand the impact of its management on the ant community, samples were carried out between April and June 2018 in 31 vineyards in this region. A total of 23464 ant specimens belonging to four subfamilies, fifteen genera and twenty-six species were identified. The genera *Aphaenogaster*, *Tapinoma* and *Messor* were the most diverse with three species in each. The species *Tapinoma nigerrimum* is the most represented with a total of 6644 individuals. Eighteen of the identified species are omnivorous. Regarding the type of vineyard management, integrated protection or biological protection, there were no significant differences regarding the abundance and richness of ants. However, I found a positive relationship between the abundance of ants and the herbaceous cover between vine rows in June. The diversity indexes showed a negative relationship with the size of the leaves of the vineyard in April. These results show that the vineyard management influences both ant abundance and species richness thus, the ecosystem services that they provide, mainly improving soil structure and the predation of some arthropod pests. With this work it is expected to have contributed to the knowledge of the diversity of ant species and their distribution in Portugal, mainly in the south of the territory.

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Analysis of vegetation dynamics for the adoption of management measures that reduce the risk of fire

M. Raposo^{1,2}, L. Nunes^{1,3} & C. Pinto-Gomes^{1,2}

¹ MED (Instituto de Ciências Agrárias e Ambientais Mediterrânicas), IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

² Departamento de Paisagem, Ambiente e Ordenamento, Escola de Ciência e Tecnologia, Universidade de Évora. Rua Romão Ramalho, n59, 7000-671 Évora, Portugal.

³ ProMetheus-Unidade de Investigação em Materiais, Energia e Ambiente para a Sustentabilidade, Escola Agrária, Instituto Politécnico de Viana do Castelo, Rua da Escola Industrial e Comercial de Nuno Alvares, 4900-347 Viana do Castelo, Portugal.

Email: mraposo@uevora.pt

Fires in Portugal in recent past decades have increased their area of occurrence, as well as their recurrence. As a consequence of these facts, there is a high number of human, material and quality losses in ecosystems. The cause of these serious impacts can be attributed to several factors, such as the rural exodus, the homogenization of the landscape, through the planting of monoculture forest stands, the lack of application of the land planning regulations, among others. However, the behavior of fire is dependent on a set of different factors, such as the atmospheric conditions, the exposition of the hillside, the type of plant material and the anthropic action. Environmental conditions are difficult to control, but the vegetation cover can be changed and managed through human action. In this work was analyzed how the dynamics of the vegetation cover can contribute to the reduction of the risk of fire, through the differentiation of the type of shrubs that exist in each serial stage. Based on the morphological characteristics of these materials, it was possible to differentiate dynamic states from the vegetation cover and separate them by vegetation classes. Pioneer shrubs have totally different characteristics in relation to forest and pre-forest shrubs, such as the heliophile character. Thus, in order to reduce the risk of fire in rural areas, selective control of the vegetation cover is proposed. The vegetation belonging to the initial stages of the vegetation series must be controlled, while the stages closest to the mature forest must be conserved. This methodology, in addition to reducing the risk of fire, allows greater efficiency of economic resources through control actions and contributes to the enhancement of ecosystem services.

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The valuation of the social dimension in the participatory process by modulating a transdisciplinarity applied to the landscape project

Myriam Vinagre ¹

¹ Socio-économiste, Université de Rennes 1, Bureau d'Etudes I.D.E.A.L., France.

E-mail: myriam.vinagre@gmail.com

Global environmental challenges linked to human activity require profound social and economic changes.

In Europe, the sustainable development project, aimed at rethinking ways of living and strengthening territorial identity, is based on participation. This requires a particular commitment from the various stakeholders, technical, political and citizens. It uses cognitive and relational mechanisms. Beyond that, it depends on organizational and structural changes. These social dynamics are nevertheless little valued. Too often reduced to the operational objective of consensus, this participation is not sufficiently inclusive and integrated to achieve these ends.

Because of its environmental and social dimensions, the landscape seems particularly favorable to dialogue in the field of planning. Can a landscape project methodology aim to enhance this dimension?

This postulate validated, the landscape project could be an object of applied transdisciplinarity. The concept, considering the co-construction of knowledge beyond the scientific framework, would allow the methodical determination of social indicators of the complexity of the participatory process. The research aims at a project methodology from which a sustainable, practical and responsive tool can be derived for the observation and evaluation of social mechanisms in the participatory process. The modulation by the systematic definition of the message processing parameters in the transdisciplinary environment of the landscape project would thus aim to sensitize the various actors and, consequently, to make effective the dynamics of change of the transdisciplinary approach.

The methodology will focus on defining indicators of the social mechanisms at work. The analysis will also consider the means, conditions and mediations of social dynamics. From a sociological approach, we will confront the theoretical framework of transdisciplinarity with the terrain of the landscape project in various territories. A documentary analysis will make it possible to select three of the most recent projects in Europe. This sample will also be defined according to a diversity of existing projects and landscapes, particularly in view of a gradient of peri-urbanization. Experimentation with the modulation of the participatory process will take the form of workshops and cross-cutting surveys of these projects.

Plenary Lecture speaker

| Name | Email |
|----------------|---------------------------|
| Mário Carvalho | mariogpcarvalho@gmail.com |

List of participants

| Name | Email |
|-------------------------------|---------------------------------|
| Laura Carreira | d47128@alunos.uevora.pt |
| Adriana Catarino | adri.catarino@hotmail.com |
| Agostinho Serranito | agostinho_p_29@hotmail.com |
| Alexandre Ribeiro Lima | carlima@ualg.pt |
| Alonso Escoto | alonso8623@gmail.com |
| Ana Faustino | ana.faustino@cebal.pt |
| Ana Galveias | acgjorge@uevora.pt |
| Ana Patrícia Cebola Poeiras | ana.poeiras@hotmail.com |
| Ana Paulino | ana.paulino@cebal.pt |
| Ana Rita Trindade | arctrindade28@gmail.com |
| André Albuquerque | d40926@alunos.uevora.pt |
| Beatriz Duarte | bzduarte@ualg.pt |
| Carla Simões | carlasimoes3@hotmail.com |
| Carla Varanda | carlavaranda@uevora.pt |
| Clarisse Mourinha | clarissemourinha_17@hotmail.com |
| Daniela Filipa Firmino Rosa | dffr@uevora.pt |
| David Guedes | dhfg@uevora.pt |
| Eduardo Ferreira | ferreira@uevora.pt |
| Emanuel Carreira | d41989@alunos.uevora.pt |
| Erika Roldão Lemos de Almeida | d49668@alunos.uevora.pt |
| Esmeralda Pereira | d39006@alunos.uevora.pt |
| Eunice Venâncio | eunycassia@gmail.com |
| Flávio Silva | fsilva@uevora.pt |
| Frederico Martins | d49669@alunos.uevora.pt |
| Gabriela Carneiro Murta | gabriela.murta@gmail.com |
| Gonçalo Almeida | goncalo.almeida@itqb.unl.pt |
| Inês Caeiro | ines_caeiro_@hotmail.com |
| Ivo Jael Lourenço Palhas | ecopalhas@gmail.com |
| Joana Rocha | d49756@alunos.uevora.pt |
| João Batista Guerreiro | d48177@alunos.uevora.pt |
| José Rico | d43337@alunos.uevora.pt |
| Lénia Rodrigues | lrodrigues-88@hotmail.com |

| | |
|--------------------------------|---------------------------|
| Letícia Fialho | leticia.fialho@cebal.pt |
| Liliana Cachucho | liliana.cachucho@cebal.pt |
| Luiza da Costa Corrêa Oliveira | luiza_cco@hotmail.com |
| Maria Couto | mpcoutho@campus.ul.pt |
| Maria Doroteia Campos | mdcc@uevora.pt |
| Maria Fernandes Félix | mrff@uevora.pt |
| Maria Inês Rouxinol | mir@uevora.pt |
| Maria João Camacho | mjoao.camacho@iniav.pt |
| Mariana Patanita | d46100@alunos.uevora.pt |
| Mauro Raposo | d39443@alunos.uevora.pt |
| Miguel Ferro | d43463@alunos.uevora.pt |
| Miguel Silvério | mavsilveriowork@gmail.com |
| Milena Brito Espinosa | mibrito.snt@gmail.com |
| Myriam Vinagre | myriam.vinagre@gmail.com |
| Natália Marques | nmarques@ualg.pt |
| Nathana Lazzarotto Cristofoli | nlcristofoli@ualg.pt |
| Nuno Pedroso | nmpsp@uevora.pt |
| Pedro Barbosa | pbarbosa@uevora.pt |
| Pedro Inácio Matias | pedrom17@live.com.pt |
| Rita Pires | d44180@alunos.uevora.pt |
| Rita Pires | rita.pires@cebal.pt |
| Sandra Caeiro | caeirosandra@hotmail.com |
| Sara Conceição | d44723@alunos.uevora.pt |
| Sara Costa | satcostaa@gmail.com |
| Sara Ricardo Rodrigues | sirr@uevora.pt |
| Sofia Trindade | m41741@alunos.uevora.pt |
| Teresa Louro | teresalouro@hotmail.com |
| Teresa Saavedra | tmr.saavedra@gmail.com |
| Tomás Magalhães | ttmagalhaes@ualg.pt |