JORNADAS MED

MEDITERRANEAN INSTITUTE
FOR AGRICULTURE, ENVIRONMENT
AND DEVELOPMENT

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BOOK OF ABSTRACTS JORNADAS MED 2019

JORNADAS MED

MEDITERRANEAN INSTITUTE FOR AGRICULTURE,
ENVIRONMENT AND DEVELOPMENT

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JORNADAS MED – PÓLO DA MITRA
27 e 28 de Junho de 2019

27 DE JUNHO

12:00H – Receção
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16:45H – **Conservação num mundo em mudança** – Diogo Alagador (CIBIO)
17:00H – **Efeitos (e propostas para a mitigação) da intensificação no olival nos serviços de controlo biológico por aves e morcegos** – José Herrera (CIBIO)
17:15H – **Estradas, Biodiversidade e Conectividade da paisagem: da monitorização à acção** - Sara Santos (CIBIO)

17:30H – **Alteração da paisagem, fragmentação de habitat e os seus impactos sobre a diversidade de fauna em ecossistemas mediterrânicos** – Pedro Salgueiro (ICAAM)
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15:45H – **Influência da data de colheita da azeitona na produção de azeite obtida por hectare** – Francisco Mondragão Rodrigues (IPP-ESAE/ICAAM)
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9:45H – **Biodiversidade e serviços de ecossistemas em vinhas**: **morcegos, aves e artrópodes, para a regulação de pragas** – Tiago Marques (ICAAM)
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9:45H – **Reproductive management and biotechnology of reproduction in Lusitano breed horses** - Elisa Bettencourt (ICAAM)
10:00H – **Operacionalização do conceito "Uma Saúde". Iniciativas Internacionais e Nacionais** - Manuela Vilhena (ICAAM)
10:15H - **Desenvolvimento de vacina edível para o controlo da doença hemorrágica viral (RHDV2) nos coelhos-bravos** - Carina Carvalho (INIAV/ICAAM)

10:30H – DEBATE
11:00H – Pausa para café

11:30H – **VALBIOTECCYNARA - Valorização económica do cardo (*Cynara cardunculus*): variabilidade natural e suas aplicações biotecnológicas** – Cristina Conceição (ICAAM)
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14:15H – **Inovação nas políticas públicas: Contributos da investigação para a construção de medidas agroambientais baseadas em resultados - caso de estudo**– Maria Isabel Ferraz (ICAAM)
14:30H – **A importância da exploração familiar no sistema alimentar regional (SALSA)** – Maria Rivera (ICAAM)
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14:15H – **Utilização da espectroscopia FT-NIR para a discriminação varietal de nozes (*Junglans regia*) - estudo comparativo entre 3 técnicas** – Ana Elisa Rato (ICAAM)
14:30H – **O uso inovador de nanoemulsões enriquecidas com óleos essenciais para retardar o amadurecimento dos frutos e prevenir os danos causados pelas baixas temperaturas de armazenamento** – Custódia Gago (MeditBio)
14:45H – **Caracterização funcional dos genes de parasitismo de *Pratylenchus penetrans* e o seu potencial no controlo do nematode** – Cláudia Vicente (ICAAM)
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15:15H - DEBATE

16:00H - MESA REDONDA: DESAFIOS DA INTERAÇÃO AGRICULTURA E AMBIENTE

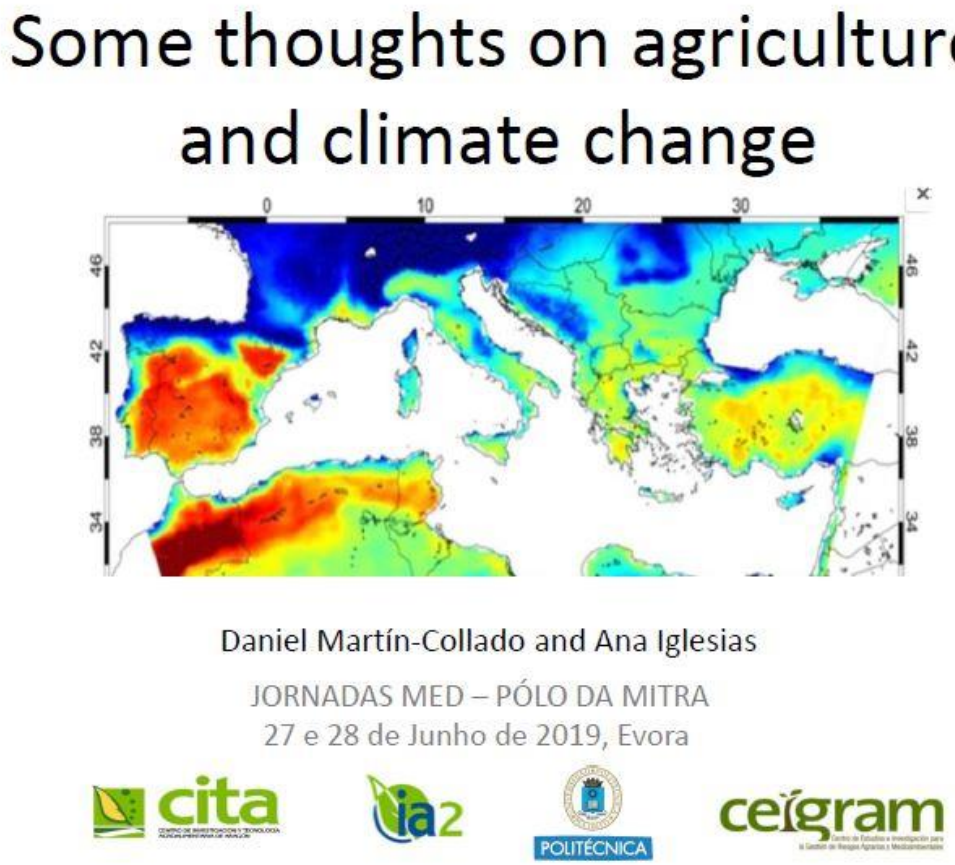
Apresentação das principais ideias e conclusões das sessões temáticas, expostas pelos Relatores de cada Linha.
Discussão aberta sobre desafios e prioridades da investigação no MED
Moderação: Teresa Pinto-Correia, Anabela Romano

17:15H – Divulgação do Melhor Poster das Jornadas e Entrega de Prémio
17:30H – Sessão de Encerramento

PLENARY SESSIONS

Sessão Plenária: Some Thoughts on Agriculture and Climate Change - Daniel Martin-Collado (CITA - Centro de Investigación y Tecnología Agroalimentaria, Zaragoza) e Ana Iglesias (CEIGRAM - Centro de Estudios y Investigación para la Gestion de Riesgos Agrarios e Medioambientales, Universidad Politécnica de Madrid).

SEE PRESENTATION WHERE



ORAL PRESENTATIONS

Invited Speaker 1

Cork Oak Genome

A. Usié[1,2], Genosuber Consortium[1,2,3,4,5,6]

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[2] Centro de Biotecnologia Agrícola e Agro-alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), Beja 7801-908, Portugal
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[5] INIAV - Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal
[6] Biocant - Parque Tecnológico de Cantanhede, Núcleo 04, Lote 3, 3060-197 Cantanhede, Portugal

The Genosuber project, a consortium formed by several Portuguese institutions, has determined the genome sequence of cork oak, one of the main forestry species in the Mediterranean, which plays a relevant biological and economic role in Portugal [1]. The outstanding advances observed in high-throughput sequencing allow the generation of substantial volumes of sequence data. However, without a sequenced reference genome these powerful datasets cannot be fully explored. With the availability of a fully sequenced and annotated genome, cork oak genomics research can now enter a new era.

In addition to the cork oak genome assembly, several omics datasets focusing on different types of sequencing have been produced in Genosuber, with the purpose of advancing cork oak genomics research applied to relevant biological systems and phenotypic traits. Large scale SNP detection projects are underway, based on whole-genome resequencing of cork oak trees, with contrasting phenotypes for cork quality, and hundreds of thousands of SNPs were identified. These SNPs are being used to perform genome-wide association studies. Moreover, structural variation and copy number variation have also been identified with these WGRS datasets. Furthermore, the selective sweeps that were identified provide the first insight regarding cork oak evolutionary and selection processes.

A multi-omics approach is being applied to comprehensively characterize tissues involved in cork formation. Within Genosuber, a comprehensive transcriptomic characterization of tissues involved in cork formation was produced identifying differentially expressed genes and targeting the non-coding transcriptome including the analysis of micro and long non-coding RNAs. The epigenomic landscape of these tissues was also determined, which added additional layers of omics information, with great potential to unravel the regulation of this complex trait.

Overall, cork oak has become a species where all types of omics studies will be possible, which will greatly enhance our ability to address the main questions regarding cork oak production and biology

Keywords: Cork oak; genome sequencing; multi-omics.

Invited Speaker 2

Pro-FlorMed: Research Group for Sustainable Management Of Mediterranean Forest Ecosystems

C. Camilo-Alves[1,2], C. Dinis[1,2], S. Saraiva Dias[1,2], A. Poeiras[1,2], J. Ribeiro[1,2,] J.A. Nunes[1,2,] J. Mota Barroso[1,2], M. Vaz[1,3,] A. Pinheiro[4,] N. Almeida Ribeiro[1,2]

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[3] Departamento de Biologia, ICAAM, Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora
[4] Departamento de Economia, Escola de Ciências Sociais, Universidade de Évora, Colégio do Espírito Santo, Largo dos Colegiais, 2, 7000-803 Évora

Mediterranean trees are of high conservation and socioeconomic value in their region. However, high mortality events have been occurring for decades, associated with lack of tree regeneration. The main objectives of our team is to produce knowledge for the comprehension of such processes and to create tools to mitigate them. The Pro-FlorMed team is expertise in several fields, from forestry, biophysics, geography, biology, agriculture, fertigation, to economics. This interdisciplinarity conjoins to produce a full comprehensive perception of the Mediterranean forest systems. Our long-term research on forestry is based on information periodically acquired in several permanent plots, using an integrated multi-level monitoring procedure. The information is gathered at the tree level – related to structure and function –at the stand level- soil, water, topography, biodiversity and interactions among components, cultural practices and meteorology. In order to obtain this information, we apply traditional methodologies as well as new and innovative technologies in partnership with other institutes. This approach allowed to model some processes such as spatial tree growth and cork production, root development, tree vitality and mortality events. We perform research about tree mortality related to biotic and abiotic factors and to management practices; research on best techniques for stand regeneration, including the improvement of fertigation techniques and its effect on tree physiology and structure (growth and cork development); models of spatial tree growth and cork production. This knowledge is processed and modeled to give tools for forest decisions makers to adequate their management for the sustainability of the system in all its aspects- tree regeneration, vitality and resilience, environmental conditions and economics. Therefore, the team established strong connections with forest producers and forest industry, in the form of research projects and provision of services, which enable the long-term research and knowledge transfer.

Invited Speaker 4

Land Classification Processes: Typologies of Montados and Related Management Units

T. Pinto-Correia[1], N. Guiomar[1], S. Godinho[1], C. Godinho[1], R. Lourenço[1], I. Ferraz de Oliveira[1]

[1]ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Formação e Investigação Avançada, Universidade de Évora. Pólo da Mitra, Ap. 94, 7006-554 Évora.

The spatial fuzziness of *Montado*, with unclear boundaries between patches with different vegetation patterns, as well as its temporal dynamics with changes in the composition and density of the different strata, are characteristics that hinder the classification and spatial delimitation of these agro-silvo-pastoral land use systems. However, there is enough and detailed data nowadays to support the classification of *Montado* in different typologies. This process is critical to the definition of well-framed and place-based strategies, only possible from a thorough understanding of the diversity of these land use systems.

The classification system to be adopted must be flexible enough to allow the achievement of several objectives and should be based on two sets of classifiers:

Horizontal and vertical structure of vegetation: what is observed as a pattern of land cover;

Geo-ecological classifiers: the biophysical characteristics that determine the carrying capacity and potential land cover of each land unit.

Although it is recognized that within the same plot or paddock there may coexist significantly different *Montado* patches, or even open patches without trees, the land classification unit will be the paddock, on which decisions are made by the landowners or by the land managers. In this classification we will not consider the cultural use of the *Montado*, i.e. the land use system. It is well known that this component is one of the factors determining the spatial pattern of *Montados*, but it is the succession of land use systems in the past that influences its present state. Moreover, obtaining such information requires to carried out with the landowners or managers, which is not compatible with a classification system to be applied to the full extent of the *Montado* land cover. The land use system in *Montado*, is evidently relevant, but as a qualifier to be used in specific cases, and not in this construction of a large-scale classification system.

Keywords: land classification systems; silvo-pastoral land use; montados; land cover, multifunctional patches

Invited Speaker 5

Birds as Indicators: from Management to Biodiversity

C. Godinho[1], P.F. Pereira[1], R. Lourenço[1], I. Roque[1], J.E. Rabaça[1]

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The world distribution of cork oak *Quercus suber* and holm oak *Q. rotundifolia* is basically restricted to the western Mediterranean basin. These two evergreen oaks are the base of the Portuguese montado occupying more than 1,000,000 ha (~34% of the national forest). This highlights the economic, social and environmental relevance of the system in Portugal. The montado is recognized as a biodiversity hotspot, holding a high number of species from different taxa, with the singularity of depending on human management. The multifunctionality of the montado is mainly associated to forest, pastoral, hunting and/or touristic activities, resulting of a heterogeneous landscape dominated by a forest matrix of oaks with open areas, small forest patches (mainly pinewood) and Mediterranean shrublands.

Montado is an important habitat for birds, holding a high diversity and abundance of species. More than one third of the species that occur regularly in Continental Portugal can be seen in montado areas. Since the distribution and conservation of these species are strictly link with the management options, birds can be used as indicators of management and conservation of the montado.

In this talk we will present an overview of our work on the role and potential of birds as indicators of biodiversity and management, specifically: (1) definition of functional guilds as indicators of montado typologies and to define High Nature Value areas, (2) systematic monitorization for support to forest certification processes, (3) calculation of bird population trends to access the conservation status of the montado, and (4) the potential role as pest controllers.

Keywords: montado; silvo-pastoral; trends; cork

Invited Speaker 6

Conserving Biodiversity in a Changing World

D. Alagador[1] & M.B. Araújo[1]

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The Grand Environmental Crisis is creating fundamental challenges for the persistence of biodiversity, ecosystems and societal welfare. Conservation efforts aimed at the protection of natural spaces and processes have had mixed success, and there is an increasing awareness that long-term protection of biodiversity requires inclusion of flexible measures and anticipative future predictions on plausible responses of species and ecosystems to scenarios of change. Novel ways to protect land in order to set aside biodiversity from local, regional and global impinging threats are critical for conservation success. The optimal location of protected areas and timings of action (or inaction) in those areas are untrivial problems that planners and scientifically-sensitive policy makers need to resolve. Upper levels of complexity are still incited when socioeconomic interests are integrated in such models, such that biodiversity benefits from opportunity windows of inter-sectorial collaborations while bottlenecks and inter-sectorial conflicts are avoided (typically linked to areas equality well-ranked for both conservation and socioeconomic development). Our work, uses spatial analysis, optimization tools and network theory to respond to these kinds of challenges. Here, we present a coherent analytical framework that empowers the identification of optimal spatial trajectories of species when following their shifting climatically-suitable areas and the likely species' persistence perspectives within those areas. Because such adaptive trajectories are characterized by time changing species occupation areas, they provide planners guiding-lights to anticipate when, currently ecologically unvalued areas, will gain relevancy and contrastingly, which areas highly-suitable in present-time will lose value, thus being sources of financial revenue for the conservation side. More than defining optimal spatial trajectories of species, the framework allows planners to find optimal landscapes to link already established protected areas. Again, this is a resource saving option as past conservation efforts are relived and made functional at long-term. Additionally, the framework allows central connectivity points to be highlighted. These are areas where both, multiple trajectories of a single (or several) species converge being therefore central to the maintenance of the whole adaptive system as climate changes. With minor rearrangements the framework here introduced permits assistance for two other relevant conservation problems: i) identification of optimal areas for successful assisted colonization of species unable to spatially adapt to current changing rates, and; ii) identification of areas that disturbing activities need to consider in order to compensate the conservation side of society from damaged habitats and species' populations states. With such a wide scope of application here we present a study in which the framework guides the identification of sets of adaptive trajectories of 30 threatened mammal species to plausible scenarios of climate change in Europe until 2080. Under the contemporary stresses derived from the negative synergies of prevalent wide-ranging societal interests and the large spatial needs of ecological processes to accomplish new equilibria states in a changing world, we trust that our work brings a glimpse of optimism, as it is aligned to reconcile the legitimate societal development with the well-functioning of species and ecosystems - a prominent issue towards a healthy, sustainable society.

Keywords: Biogeography; Climate change; Dynamic systems; Network analysis; Optimization; Prioritization

This study was funded by FEDER funds through the Fundação para a Ciência e a Tecnologia (FCT) and Programa Operacional Factores de Competitividade – COMPETE – through the project RECONCILE PTDC/AAG-GLO/3979/2014 (ref. 9471-RIDTI).

Invited Speaker 7

Effects of (and mitigiations proposals for) Management Intensification on Biocontrol Services Provided by Birds and Bats in Olive Groves

José M. Herrera[1]

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Land conversion for agricultural purposes is a major driver of biodiversity loss worldwide. Paradoxically, this compromises the provision of key ecosystem services supplied by wild species with direct implications in crop production. Biocontrol services, is one of such services, which can be defined as the impact of natural enemies on the population density of crop pests.
Biocontrol services are indeed increasingly recognibed to provide undeniable incentives for biodiversity conservation within production landscapes. To a large extent, this is because a burgeoning research literature demonstrates that some species (particularly vertebrates such as birds and bats) perform a pivotal role as biocontrol agents against a wide variety of crop pests.
In the present work, I show how the intensification of farming practices strongly compromises the provision of biocontrol services supplied by birds and bats, using Mediterranean olive farms as study case. While empirical conclusions are obtained from the region of Alentejo, I make the exercise of extrapolating them to the whole of Portugal.

Keywords: biocontrol services, conservation management, olive groves pests, vertebrates

This work was funded by the Portuguese National Funding Agency for Science, Research and Technology (FCT) through the project PTDC/AAG-REC/6480/2014 the contract IF/00001/2015.

Invited Speaker 8

Roads, Biodiversity and Lansdscape Connectiviy: From Monitoring to Action

S. M. Santos [1,2], A. Mira [1,2]

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[2] UBC – Unidade de Biologia da Conservação, Departamento de Biologia, Universidade de Évora. Pólo da Mitra 7006-554 Évora

Roads are man-made structures widespread through the world and vital to modern human societies, connecting people and goods. However, these linear infrastructures have several negative effects on the environment. The discipline of Road Ecology emerged 20 years ago at a time when the ecological consequences of roads were not taken into account, and since then this field has advanced rapidly. Roads affect wildlife by increasing habitat fragmentation, modifying animal behaviour and movements, and increasing mortality as a consequence of road-killing. The collision with vehicles is the most visible impact of roads, with millions of individuals from a wide range of taxonomic groups being killed every year. These collisions can be particularly detrimental, because the effect of these mortality numbers on the long term persistence of most affected species is still uncertain. Therefore, mitigation of negative impacts should be based on a thorough understanding of the species most vulnerable to road mortality, which is generally very limited.

We will present some of our results from the last 13 years of Road Ecology studies. Our group addresses themes such as the spatial and temporal patterns of collisions, the factors influencing it, the identification of most critical areas for collisions, disturbance and barrier effects on species activity and abundance, the role of road verges as movement corridors or alternative habitats, the impacts of roads on landscape connectivity, the effects of road proximity on movement patterns of species, and the improvement of monitoring methods. We use diverse vertebrate models to understand these effects, namely amphibians, passerine, owls, bats, small mammals, and carnivores.

Until 2015 there was no specific finantial support for the MOVE project (Evaluation of the effects of roads on terrestrial vertebrates). However, the data gathered in this period (10 years) allowed the approval of two funded projects. The POPCONNECT project (PTDC/AAG-MAA/0372/2014) which combines microsatellite markers, telemetry, roadkill data and modelling to assess the effects of roads on population parameters of species and landscape functional connectivity. The project LIFE LINES project (LIFE14 NAT/PT/001081) which aims to essay, evaluate and disseminate practices directed at the mitigation of negative effects from transport infrastructures in wild fauna and simultaneously promote the creation of a Green Infrastructure.

Invited Speaker 9

Landscape Change, Habitat Fragmentation, and its Long-lasting Effects on Animal Diversity in Mediterranean Ecosystems

P.A. Salgueiro[1,2,3,5], A. Mira[1,4,5], J.E. Rabaça[2,4,5], S.M. Santos[1,4,5]

[1]UBC – Conservation Biology Lab;
[2]LabOr – Laboratory of Ornithology;
[3]CIBIO-UE – Research Center in Biodiversity and Genetic Resources, Pole of Évora;
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Human-induced landscape changes are accounted as having long-lasting effects on animal diversity caused by either habitat loss or fragmentation. Understanding how changes on landscapes' spatial arrangement can alter population structure and ecological processes (e.g., connectivity) is a priority in order to estimate the extent of its impacts. However, most of the research in this field has set its foundations on discrete conceptualizations of landscapes, which assume that species perceive landscape similarly as patches of suitable and unsuitable habitat. In this study, we provide an insight on how bird species perceive and distribute along fragmented Mediterranean 'montados'. Firstly, we aim to define the thresholds of occurrence of a bird community inhabiting a tree canopy gradient. We modeled species-specific responses to the gradient, which enabled us to identify species richness and turnover patterns marking strong changes in community composition. Secondly, we tested different conceptualizations of the 'montado' landscape in order to evaluate how gradients capture landscape complexity comparing with site-scale variables and discrete land use mapping based on unrealistic discontinuities in the landscape.

The tree canopy gradient is responsible for major changes in bird community acknowledging the loss of vegetation vertical strata and canopy enclosure. Maximum species richness is reached at 10% canopy cover while total turnover rate shows a distinct peak around 1% canopy cover and a smaller one at 20%. Typical Mediterranean 'montados' promote higher regional levels of bird richness. However, different cover densities may provide uniqueness and function (connectivity). Regarding landscape conceptualization, the suitability of a given conceptual model depends on species habitat specialization, and the ability of each conceptualization to capture spatial heterogeneity. Gradient-based models provide better information on resource allocation, while patch-based models offer a simplified perspective on landscape attributes. Future research should integrate different sources of information in order to avoid bias from inadequate landscape conceptualizations, which may lead to ineffective strategies for conservation.

Keywords: bird community; 'montado' silvo-pastoral system; tree canopy cover gradient; landscape conceptualization; ecological thresholds; spatial heterogeneity

PAS and SMS were funded by grants of the Portuguese Science Foundation (reference SFRH/BD/87177/2012 and SFRH/BPD/70124/2010, respectively). Fieldwork was supported by Integrated Program of IC&DT (1/SAESCTN/ALENT-07-0224 FEDER-001755).

Invited Speaker 10

Mediterranean Temporary Ponds Restoration Approaches

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Mediterranean Temporary Ponds (MTP) are seasonal freshwater habitats classified as priority (3170*) by the European Habitats Directive. These ecosystems are strongly dependent on rainfall and annuallyexperience a flood / dissection cycle. Living beings that colonize them are specialized in this alternation of ecological conditions.
MTP are nowadays subjected to strong anthropogenic pressures, such as deep soil turning, drainage, flattening the surface topography or transformation into permanent reservoirs for irrigation. On the other way pond conservation status decay is also due to the lack of grazing and even some superficial tilling, resulting to grass encroachment that led to the disappearance of rare/less abundant flora species, and to the invasion by opportunistic heliophytic plants. Therefore, it is urgent to act in order to halt this decline in area and quality.
When restoring MTP habitat we must bear in mind that this habitat results from a long human presence in the Mediterranean region, as it's persistence has not only been compatible but favored by extensive human activities
We simulated this traditional land-use regime in 8 MTP in the Southwest Coast of Portugal in Spring 2018 to recover, among others, Apium repens(Jacq.) Lag., a priority species for conservation under Habitats Directive. The methods proved to be efficient as plant species richness increased resulting from bringing to the surface the seeds that were still in the soil seed bank and thus providing them with enough light to germinate.
The recovery, in terms of topographic profile of the basin, is challenging and has to be carried out having in mind specifically the characteristic water regime recovery. We carried out physical replacement of pond basin topography, invasive vegetation control and plant communities' enhancement. The restoration results are positive, with a reinforcement of the spatial differentiation of the floristic communities and the increase of characteristic / indicator plant species. Regarding the control of invasive species (e.g. Acaciaspp.) the results encouraged us, since the removal of the vegetal mass allows the germination of the characteristic small annual species.

Keywords: Vernal pools, Plant species, Ecological restoration, grazing

Invited Speaker 11 - Thematic Conference

The Cultivation In Hedge in Dry Land: The True Opportunity Of The Model

Rubén Márquez Jiménez

AGROMILLORA IBERIA SLU. - DELEGADO COMERCIAL PORTUGAL – EXTREMADURA

The unirrigated olive grove represents more than 77% of the total area devoted to the cultivation of olive trees in the world. Spain and Portugal are a faithful reflection of the previous statement since, more than 72% of its olive grove area was developed under rainfed conditions.

The continuity of rainfed olive groves in time seems assured given the growing demand for oil by the market and the limitations of
current water resources that make it difficult to replace these hectares with other irrigated ones. Its low profitability, however, means that its economic viability is closely linked to the oscillation of the prices of olive oil, and that in conditions of low prices are the first plantations to be abandoned or remain in a situation of "semi-abandonment".

The cultivation in hedge in dry land: the true opportunity of the model

The results obtained over these years with this model have been more than satisfactory, since the reduction in costs that comes with mechanical harvesting in itself, significantly improved the profitability of the rain-fed olive plantations.

Keywords: superintensivo secano
References:
1.AGROMILLORA. OLINT.
2.JOSE MARIA ROCA

Invited Speaker 12

Evaluation of Different Pruning Solutions for Superhigh Density Olive Orchards

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In the super high density olive orchards, manual pruning is the current practice used by olive growers. This option requires a large amount of labour. Although disc saw pruning machine allow high work rate, it would be necessary to evaluate the influence of the use of the pruning machine in olive yield in order to establish a pruning strategy.
In 2015 a trial was established in a commercial orchard of Arbequina cultivar. The orchard was planted, in the year of 2006, according to 3,75 meters between rows and 1,35 meters between trees in the row. The trees were trained according to the central leader system. In this trial, in a randomised complete block with three replications, three treatments (T1; T2; T3) are being compared leading to 9 plots with 480 trees per plot. The treatments under study are: T1 - manual pruning using chain saws; T2 - mechanical pruning + manual pruning; topping the canopy parallel to the ground and hedging on the two sides of the canopy in 2015; topping the canopy parallel to the ground twice in 2016 (winter pruning and summer pruning); manual pruning in the winter of 2017 and topping the canopy parallel to the ground in the summer of 2017; hedging the two sides of the canopy in the winter of 2018 and topping the canopy parallel to the ground in the summer of 2018; T3 – mechanical pruning + manual pruning; hedging on the East side of the canopy in 2015 with an oblique cut; manual pruning in West side of the canopy in 2016; manual pruning in the East side of canopy in 2017 and hedging on the West side of the canopy in 2018 with an oblique cut.
The topping parallel to the ground in the summer could be useful to control suckers in the top of the canopy.
Significant differences in olive yield between years were found: Treatment 2 obtained more regularity on olive yield among years.
More years of field work are essential to define the better pruning strategy.

Keywords: mechanical pruning; super high density olive orchard; work rate; olive yield; pruning cost.

Invited Speaker 13

Influence of the Olive Harvest Date on the Quantity of Olive Oil Obtained per Hectare

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Within the framework of the OLEAVALOR project - Valuation of Portuguese olive varieties (Operation ALT20-03-0145-FEDER-000014), started in 2016, work has been developed in olive groves in production to evaluate the phenological, vegetative and reproductive behavior of the varieties included in the project. It has also been evaluated the cultural operations carried out by olive growers and their repercussion on the productivity and economic yield of the crop. It is argued that harvesting should occur when stabilizing the Fat content measured in Dry Matter (FDM), because this parameter determined in this way is not influenced by the decrease of moisture of the fruit as the winter cold increases, due to dehydration. In the present study the data for the years 2017 and 2018 and the varieties 'Cobrançosa' and 'Galega vulgar' are presented from an olive grove at Monforte. To reduce the use of laboratory tests (which should not be completely waived to obtain the exact value of FDM), we should make use of the high correlation between FDM and the Maturation Index (MI). In the 'Cobrançosa' the FDM value stabilizes when the MI presents values from 2,1 (2018) to 2,2 (2017) and for 'Galega vulgar' stabilizes for IM values from 3,6 (2018) to 3,9 (2917). In both varieties this stabilization occurred earlier in 2017 (2 November) and later in 2018 (19 November). There appears to be a strong relationship between the evolution of FDM and MI over the years and for each variety there is a "characteristic" MI value (or short range of values) corresponding to the FDM stabilization time. Once the FDM stabilizes, the olives must be harvested, as in 2017 the 'Cobrançosa' lost 180 kg of olive oil per hectare in the following week and 45 kg / ha of olive oil in 'Galega vulgar' in the same period, in both cases by falling olives. In other words, harvesting later does not mean more oil per hectare; instead, it increases the probability of harvesting less olive oil and of poorer quality (by altering the fatty acid profile and decreasing polyphenols). These results will have to be confirmed with studies on more olive tree varieties and campaigns.

Keywords: harvest; fat in the dry matter; olive; maturation index.

Invited Speaker 14

Side-Row Continuous Olive Harvester. Evaluation of 2 Consecutive Passages in the Same Olive Grove

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Since 2009, the University of Évora has been involved in the development of equipment for continuous harvesting of high-density olive groves (285 to 400 trees per hectare). Three research programs (ADI/CREN, n.º 3457 – "*Continuous Olive Harvesting Machine* – 2009/2012; PRODER 55344 "*Evaluation of the Performance of the Continuous Olive Harvesting Machine*" – 2014/2017 and GO PDR2020-101-031893 "*Mechanical pruning and Continuous Olive Harvesting of olive groves of Portuguese varieties*" – 2019/20121 established the concept, build and test the prototype of the COHM.

The work presents a brief description of the COHM in its current Mk III development and reports the test in which the COHM was harvesting a line of trees in two consecutive passages. The experimental design was based on a randomised complete block with two treatments and three replications, leading to 6 plots with 15 trees per plot.

Results of work rate, olive detachment efficiency and olive collection efficiency are presented.

The test simulates the working capacity for 1 hectare of olive grove and estimates the cost of harvesting, which is compared with the equivalent value of another common harvesting solution.

Keywords: High-density olive groves; farm machinery; work rate; operation costs.

Invited Speaker 15

New Approaches for the Control of Anthracnose in Olive

Patrick Materatski[1], Carla Varanda[1], Teresa Carvalho[2], António Dias[1], M. Doroteia Campos[1], Luis Gomes[1], Tânia Nobre[1], Fernando Rei[1], Maria do Rosário Félix[1]

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The olive tree (*Olea europaea* L.) is affected by several diseases, including anthracnose, a disease of major concern in most olive-producing countries, that is able to destroy an entire production. Olive anthracnose is caused by diverse species of *Colletotrichum*; in Portugal, most of them belong to *Colletotrichum acutatum* complexes. Our studies have addressed many aspects of olive and *Colletotrichum* spp. interactions such as: 1) *Colletotrichum* spp. colonization and primary infection in olive trees of three important olive cultivars, 'Galega vulgar', 'Cobrançosa', and 'Azeiteira'; 2) spatial and temporal distribution of endophytic communities in olive cultivars with different degrees of susceptibility to anthracnose; 3) level of infection and variability of *Colletotrichum* spp. isolated from fruits of the major Portuguese olive cultivar 'Galega vulgar' grown under different modes of management. We detected *C. godetiae* for the first time in Alentejo region and our results confirmed that *C. nymphaeae* is the key pathogen in olive anthracnose in this region. We also verified that the cultivar 'Galega vulgar' presents a significant higher number of infected trees and higher percentages of infected organs when compared to 'Azeiteira' and 'Cobrançosa'. Our results showed that one particular isolate of *C. nymphaeae* was present in different organs of the same tree, suggesting that the fungus may travel from the stems to other parts of the plant in a systemic movement. In addition, spatial-temporal analysis of endophytic communities revealed that cultivar 'Galega vulgar' and season autumn present significant higher values in terms of fungal richness and diversity. Lastly, our advances suggest that the application of fungicides may have increased the selection pressure of *Colletotrichum* spp., since we observed that the fungicide treatment decreases the number of trees positive to *Colletotrichum* spp., but those that remain positive show a higher number of fruits infected. Overall, our results show the different impact anthracnose has in different olive cultivars and the importance of developing alternative strategies for the effective and timely management of the disease. It urges to stop the use of unnecessary fungicide applications that no longer show effect on many emerging resistant and highly virulent *Colletotrichum* spp. isolates.

Keywords: Anthracnose; Control; *Olea europaea* L.; Endophytic fungi; Fungicides resistance

This work was supported by the project "INIMIGOSCHAVE" (ALT20-03-0145-FEDER-000029), co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020. P. Materatski received a post-doctoral fellowship in the cited project above and was also funded by the project "GAFAPROTECT" (ALT20-03-0145-FEDER-028263), and M. D. Campos was funded by the project "TOMVIRPROTECT" (ALT20-03-0145-FEDER-028266), both projects financed by the Regional Operational Program of Alentejo and Algarve supported by the FEDER and FCT/MCTES – OE. This work is also funded by National Funds through FCT under the Project UID/AGR/00115/2019.

Invited Speaker 16

Insect Vectors of Xylella fastidiosa in the Alentejo Region: a Threat to Olive Orchards?

Ana Carina Neto[1], Ganna Popova[1], Luís Gomes[2], Patrick Materatski[2], Maria Rosário Félix[2], Maria Teresa Rebelo[1] & Fernando Trindade Rei[2]

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The bacterium *Xylella fastidiosa* (Xf) Wells et al. is responsible for the olive quick decline syndrome, identified in southern Italy, forcing several thousands of infected olive trees to be removed as a prophylactic measure to contain the disease. The main transmission of *Xf* between plants, at short distance, is mainly carried out by phytophagous insects belonging to Hemiptera order, which feed on the xylem and acquire *Xf* previously from an infected plant. According to this and to prevent the occurrence and spread of the disease, it is important to understand the presence of the potential *Xf* vector insects in our country, especially in the main olive growing region, located in Alentejo. In the autumn of 2016 and in the spring of 2017, potential *Xf* vectors were prospected and identified in olive canopies and in proximal spontaneous vegetation, in 116 sites, randomly distributed on a grid of 18 squares with 30x30 km. The possible presence of the bacterium inside the insects was also tested. In parallel, the presence of functional arthropodofauna, was similarly evaluated in the habitat of the captured potential *Xf* vectors. The obtained results revealed the presence of five potential *Xf* vector species: *Cercopis intermedia*, *Lepyronia coleoptrata*, *Neophilaenus campestris*, *Philaenus spumarius and P. tesselatus*, the most captured species. In the autumn, 25 individuals were captured, mostly in the spontaneous vegetation, and 67 individuals in spring, 30 in olive trees and 37 in spontaneous plants. The presence of *Xf* was not detected in any of the collected individuals during both sampling periods. Olive grove spontaneous vegetation cover was confirmed as an important host for the potential *Xf* vectors, especially in autumn, when they perform their oviposition in this herbaceous cover. Although the occurrence of severe summer climatic conditions, prior to insect sampling, comprising several periods with maximum records of high temperatures associated to reduced precipitation, the presence of potential *Xf* vectors during the fall of 2016, suggests the high resilience of these insects to such conditions. According to this, even in the predictable climate change scenario for the Alentejo region, due to the global warming effect, the threat to the Alentejo olive groves, associated to these potential *Xf* vectors, remains. Nevertheless, the abundance of beneficial arthropods during the autumn of 2016 (3000 specimens belonging to 7 groups of predators and parasitoids) in the spontaneous vegetation (60% of the captures), and the observation of a parasitized *N. campestris* specimen, indicates the existence of established natural suppression relationships, even under severe climatic conditions, which can be future exploited in a context of biological control of the *Xf* vectors.

Keywords: Auchenorrhyncha, Aphrophoridae, pest management, phytopathogenic bacterium, functional diversity, global warming.

Invited Speaker 17

Chemical Characterization of Olive Oil – Seven Portuguese Olive Tree Cultivars Under Study

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Extra virgin olive oil (EVOO) represents the primary source of fat intake in the Mediterranean diet, is well known for its health benefits. Phenolic compounds (PC) are among the health-promoters present in EVOO, highlighted by the European Food Safety Authority in 2012, with a health claim for virgin olive oils PCs. The increasing demands for high-quality EVOO was driven research to further explore distinct chemical profiles associating to agronomic traits and it to beneficial health. The objective of this study was the chemical characterization of seven Portuguese olive tree cultivars: 'Galega Vulgar', 'Cobrançosa', 'Verdeal Alentejana', 'Cordovil de Serpa', 'Azeiteira', 'Blanqueta' and 'Carrasquenha de Elvas', with a particular focus on their phenolic profiles. Each monovarietal EVOO was obtained by ABENCOR and physical and chemically analyzed; phenolic profiles were obtain in EVOO extracts characterized by HPLC-UV with Kinetex Biphenyl column [1].
The phenolic profile of these EVOO were compared for consecutive years, showing unique cultivars profiles. Hidroxytyrosol and Tyrosol presents higher amounts in 'Cordovil de Serpa' and 'Verdeal Alentejana'. Shelf time was also assessed in order to monitor the behavior of these EVOO profiles over time under controlled conditions. Other physical and chemical parameters were also evaluated. Results strongly suggest that the variability of the phenolic profile contributes for the oxidative stabilization, and consequently, organoleptic stabilization.

Keywords: Extra virgin olive oil, chemical and physical EVOO properties, phenolic compounds

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Invited Speaker 18

Authenticity of Portuguese Olive Oils assured by the use of Molecular Markers

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Olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) is one of the oldest tree crops in the Mediterranean basin which has recently be gaining attention due to the nutritional and health properties of its fruit and derived oil. The demand for high quality olive oils have been directly implicated on the increase of typical marks, awarded to high-quality olive oils produced from local varieties grown in well-defined geographical regions. To protect the typicity of those regional products -and consequently protect both consumers' expectations and producers' profits- several Protected Denomination of Origin (PDO) regions have been established by legislation in different olive oil producer countries. Portugal has registered six PDO regions regarding virgin olive oils, produced from eleven Portuguese varieties and including blend and monovarietal products. Their authenticity should to be guarded, particularly with the increase in the use of foreigner varieties in recently established orchards under intensive management regimes. In this sense, it is mandatory to find methodologies that enable the detection of adulteration and/or fraud, namely the improper use of fruits from those varieties in PDO olive oils production. We have established a DNA-based molecular tool to be applied in olive oils traceability. The established tool is based on Single Sequence Repeats (SSRs) markers to assess genetic variation across five representative Portuguese olive varieties ('Cordovil de Serpa', 'Cobrançosa', 'Galega vulgar', 'Carrasquenha' and 'Verdeal Alentejana') and two non-Portuguese varieties ('Arbequina' and 'Picual' as the varieties most used in new orchards). Selection of the most polymorphic SSRs was conducted by High-Resolution Melting technique. From the 31 SSRs screened by HRM, six were selected for varieties identification through fragment length analysis. As part of olive oil authenticity checking, the applicability of the previous selected SSRs on olive oils was demonstrated. This tool has the potential of being further developed into a fraud screening system to support Portuguese olive oil certification.

Keywords: single sequence repeats (SSRs), olive genotyping, allele size diversity, olive oil traceability, High-Resolution Melting, fragment length analysis

This work was financially supported by FCT (Foundation for Science and Technology) under the Project Por3O – Portuguese Olive Oil Omics for traceability and authenticity (PTDC/AGRPRO/2003/2014) and by national funds through UID/AGR/00115/2019.

Invited Speaker 19

Discussing more Innovative and Sustainable Governance Options to Tackle Complex Changes in Olive Groves and the Olive Oil Sector in Alentejo (Portugal).

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Olive groves and the olive oil sector in Alentejo (Portugal) are currently undergoing a rapid and arguably unsustainable processes of intensification, financialisation and globalisation which especially affect the most productive agricultural land with access to irrigation permits and infrastructure. In parallel, least productive land is either maintained for traditional and extensive olive-based farming systems that are generally less advantageous financially or even at times abandoned. This results in a complex mosaic of rural land-use systems with high degrees of social-ecological and territorial heterogeneity across scales. Such a complex mosaic can potentially provide with a number of ecosystem and landscape services that need to be enhanced, and also with disservices that need to be avoided. This demands devising more innovative governance structures and instruments. Problems detected in the current governance framework include the fragmentation and lack of coherence among the key land-use policy instruments affecting olive governance, the absence of an overarching strategic and operational framework for spatial coordination and planning of olive grove expansion across spatial-temporal scales and institutional levels, and the many conflicts arising between those actors at the extreme ends of the policy and market-power scales. This is then complicated even more by the prevalence of a productivist discourse that largely permeates media, policy and the general public and that largely ignores the importance for sustainability of maintaining a balance between extensive and intensive systems of production. Generally speaking, intensive olive oil producers are more open to adopt technological innovation, whilst they are not as eager as traditional olive grove farmers to embrace interactive or governance innovation. In this poster, we will present the overall picture of governance institutions and tools driving rural land-use change in olive sector in Alentejo. Then we will also identify the main gaps preventing this governance framework to help the olive sector contribute to achieve UN´s sustainable development goals 2 (zero hunger), 13 (climate action) and 15 (life on land). We will then close up by proposing a set of governance options for the future that can help tackle the gaps identified and that will be defined on the basis of 4 different scenarios: International Competition, Market Segmentation, Euorpeization and Ecologization.

Keywords: Sustainability; Farming Systems; Olive Groves; Olive Oil; Alentejo; Governance

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Invited Speaker 20 - THEMATIC CONFERENCE

Grapevine Cultivars Resistant to Powdery and Downy Mildew; a Dubious Past For a Promising Future?

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On the second half of the XIX century, new pests and diseases arising from America (Downy mildew, 1845; Phylloxera vastatrix, 1863; Powdery mildew, 1875), strongly affected the European vines (*Vitis vinfera*). The problem of phylloxera was solved using hybrid rootstocks and the problem of downy mildew was attenuated using copper. Nowadays, the vineyard uses in Europe more than 50% of the marketed fungicides, situation that is neither economically nor environmentally sustainable. Shortly after the phylloxeric crisis, hybrids plants, initially obtained from crossbreeding between American species (American hybrids), and, later, from crossbreeding between *V. vinifera* and American species (French hybrids), were released. These first-generation hybrids have produced wines of doubtful quality (methanol, 'Foxy' aromas, high acidity, blued shades in the reds wines), presenting as the sole advantage the simultaneous tolerance to phylloxera, powdery and downy mildew. Despite its poor quality, they were protagonists in a plant breeding strategy, offering today new hybrid cultivars, product of complex crossbreeding between resistant species, and backcrosses with European cultivars. Throughout this time, relevant information on the genome of the resistant plants was acquired, with the identification of several resistance *loci*. In *Muscadinia rotundifolia*, for instance, it is known today that the resistance is mainly associated with a *locus* on chromosome 12, which contains a family of seven TIR-NB-LRR genes. Two of these genes (RUN1 – Resistance Uncinula Necator and RPV1 – Resistance Plasmopara vitícola), presenting a high level of homology (86% of amino acid similarity), confer high resistance to powdery and downy mildew respectively. These two genes were cloned into transformation vectors and used to obtain genetically modified *V. vinfera* plants, whose behavior for resistance is now under evaluation. Some *loci* responsible for production of MLO (Mildew Resistance Locus) proteins were also identified and may be related with the greater susceptibility of the *V. vinifera* to downy mildew infection. Its silencing, or sub-expression, may by itself increase the species resistance. In Portugal, hybrids produced in German breeding programs, are being evaluated for resistance and quality since the 90's. As a result, a cultivar with the designation 'Defensor', was proposed for registration in the National Catalogue of Vine Varieties. Hybridization using German mono resistant *loci* cultivars and the Portuguese ones, began in 2001, under the scope of an AGRO2 project. This project, promoted by the nursery company Viveiros Plansel and the University of Évora, have now continuity with the recent approval of a PDR2020 proposal. Sixty-two hybrids previously obtained, are under observation, and, new crossbreeding based on cultivars with multi resistance loci, are ongoing.

Keywords: Downy mildew; Grapevine; Powdery mildew; Resistance

Work developed under the scope of the project 784-042746 (Programa de Conservação e Melhoramento Genético da Videira), supported by national and community funds, through the PDR2020.

Invited Speaker 21

Plant Parasitic Nematodes of the Family *Longidoridae* Associated with Grapevine in Portugal

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'Dagger' and 'needle´ nematodes, commonly known as longidorids, are one of the most economically important parasitic nematode groups in grapevine worldwide. They are polyphagous root ectoparasites causing severe damage to plants by their direct feeding, and in addition some species can transmit plant viruses. *Xiphinema index* is one of the most significant plant parasitic nematodes because it is the vector of Grapevine fanleaf virus (*GFLV*), one of the most destructive viral diseases affecting grapevine. Surveys were conducted since 2015 to 2018 during the spring and autumn seasons on a representative number of vineyards in the main grapevine-growing regions of Portugal. Our research activity was focused on establishing the biodiversity and occurrence of longidorid nematodes infesting grapevines in Portugal, as well as their dissemination and current distribution worldwide. An integrative taxonomy strategy based on morphometric measurements and morphological characterizations, combined with molecular analysis using ribosomal DNA (rDNA) sequences from ITS regions, partial 18S and D2–D3 expansion segments of the 28S gene and their phylogenetic analyses, was useful for species delimitation and identification. *Xiphinema pachtaicum* and *X. index* are the most frequent dagger nematodes found, but other dagger species include *X. santos*, *X. opistohysterum*, *X. baetica* and *X. vallense* (1). Needle nematodes were the least abundant and diverse longidorid nematodes found, including only *Longidorus vinearum* and *Paralongidorus lusitanicus* (1, 2). The high prevalence of *X. index* found in the oldest grape-growing regions in Portugal prompted a collaboration with an international research team in a phylogeography study of this nematode based on mitochondrial and microsatellite markers (3). The results suggested that Middle- and Near-East regions included the native area of *X. index* (3). In addition, east-to-west nematode dissemination appears to match that of its domesticated grapevine host during Antiquity (3). Our research activity provides an improved knowledge on this nematode group for establishing alternative management strategies for the control of these important phytopathogens.

Keywords: *GFLV*, longidorids, mitDNA, rDNA, virus vector, *Xiphinema index*

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Invited Speaker 22

Biodiversity and Ecosystem Services in Vineyards: Bats, Birds and Arthropods for Pest Consumption

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Traditional agriculture systems have been subject to marked intensification in recent decades. This process has several environmental impacts that contribute to the biodiversity loss in agricultural areas. However, within the species that occur in farmland some can provide ecosystem services such as pest consumption. Thus, it is of utmost importance to identify which species have the potential contribute to a more environmental friendly agriculture production and, which management pratices improve biodiversity and the services it provides. Our objectives are: (i) to identify which species of bats, birds and arthropods feed on pests in vineyards (ii) to assess which vineyard practices and landscape features influence the abundance and species richness of these biological groups. We sampled 32 sites in vineyards across the Central Alentejo for species richness and abundance of bats, birds and arthropods. We also recorded the management practices, land use in the vicinity and ecological structures in the landscape arround the vineyards. We did diet studies of bat and bird species to identify which species can consume arthropods that can do economic damage to vines. In the vineyards we surveyed 10 species of bats, 73 species of birds and 32 species of carabid beetles. For both bats and birds the landscape features of the surrounding areas, such as the presence of riparian vegetation and montados, had a higher influence on species richness and abundance than the vineyard management. However, when there was grass between the vineyards lines it had a positive effect on all the three analyzed groups. Our results indicate that low impact farming practices can support biodiversity values in vineyards and protect the provisioning of ecosystem services. Nevertheless, because of the higher influence of the semi-natural areas near the vineyards, an effective strategy to promote biodiversity and ecosystem services must preserve ecological structures heterogeneity and the conservation of “montados” and riparian vegetation.

Keywords: sustainability; resource tracking; nature-based solutions
Acknowledgements to all members of the Project team of 2017, members of the CIBIO - Porto: Sónia Ferreira, Pedro Beja, Joana Paupério and Vanessa Mata. I would also like to thank to the landowners and vineyard managers for access to their properties.The funding for the

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Invited Speaker 23

"Solar IrRAdiance impact tHrough UVs on grape quality in Portugal (SIRAH)."

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Meteorological parameters affect the way solar radiation reaches the surface of the earth, thus influencing plant photosynthesis [1].
In Portugal, wine is one of the most exported products. Portugal is the 11th biggest wine producer in the world, contributing to around 2-3% of the world production with ~750M€ of exports in 2017 [2].
Moreover, it is empirical known that UV band of solar radiation has a significant impact on the grape quality through to the prevalence of clouds, with cloudy springs resulting in grapes of better quality. Nevertheless, no statistically validation was made on this observation.
With these two reasons in mind, SIRAH combines efforts in two areas: a well-prepared meteorological database, especially global horizontal solar irradiance and UV band and long-term data of grape quality parameters. The objective is to find a correlation factor between UV and fruit quality enabling a reliable annual forecast of wine quality based on meteorological observations.

Keywords: Solar radiation, UV band, Agrometeorological impact, Grapevine.

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Invited Speaker 24

Development of New Technologies for the Ageing of Wine Spirit

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The freshly distilled wine spirit has a high concentration of ethanol and richness of volatile compounds, but is devoid of phenolic compounds other than volatile phenols. Therefore, the wood contact during the ageing process is crucial to the beverage's enrichment in wood compounds namely those of phenolic nature, which are positively correlated with quality. The underlying changes are closely related to the action of factors ruling the ageing process such as the ageing technology (traditional and alternative) and the kind of wood used [1]. Traditional technology consists of ageing in wooden barrels. Despite the high quality achieved by the wine spirit, it is a time-consuming and costly process, and the capital invested in wine spirit and wood is immobilised for a long period. Besides, it involves the use of a large amount of a natural resource, the wood, whose availability is limited. For these reasons, alternatives have been searched toward ageing sustainability. This challenge has been the main driver of pioneering research conducted by our team in the last 12 years, based on the *Lourinhã* wine spirit. In this communication, an overview of such investigation is presented, highlighting the impact of new ageing technologies using wood pieces in the beverage stored in stainless steel tanks on the phenolic composition and related features of the aged wine spirit [2, 3].

Keywords: wine spirit; ageing technology; physicochemical characteristics; sensory properties.

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Invited Speaker 25

Amphora Wines - Volatile Composition and Mineral Elements

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Amphora wines also known as Vinhos de Talha are typical of Alentejo, in the south of Portugal. Crushed grapes are fermented in clay vessels, regardless being white, red or a mixture of both grapes, with a minimalist intervention, resulting in white, red or palhete wines. Due to the technological differences associated to the winemaking process of these wines, the present research aimed to evaluate the volatile profile of amphora wines (red, white and palhete wines) and evaluate the mineral characterization these wines with the purpose of ensuring consumer food safety.
To achieve these objectives, the volatile fraction of the different types of amphora wines was analyzed using headspace solid-phase microextraction (HS-SPME) followed by gas chromatography mass spectrometry (GC/MS). The inductively coupled plasma mass spectrometry (ICPMS) was used to assess their mineral composition.
A total of 117 volatile compounds in the different wines analyzed were tentatively identified and principal component analysis showed a clear separation among the different types of wine based on their volatile composition. Additionally, data shows differences among red, white and palhete wines regarding the multi-element composition, and linear discriminant analysis allowed a classification according to the wine type and geographic origin. From all samples, only one red wine presented concentrations of Zn higher than the value published by International Organisation of Vine and Wine [1](OIV, 2015).

Keywords Amphora wines; volatile profile; mineral composition.

OIV. (2015). Maximum acceptable limits of various substances contained in wine. Code International des Pratiques OEnologiques. Fiche Code OIV - edition 01/2015.

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Invited Speaker 26

Strategic Objectives of the Thematic Line - Irrigated Agriculture

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Portuguese agriculture accounts for about 3.5% of gross domestic product (GDP) and 1.4% of national gross value added (GVA). This weak contribution of the sector to the GVA results from an intense use of factors, whose utilization efficiency has decreased about 28% in the last 30 years. If the economic importance of the sector is small, agriculture plays an indispensable role in sustaining the territory and the use of irrigation has to take into account this aspect.
Analysing the Portuguese climate and possible climatic changes, it is necessary to recognize that the country has better conditions for the production of fodder and pasture than for the production of arable crops. This reality is reflected in the change in land use (transfer of 50% of the area classified as arable land for pasture) which the successive reforms of the Common Agricultural Policy (CAP) imposed, by disengaging the aid from the activity.
Considering the area equipped for irrigation (15% at national level and 13% in the Alentejo) it is evident that the sustainability of the territory will depend on the viability of the rainfed agriculture, which in turn has an obvious forest-pastoral vocation. The vulnerabilities of livestock systems are related to the need for good quality and low prices of conserved forage and the threat of periodic droughts. It is in the reduction of these vulnerabilities that irrigation can assume a strategic role in sustaining the territory.
Considering the scarcity and cost of water, its productivity is critical. In this perspective its use in autumn / winter or spring / summer crops has to be evaluated, as well as the use of techniques that increases irrigation efficiency. Irrigation increases the pressure on soil conservation, which also has to be addressed in the concerns of this strategic line.

Keywords: Agriculture importance; irrigation; water productivity; soil conservation

Invited Speaker 27

Conservation Agriculture as a Tool to Improve Water Use Efficiency

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The main objective of the ACUAsave project ((ALT20-46-2016-06) was to assess the impact of the principles of Conservation Agriculture (CA), namely minimum soil disturbance and soil cover, regarding its potential to improve water use efficiency in a maize crop. For this purpose, a prototype of a single grain no-till planter was developed, based on the use of a recently developed, strongly inclined disc opener in order to guarantee crop establishment through a thick cover of crop residues without exposing bare soil to the atmosphere. Thus, it was expected to minimize evaporation losses from the soil.
On two different soil types (sandy soil and a clay loam) three levels of surface cover/soil management were established: a) traditional tillage soil preparation without cover; b) no-till with cover crop stubble, and c) not-till with full retention of cover crop residues. Soil moisture was measured throughout the season and crop performance was assessed at the end of the cropping season.
The results from both sites confirm that crop establishment with no-till allowing for the complete cover of the soil surface after planting contributes to considerable savings in water application through irrigation. Together with cost reductions during crop establishment, the water savings in combination with the similar crop performance make the CA approach extremely interesting for irrigated summer crops under the conditions of the Alentejo region.

Keywords: no-till, soil cover, cover crop, residues, precision no-till drill

The ACUAsave project is co-financed by Alentejo 2020, Portugal 2020 and European Union - European Regional Development Fund.

Invited Speaker 28

Sustainable Soil and Water use to Improve Crop Productivity in Irrigated Areas

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[3] Departamento de Física, Faculdade de Ciências, Instituto Dom Luíz (IDL/ULisboa).
[4] Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV); project RC.

Irrigation is basic to improve agricultural productivity in the Mediterranean regions, characterized by hot dry summers. However, irrigation also increases evapotranspiration and salts accumulation in the soil profile, decreasing crop yields and, ultimately, leading to land desertification and abandonment.
Salinization and nutrient leaching control, associated to water economy, are sufficient reasons for establishing sound management options to improve crop yields and minimize soil degradation. These basic objectives for sustainable soil management are being applied to agricultural fields located in Roxo's irrigation district (Alentejo), adapting the irrigation to the soil properties through hydrological modelling (10 parcels with representative soils) and crop development (olive tree and pomegranate).
This project aims: 1. to identify and monitor soil salinization susceptible areas in the irrigation district; 2. to study the effect of irrigation water quality on crop development using physiological indicators and, relating them with yield; 3. to improve irrigation water management and nitrogen fertilization using modelling tools which will help minimizing soil salinization risks; 4. to develop a WebGIS platform for soil degradation awareness and promotion of best agricultural practices.
The working program necessary to achieve these objectives will include the monitoring of irrigation water quality of the Roxo reservoir, and the study of the spatial distribution of soil properties using electromagnetic induction and geostatistical methods. The WebGIS platform will integrate several modelling results (soil water content, soil salinity, nitrate leaching and crop growth) allowing to choose sustainable soil management solutions for different tailor made scenarios.
This project will also contribute to improve the Portuguese tools available for the implementation of the Nitrate Directives and the Water Directive, and for soil conservation. To achieve its several objectives this project has a multidisciplinary team from four institutions.

Keywords: soil salinity, solute transport, nitrate leaching, modelling, HYDRUS, MOHID-Land

Project PTDC/ASP-SOL/28796/2017 “Sustainable use of soil and water for improving crops productivity in irrigated áreas” (SOIL4EVER).

Invited Speaker 29

AWARTECH - Animal Welfare Adjusted Real Time Environmental Conditions of Housing

V. Fitas da Cruz[1], F. Baptista [1], R. Charneca[2], T. Morgado[1], J.C.S. Rico[1], C. Martins[3], D.Botas[4], D. Coelho[3]

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The world is demanding for high quality food, produced according to animal welfare regulations and ethical principles, and both social and environmental responsibility. This requires a special care with cost rationalization and increased efficiency in the use of production factors. Pig production is an important sector in Portuguese farming economy, and almost half of the national pig herd is produced in Alentejo region. This region has a very extreme climate, where summer temperatures (T) can reach the 40ºC and relative humidity (RH) can be of 50%, whereas in the winter T can be around 5ºC and RH values of about 80%. This means that the facilities must be equipped with environmental control systems. However, nowadays the environmental control systems are limited to actuate on the ventilation, cooling and heating systems, based on the analysis of the air temperature and humidity. These control systems do not take in consideration the real impact in animal welfare. Therefore, there is a need to incorporate new variables to be monitored, in real-time, that are related to animal welfare, like physiological indicators of welfare. Within this framework we have developed the AWARTECH project that aims to create and develop a precision animal husbandry tool that supports the sustainability of the swine value chain. The intention is to create a platform that collect and organize the data, that evaluate a set of environmental and physiological variables and controls in real time the environmental conditions based on the animal behaviour.

Keywords: Animal Welfare, Environmental Control, Pigs, Smart-Farming

This project is funded by FEDER, program Portugal 2020 and Alentejo 2020.

Invited Speaker 30

GEN-RES-ALENTEJO – Use of Genomics Methodologies to Assist Selection of Sheep Resistant to Footrot and Gastrointestinal Parasites in the Alentejo Region

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6 – Bolseira do projeto Universidade de Évora

Footrot and parasitism by gastrointestinal nematodes have a significant economic impact on sheep production in the Alentejo region. The main objectives of the project are: (i) a characterization of diseases in sheep farms in Alentejo; ii) the use of genetic methodologies for the identification of genetic markers associated with the resistance to the disease to be used in the genetic selection of animals. To achieve these proposed objectives, following steps were implemented:
a) Identification of risk factors associated with footrot and parasitism by gastrointestinal nematodes in sheep;
b) Characterization of diseases and development of diagnostic methodologies that allow the identification of resistant and susceptible animals. Use of metagenomics for the identification and characterization of the footrot agent.
c) Evaluation of the economic impact of footrot and parasitism by gastrointestinal nematodes on sheep farms in Alentejo;
d) Use of genomic studies for the identification of genetic markers associated with footrot and parasitism resistance by gastrointestinal nematodes;
(e) an assessment of the economic impact of the project on control of footrot and parasitism by gastrointestinal nematodes and on improving the productivity and profitability of sheep farms;
f) Dissemination of project results.

The leading Instituition is Associação de Agricultores do Sul (ACOS) and partner Instituitions are Universidade de Évora (UE), Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) and Instituto Nacional de Investigação Agrária e Veterinária (INIAV). The project started on 1st September 2016 and will last for36 months.

Keywords: footrot; sheep; genomics; parasites; assist selection

This project was funded by European Union's Portugal2020 (Regional Operational Program of Alentejo).

Invited Speaker 31

Innovative Studies on Alentejano, Bísaro and Ribatejano Pigs

R. Charneca, J.M. Martins, A. Freitas, J. Neves, J. Nunes, M. Laranjo, A. Albuquerque, M. Elias

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The ICAAM/MED team focused in swine science integrates researchers with skills and knowledges in different areas, namely: production systems, genetics and genomics, reproduction, nutrition, specific aspects of physiology (lipid metabolism, lactation, newborn piglets), and carcass, meat and meat products quality. The main research focus has been the local Alentejano (AL) breed produced in extensive systems although some works have been made in intensive production systems with modern improved genotypes. The facilities available at ICAAM/MED in the University of Évora allow animal field studies, collection and preservation of samples, as well as their laboratory analyses. One good example of the team multidisciplinarity is our participation in the recently finished TREASURE project (https://treasure.kis.si/), that allowed the acquisition of new data on Portuguese AL and Bísaro (BI) local breeds, along with innovative information about the cross between those breeds, the Ribatejano (RI) pig. Our main task during the project was to compare AL, BI and RI pigs concerning their performance, carcass, and meat and traditional dry-cured meat products ("Paios") quality. Preliminary results of these works showing the potential interest of RI pigs and their products were presented in international scientific meetings, two scientific papers have been published and three manuscripts are being prepared for publication. Genetic studies in collaboration with INIA (Spain) also provided new information about the genetic diversity associated to production and quality traits in AL pigs. Some main results of these studies were published [1] and revealed few genes with mutational variability that would allow marker assisted genetic improvement programs. Furthermore, transcriptomic analyses of AL and BI muscle and fat tissues done in collaboration with INIA are being analysed. The initial outputs show differences in gene expression between these two breeds of different genetic origin. The recognized successful participation in this project, together with its achieved and expected outputs, encourage the team to proceed their research on these topics.

Keywords: swine; Treasure project; local breeds; performance; meat and dry-cured meat products quality; genomics

1.Muñoz, María, Bozzi, Riccardo, García, Fabián, et al. (2018) Diversity across major and candidate genes in European local pig breeds. PLoS ONE 13(11): e0207475.

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Invited Speaker 32

Reproductive Management and Biotechnology of Reproduction in Lusitano Breed Horses

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Invited Speaker 33

Operationalization of the "One Health" Concept. International and National Initiatives

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The importance of the "One Health" concept as a working tool is exposed and contextualized taking over the principles of Sustainable Development of the United Nations Charter.
The need for cross-disciplinary knowledge is essential when prevention health measures are to be developed and implemented. It was nature, with the emergence of BSE in 1984, responsible for demonstrating the urgency of interconnecting and sharing knowledge and information on human health, animal health and the environment. The 1997 H5N1 pandemic demonstrated the strength of the One Health concept to combat and preventing potentially catastrophic health events.
We need to handle knowledge, including at the level of wildlife health, as an essential component of global disease monitoring, surveillance, control, prevention and mitigation.
The importance of ecosystem resilience in health, the impact of poor decisions on land and water use, allow changes in the patterns of occurrence and dissemination of phenomena linked to disease transmission.
In 2008, United Nations organizations with responsibilities in the areas of human, animal and environmental health adopted the concept of "One Health" as a working tool [1]. In 2018, the World Bank published the document "Operational framework for strengthening human, animal and environmental public health systems at their interface" [2]
We present the main international projects where this theme was worked out, as well as the results obtained, such as the COST TD 1404 Network for Evaluation of One Health (NEOH), which identified and validated the variables that allow us to measure and classify different **One Health** initiatives [3]. The initiatives of the University of Évora in this area, both past and future, are also talked.

Keywords: Public Health; One Health; Environment; Food security

1. The Tripartite's Commitment Providing multi-sectoral, collaborative leadership in addressing health challenges. FAO, OIE and WHO, 2017 Food security
2. Berthe, Franck Cesar Jean; Bouley, Timothy; Karesh, William B.; Le Gall, Francois G.; Machalaba, Catherine Christina; Plante, Caroline Aurelie; Seifman, Richard M.. 2018. *Operational framework for strengthening human, animal and environmental public health systems at their interface (English)*. Washington, D.C. : World Bank Group.
3. Integrated approaches to health. A handbook for the evaluation of One Health. edited by: Simon R. Rüegg, Barbara Häsler, Jakob Zinsstag. Wageningen Academic P u b l i s h e r s

Invited Speaker 34

Project FIGHT-TWO - Edible Bait Vaccine for Rabbit Haemorrhagic Disease Virus 2 (RHDV2) Control in Wild Rabbits

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Rabbit haemorrhagic disease (RHD) is a highly contagious and often lethal systemic infection in the European rabbit (*Oryctolagus cuniculus*). Presently, the condition is caused by the rabbit haemorrhagic disease virus 2 (RHDV2), which replaced the classical RHDV genogroups (G1-G6) after its emergence in 2010, and continues to cause great concerns on the conservation of the diminished Iberian wild rabbit populations, directly impacting on several endangered species that depend on the rabbit for survival.
The commercial RHDV2 vaccines available to date are inactivated, obtained from infected animal liver extracts and the route of administration is usually subcutaneous, requiring handling. Further than the risks associated with incomplete virus inactivation and the inadvertent release of infectious virus, these vaccines are not suitable for wild rabbits, requiring capture for inoculation. The immunity is short and, hence, the protection transient. The previous commercial RHDV vaccines, most also inactivated, were shown to be ineffective in conferring cross protection against RHDV2.
The *FIGHT-TWO* strategic framework is the development and production of a new edible vaccine against RHDV2 which is presently disseminated in the entire territory of Portugal, including the insular regions. The safe, pathogen-free RHDV2 oral vaccine is to be distributed in the field as bait or included in dry feed. This non-invasive immunization has the potential to protect a broad proportion of the populations, crucial to stop virus transmission and control the infection, while overcoming the need of capture and manipulation.
The virus-like particle (VLP)-based vaccine will be produced in insect cells-baculovirus expression vector system (IC-BEVS) and updated accordingly to RHDV2 evolution (open system). To accomplish FIGHT-TWO objectives, INIAV, the reference laboratory for animal diseases, join forces with IBET, UE and FMV.
FIGHT-TWO will allow to proceed with one of the 12 measures specified in the Action Plan for the Control of Rabbit Hemorrhagic Viral disease in Rabbits (Dispatch 4757/17 of 31 May). Obtaining this vaccine will be crucial to support more generalist wild rabbit management policies towards the recovery of population densities and disease control, the recovery of ecosystems where the rabbit is keystone and the reactivation of hunting activities in Portugal.

Keywords: *Oryctoalgus cuniculus algirus*, wild rabbit, RHDV2, oral vaccine, VP60-VLPs,
PTDC/CVT-CVT/29062/2017-PT2020; Partnership +Coelho

Invited Speaker 35

ValBioTeCynara - Economic Enhancement *Cynara cardunculus*: Natural Variability and its Biotechnological Applications

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ValBioTecCynara aims to address the natural *C. cardunculus* L. genetic, molecular, morphologic, and biochemical variabilities, as a combined strategy to identify individuals with certain and specific required profiles. *C. cardunculus* L. is used as a coagulant for cheesemaking where the respective use is mandatory in certain cheeses benefiting from Protected Designation of Origin (PDO)(1). The potential of 15 different *C. cardunculus* L. natural occurring populations within Alentejo region (south of Portugal), were evaluated on flower technological properties, being three of them studied regarding chemical, rheological and sensorial characteristics within the three PDO Alentejo cheeses (Évora, Serpa and Nisa). A significance variability on flower technological properties was found, based on milk clotting activity, gel firmness, micellar aggregation rate and proteolytic activity and it was possible to identify 5 different groups of C*. cardunculus* L. populations (2). The action of coagulant populations was specific according the cheese matrix. Nevertheless, a common pattern of cheese proteolysis, rheology and sensory characteristics was verified, allowing to highlight the cheeses manufactured with two of the three *C. cardunculus* L. populations. Proteolysis was more intense in early stages of ripening, influenced by cheese moisture content and raising to caseins fractions, β-caseins were less susceptible to proteolysis than α_S-caseins with influence in cheese sensory characteristics. These results have a very stimulating uses concerning specific target, such as uncertainty in the cheese manufacture, contributing to the incentive of the cheese production systems promoting the milk production of small ruminants.

Keywords: *Cynara cardunculus* L., proteases, milk, PDO Évora, Serpa, Nisa cheeses

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This project was funded by European Regional Development Fund (FEDER), ValBioTecCynara (ALT20-03-0145-FEDER-000038)—Economic valorisation of Cardoon (*Cynara cardunculus*): study of natural variability and biotechnological applications.
The authors acknowledge FCT for PhD grant to Teresa Brás (SFRH/BD/110969/2015).

Invited Speaker 36

Alternative Feed Resources – Utilization of *Cistus Ladanifer* and its Constituents in Ruminant Diets

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In the Mediterranean region are available several feed resources, such as agro-industrial by-products, shrubs and tree foliage that are suitable for ruminant nutrition. Such feed resources may constitute an alternative to some conventional raw-materials used in ruminant diets, providing energy and nutrients. Moreover, they are also sources of several bioactive compounds with potential benefits on animal health and welfare, quality of products and environmental impact of ruminant production. Within this context, in the last years and more recently under de CistusRumen project (http://cistusrumen.pt/) our research team developed an extensive work on utilization of *Cistus ladanifer* L. (Esteva) in ruminant diets. A multidisciplinary team has performed a wide range of studies, focused on chemical composition and nutritional value of *Cistus ladanifer*, extraction procedures of interest compounds, and impact of application of *Cistus ladanifer* or its condensed tannins extract in ruminant diets on feed efficiency, animal health, productivity and quality of products. Although it is considered as a feed with poor nutritional value, our results showed that in specific conditions aerial part of *Cistus ladanifer* (soft stems and leaves) can be used as component of ruminant diets without compromise the animal performance. Moreover, *Cistus ladanifer* showed to be able to modify the ruminal biohydrogenation, promoting the ruminal synthesis of the healthy fatty acids with improvement of the nutritional value of lipids from lamb meat. On the other hand, when incorporated into ruminant diets, *Cistus ladanifer* also limits the meat lipid oxidation. So, taken into account the promising results on utilization of *Cistus ladanifer* in ruminant diets, we intend to continue with the work on this topic, in order to optimize the conditions of use, explore other benefits and elucidate the action mechanisms.

Keywords: CistusRumen project, alternative feed resources, small ruminants, productivity, products quality

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Invited Speaker 37

Rural Dynamics and Governance – How this Research Line is Linked with the other Research Lines in MED

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Agriculture activity, is its broadest sense, is an economic activity which particularity is its linkage to territory and landscape. This linkage changed a lot over time as agriculture lost weight in society and economy, with a progressive abandonment of territory and production efficiency increased.
In the last Agricultural Census (2009) agricultural surface was 70% of 1979 agricultural surface and the employed population in the primary sector, relative to the total population (SLIDE), fell from about 31% in 1979 to about 6% in 2018 (1). Overall, in the middle of last century the relationship between people and territory went through agriculture and the rural world developed from this relationship. Today in many countries (including Portugal) there is more land then the used by agriculture and the activity of those living and working in rural world no longer mediates the relationship between people and the territory (2) – we have neo-rurals, different uses for landscape and agriculture can be managed just a click away.
The others' ICAAM research lines are concerned about productive models – in olive, vineyards, horticulture, animal production, etc. But these productive models are framed by their context. Although In the last years technology updates contributed to the technological development of production systems, rural development needs to consider also the changes in relationship patterns among those in rural areas – to understand the evolution we need to take into account the technological evolution but also sociological elements and the ecological sustainability of rural world.
So, in this research line we analyze how new demands and determinants of rural, in its relation with urban, can be combined with production activities trough innovative governance models, what synergies exist or can be created and which tensions should be overcome. The high value and multifunctional nature of many Mediterranean agricultural systems as well as the hybrid nature of social relationship are the main capital of this research line.

Keywords: Governance, rural dynamics

Pordata – Base de dados Portugal Contemporâneo (Fontes de Dados: INE - Recenseamento Geral da Agricultura | Inquérito à Estrutura das Explorações Agrícolas) https://www.pordata.pt/ Accessed June 2019.
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Invited Speaker 38

Co-production of Knowledge Between Science and Society: What Is It and How Can It Work

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We witness a persistent tension between established ways of knowledge production through disciplines, and the urgent need to widen and change, both the production of knowledge and its organization, not least, in order to be able to understand and address the future and its challenges. One of the goals of these line of research is to put transdisciplinarity (i.e. co-construction) in practice and assess how far can research go with such an approach within the academic realm.
Pohl (2011) defines transdisciplinarity by the specific aims that such knowledge production tries to achieve. Accordingly, transdisciplinary research frames, analyzes, and processes an issue so that (1) the issue's complexity is grasped, (2) the diverse perspectives on the issue are taken into account, (3) abstract and case-specific knowledge are linked, and (4) common good-oriented descriptive, normative, and practical types of knowledge to address the issue are developed. In a transdisciplinary research project, representatives of different disciplines, of the private and the public sectors, as well as of civil society, co-produce knowledge on an issue, trying to match items (1)–(4). Going beyond definitions and putting transdisciplinarity in practice requires a theoretical background that directs its application. A great source of inspiration is the work by Elinor Ostrom (nobel prize winner in 2009). Early exploration of environmental problems often cast them in terms of the inescapable tragedy of the commons (Anderies and. Janssen, 2012). The ''tragedy'' refers to a fundamental disconnection between what is good for the individual and what is good for the group. In trying to serve their own self-interests, individuals end up hurting themselves—and the public good—in the long run. Ostrom's work in experimental economics showed that humans might be better labelled as *Homo cooperaticus*, who are naturally willing to work together but may quit if they are suckered, rather than *Homo economicus*, who are rational, narrowly self-interested, and never willing to cooperate.T he importance of true communication and trust building substantiate the current stage of this research line. In this communication we will give the example of Tertúlias do Montado (http://tertuliasdomontado.blogspot.com/ to show how a transdisciplinary research approach can be put in to practice, tested and replicated. Further, we will also discuss the urgency of building capacity of young researchers that want to use such form of knowledge production within their academic career. *"We cannot resolve any of the big challenges we face in the future with just people who have sat in silos coming together. We need young professionals who have come up in this way… to see the interconnections"* (interdisciplinary doctoral supervisor, 2009, cited in Lyall, 2019).

Keywords: Knowledge production, governance, transdisciplinarity

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Invited Speaker 39

Innovation in Public Policies: Research Contributions to the Construction of a Montado Results-based Agri-environmental Scheme – A Case Study

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The Montado is a human shaped sylvo-pastoral system, composed of complementary elements and activities that occupies about 1M hectares in Southern Portugal. The Montado is a highly multifunctional system, but despite the public goods it supports and its role on new economies in the region, the Montado has been in decay in the last 3 decades (Godinho et al., 2016). Both decreases in tree density and lack of tree renewal result in a reduction in the total extension occupied. New solutions are needed if this unique system is to be maintained. The results-oriented payment approaches, foreseen in the coming CAP (Common Agricultural Policy), are a means to deliver ecological outcomes in agri-environment schemes. This approach opens new possibilities for the much required adaptive management to promote production and conservation in farming systems. Still it represents a change in paradigm in relation to the agri-environmental schemes known so far in the Southern European context. In fact in a recent survey, from the 36 results payment schemes identified (in 9 European countries) half of them were implemented in Germay and most of them in Northern Europe (Herzon et al., 2018).
We present an innovative process of co-construction of a proposal for a result-based scheme for the Montado, involving farmers, public officers and researchers in the different steps of development of the scheme. We also present the structure and details of the proposal under construction. The proposal aims for application as a pilot case, at a local scale, in a Natura 2000 site and its surroundings, in Alentejo. The approach followed is inspired by the "Burren farming for conservation program" (http://burrenprogramme.com/the-programme/), applied with success for more than 20 years in The Burren, Ireland. The on-going co-construction process for the result-based scheme for the Montado has been developed under HNV-Link, an H2020 CSA (Coordination and Support Action) on "High Nature Value Farming: Learning, Innovation and Knowledge".

Keywords: Results-based scheme, Multiactor aproach, Multifunctional Montado

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Invited Speaker 40

The Importance of Small Farms in Regional Food Systems (SALSA)

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Small farms exist across an extensive variety of cultures and landscapes in Europe, and this diversity results in different responses of small farmers and communities, both between and within different regions and countries. Understanding this diversity is essential for developing policies that can respond to the challenges of farmers with different assets, skills, life histories, strategies and future ambitions. Typologies can provide a useful—if simplified—way to capture this variability and richness. In SALSA project we have developed a typology of small farms in Europe to target and identify the appropriate interventions needed by different farms, as well as to shed light into the world of small farms and their households. We combined participatory approaches (face to face interviews to small farmers) with multivariate statistics (PCA analysis and hierarchical clustering), with a sample of over 800 small farms in 25 European regions. Results show five main and distinct small farm typologies, differentiated largely along the dimension of market integration.

Invited Speaker 41

Research and Circular Economy Practices in the Agri-Food Sector

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In a world of increased lack of resources, it is imperative to promote the transition to a more circular economy, seen as an alternative business model to promote competitiveness, reducing simultaneously the consumption and dependency of raw materials and energy.
To streamline this transition Évora University (ICAAM) and ISQ promote the "Alentejo Circular" project whose was to raise awareness and mobilize Alentejo' s agrifood sectors to adopt the circular economy model, aiming to create value for enterprises, and consequently for the region, by transferring knowledge on the best practices and technologies for efficient resource use and waste valorisation. The work was focused on the wine, olive oil and pig farming industries, due to their current and growing importance for the regional economy.
Through national and international technical site visits and bibliographic research, a current diagnosis of circularity in the addressed regional industries was as carried out, as well the assessment of circular economy best practices, at national and international level. The resources and wastes were characterized and quantified improving the knowledge of recovery cycles with a list of best practices being gathered as priority for actions to be taken by the regional stakeholders, in order to support the transition towards a circular economic model.
As a result of this study, it was possible to conclude that the best available practices in these sectors, such as, smart farming and the use of renewable energy are already in use across the region, however these practices are only implemented by a limited number of the regional companies.
By means of several public sessions across the territory and a web 2.0 based platform, the interaction between stakeholders, including companies, public authorities and the scientific community increased, resulting in shared experience and knowledge on alternative use of resources, thus settling the conditions for the development of future synergies.
"Alentejo Circular" project was also the pillar project for the regional authorities to launch FECA (Forum de Economia Circular do Alentejo) which is the regional forum for discussion of the circular economy with the participation of the authorities of public administration, companies and of units of the scientific-technological system (universities and research centers).

Keywords: resources, sustainability, agri-food, efficiency

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Invited Speaker 42

Benefits From the Use of Organic Compounds and Wastes in Agriculture

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Composting is a solid waste treatment method, where organic components are biologically decomposed under controlled conditions, achieving a state in which it can be handled, stored or applied to the soil without affecting the environment. The use of synthetic fungicides has caused several problems, such as fungi resistance, ecosystem imbalance by toxic effects of residues and human and animal health hazards (1). Besides its traditional use as soil fertilizer, composts can be used to control soil diseases (2) nematodes, or weeds. A number of composts have been produced at the UALg, through the composting of agro-industrial wastes, and tested as plant substrates, soil mulches or fertilizers. Microbiota from composts were isolated and identified, in order to identify soil diseases antagonists (3). Nearly thirty potential antagonistic species activity were isolated and evaluated in vitro an in pot trials. The tested composts effectively controlled several diseases, such as Fusarium spp., Bionetria (4), Sclerotinia homoeocarpa (5) and Rizoctonia solani.

Keywords: antagonism, biological control, fungi, molecular biology, turfgrasss.

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Invited Speaker 43

Plant Nutrition in Mediterranean Environment: Iron Metabolism

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Iron deficiency is an important nutritional disorder that affect 20% to 50% of fruit trees in the Mediterranean region. It occurs in calcareous soils due to the presence of the bicarbonate ion and high soil pH, which may reach 8.5. This deficiency normally triggers limited plant growth, nutritional imbalances, poor fruit quality, and yield decrease [1]. Plants develop several morphological and physiological mechanisms to cope with this deficiency. These mechanisms include morphological root changes, enhancement of Fe uptake and transport, and the production of organic acids, among others. Several important crops, such as Citrus and strawberry, may be affected by this nutritional disorder, but there are others like carob-tree, that under calcareous soils conditions do not show symptoms of iron deficiency. These contrasting responses deserve particular attention and may provide additional information for the study of this problem.
Currently, a major concern is the correction of iron deficiency and chlorosis. Farmers applied large amounts of synthetic iron chelates to soil, but this treatment has to be repeated each year. Sprays with several compounds have been tested, but it was possible to produce a grass clipping extract, which can correct Fe deficiency in strawberry and recover plants from moderate chlorosis [2]. The role of this extract and its mode of action are now under study.

Keywords Fruit tree crops; calcareous soils; micronutrients; iron chlorosis.

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Invited Speaker 44

Varietal Discrimination of Walnuts Using Near Infrared Spectroscopy

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The origin of the Persian walnut (*Juglans regia* L.) lies in Central Asia where this tree grows in a semi-cultivated state. However, it was only during the Roman period that walnut tree was widely cultivated in western Europe. During centuries the walnut was the most important nut in the ancient Mediterranean World and its medicinal virtues are detailed in many Greek and Roman medicinal writings [1]. Nowadays many health benefits are attributed to walnut consumption. Walnuts are rich in polyunsaturated fatty acids and tocopherols, being linoleic acid the most abundant fatty acids. Moreover, bioactive compounds with potential health benefits, such as dietary fibre, folic acid, polyphenolic compounds and other antioxidants, are also present in walnuts [2]. There are many different varieties of walnuts that vary in physical attributes, fatty acid composition and taste.
In small producers, during harvest and walnut processing, there are some fruit mixture resulting in heterogenous batches. The main objective of this work was to evaluate 5 different walnut varieties produced in Portugal, using near infrared spectroscopy in order to obtain an accurate model for varietal discrimination.
In this feasibility study, 200 walnut samples of 5 different varieties, 'Chandler', 'Franquette', 'Howard', 'Lara' and 'Tulare' were collected and their NIR spectra were recorded with 3 different devices: a benchtop FT-NIR spectrograph, a hyperspectral imaging camera and a portable NIR device. Discriminant analyses were applied and different methods for the varietal discrimination of walnuts were obtained and compared.
Up to 96 and 84% of correct identification in internal and external validation were obtained. Better results were obtained covering the entire shell surface than collecting a unique random spectrum per sample. Moreover, FT-NIR and hyperspectral produce better models than the portable NIR one.

Keywords: near infrared, walnuts, hyperspectral imaging, chemometrics, discriminant analysis, *Juglans regia* L.

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Invited Speaker 45

Innovative Use of Nanoemulsions Enriched With Essential Oils to Delay Ripening and Prevent Chilling Injuries During Cold Storage

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Cold storage is one of the most effective technologies to control ripening and quality of postharvest fruit. However, storage of cold sensitive fruits at low temperature is limited by the great risk of chilling injury (CI), which is expressed as superficial scald in ´Rocha' pear or pitting in ´Rohde´ navel oranges.
Some studies are showing the great potential of nanocoating enriched with essential oils to maintaining fruit quality during fruit storage and shelf-life [1, 2]. However, as far as we know the possibility of coatings to avoid chilling injury has never been explored. Therefore, the objective of our work is to find out edible coatings-nanoemulsion able to raise the quality of fruits (e.g. pears and oranges), during storage. For achieving these goals, the group has studied the effects of the edible coatings-nanoemulsion based on sodium alginate (2.00% w/w) with incorporation of lemongrass (1.25 and 2.50% w/w).
In what concerns the 'Rocha' pears, the results have demonstrated that the utilization of these types of nanoemulsions retard the ripening process and significantly reduced superficial scald, keeping sensory quality for up to 6 months plus 7 days of shelf-life. Moreover, these results are highly dependent on the concentration of the lemongrass essential oil used.
Concerning oranges, during storage time, firmness decreased in all coated and control orange fruit, but the latter remained firmer. Control and lemongrass 1.25%-coated fruit presented less symptoms of chilling injury although commercially unacceptable.
Keywords: Superficial scald, internal disorders, firmness, taste, lemongrass.

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Invited Speaker 46

Functional Characterization of Parasitism Genes of Pratylenchus Penetrans and its Potential for Nematode Control

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Root lesion nematodes (RLN), *Pratylenchus* spp., are the third-ranking worldwide economic impact group of plant-parasitic nematodes (PPN) and a serious issue in crop protection and productivity. *Pratylenchus penetrans* is considered one of the most damaging species of this group, with over 400 plant hosts recorded, and a limiting factor for the production of important agronomic crops (e.g. corn, potato), ornamental plants (e.g. lily, roses) and fruit trees (e.g. almond, cherry orchards). In Europe, and in particular in Portugal, this species is widely detected in different potato growing areas. As other PPN, *P. penetrans* secretes a repertoire of specific parasitism proteins called effectors. PPN effectors once delivered into the plant can interact with plant molecules (e.g. receptors or intermediates), either in the apoplast or cytoplasm, triggering and manipulating in their benefit host molecular processes [2]. Cell wall-degrading enzymes and other PPN homologous effectors of *P. penetrans* have been described [1, 3-6]. However, little is known about the function of pioneer candidate effectors recently identified for this particular species [5]. In this sense, gaining insights into the mode of action of these new effectors and their interacting host targets can provide useful knowledge for the development of nematode-resistant plants [1]. Furthermore, the impact of *P. penetrans* in interaction with other plant pathogens (e.g. bacteria and fungi) in the affected cultures emphasizes an urgent demand for the development of new and sustainable control approaches as an alternative to common agriculture chemicals. Given the importance of nematode effectors on plant-nematode interaction [2], this work intends to explore novel biotechnological tools (e.g. RNA interference) to understand in detail the importance and function of specific effectors of *P. penetrans*, which can be used for strategic improvement of crops against this resilient group of nematodes.

Keywords: *Pratylenchus penetrans*, plant parasitic nematodes, parasitism, effectors, biotechnology, potato.

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Invited Speaker 47

Identification of Disease Resistance Genes in *Brassica oleracea* L. and *Pisum sativum L.*

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Downy mildew elicited by the oomycete *Hyaloperonospora* Constant. *parasitica* (Pers.:Fr) Fr. is one of the most devastating diseases in *Brassica oleracea* L. After the identificationof a broccoli accession highly resistant to the downy mildew isolate Pp523, at the Instituto Superior de Agronomia (Lisbon), a collaborative project with the Laboratory the Laboratory of Genomics and Genetic Improvement (LGGI) of the Universidade do Algarve, was initiated aiming at the identification of the locus (*Pp523*) responsible for this dominant and monogenic resistance. Based on a specifically constructed F2-based genetic map, the resistance locus was located in the chromosome 8 of this horticulture species. The analysis of *B. oleracea* genomic libraries constructed at the University of Georgia, USA, and of a genomic BAC-library constructed in our lab for a S4 line of the resistant accession, allowed the physical mapping of the locus *Pp523* in a *contig* of 13 BAC clones. Nevertheless, the sequencing of this BAC-*contig* revealed the presence of multiple resistance-like genes in this genomic region, a circumstance that, so far, hampered the exact and unequivocal identification of the locus *Pp523.*

Treatments of pea (*Pisum sativum* L.) with the alkylating agent ethylnitrourea (ENU), in the frame of a mutation breeding collaborative project with the Estação de Melhoramento de Plantas (Elvas), resulted in the induction of two mutant lines immune to powdery mildew (*Erysiphe* pisi Syd.). The complementation crosses performed between the two mutant lines and between them and a line carrying the naturally occurring recessive resistance locus *er1*, revealed that both recessive mutations had affected the same locus and that this locus was *er1*. The identification of the expressed sequence of this gene by another research group, was immediately followed by the identification of total genomic sequence in our lab. The analysis of the genomic sequences allowed the identification of a different point mutation (SNP) in each mutant line, which, in both cases, create early stop codons and lead to the truncation and loss of function of the coded protein (PsMLO1). The identification of a highly polymorphic microsatellite sequence in the fifth intron allowed the establishment of a SSR marker able to discriminate among a large multitude of alleles of this resistance gene.

Keywords: Downy mildew; Powdery mildew; Genetic resistance; *Pisum*; *Brassica*.

POSTERS COMMUNICATION

POSTER NUMBER 1

Are Innovative Approaches Important to Sustain High Nature Value Farming (HNVf) Systems? The Montado Case Study

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High Nature Value farmland (HNVf) can be defined as "those areas in Europe where agriculture is a major land use and where that agriculture supports or is associated with either a high species and habitat diversity or the presence of species of European conservation concern, or both" (Andersen *et al*., 200). HNV-Link is an H2020 recently closed project (March 2019) that ultimately aimed at improving the socio-economic and environmental sustainability of HNVf across the European Union by promoting innovation. The project created a community of practice and knowledge by linking 10 areas throughout European where HNV farming systems are prevalent. These "learning areas" (LA) were used to evaluate innovation examples and gaps relevant to HNV farming systems. In this paper we used the Portuguese LA – the Silvo-pastoral system Montado located in "Sítio de Monfurado" (SM) to illustrate the approach made in the project. Therefore, we identified innovative solutions of technical, commercial, social, institutional, and policy nature that work and are relevant for HNVf. We then highlighted gaps of innovation (the innovations that are needed but not yet implemented) and promoted the exchange and uptake of innovative practices in support of HNVf. We concluded that within the Montado of SM, examples of innovation are not frequent and are mostly related to individual rather than collective initiatives. At present the main challenges identified for the Montado were related to: the low soil fertility; lack of new oak trees; undifferentiated products; resistance to management for multifunctionality; lack of investment on new management practices; dominant regime supporting productivism and specialization in farming. The way forward within the framework of the project was the exchange of innovation approaches existing in other areas within the project. The co-construction of results based agri-environmental schemes in the Burren LA located in Ireland, was elected as an inspiring example for supporting the improvement of the economic and environmental sustainability of the Montado as an HNVf system.

Keywords: High Nature Value farming; Innovation; Sustainability; Locally-adapted

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POSTER NUMBER 1

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

As soluções inovadoras são importantes para a sustentabilidade de sistemas agrários de elevado valor natural? – O montado como caso de estudo

AGRO-SILVO-PASTORAL SYSTEMS

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1. Objetivo

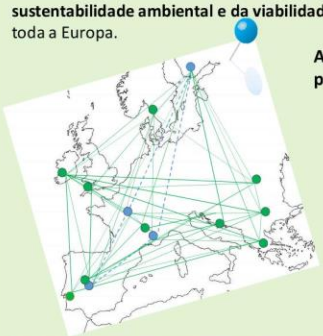
Identificar, desenvolver e partilhar estratégias e práticas inovadoras de natureza técnica, comercial, social e normativa que contribuem para melhorar a viabilidade socioeconómica e a eficiência ambiental do "Montado" como sistema silvopastoril complexo.

2. Enquadramento

✓O que são Sistemas de Elevado Valor Natural (SEVN)?

✓O projeto HNV-Link

Desenvolvimento e intercâmbio de inovacões para abordar os desafios da **sustentabilidade ambiental e da viabilidade socioeconómica** das **zonas de SEVN** em toda a Europa.



Abordagem territorial da inovação para áreas de EVN.

Dartmoor (Reino Unido)
Sítio de Monfurado (Portugal)
Dalmatian Islands (Croacia)
Eastern Hills of Cluj (Roménia)
Western Stara Planina (Bulgária)
Västra Götaland (Suecia)
The Burren (Irlanda)
Thessalia-Pindus (Grecia)
Causses et Cévennes (França)
La Vera, Extremadura (Espanha)

✓A área de aprendizagem Portuguesa

Sítio de Monfurado (SM)

Sistema agro-silvopastoril complexo, moldado pelo Homem, dominado por coberto arbóreo de *Quercus suber*, com algum *Quercus rotundifolia* e pontualmente outras espécies de Quercus.

3. Metodologia

✓Abordagem multiactor

Diagnostico	• Análise da situação atual – "Baseline assessment" • Estudo da evolução do sistema socio-ecológico no SM • Identificação de fatores diretos e indiretos de mudança
Visão	• Construção de uma visão de EVN para o Montado do SM com produtores e outras partes interessadas • Recolha de informação de projetos anteriores • Realização de entrevistas e workshops com diferentes actores
Soluções inovadoras	• Identificação de estratégias de inovação locais de natureza tecnológica, de produtos e de mercado, social, institucional e política • Deteção de obstáculos à inovação e lacunas de inovação
Intercâmbio e aprendizagem	• Realização de visitas cruzadas • Promoção de intercâmbios e adoção de práticas inovadoras

4. Resultados

✓Os desafios para a sustentabilidade do Montado do SM

Baixa fertilidade do solo	Ausência de regeneração florestal
Produtos indiferenciados	Resistência à multifuncionalidade
Agenda económica: produtivismo e especialização	Falta de investimento em novas práticas de gestão

✓Questões prioritárias identificadas no SM

Necessidades de inovação prioritárias	Caminhos possíveis
1. Maneio do solo para restaurar e melhorar a fertilidade	-Capacitação -Aconselhamento agronómico
2. Mecanismos para promover e proteger a regeneração de sobreiros e azinheiras	-Uso de protetores de árvores
3. Promoção do sistema e dos seus produtos únicos de elevada qualidade	- Criar uma marca territorial

5. O caminho a seguir

Esquemas agro-ambientais baseados em resultados

- Resiliência do Montado : **Regeneração** das árvores
- Conservação de **pastagens Mediterrânicas biodiversas**
- Conservação do **solo** saudável e funcional
- Preservação de **habitats** com interesse de conservação

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HNV Link

POSTER NUMBER 2

Conserving Bat Mediated Biocontrol Services in a Climatically Changing World

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Climate change is widely recognized as being one of the biggest threats to global terrestrial biodiver sity [1] and the phenology of species are shifting as a consequence of climatic variations [2]. Climate change has therefore the potential to disrupt ecosystem processes through temporal (phenological) mismatches between ecologically interacting species [3]. Far from being trivial, alterations to the networks of interactions among species have significant negative consequences on the maintenance and stability of key ecosystem processes, including some of which human well-being relies on (the so-called ecosystem services). Biocontrol services is one of such ecosystem services and can be defined as the impact of wild species (biocontrol agents) on the population density of pests [4]. However, the potential impact of climate change on biocontrol services through phenological mismatches between biocontrol agents and pests is still far from being understood, limiting our ability to plan their management and conservation. The overarching goal of this research is to determine the impact of climate change on bat-mediated biocontrol services in mediterranean olive groves in the Alentejo region, the largest and most important olive production region in Portugal. To achieve this challenging goal, we will investigate the environmental mechanisms underlying the flight phenologies of insectivorous bats and a major olive pest, the Olive fruit moth (*Prays oleae* Bernard 1788) in the study region, the synchrony (or degree of overlap) between the flight phenologies of bats and pests, the temporal dynamic of the interaction between bats and pests using an interaction network approach, and the regional impacts of climate trends on bat-mediated biocontrol services. The overall hypothesis is that climate change will lead to a phenological mismatch between the flight phenologies of insectivorous bats and olive insect pests, which will be expressed in a non-negligible impact on bat-mediated biocontrol services.

Keywords agricultural pests; biocontrol services; phenology; climate change

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POSTER NUMBER 2

Jornadas Med 2019
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CONSERVING BAT MEDIATED BIOCONTROL SERVICES IN A CLIMATICALLY CHANGING WORLD

AGRO-SILVO-PASTORAL SYSTEMS

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Introduction

Climate change is widely recognized as being one of the biggest threats to global terrestrial biodiversity. Studies have shown that the phenology of species are shifting as a consequence of climatic variations, potentially disrupting ecosystem processes through temporal (phenological) mismatches between ecologically interacting species. Nevertheless, subsequent impacts on interaction-mediated ecosystem processes are however surprisingly scarce. Far from being trivial, alterations to the networks of interactions among species may have significant negative consequences on the maintenance and stability of key ecosystem processes, including some of which human well-being relies on (the so-called ecosystem services). Biocontrol service, i.e. the impact of wild species (biocontrol agents) on the population density of pests is one of such ecosystem services. By reducing crop losses due to pests and the need for agrochemicals, biocontrol services provide significant economic, environmental, and societal benefits, thus reenforcing the need for the conservation of wild species in agroecosystems as a reliable pathway to guarantee food production and security under more environmentally sensitive farming practices. In the European Union, particularly in southern countries which will be especially hit by climate change, the conservation management of production landscapes based on the role of biodiversity as insurance for biocontrol services is particularly urgent, especially in Mediterranean olive (Olea europaea L. 1753) farms, where olive pests (mainly insects) cause huge economic losses estimated at hundred of millions of euros per year, with additional costs related to agrochemicals inputs. To date though, the driving role of climate change in phenological mismatches between biocontrol agents and pests, or its implications for the provision of biocontrol services in this particular cropping system is poorly understood.

Objectives and Methods

The overarching goal is to determine the impact of climate change on bat-mediated biocontrol services in Mediterranean olive groves in the Alentejo region. To achieve this challenging goal, research will focus on: (1) the environmental mechanisms underlying the flight phenologies of insectivorous bats and a major olive pest, the Olive fruit moth (Prays oleae Bernard 1788), (2) the synchrony (or degree of overlap) between the flight phenologies of bats and pests, (3) the temporal dynamic of the interaction between bats and pests using an interaction network approach and, (4) the regional impacts of climate trends on bat-mediated biocontrol services. Four tasks were defined to achieve this goal.

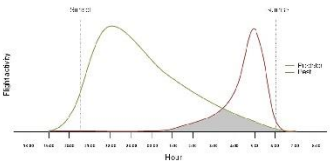
Task 1. Determining the environmental mechanisms underlying the flight phenology of bats and pests. Flight phenologies of insectivorous bats and the Olive fruit moth will be monitored within olive farms. Automatic acoustic bat detectors will be used to determine the flight phenology of bats. For pests, Delta-traps baited with species-specific sex pheromone will be used. A camera module attached to each Delta-trap will take photos every 30m. Recordings and photos will be analyzed with artificial neural networks. Temperature and humidity will be obtained using portable data-loggers.

Task 2. Determining the synchrony between flight phenologies of bats and pests. The overlap period between the flight phenology of bats and pests will be used as a proxy of the potential of bats to consume insect pests. Thus, higher the daily and seasonal overlap, the higher the role of bats as biocontrol agents. The degree of overlap will be estimated both daily and seasonally, as flight phenologies of many taxonomic groups have been shown to vary in response to climate change at both temporal scales. This approach will allow to determine the match between the flight phenology of bats and pests at both temporal scales. To test the assumption that variations in the overlap between flight phenologies lead to variations in pests consumption by bats (Schematic 1), the occurrence of prey species in bat faecal pellets will be obtained using state-of-the-art molecular techniques, i.e., metagenomics.

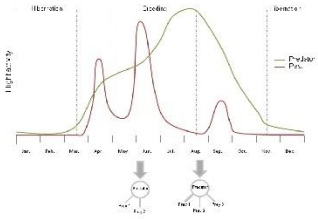
Task 3. Characterizing predator-prey interaction networks. The interaction predator-prey will be characterized using a network approach. Networks will be calculated in periods within and in-between peaks of highest pest activity. Network structure will be summarized using common metrics and modeled against environmental variables to determine their underlying mechanisms. Thus, this will give a better understanding of the impact that environmental changes may have, in different seasons of the year, on pest consumption by bats (Schematic 2).

Task 4. Modeling the impact of climate change on bat-mediated biocontrol services.

Knowing the environmental mechanisms driving the daily and seasonal flight phenology of bats and insect pests (Task 1) and their consequences for biocontrol services (Task 2-3), Task 4 is aimed to model the potential regional impact of projected climate change scenarios on bat-mediated biocontrol services provision. The impact of climate change will be estimated as the relative match/mismatch between the flight phenologies of biocontrol agents and insect pests. Three projected climate change scenarios for 2050 and 2080 will be considered: (1) "Business As Might Be Usual (BAMBU)", (2) "GRowthApplied Strategy (GRAS)" and (3) "Sustainable European Development Goal (SEDG)".



Schematic 1 - Daily flight curves of a bat species and Prays oleae. In gray is shown the overlap in flight activity.



Schematic 2 - Yearly flight curves of of a bat species and Prays oleae, with predator-pray interaction networks in different phases of pray year cycle.

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POSTER NUMBER 3

Effect of Different Delignification Processes on *Cistus ladanifer* Distillery residues

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Lignin is a very important source of phenolic compounds with functional activities and established/emerging new applications in the cosmetic, pharmaceutical and food industries. In the last years there has been a renewed interest in the study of delignification processes, not only because they facilitate cellulose upgrade, as they significantly increase cellulose digestibility, but also due to the possibility to obtain value added products from lignin. In this work, the residues of *Cistus ladanifer*, a native species of the Mediterranean region, were explored as a source of added-value lignin derived compounds in the framework of its upgrade within the biorefinery. These residues after extraction and hydrothermal treatment were submitted to two different delignification (organosolv) processes using as solvent ethanol/water mixtures or alkaline-catalyzed glycerol (AGO) under diverse reaction conditions in order to maximize the recovery of the lignin-derived phenolic compounds and to produce carbohydrate-rich solids. For comparative purposes, an aqueous alkaline process was also tested (ASP).
The obtained results indicate that ASP (4% NaOH for 2h) lead to a higher delignification (87%) and enzymatic saccharification (79%). A delignification of ~76% and enzymatic hydrolysis yields of 72% were obtained for AGO. EO was much less efficient (maximum lignin removal 30%). The main phenolics found in the liquors obtained from ASP and AGO were vanillic acid and epicatechin, while gallic acid was the main phenolic obtained from EO.
The results obtained showed that *C. ladanifer* residues can be a viable option for the production of added-value lignin-derivatives and glucan-rich solids to be used as feedstock for subsequent bioconversion processes.

Keywords: biorefinery, delignification, enzymatic hydrolysis, lignin-derived products

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POSTER NUMBER 3

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Mediterranean Institute for Agriculture, Environment and Development

Effect of different delignification processes on *Cistus ladanifer* distillery residues

AGRO-SILVO-PASTORAL SYSTEMS

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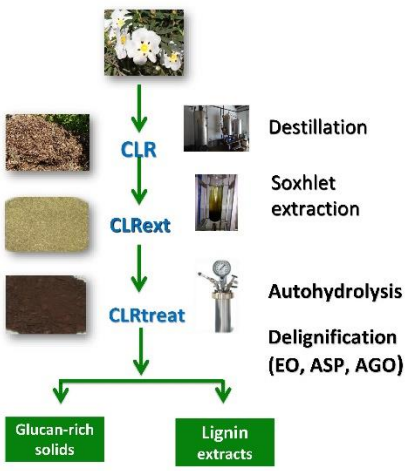
INTRODUCTION

Cistus ladanifer, a native species of the Mediterranean region, has an incipient industrial use as a source of added-value essential oils. The residual biomass has been demonstrated to be able to produce significant amounts of marketable phenolics and oligosaccharides by a water ethanol extraction strategy followed by autohydrolysis. The remaining solid biomass (**CLRtreat**) is enriched in cellulose and lignin and is here studied not only as a source of digestible cellulose, but also of lignin-derived compounds with functional activities and potential applications in cosmetic, pharmaceutical and food industries.

Three organosolv processes: ethanol/water mixtures (**EO**), alkaline-catalyzed glycerol (**AGO**), aqueous sodium hydroxide process (**ASP**), are optimized and the recovery of the lignin-derived phenolic compounds and production of cellulose-rich solids is compared aiming to contribute to the development of small-scale and locally adapted biorefineries that will strengthen local economies, thereby providing a new perspective for the use of this species.

METHODOLOGY



Organosolv and alkaline processes were carried out in a Parr reactor, or autoclave, under temperature/solvent mixture/catalyst according to table below

Chemical composition of CLRtreat was 35% glucan, 9% xylan, 1.4% acetyl groups and 45.8 % lignin

Chemical characterization of (CLRtreat) and delignified solids, and enzymatic saccharification were carried out according to NREL LAPs

Phenolic profile was characterized by capillary zone electrophoresis and the total phenolic content was determined by the Folin–Ciocalteu method expressed in gGAE/L (GAE=Gallic Acid Equivalents)

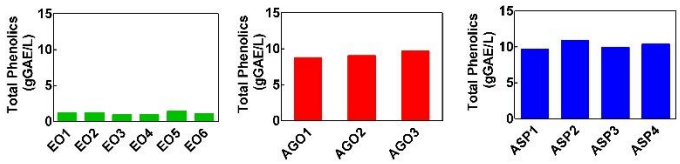
Experimental conditions

Treatments					
Process	Nr	Reagent	Reactor	Temperature (°C)	Time (h)
EO	1	50% EtOH	Parr	170	NI
EO	2	50% EtOH	Parr	180	NI
EO	3	50% EtOH	Parr	190	NI
EO	4	50% EtOH	Parr	200	NI
EO	5	50% EtOH	Parr	210	NI
EO	6	50% EtOH	Parr	220	NI
AGO	1	50% GlyOH+1% NaOH	Autoclave	130	1
AGO	2	50% GlyOH+2% NaOH	Autoclave	130	1
AGO	3	50% GlyOH+4% NaOH	Autoclave	130	1
ASP	1	2% NaOH	Autoclave	130	1
ASP	2	2% NaOH	Autoclave	130	2
ASP	3	4% NaOH	Autoclave	130	1
ASP	4	4% NaOH	Autoclave	130	2

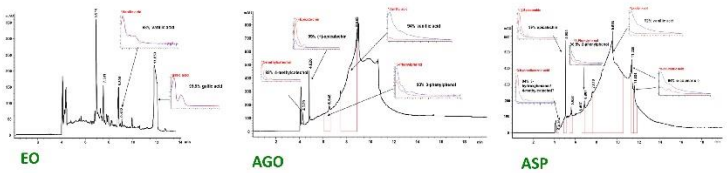
EO–Ethanol/water mixtures; AGO-alkaline-catalysed glycerol; ASP- aqueous sodium hydroxide process; EtOH – ethanol; GlyOH – glycerol; NI: non-isothermal.

RESULTS

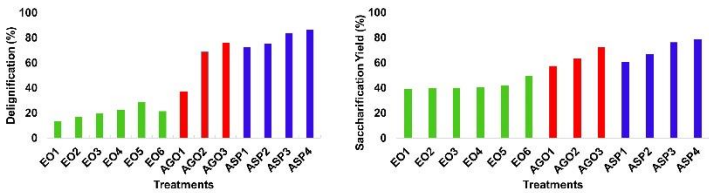
- Ethanol organosolv process yield lower total phenolics content, between 0.9 and 1.4 g GAE/L, than alkaline treatments that resulted in phenolic concentrations between 8.75 and 10.8 g GAE/L



- The type of delignification process affects the phenolic profile and purity of phenolic extracts
- The main phenolics in the ASP and AGO liquors were vanillic acid and epicatechin, while gallic acid was the main phenolic in the EO liquors



- The results indicate that **ASP** (4% NaOH, 2 h) led to a higher delignification (87%) and enzymatic saccharification (79%)
- A delignification of 76% and saccharification yields of 72% were obtained for **AGO** (4% NaOH, 1 h)
- **EO** was a much less efficient process (maximum lignin removal 30%)



CONCLUSIONS

- Alkaline catalyzed processes induced higher delignification and were more effective on the production of soluble phenolics
- These processes are carried out at milder temperature conditions than ethanol organosolv process, therefore presenting also a potential higher energy efficiency
- The integration of delignification in the *C. ladanifer* upgrade strategy can be a valuable step for the economic viability of a small-scale, specialty-based biorefinery

Acknowledgements

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POSTER NUMBER 4

ECOMONTADO XXI - Agroecology applied to the design of the New Montado System

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The Montado ecosystem is losing its vitality and productivity, consequence of soil and biodiversity depletion, aggravated by the effects of climate change. The work to be carried out by the operational group ECOMONTADO XXI consists in the implementation and monitoring of a new forest management practice based on the Keyline system. This system is thought to promote the retention and redistribution of water in the soil, as well as the active construction of soil in cleared areas or with a low degree of forest cover. It can be used for pasture installation, as well as of new Montado areas or any other agroforestry system. ICAAM is collaborating with Herdade do Freixo do Meio and Herdade da Machoqueira do Grou in the implementation and monitoring of the effectiveness of Keyline in these farms. The monitoring focuses on the evolution of moisture in the soil profile, the evolution of the pasture species and the evolution of tree-growth until the end of 2021. Another task of ICAAM is to disseminate this system of soil construction and water management that can be very adequate for the Mediterranean systems and a measure to mitigate the effects of the climatic changes on the Montado.

KeyWords: Keyline, soil building, water management, Montado, mitigation of climatic changes

The authors are grateful for the postdoctoral fellowship awarded under the project PDR2020-101-FEADER-03114, as well as the availability of the different researchers to participate in this challenging interdisciplinary work.

POSTER NUMBER 4

Jornadas Med 2019
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ECOMONTADO XXI - Agroecology applied to the design of the new Montado - PDR2020-101-FEADER-03114

AGRO-SILVO-PASTORAL SYSTEMS

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RDP Financing - Priority P4 - Restore, preserve and improve the ecosystems linked to agriculture and forestry in Operation - 1.0.1 of the Operational Groups

Total amount of finance € 338 482.56

Approved eligible investment to the University of Évora 153 317,69 €

To run between 01-01-2017 and 12-131-2021

Partners

THE PROBLEM:
Loss of vitality and productivity in Montado:
- Inefficient use of water and soil,
- Soil loss
- due to climate change effects

PROPOSED SOLUTION:
Implementation of a novel forest management practice aimed at recovering the Montado using the Keyline system, which is based upon the integrated management of soil and water resources.

ECOMONTADO XXI ACTIVITIES:

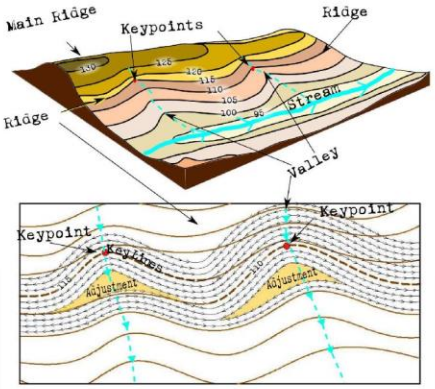
1. Implementation of the Keyline system at the Herdade do Freixo do Meio and Herdade da Machoqueira do Grou.

2. Monitoring of key components of the system, namely:
   - Moisture, composition and texture of the soil,
   - Organic Carbon Content,
   - Growth and diversity of vegetation,

3. Dissemination of this practice and its effective implementation across other holdings.

KEYLINE:
Topographic line drawn using as reference a point on the slope of the terrain where the water, upon descending, abruptly slows down, resulting in an area of accumulation of water.

KEYLINE SYSTEM:
Technique of intervention at soil and water level that makes use of the fact that topographic lines are slightly uneven (about 1% with respect to Keyline), for redistribution of water at the surface and / or water storage.



THE PURPOSE OF THE KEYLINE SYSTEM?
The KEYLINE system aims at rehabilitating degraded agricultural land, both dry and seasonally subject to forest fires.
It allows to conserve the rainwater that falls on a given piece of land, distributing it inside the soil and progressively leading it from the valley to the ridges. In this way the rate of infiltration is increased while reducing the surface runoff and the evaporation rate, allowing a significant improvement of the fertility and structure of land.
By improving the distribution of soil moisture, and thus significantly increasing the total organic matter content, biological activity is promoted.

SOIL WORK:

The Yeomans plow was developed to make small tunnels in the soil according to the orientation of the Keylines. Its work allows some aeration but does not cause the mixing of horizons so that the existing organic matter is not subject to mineralization. The low working depth of this plow is essential so that the roots of the already installed plants can colonize the new aeration channels. Its work can be accompanied by direct sowing and also by the application of slurries or biofertilizers. In some situations the Yeomans plow has been replaced by an adapted subsoiler. The work of this equipment disturbs the ground a little more, but allows the same distribution of water across land surface.


Yeomans plow


Keyline (Plot 2)

MONITORING PLAN:

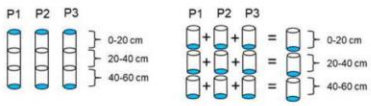
SOIL:

Monthly: Measurement of soil moisture up to 60 cm depth (with 10-by-10 cm data) in the 57 defined points across the 5 plots of the project.

At the beginning and at the end of the project: Soil analyses in the sampling plots at the following depth: 0-20 cm, 20-40 cm, 40-60 cm.

Field texture	1 analysis at the beginning of the project
pH (H_2O) pH (KCl) Conductivity Organic matter Extractable Phosphorus (P2O5) Extractable potassium (K2O) Removable calcium Extractable magnesium Manganese	1 analysis at the beginning of the project and 1 analysis at the end

SOIL SAMPLING:



MEASUREMENT OF SOIL MOISTURE:

Equipment - Diviner 2000 with probes installed on the ground

57 tubes were installed for monthly measurement of moisture at the center of the pixels (10 m resolution) corresponding to each point, so that the SENTINEL I, the European satellite that collects data for agriculture, can be used.

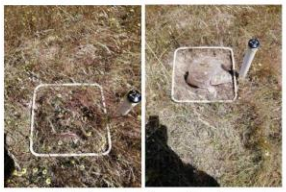

Probes (Plot 3)


Measuring the moisture (Plot 4)

GEOREFERENCING AND MONITORING OF TREE GROWTH:

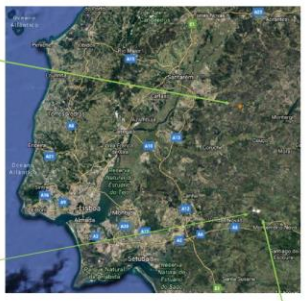
- Measuring the height of new trees after each growing season, twice a year.
- Measurement of adult tree height, diameter at breast height and height of crown base.

GRASSLAND BIODIVERSITY:


Frame 29 before and after cutting for species identification and determination of its biomass


Classification and storage of plant species


Plot 4 (above - with Keyline) and Plot 5 (below - without Keyline) – Machoqueira do Grou




Plot 2 (with Keyline) and Plot 3 (without Keyline) – Freixo do Meio


Plot 1 – Freixo do Meio


http://www.ecomontadoxxi.uevora.pt/

Instituto de Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

PROGRAMA DE DESENVOLVIMENTO RURAL 2014-2020 · PORTUGAL 2020 · UNIÃO EUROPEIA

POSTER NUMBER 5

Food Technology, Quality and Safety

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The *Food Technology, Quality and Safety* group (in Portuguese "*Tecnologia, Qualidade e Segurança dos Alimentos*"-TEQSA) promotes research in food characterisation, processing and preservation, in order to ensure food quality and safety, mainly of foods characteristic from our Mediterranean diet. Furthermore, they pursuit food innovation through the development of added value food products, operating new preservation methodologies, including the preservation of foods using aromatic and medicinal plants, as well as their essential oils, and also evidencing environmental concerns both by the use of edible films and biodegradable packages or just by reducing food loss and food waste. The group currently works under several thematic lines, such as food quality and safety, food technology, development of new food products, shelf-life definition, heathier foods, quality standardisation (for example using starter cultures), biodegradable packages, and food loss and waste.
To achieve the proposed goals the group works in different infrastructures and laboratories, namely the Microbiology Laboratory, the Technology and Postharvest Laboratory, the Sensory Room, and the Experimental Mill.
In our projects, microbiological, as well as physicochemical, biochemical, rheological and sensory analyses are performed in different food matrices, such as meat and meat products, fruits, and cheeses, among others.
Presently, the group is involved in three ongoing projects, all in consortia with food industries: two in meat and meat products, specifically one in bovine meat and another in pork fermented meat products, and one in fruits, namely table grapes.

Keywords food science and technology; food quality and safety; starter cultures; Mediterranean diet; biodegradable packages; Food Loss and Waste

This work is funded by national funds through *Fundação para a Ciência e a Tecnologia* (FCT)/MCTES under project UID/AGR/00115/2019, and supported by projects PDR2020-1.0.1-FEADER-031373, PDR2020-1.0.1-FEADER-030803 and ALT20-03-0247-FEDER-024248, funded by national funds through FCT and co-funded through the European Regional Development Fund (ERDF).

POSTER NUMBER 5

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Food Technology, Quality and Safety

VINEYARDS AND WINE

AGRO-SILVO-PASTORAL SYSTEMS

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The ***Food Technology, Quality and Safety*** group (in Portuguese "***Tecnologia, Qualidade e Segurança dos Alimentos***"-**TEQSA**) promotes research in food characterization, processing and preservation, in order to ensure food quality and safety, mainly of foods characteristic from our Mediterranean diet.

CURRENT THEMATIC LINES

-food quality and safety
-food technology
-development of new food products
-shelf-life definition
-aromatic and medicinal plants and their essential oils
-healthier foods
-quality standardization (e.g. using starters and standardizing processes)
-biodegradable packages
-food loss and food waste

ASSOCIATED INFRASTRUCTURES

-Microbiology Laboratory
-Technology and Postharvest Laboratory
-Sensory Room
-Experimental Mill

FOOD MATRICES

- meat and meat products
- cheeses
- table grapes
- olives
- other food products




ONGOING PROJECTS

-"***Estudo da qualidade da carne de bovinos da raça Cachena***". Project PDR2020-101-030803. PI-Miguel Elias.

-"***Criação de um núcleo de I&D para a geração de novo conhecimento relacionado com a uva de mesa sem grainha Vale da Rosa***". Project ALT20-03-0247-FEDER-024248. PI (UEVORA)-Ana Cristina Agulheiro-Santos.

-"***Segurança & Qualidade dos Produtos Cárneos Transformados***". Project PDR2020-101-031373. PI (UEVORA)-Miguel Elias. https://www.safemeatprod.com/

All in consortia with food industries: two in meat and meat products, specifically one in bovine meat and another in pork fermented meat products, and one in fruits, namely table grapes.

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A Europa investe nas Zonas Rurais

ALENTEJO 2020

PORTUGAL 2020

UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional

POSTER NUMBER 6

Functional Characterization of Putative Genes Involved in Suberin Biosynthesis

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Cork oak (*Quercus suber*) is a tree species native to the western Mediterranean regions with a relevant ecological and socioeconomic importance for Portugal. Cork accounts for 33% of all forest products at a national level, placing Portugal as a world leader in its production, industrial processing, and exportation. Cork or phellem is a tissue derived from the meristematic activity of the phellogen characterized by a layered deposition of suberized death cells. The high content in suberin provides cork with unique insulator and elastic properties that translates into a large variety of industrial applications. [1]. *QsMYB1* gene, a member of the R2R3-MYB transcription factor family has already been associated to the regulation of suberin biosynthesis, in cork oak [1,2].
During this work, a system for functional characterization of *QsMYB1* gene was developed using a reverse genetic approach, in *Solanum tuberosum* (potato), which has been used as a model to periderm studies. After the establishment of in vitro potato plants, the protocol to produce plants genetically transformed for overexpression of the *QsMYB1* gene was optimized using *Agrobacterium tumefaciens*. The shoot regeneration efficiency after co-cultivation with *A.tumefaciens* varied from 29 to 80%, according to the type of explants and vectors used. The root formation rate was higher than 89%, except in the internodal stem explants, using the *QsMYB1* vector. The confirmation of transformation efficiency are being performed by RT-qPCR. The chemical composition of microtubers periderm produced from transformed lines will be evaluated.
This work will contribute to the elucidation of the regulatory networks of genes and metabolites involved in in the suberin biosynthetic pathway.

Keywords: Suberin; Cork oak; *Solanum tuberosum*; Genetic reverse; *QsMYB1*.

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POSTER NUMBER 7

Identifying Potentially Bioactive Substances in Phlomis Purpurea Challenged with Phytophthora Cinnamomi

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We recently reported that *Phlomis purpurea* (Lamiaceae), indigenous to the southern Iberian Peninsula and Morocco, has antagonistic potential in the rhizosphere of *Quercus suber* L.(cork oak) and *Q. ilex* L. (holm oak) against the highly aggressive soil-born oomycete *Phytophthora cinnamomi* [1]. In preliminary biological screenings, the 70% aq. EtOH extract of the rhizome of *Phlomis purpurea* inhibited mycelial growth of *P. cinnamomi* and protected *Q. suber* seedlings from infection by *P. cinnamomi*. A novel nortriterpenoid was isolated from the root extracts and its structure elucidated. It was shown to be exsuded by roots and to exhibit antiproliferative activity against five *Phytophthora* species, and tumor HeLa and L929 cell lines [2]. Moreover, the mRNA expression profiling of *P. purpurea* when challenged with *P. cinnamomi* revealed genes putatively involved in structural barriers and induced defence responses against the pathogen [3].
Crossing the metabolome and transcriptome data is of major importance to understand the mechanisms of resistance to this pathogen. 55 root and 55 leaf samples (challenged and controls) were analysed by LC-MS and mass spectra were generated comprising 8727 m/z values. Separate data corresponding to m/z masses from 50 to 1999, and intensities present in all the spectra were used to originate a data matrix which is presently being analysed for metabolite identification. Preliminary results will be disclosed.

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2. Mateus, M.C.; Neves, D.; Dacunha, B.; Laczko, E.; Maia, C.; Teixeira, R.; Cravador, A. 2016. Structure, anti-*Phytophthora* and anti-tumor activities of a nortriterpenoid from the rhizome of *Phlomis purpurea* (Lamiaceae). Phytochemistry, 131: 158-164.
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POSTER NUMBER 7

Identifying potentially bioactive substances in *Phlomis purpurea* challenged with *Phytophthora cinnamomi* A metabolome study

AGRO-SILVO-PASTORAL SYSTEMS

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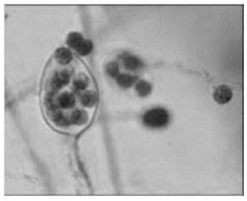
Introduction

Phlomis purpurea, a member of the family Lamiaceae is a spontaneous evergreen shrub, autochthonous to southern Iberian Peninsula and Morocco and is present in cork oak and holm oak forest habitats. It was found to be resistant to *Phytophthora cinnamomi*, a highly aggressive wide-range host root pathogen. *Phlomis purpurea* has revealed antagonistic effect in the rhizosphere of *Quercus suber* and *Q. ilex* against *P. cinnamomi*[1]. Its roots produce bioactive compounds exhibiting antitumor and anti-*Phytophthora* activities with potential to protect susceptible plants[2]. Data from the de novo assembly of transcriptome using short reads suggest a combination of a constitutive resistance and an increased transcriptional response from *P. purpurea* when challenged with the pathogen[3]. To further investigate bioactive natural products involved in the resistance of the plant and/or responsible for its antagonist effect, a metabolome study of *P. purpurea* challenged with *P. cinnamomi* has been undertaken.

Experimental design

Seeds were collected in the field, across the Algarve, germinated in *Petri* dishes until the radicles were 2–3 cm long and then transferred into cylindrical soft black plastic tubes (25 cm × 3 cm) containing vermiculite.

Three-month-old seedlings were carefully removed from the tubes, washed to remove the substrate and were challenged with *P. cinnamomi* zoospores in glass tubes in the dark at 22 °C.



Replicas

20 plants control **20 plants challenged with *P. cinnamomi* zoospores**

time points: 0, 6, 12, 24, 48 and 72 h

experience repeated 5 times ⟶ **55 root + 55 leaf samples**

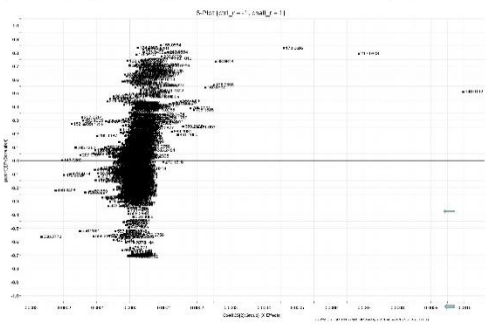
Ultra performance liquid chromatography/mass spectrometry analysis of *Phlomis purpurea* root and leaf extracts

Phlomis purpurea root and leaf samples (55 of each) were extracted with MeOH and metabolite profiling was performed using a LC-MS system (Waters Synapt G2 HDMS), according to established standard workflows. Lipids and slightly polar metabolites were separated using reversed phase chromatography (RP) with BEH C_{18} column and a HSS T3 C_{18} column.

Preliminary Results

Mass spectra were generated comprising 8727 m/z values. Separate data corresponding to m/z masses from 50 to 1999, and intensities present in all the spectra were used to originate a data matrix which is now being analysed. Group differences between control and challenged *P. purpurea* in the root methanol extracts for the 5 replicates, using OPLS-DA showed 4 possible biomarkers, m/z: 149.0112; 173.0692; 217.0903 and 556.2778 (below right). In leafs 2 potential biomarkers arose at m/z 149.0112 and 198.0393.

Metabolomic Data Analysis of aerial parts with MetaboAnalyst 2.5 showed differential produced metabolites at 6 hpi (m/z 1494.048 and m/z 742.4841, $P<0.001$) at 48 hpi (m/z 414.7606, P=0.01) and at 72 hpi (m/z 920.2796, P=0.002, and m/z 845.3147, P=0.01).



Discussion

The use of transcriptomic and metabolomic approaches will allow a more complete overview of the resistance process and thus determine potential genes of importance to understand the basis of resistance towards *Phytophthora* and further define resistance markers. Data generated in the present work open good prospects that are presently being explored using MetaboAnalyst 4.0 and MassTRIX for high precise MS data annotation and mass translation into pathways

FCT Fundação para a Ciência e a Tecnologia
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PTDC/AGR-CFL/100217/2008-METRANSPHLOMIS

Fundação Calouste Gulbenkian Academic Mobility Program for PhD Students (project 122180)

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POSTER NUMBER 8

Impacts of Management Intensification on Soil Invertebrate Communities of Olive Groves in the Alentejo

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The ecological impacts of management intensification on biodiversity and agroecosystem functions is, currently, a major concern in applied ecology and conservation. Conventional agricultural practices have been shown to adversely affect animal communities and their associated ecosystem processes, through the conversion of complex natural ecosystems into simplified, cultivated crops, and the intensification of management practices on such converted areas. Most studies focus on intensification impacts on predatory vertebrates or arthropods considered as natural enemies, although ecological research on how the intensification of agricultural management practices can affect entire communities, and specially soil communities, is still scarce. Here we investigated the effect of agricultural intensification on soil invertebrate communities of Mediterranean olive groves from the Alentejo region. We found a major decline in the total abundance of soil animals with management intensification, as well as on some particular functional groups, such as microbivores and fungivores. Animal body sizes were also affected by intensive management practices, which can lead to dramatic consequences on food-web structure and dynamics. Our results suggest that conventional agricultural practices (use of herbicides and pesticides, irrigation, etc.) may severely impact soil food web structure, potentially altering associated ecosystem functions in Mediterranean olive groves.

Keywords: Soil invertebrate communities; agricultural intensification; management practices; Mediterranean olive groves; functional groups; conservation

This work was funded by the Portuguese National Funding Agency for Science, Research and Technology (FCT) and the projects PTDC/AAG-REC/6480/2014 and ALT20-03-0145-FEDER-000008.

POSTER NUMBER 8

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Impacts of management intensification on soil invertebrate communities of olive groves in the Alentejo

AGRO-SILVO-PASTORAL SYSTEMS

N. Melguizo-Ruiz, G. Jiménez-Navarro, B. Silva, S. Barreiro, S. Vasconcelos, J.M. Herrera

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Department of Ecology, Swedish University of Agricultural Sciences, PO Box 7044, SE-750 07 Uppsala, Sweden.

Introduction

A major aim in applied ecology and conservation is to investigate the ecological impacts of management intensification on biodiversity and agroecosystem functions. Ecological research on how the intensification of agricultural management practices can affect entire communities, and specially soil communities, is still scarce. Here we investigated the effect of agricultural intensification on soil invertebrate communities of Mediterranean olive groves from the Alentejo region.

Materials and methods

Study area

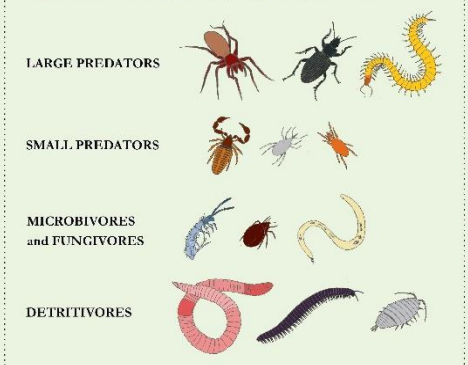
45 sites located in 29 olive groves in the Alentejo region, conforming an intensification gradient from organic to super-intensive olive groves.

ORGANIC | TRADITIONAL | INTENSIVE | SUPER-INTENSIVE

Animal sampling

Basic units: 2 soil samples (2L from the upper soil layers) per site; i.e., 90 samples brought to the lab.
A total of 8890 individuals identified, counted and measured.

Taxonomic and functional classification



Results

The total abundance of fauna significantly differed among management systems (Fig. 1a), with higher numbers of soil animals in organic olive groves. Body size was also affected by management intensification (Fig. 1b); animals found in organic and traditional olive groves were larger than those inhabiting intensive and super-intensive groves. The use of herbicides (Fig. 2a) and pesticides (Fig. 2b) had a negative impact on the abundance of all functional groups, especially microbivores and fungivores. Pesticides may have a direct effect on soil invertebrates, while herbicides may indirectly affect animal abundances through the resulting absence of herbs and schrubs.

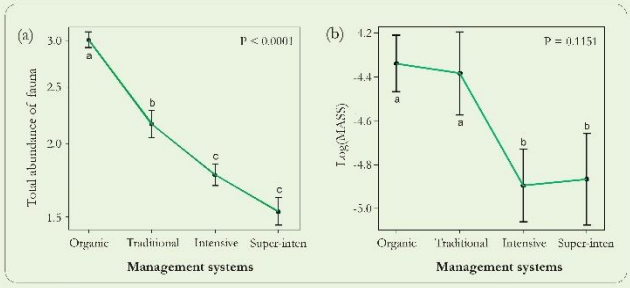


Figure 1. Total abundance of fauna (a) and body mass (b) of soil fauna among management systems (Organic, Traditional, Intensive and Super-intensive).

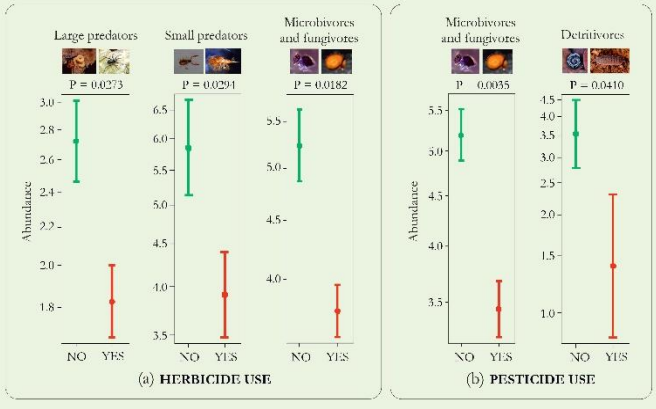


Figure 2. Response of soil functional groups to (a) Herbicide use and (b) Pesticide use.

Conclusions

- Significant differences in the total abundance of soil fauna among management systems, with a **strong decline** in the most intensively managed olive groves.
- **Smaller animals** found in intensive and super-intensive olive groves, compared to traditional and organic groves.
- All functional feeding groups **negatively affected by agrochemical use**, especially microbivores and fungivores.
- Conventional management practices associated with agricultural intensification appear to severely impact soil invertebrate communities.

so...

What is the impact of management intensification on the ecosystem processes and services provided by such animal communities?

Acknowledgements

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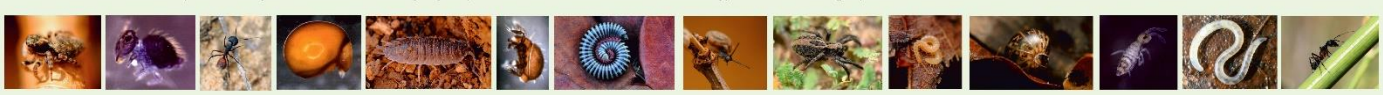

POSTER NUMBER 9

Improvement of Pasture Production in the Montado System: Chemical and Biological Approach to Overcome the Problems of Acid Soils

J. R. da-Silva, E. Menéndez, L. Alho, C. Brígido, A. Alexandre, I. Brito, M. Carvalho

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Soil acidity is one of the most important stress factors negatively affecting crop production worldwide. Approximately, 70% of the World's potentially arable land and 85% in Portugal are acid soils, displaying manganese (Mn) and aluminum (Al) toxic levels. Dolomitic limestone can overcome Mn toxicity of the granitic soils in Portugal [1], but might be expensive. A complementary or alternative approach to chemical correction is the use of beneficial microorganisms, which protect plants against toxicity of metal ions. In addition, these microorganisms can also increase nutrient availability and thereby help to overcome the low fertility problems, which are common in the Montado system. Among other benefits, mycorrhizal symbiosis expands the plant uptake of nutrients and protects the host against abiotic stresses [2], while rhizobia symbiosis allows the conversion of atmospheric nitrogen into inorganic nitrogen compounds that become available to legume host. Therefore, the exploitation of the tripartite symbiosis (Arbuscular Mycorrhizal Fungi–Rhizobia-Legume host) might be a possible approach for plant growth improvement [3]. This biological approach is particularly relevant in the present days, considering that there is an urgent need for a sustainable intensification of agricultural systems.

The main objective of this project is to increase productivity in soils with Mn toxicity through chemical and biological approaches. In order to achieve this objective, several strategies were used, such as monitoring the rhizobial population dynamics after soil correction (chemical approach) and isolation of rhizobia adapted to the most prevailing soil conditions. This will lead to the formulation of efficient biofertilizers based on consortia of native rhizobial strains, which will be effective and will improve pasture productivity. Moreover, exploiting tripartite symbiotic associations (biological approach) under toxicity conditions will contribute to increase system productivity and to avoid partially or completely the application of dolomitic limestone as soil correction, which is expensive and scarce in many areas of the World. Overall, these strategies will contribute to increase the pasture productivity and lead to a more sustainable and efficient exploitation of the Montado agrosilvopastoral systems.

Keywords: acidity, arbuscular mycorrizal fungi, rhizobia, symbiosis

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Goss, M.J., Carvalho M., Brito I., Alho L. and Kadir S. Academic Press, 2017. 81-109.

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POSTER NUMBER 9

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AGRO-SILVO-PASTORAL SYSTEMS

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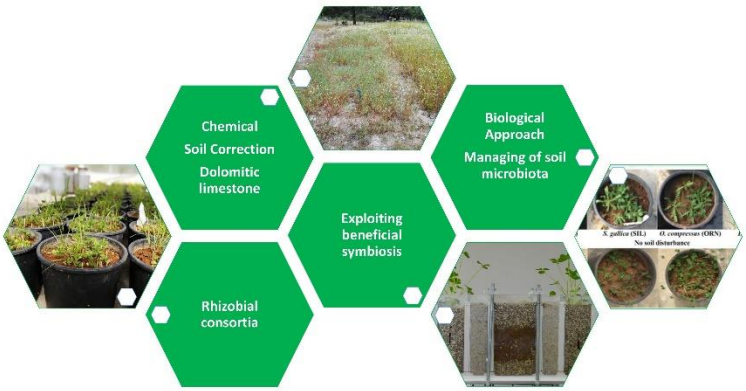

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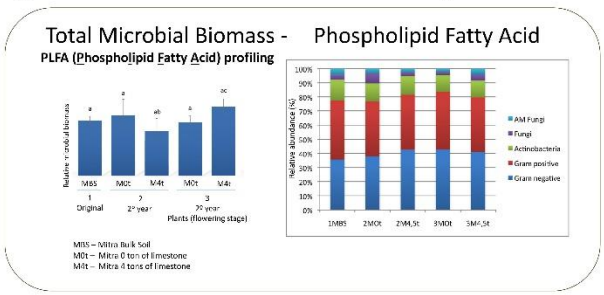
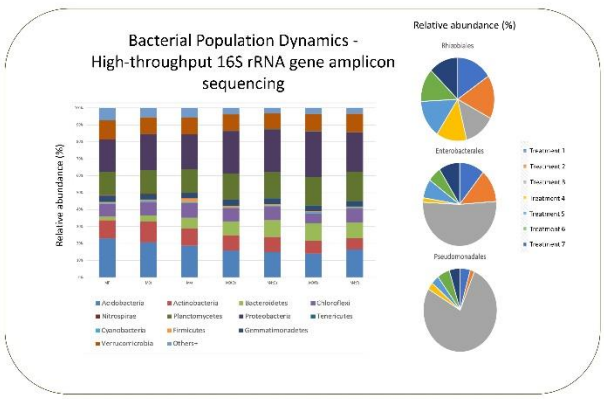
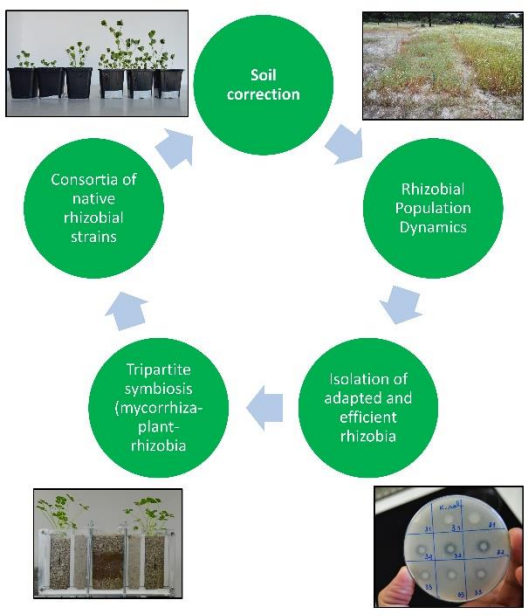
Several strategies were used, such as monitoring the rhizobial population dynamics after soil correction (chemical approach) and isolation of rhizobia adapted to the most prevailing soil conditions. This will lead to the formulation of efficient biofertilizers based on consortia of native rhizobial strains, which will improve pasture productivity. Moreover, optimizing the beneficial symbiotic associations (biological approach) under toxicity conditions will contribute to increase system productivity and to avoid partially or completely the application of dolomitic limestone as soil correction, which is expensive and scarce in many areas of the World.







Overall, these strategies will contribute to increase the pasture productivity and lead to a more sustainable and efficient exploitation of the Montado agrosilvopastoral systems.

Instituto de Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

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POSTER NUMBER 10

Micopartners – Symbiosis are the Future

R. Louro1, B. Natário1, T. Nobre [2], C. Santos-Silva1

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Desert truffles are of considerable interest for ecological, agroforestry and commercial purposes. They represent a key component of the mycobiota, in arid and semi-arid regions, due to their important role as symbiotic partners of diverse host plants, most often members of the *Cistaceae*. Plus, their fruitbodies constitutes a potentially important food source for animals and humans, rich in proteins and poor in carbohydrates and lipids. Desert truffle increasing demand has recently boosted more research, aiming to achieve new strategies to enable their medium-large-scale cultivation. Hence, this study aims to expand the current knowledge on the mycorrhizal associations of *Cistus* spp. with *Terfezia* and develop strategies to allow its application towards desert truffle cultivation and production of *Cistus* mycorrhized plants. As for results, we have successfully developed rapid and efficient micropropagation protocols for both *Cistus ladanifer* and *Cistus salviifolius* [1]. Furthermore, we effectively infected, *ex-vitro*, the roots of micropropagated *C. ladanifer* and *C. salviifolius* seedlings with *Terfezia arenaria* inoculum. Using the ex-vitro approach we obtained an elevated infection rates (80 %) and observed the formation of ectomycorrhizae with partial shead on the fine roots of both *Cistus* species [2]. One of the most important output from this study was the successful isolation and subculture of various *Terfezia* strains/species (11 strains/ 4 species) on a novel agar media (patent pending) developed to overcome the broadly known difficulty of isolation of many *Ascomycota* and some *Basidiomycota*.
These results are promising, as they show that in the near future, this and other biotechnological research might enable the cultivation of a myriad of wild edible mushrooms and truffles that so far cannot be cultivated.

Keywords: *Terfezia arenaria*; *Cistus*; Mycorrhization process; desert truffles

Louro R., Peixe A. & Santos-Silva C. 2017. New Insights on *Cistus salviifolius* L. micropropagation. Research & Reviews: Journal of Botanical Sciences, 6(3): 10-14; Santos-Silva C. 2018. Project ALT20-03-0145-FEDER-000006 Annual report. 6pp.

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POSTER NUMBER 10

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

MICOPARTNERS
SYMBIOSIS ARE THE FUTURE

AGRO-SILVO-PASTORAL SYSTEMS

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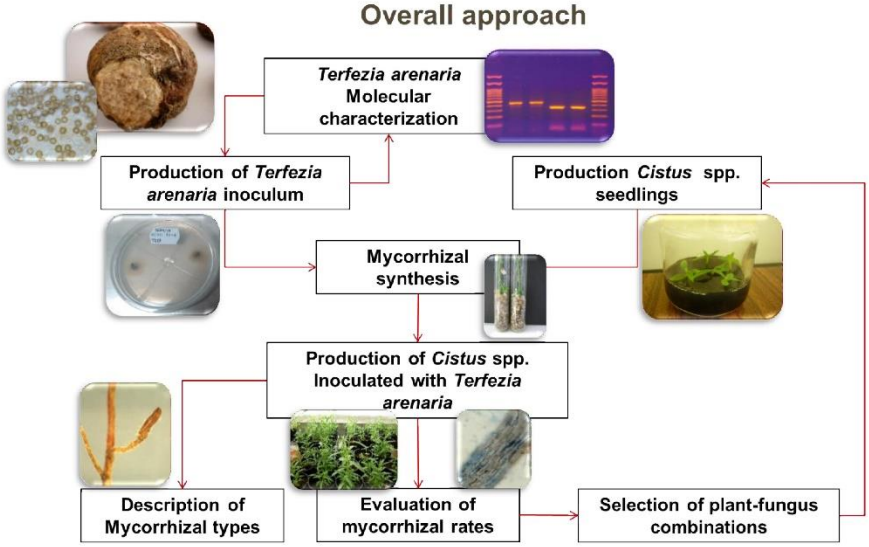
Overall approach



Experimental plots

These results are promising, as they show that in the near future, this and other biotechnological research might enable the cultivation of a myriad of wild edible mushrooms and truffles that so far cannot be cultivated.

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POSTER NUMBER 11

Microrheology of Novel Cellulose Stabilized OIL-IN-WATER Emulsions

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[1]*Faculty of Sciences and Technology (MeditBio), Ed. 8, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*
[2]*FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden*

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Diffusion wave spectroscopy (DWS) is a powerful optical technique suitable to investigate turbid samples in a nondestructive and reproducible way, providing information on the static and dynamic properties of the system. This includes the relative displacement of emulsion droplets over time and changes in the viscoelastic properties. Here, novel and promising cellulose-based oil-in-water (O/W) emulsions were prepared and studied, for the first time, by DWS [1]. Cellulose plays the role of a novel eco-friendly emulsifying agent. The hydrolysis time of cellulose was observed to affect the average size of the emulsion droplets and their stability; the longer the hydrolysis time, the more dispersed and stable the emulsions were found to be. Additionally, a good complementarity between the microrheology (DWS) and macrorheology (mechanical rheometer) data was found. Our work suggests that DWS is a highly attractive method to investigate the stability, aging and microrheology properties of cellulose-based emulsions, providing valuable insights on their microstructure. This technique is thus highly appealing for the characterization and design of novel emulsion formulations.

Keywords: Diffusing-wave spectroscopy; Cellulose; Emulsions; Microrheology

Medronho, B.; Filipe, A.; Costa, C.; Romano, A.; Lindman, B.; Edlund, H.; Norgren, M. Microrheology of novel cellulose stabilized oil-in-water emulsions. J. Colloid. Interf. Sci. 2018, 531, 225-232.

The Portuguese Foundation for Science and Technology (FCT) is acknowledged through the projects PTDC/AGR-TEC/4814/2014 and project n° 030619 (02/SAICT/2017) and researcher grant IF/01005/2014. Financial support from the Sodra Skogsagarnas Stiftelse, Stiftelsen Nils och Dorthi Troedssons forskningsfond, Swedish Research Council VR, grant no. 2015-04290 are gratefully acknowledged. Dr. Mathias Reufer (LS Instruments AG) is acknowledged for all the technical support with the measurements.

POSTER NUMBER 11

Microrheology of novel cellulose stabilized oil-in-water emulsions

Medronho, B.[1,2*], Filipe, A.[1]; Costa, C.[2]; Romano, A.[1]; Lindman, B.[2]; Edlund, H.[2] and Norgren, M.[2]

[1]Faculty of Sciences and Technology (MeditBio), Ed. 8, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; [2]FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden
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Introduction

Emulsions are widely used for pharmaceutical, food and cosmetic applications and their flow properties are among the most important physical characteristics, either during their production (e.g. mixing and pumping) or as an end product (pouring or extrusion from containers, draining, etc). Therefore, in order to guarantee that their critical quality attributes meet specifications, it is desirable to monitor the emulsion manufacturing process. However, finding of a suitable process analyzer has so far remained challenging. In this work, Diffusion wave spectroscopy (DWS) is highlighted as a powerful optical technique suitable to investigate turbid samples in a nondestructive and reproducible way, providing information on the static and dynamic properties of the system (Figure 1). A novel oil-in-water emulsion system stabilized by dissolved cellulose is introduced and studied by DWS.

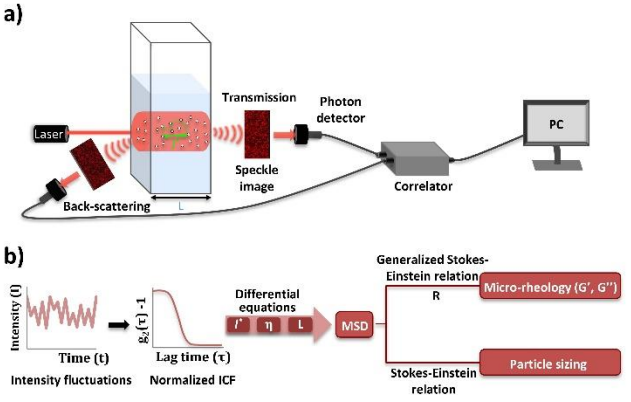
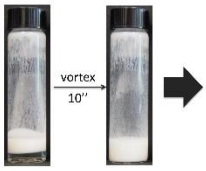


Figure 1. DWS setup: DWS-based microrheology (a) and particle sizing (b)

Materials and Methods

Three samples of O/W emulsions, composed of 7.5 % (v/v) paraffin oil, were prepared using cellulose (commercial sulfite dissolving pulp from Domsjö Fabriker, Sweden) as the emulsifier agent. The samples were named as *24h*, *48h* and *96h*, according to the respective hydrolysis times of cellulose in the phosphoric acid solution.



- **Diffusing-wave Spectroscopy**

DWS RheoLab (LS Instruments AG)

Backscattering mode: droplet size estimation

Forward geometry: micro-rheology

- **Mechanical Rheology** (macro-rheology)
- **Optical light microscopy** (droplet size estimation)

Conclusions

- **Cellulose proved to be an efficient eco-friendly emulsifier which is an important step towards the development of more sustainable emulsion-based products.**
- **The longer the hydrolysis time of cellulose the smaller (and more stable) the droplets formed.**
- **$t_{2/3}$ and *I** demonstrated to be suitable indicators of the emulsions long term stability.**
- **Remarkable complementarity between the optical microrheology and common mechanical macrorheology.**
- **DWS assays enabled the determination of the droplet size, microrheology and monitoring real-time changes in the emulsion system which is of major importance for an efficient and reliable manufacture, optimization of formulations and storage control. Valuable insights on their microstructure.**

Results

Emulsion size via DWS and Optical microscopy

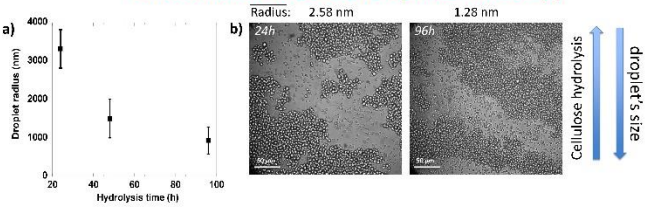


Figure 2. Characterization of the droplet size: (a) variation of the average droplet radius with the different times of cellulose hydrolysis, measured with DWS; (b) optical microscopy images.

Micro and macrorheometry

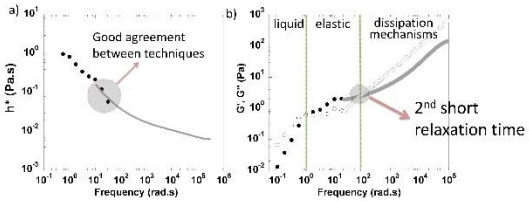


Figure 3. Viscoelastic parameters of the *96h* sample determined by mechanical rheometry (black symbols) and DWS (grey symbols) at 25 ºC: a) complex viscosity and b) G' (full circles) and G'' (empty circles) as a function of frequency.

Cellulose hydrolysis effect on the viscosity

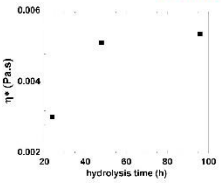


Figure 4. Variation of the η^* of the emulsions with the cellulose hydrolysis time at a constant frequency of 6000 rad/s by DWS.

The decrease of the droplet size (Fig.2) results in an increase in the number of droplets in solution

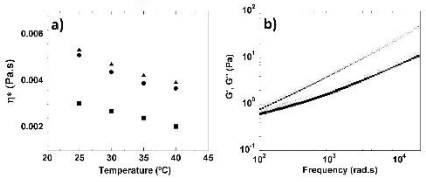
↓

Interactions among of the oil droplets (Fig. 2b)

↓

Increase in the η^*

Temperature effect on the viscoelastic properties



The temperature effect is essentially reversible

Figure 5. a) Variation of η^* with temperature for the three emulsion samples: *24h* (full black squares), *48h* (full black circles) and *96h* (full black triangles), at a constant frequency of 6000 rad/s. b) G' (squares) and G'' (circles) of the *24h* measured at 25 ºC, before (full symbols) and after being heated to 40 ºC (empty symbols).

Aging and stability: effect of cellulose hydrolysis time

Larger oil droplets are formed with time

↓

ICF decays at higher lag times

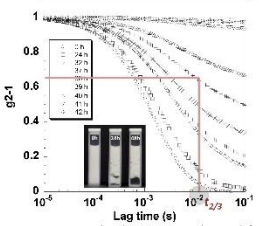


Figure 6. Normalized ICF curves obtained for the *96h* sample at different aging times (25 ºC)

$T_{2/3}$ and I* increase after certain critical aging

↓

Dramatic changes in the droplet's size

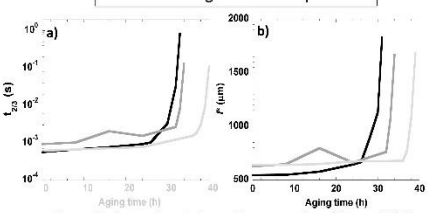


Figure 7. Comparison between the parameters a) $t_{2/3}$ and b) *I** for the *24h* (black line), *48h* (dark grey line) and *96h* (light grey line) samples, monitored during 40 h of aging at 25 ºC.

UAlg Universidade do Algarve · MeditBio Centre for Mediterranean Bioresources and Food · FCT Fundação para a Ciência e a Tecnologia · FSCN part of Mid Sweden University · Mittuniversitetet Mid Sweden University

POSTER NUMBER 12

PineEnemy - Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease

C. Vicente[1,2], H. Bragança[1], E. Sousa[1], P. Naves[1], M. Mota[2,3], F. Nóbrega[1], M.L. Inácio[1]

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Pine wilt disease (PWD) is one of the most important threats to conifer forests in Europe [1] and worldwide [2], causing severe economic and environmental damages. This complex disease results from the interaction between three biological elements: the pathogenic agent *Bursaphelenchus xylophilus*, also known as pinewood nematode (PWN); the insect-vector (cerambycid beetles of the genus *Monochamus*) [3], and the host tree, mostly from *Pinus* spp. [4]. In 1999, *B. xylophilus* was firstly identified in maritime pines (*Pinus pinaster*) in Portugal and in Europe [1, 5]. Despite a nationwide monitoring system, enforcement of strict phytosanitary measures and constraints implemented after PWN detection, this devastating problem has spread quickly in Portuguese mainland, Madeira Island [5] and Northern Spain [6].

As a migratory endoparasite, once inside susceptible tree, PWN has the ability to feed on the living parenchyma of the epithelial cells in the resin canals, causing a reduction in water flux and ultimately cessation of resin flow. In the later stages of the disease, PWN assumes a mycetophagous phase. The development of the PWN population appears to be strongly associated with fungi that colonize the declining trees [3]. Fungi harboured in weakened pines are seen as essential for the ongoing development and completion of the PWN life-cycle, affecting not only PWN reproduction [8] but also the number of individuals carried by the insect-vector [9-10].

Through a spatio-temporal analysis, PineEnemy will focus on the characterization of the structure and dynamics of the nematode-fungi interactions through culturable and non-culturable approaches with special emphasis in metagenomics analysis. Our aim is to understand if PWN-associated mycobiota plays a key-role in the development of PWD, in interaction with PWN and insect-vector, and into which extend can be targeted to disrupt the disease cycle.

Keywords: *Bursaphelenchus xylophilus*, Metagenomics, *Monochamus* spp., Mycobiota, Pine Wilt Disease.

This work was supported by the the national project PineEnemy: Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease (LISBOA-01-0145-FEDER-028724) which is cofunded by Fundação para a Ciência e Tecnologia / Ministério da Ciência e do Ensino Superior, through national funds, and by FEDER under the PT2020 Partnership Agreement.

POSTER NUMBER 12

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

PineEnemy - Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease

AGRO-SILVO-PASTORAL SYSTEMS

C. Vicente[1,2], H. Bragança[1], E. Sousa[1], P. Naves[1], M. Mota[2,3], F. Nóbrega[1], M.L. Inácio[1]

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A doença da murchidão do pinheiro (DMP) é uma das principais ameaças às florestas mundiais [1 e 2], causando danos ambientais e económicos severos. Esta doença complexa resulta da interação entre três elementos biológicos: o nemátode da madeira do pinheiro (NMP) *Bursaphelenchus xylophilus*; o insecto-vector do género *Monochamus* [3]; e a árvore hospedeira, coníferas do género *Pinus* [4].

Como endoparasita migratório, o NMP tem a capacidade de se alimentar de células vivas nos canais de resina, provocando a redução no fluxo de água e cessação do fluxo de resina (fase fitófaga). Nos estágios mais avançados da doença, o NMP tem a capacidade se tornar micetófago, alimentando-se de fungos das árvores em declínio (fase micetófaga). O desenvolvimento da população do NMP parece estar fortemente associado aos fungos que colonizam as árvores afetadas [3], influenciando não só a reprodução do nemátode bem como o número de indivíduos transportados pelo insecto-vector [5 -7].

Através de uma análise espacial e temporal, o projeto **PineEnemy – Exploring the NEmatode-Mycobiota interactions in Pine Wilt Disease (**LISBOA-01-0145-FEDER-028724) (Figura 1) pretende caracterizar a estrutura e a dinâmica das interações nemátode-fungo de modo a compreender o papel da micobiota associada ao NMP no complexo da doença, na interação com o NMP e com o insecto-vector e de como pode ser utilizada no desenvolvimento de medidas de controlo da doença.

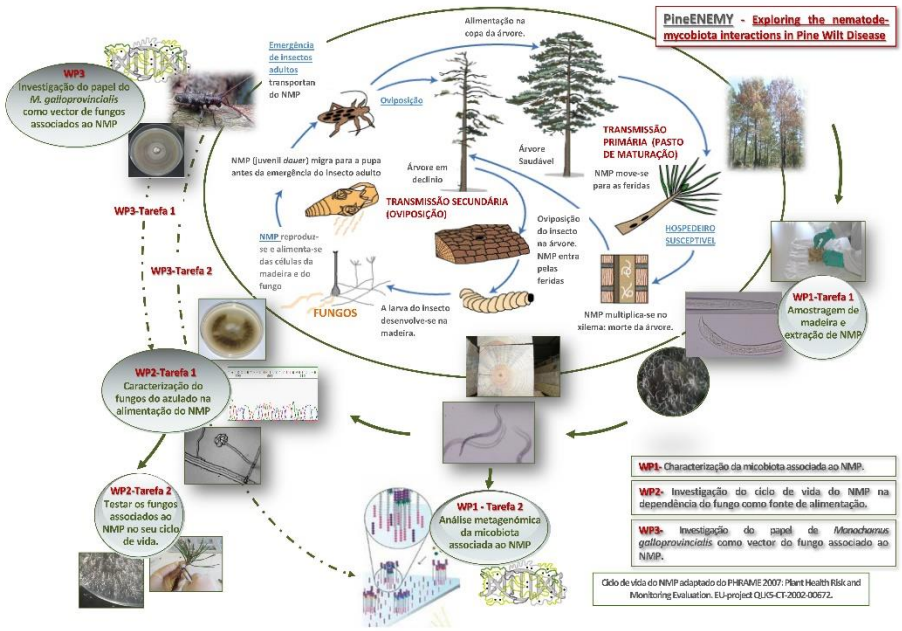

Figura 1 – Apresentação dos objetivos principais do projeto PineEnemy no contexto do ciclo de vida do nemátode da madeira do pinheiro na doença da murchidão do pinheiro (diagrama adaptado de PHRAME 2007: Plant-Health Risck and Monitoring Evaluation EU – Project QLK5-CT-2002-00672).

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POSTER NUMBER 13

Porosity as the Main Parameter of Cork Quality - A Histological and Transcriptomic Approach

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Cork is one of the most valuable non-wood forest products, which takes part of the most important industrial sectors in Portugal, playing also an important economic, social and ecological role in the Mediterranean region. It is periodically harvested, every 9 years, from cork oak (*Quercus suber* L.) during the tree's lifespan. Cork tissue results from the activity of a secondary meristem called phellogen. Sometimes, the cumulative layers of cork are crossed by lenticular channels, named lenticels or cork pores, which are produced by a specific meristem, the lenticular phellogen [1]. Cork porosity, considering the number, dimension and distribution of lenticular channels, is the major contributor to the cork quality. Large and abundant pores are unwanted in industry as they interfere in cork properties [2]. Meanwhile, the controlling factors for the formation of lenticels are still unknown. During the Lentidev project, a histological study of lenticular channel development was performed in young branches together with a transcriptomic analysis of these structures. In this context, the isolation of single cells of those specific meristems was optimized in *Q.suber* tissues using laser microdissection technique. RNA extractions for RNA-seq analysis have been also optimized in the suberized cells. This work will contributed to increase the knowledge of the lenticular development and the genetic role of these structures in cork quality.

Keywords: Phellogen; Lenticel; Single cell; Laser Microdissection; RNA-Seq; Cork oak.

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This work was supported by Alentejo2020, through FEDER under Lentidev-ALT20-03-0145-FEDER-000020 project.

POSTER NUMBER 14

Project Water Management and Ecological Security - The Nanxi River Study Case - Zhejiang, China

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ICAAM participates in the Water Management and Ecological Security project funded through the EU Partnership Instrument, specifically through the development of the River Basin Restoration Plan of Nanxi River (Yongjia county, Zhejiang, China). The watershed of the Nanxi River is rich in natural and cultural resources. The objective of the project is to analyse the ecological status of the Nanxi River, adapting to the Chinese context the methodologies developed for the implementation of the Water Framework Directive (WFD). This objective involves analysis of physical-chemical parameters, hydromorphology, macrophytes, riparian vegetation, macroinvertebrates, fish and birds. The pressures and changes that the river basin is subject to will also be analyzed. The collection of these elements and evaluation of the data collected will allow the development of the mitigation and restoration plan. During the spring of 2019 a sampling campaign was conducted. The information gathered will serve as a basis for improving the adaptation of the WFD protocols to the Chinese reality. In the future, more information will be collected and later used for the development of future scenarios to elaborate the restoration plan, involving the needs of the community of stakeholders. We hope that this work can contribute to the development of policy recommendations that can be replicated in Chinese rivers. For additional information and involvement in future activities of the project contact: aimendes@uevora.pt

Keywords: China, River, Freshwater, Restoration, Water Framework Directive

This project is funded by the European Union trough the Partnership Instrument. The project partners are solely responsible for the contents, which do not necessary reflect the views of the funding parties.

POSTER NUMBER 15

RECONCILE: Spatial Patterns of Socioeconomic Drivers in Europe, Opportunities and Bottlenecks for Effective Conservation Planning

Fernando Chacón-Prieto[1], Diogo Alagador[1].
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Conflicts between socioeconomic and biodiversity conservation goals have produced a gloomy legate that are being currently upgraded under the Global Environmental Crisis. The direct anthropic impacts over biodiversity are now adding up to a myriad of other threats, thus putting biodiversity under stressful conditions. In a globalized world, the effects of grand environmental processes (e.g. climate change) instigate severe pressures over biodiversity equilibria, resulting in fully-dynamic ecological systems in which biological features (as species) spread spattialy. The enlargment of space required to processes operate leads to increased interactions of ecological elements with established societal processes (e.g. pervasive socioeconomic activities). Under this context, cutting-edge conservation policies need to be guided by a new set of rules that balance-out the legitimate, aspirational outputs of modern-day societal development with healthy biodiversity conditions, persistent at long-term. Curiously, this double-sided reconciliatory portrait has been compellingly supported by strategic, policy-driven, global sustainability treaties (e.g. Convention on Biological Diversity, the UN 2030 Sustainable Development Agenda,).
Using the 15th (Life on Land) and 17th (Partnerships) goals of the 2030 UN Agenda as benchmarks, we developed a European- wide spatial dataset of potential socioeconomic area-atractiveness with the aim of identifying opportunity areas for collaborative partnerships (win/win frameworks) between conservation and established socioeconomic activities and, parallely, to pinpoint bottlenecks of such reconciliatory approaches, where biodiversity conservation tends to lose societal inclusiveness (thus effectiveness). A set of informative socioeconomic maps are presented, where a diverse array of socioeconomic information collected in institutuional, open-access web databases is assessed. These maps are categorized in three classes according to their conservation planning utility: i) maps of (financial and legislative) conservation incentives; ii) maps of societal factors likely to facilitate absorsion of conservation actions at local scales (e.g. educational programmes, environmentally-concerned legislation), and iii) maps about opportunity costs, defined as the required financial efforts that conservation plans incur to compensate established conflictual actitivities for their foregone returns when these abate (or cease) their negative impacts or when they are relocated in other regions. Additionally, summary maps were also developed, in which the three utility datasets are standardized by their monetary valuing at a 10 km2 grid resolution. These ensemble maps deliver a first-stage socioeconomic layer to be used in implementaion and policy-driven stages of conservation plans in Europe at a continental scale.
Science-based conservation planning requires proper explanatory data of biotic, abiotic and societal realities. With the two former factors already well established in typical conservation approaches, larger gaps still exist to inform on the socioeconomic dimension. This study brings a modest contribution in the provision of a potential socioeconomic fingerprint across Europe. As in war games, learning and studying enemy patterns and strategies enables one to best respond to their dissents and, ideally to build bridges for dialogue and reconciliation. In this environment, there are space for both parties to win. Under such an idilic vision, the well functioning of bioversity and ecosystems promotes human welfare under a sustainability paradigm of development, capable to thrive the global flowing stresses from climate change.
Keywords: *biodiversity; biosocioeconomic systems, climate change; optimization; protected areas; spatial conservation planning.*

POSTER NUMBER 15

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

RECONCILE: Spatial patterns of socioeconomic drivers in Europe, *opportunities and bottlenecks for effective conservation planning*

Fernando Chacón-Prieto[1], Diogo Alagador[1].

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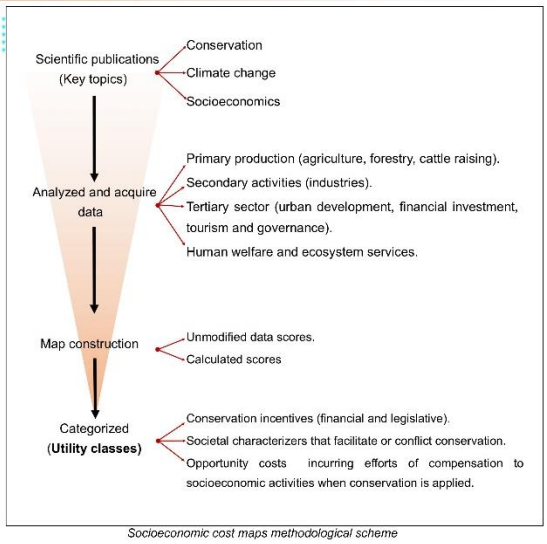
AGRO-SILVO-PASTORAL SYSTEMS

Conflicts between socioeconomic and biodiversity conservation goals have produced a gloomy legate currently being upgraded under the Global Environmental Crisis, where anthropic impacts over biodiversity add up to a myriad of other threats (e.g., climate change). Stressed biodiversity conditions compromise its equilibria, resulting in fully-dynamic ecological systems in which biological features (as species) spread spatially [1]. Under this context, conservation policies need to be guided by a set of rules that balance-out the aspirational outputs of modern-day societal development with healthy biodiversity conditions at long-term [2]. Curiously, this double-sided reconciliatory portrait has been compellingly supported by strategic, policy-driven, global sustainability treaties (e.g., Convention on Biological Diversity, the UN 2030 Sustainable Development Agenda).

AIM & RATIONALE

A pivotal step towards the integration of socioeconomic opportunities and constraints in conservation planning is to assess the spatial distribution of societal-relevant socioeconomic activities and the type costs they convey [3,4]. Here, we created a reliable set of maps characterizing three utility classes of socioeconomic factors likely to impact conservation area prioritization. Analogous analyses were reviewed; data were gathered from open access institutional data, and; financial analytic procedures provided the basis for their comparison and mapping.

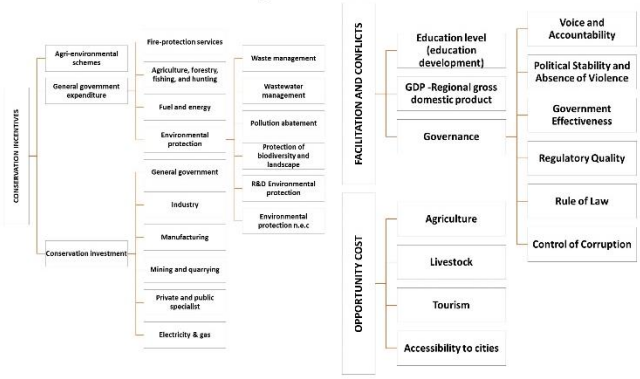
SOCIOECONOMIC OVERVIEW



Socioeconomic cost maps methodological scheme

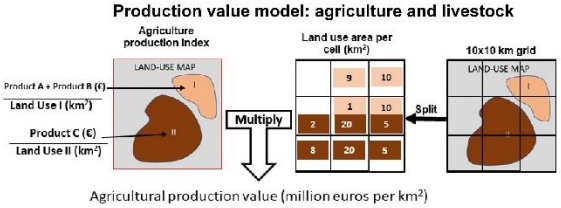
Maps produced constitute European countries for which (comparable) data are available. Cost scores are represented as original data, such as indexes (governance and education) and economic indicator (agri-enviroment, GDP), and production value (Agriculture and livestock), standardized by their monetary valuing at a 10x10 km grid resolution. These ensemble maps deliver a first-stage socioeconomic layer to be used in implementation and policy-driven stages of conservation plans in Europe at a continental scale.

Utility classes framework



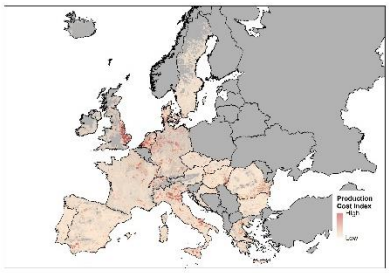
The framework used for selected socioeconomic information based on the effect of utility classes in biodiversity conservation. Data was further used for mapping.

Production value model: agriculture and livestock

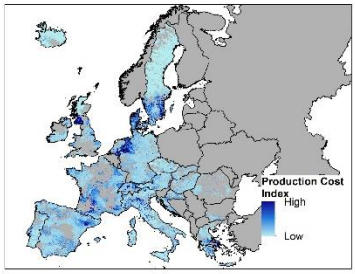


The total production value of an asset in each NUT2 is distributed among cells accordingly to the proportional area of the respective land use(s).

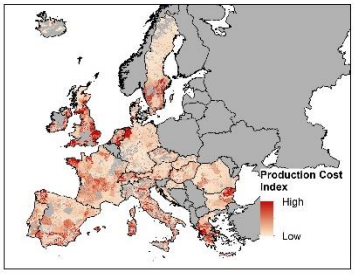
RESULTS



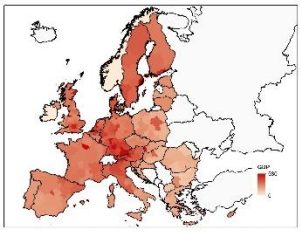
Production value distribution of agriculture types



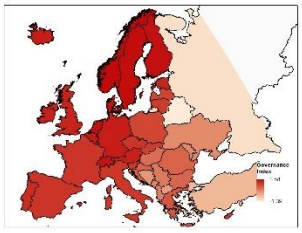
Livestock extensive farming: pigs and poultry



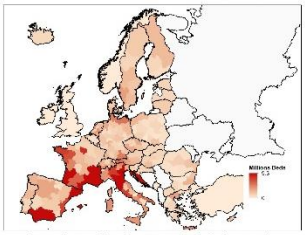
Livestock intensive farming: pigs and poultry



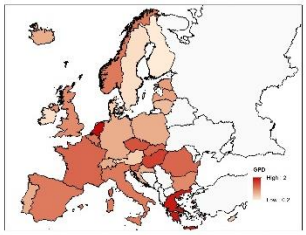
Gross Domestic Product (GDP)



Governance: Control of corruption



Tourism: Beds on establishment



Expenditure: Environmental protection

CONCLUSION

This study brings a modest contribution to the provision of a European-wide socioeconomic fingerprint that needs to be accounted when planning for biodiversity conservation. The broadening of the conservation spectrum from a sole natural to a bio-socioeconomic system paves the way towards a supportive society, where conservation cost-effectiveness (i.e., ecological gains from euros spent) is empowered. When setting realistic conservation objectives, the consideration of coordinated European wide conservation policies to sustain biodiversity and functioning ecosystems, and societal aspirations endorsement, are two major goals (the 15th and 17th, respectively) configured within the UN Sustainable Development Agenda for 2030.

Acknowledgments

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POSTER NUMBER 16

Rhizobia as Key Partners in Sustainable Agriculture: Understanding the Molecular Basis of Stress Tolerance

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The benefits of using legume crops in agriculture have been known for a long time. Most legume species used for food and forage share the ability to establish nitrogen-fixing symbiosis with a particular group of soil bacteria, named rhizobia. Inside the nodules, rhizobia convert atmospheric nitrogen (N_2) into ammonia (NH_3), which is then assimilated by the host plant and ultimately this nitrogen can be transferred to the soil. A better understanding of the molecular bases of rhizobia stress tolerance will allow us to maximise the nitrogen input provided by these symbioses. This is particularly important considering common soil constraints, such as acidity or salinity problems, as well as the current predictions of climate change (towards higher temperatures in the Mediterranean region) [1,2].
Our work showed that natural rhizobia populations are diverse in terms of tolerance to abiotic stresses, even when isolates from the same species are compared [3-5]. In addition, these studies showed that, in some cases, a higher induction of major stress response genes seems to be associated with a higher tolerance to stress. Nevertheless, a stress tolerant phenotype probably relies on a more complex gene expression control. Therefore, a global transcriptomic approach was used to identify genes responding to heat, acidity and salinity [6-8]. Functional studies on individual stress response genes showed that besides their influence on the stress tolerance phenotype, these genes may also improve the symbiotic performance of rhizobia [9-12]. Thus, stress response genes represent a promising tool to optimise rhizobia-legume symbioses, in order to provide higher nitrogen levels to the ecosystem and consequently reducing the amount of synthetic nitrogen fertilisers used in agriculture.

Keywords: symbiosis; legumes; biological nitrogen fixation; temperature; salinity; acidity

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AGRO-SILVO-PASTORAL SYSTEMS

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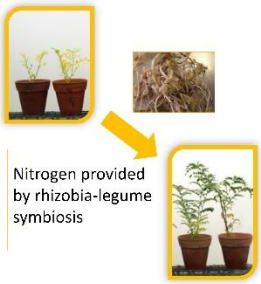
Rhizobia are soil bacteria able to metabolize atmospheric nitrogen and provide it to the host plant

- Maximizing these rhizobia-legume symbioses will help to reduce the use of synthetic N-fertilizers

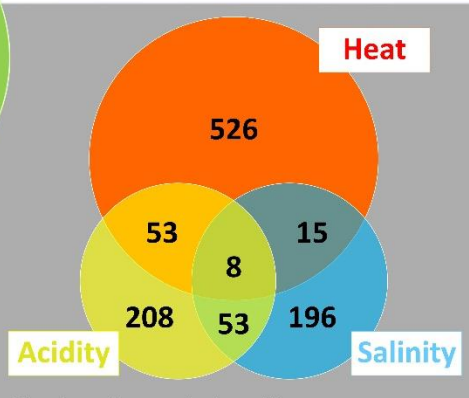
- A better understanding of the molecular bases of rhizobia stress tolerance is particularly important considering common soil constraints, such as acidity or salinity problems, as well as the current predictions of climate change (towards higher temperatures in the Mediterranean region)



Does the transcriptional response to different stresses involve common pathways ?

Transcriptomics approach

***Mesorhizobium japonicum* MAFF303099 (7231 genes were analysed)**



Number of genes induced in response to stress

A surprisingly low number of genes induced in reponse to the three stresses

General stress response genes apparently belong to the RpoE2/EcfG1 regulon

The heat shock changed the expression of a large number of genes (extensive downregulation)

Cross protection was only detected when a mild acidity shock was applied prior to the heat shock

Are stress response genes directly involved in the symbiosis?

Functional analysis of specific genes

Different *Mesorhizobium* strains were genetically modified

Control

Higher *clpB* expression levels

A - ST2pRK415 and B - ST2pRKgroEL strains tagged with GFP (green)

ST2pRK415 (red) and ST2pRKgroEL (green) strains co-inoculated

Higher expression levels of the *clpB* gene led to a higher frequency of root hair curling and also improved nodulation ability and symbiotic effectiveness

Higher expression levels of the *groEL* gene improved symbiotic effectiveness, but it did not affect the early stages of the infection process

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POSTER NUMBER 17

Roads and Forest Plantations Affect Badger Occupancy in Intensive Mediterranean Farmland

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Understanding how to retain wildlife in agricultural landscapes has become a main endeavour to further promote and strengthen global biodiversity conservation efforts. Here we address this topic, focusing on the European badger (*Meles meles*) in Mediterranean farmland, where this generalist mammalian carnivore may occur at low density in landscapes dominated by intensive agriculture (and associated human infrastructure development), with little or no cover by its preferred habitats (native woodland and shrubland). Based on presence sign surveys conducted across SW Portugal, we test the hypothesis that badgers in these landscapes may be favoured by substitute refuge habitats and shelters such as forest plantations, while avoiding areas more heavily used by humans such as paved roads. Occupancy-detection models were used to quantify the effect of environmental variables on badger occupancy probability, while controlling for imperfect detection. Badger detection probability was positively affected by the density of dirt roads, possibly reflecting a preference of badgers to mark along those structures. According to predictions, badger occupancy significantly increased with the amount of forest plantations and arboreal hedgerows, while decreasing with increasing paved road density. Overall, these results suggest that the conservation of generalist carnivores such as the European badger in intensively used Mediterranean farmland requires the protection of areas with low paved road density, and the retention of wood cover, even where these are highly-modified habitats such as forest plantations and arboreal hedgerows.

Keywords: Agricultural landscapes; Carnivore conservation; Exotic tree plantations, Habitat loss and fragmentation; Hedgerows; Road effects

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Roads and planted forests affect badger occupancy in Mediterranean farmland

AGRO-SILVO-PASTORAL SYSTEMS

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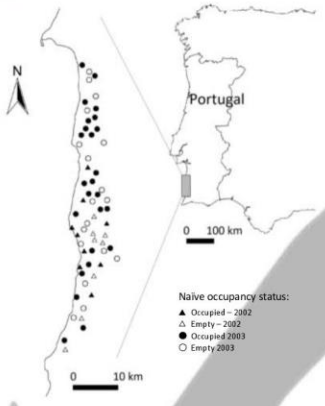
Introduction

- Expanding biodiversity conservation to human dominated landscapes has become a central issue in conservation biology. In particular, there is increasing interest in understanding how to retain wildlife in intensively used agricultural landscapes, where major declines in biodiversity have been identified worldwide (Haddad et al., 2015).
- Here we address this topic, focusing on the European badger (*Meles meles*) in intensively used Mediterranean farmland, where this generalist mammalian carnivore may occur at low density in landscapes dominated by intensive agriculture, with little or no cover by its preferred habitats (native woodland and shrubland).
- We hypothesised that badgers in these landscapes are favoured by substitute habitats such as forest plantations, while avoiding areas more heavily used by humans such as paved roads.



Methods

Study Area and Sampling Sites:

The study was conducted in SW Portugal, which is characterized by Mediterranean climate. The landscape is mostly devoted to agriculture and beef cattle production. Forest cover is largely restricted to some woodlots and hedgerows of planted eucalyptus and pines delimiting agricultural fields.

Surveys were conducted in 60 circular areas (sites) with 1km diameter (≈3.14 km²).

Badger and Land Use Surveys:

At each site, badger's typical presence signs were surveyed 3 times between March and September, along fixed transects totalling between 1.6 and 2.8 km (mean ± SD = 2.2 ± 0.2 km) per site (Pita et al., 2009).

We also estimated a set of explanatory variables describing land-use composition and configuration possibly affecting badgers (e.g. Virgós, 2001; Rosalino et al., 2008).

Analyses:

We used single-season occupancy-detection models (Mackenzie et al., 2002), to examine how landscape variables affected badger occupancy probability, while controlling for imperfect detection and the factors affecting it.

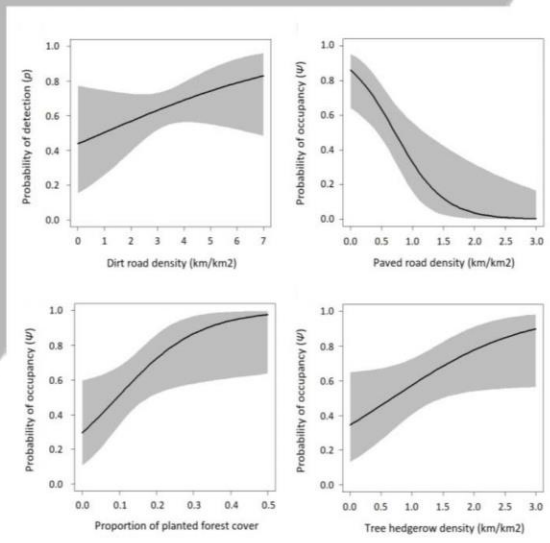
Variable	Mean ± SD	Range
Arable land (%)	67.1 ± 17.4	31 – 93
Planted forests (%)	15.4 ± 9.9	0.6 – 42
Semi-natural habitat (%)	14.8 ± 14.2	0.1 – 65
Urban areas (%)	2.0 ± 1.9	0 – 9.6
Mean patch size (ha)	3.9 ± 1.9	1.0 – 9.5
Tree hedgerows (km/km²)	1.2 ± 0.7	0.1 – 2.8
Shrub hedgerows (km/km²)	1.0 ± 0.8	0.1 – 4.5
Dirt roads (km/km²)	3.2 ± 1.1	1.4 – 6.2
Paved roads (km/km²)	0.5 ± 0.4	0 – 2.3

Results

A total of 120 badger presence signs were recorded in 34 of the 60 sites surveyed in at least one of the 3 sampling periods (naïve occupancy of 56.7%).

Mean+SD detection probability per survey was 64.7 ± 5.4%, while mean occupancy probability was 59.3 ± 6.8%.

The best model indicated a positive effect of dirt road density on badger detection probability. Occupancy was negatively effected by paved road density, and positively effected by planted forest cover and tree hedgerow density



Discussion

Our study provides evidence that where native woodland habitats are rare, planted forest woodlots and hedgerows may become important habitats for wood-dwelling generalists such as the European badger. Although we acknowledge that further research incorporating other variables not assessed here is needed, we believe our study may provide critical guidance for land-use management planning towards the retention of badgers and other generalist carnivore species in intensively used Mediterranean farmland.

Specifically, our results suggest that in intensively used Mediterranean farmland, planted forest cover above 10% may increase badgers occupancy probability to > 0.5, highlighting the likely functional importance of planted tree habitats in open farmland (Pita et al., 2009; Simonetti et al., 2013; Teixeira et al., 2017).

Likewise, we also found that tree hedgerow densities greater than 0.67km/km² likely contribute for increasing the chances of badger occupancy to > 0.5, confirming the idea that linear habitats are key elements for conserving medium-sized carnivores within the agricultural matrix (e.g. Pereira and Rodríguez, 2014).

Our study also provides a quantitative indication of paved road density effects on the likelihood of badger occupancy, showing that road density values above 0.71 km/km² were associated to probabilities of badger occupancy of <0 .5.

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FCT COMPETE 2020
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POSTER NUMBER 18

Study of the Relationship Between Animal Grazing, Soil and Pasture in the Ecosystem Montado

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The work associated with ECO-SPAA, a project to be held at Mitra farm (Évora University), has as its main objective the monitoring of the Montado ecosystem (ECO) by monitoring and studying the interactions between soil, pasture, trees and animals (SPAA). After the fourth year, and after correction and fertilization of the soil (2ha), the component related to the dynamic management of grazing will be implemented between November 2019 and June 2021. Thus, in two plots with about 2ha each, 12 trees (holm oaks) 6 were selected in a low area and 6 in a high area, 4 exclusion boxes, 2 under the canopy and 2 outside the canopy having been placed for each of the shafts. In one of the plots a soil pH correction was carried out, while in the other, the natural conditions of the soil were maintained, with only one annual fertilization with phosphate fertilizer, as in the first plot. The following measurements are taken every month (between September and June): 1) NDVI, which is correlated with the amount of chlorophyll and with the vegetative vigor of the pasture; 2) Capacitance, which is correlated with pasture production (Kg MS / ha); 3) Soil moisture; 4) Thermographic photographs, which measure the temperature of the pasture5) Pasture height; 6) Pasture crops, for percentage of dry matter, crude protein and NDF. In addition to these procedures, grazing is carried out by ewes, which are supplemented during lack of grazing periods (quantity and/or quality).
Following this, a doctorate will be held, with the following questions: 1) Why are there differences in pasture growth under and outside the canopies?; 2) After correction of soil pH there are differences in productivity under and outside the canopy?; 3) What is the productivity of animals in this system?; 4) What is the effect of grazing on pasture productivity?. We will have 4 plots, 2 we will have a fixed header and continuous grazing and in the other 2 there will be variable biotic loads and rotational grazing, depending on the pasture availability. To study the subject and try to answer the questions the methodology is as follows: 1) monthly performance of the measurements described above; 2) weighing and body condition of the sheep; 3) pasture bromatological analyzes. On the one hand, the soil-pasture interaction will be evaluated, on the other, the pasture-animal interaction.

Key words: Grazing; Pasture; Animals; Soil; Montado.

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Study of the relationship between animal grazing, soil and pasture in the ecosystem *montado*

AGRO-SILVO-PASTORAL SYSTEMS

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Introduction:

Montado is an agro-silvo-pastoral ecosystem, multifunctional, consisting of several subsystems and integrated and interdependent production systems. It is based on extensive production, that has been shown not aggressive to the environment, provided that the necessary rules are respected maintenance of equilibrium in the various subsystems, that make it up. The complexity associated with the *montado* results largely from the interaction between climate, the relief and the diverse elements that constitute this ecosystem: soil, pasture, trees and animals.

Hypotheses:

(i) Will there be differences in pasture growth and floristic composition under and outside tree canopy?
(ii) After correction of soil pH, will there be differences in productivity under and outside tree canopy?
(iii) Can the different pasture dynamics provide differences in the development cycle of the species that make up the pasture?
(iv) Can the different grazing pressure and periodic grazing allow for a higher accumulated nutritional value of the pasture?
(v) Can increased grazing pressure be advantageous in the balance between grazing species by reducing selectivity or instead reducing seed production with effects on subsequent development cycles?

Methodological Proposal:

Technological approach for monitoring soil-trees-pasture + animals

+

Animal component:

- Weighing and body condition; Fixed biotic load vs. variable biotic load;
- Continuous grazing vs. rotating grazing; Selective grazing vs. non-selective grazing.

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POSTER NUMBER 19

The use of GPS Collars to Manage Grazing Pressure

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Extensive grazing systems are an integrated combination of animals, soils, plants and procedures, used to address animal production in ever changing environments (Sales-Baptista et al., 2015). To manage such system for maximization of livestock production on a sustainable basis is challenging, as both overgrazing and undergrazing may occur and negatively impact the ecosystem. Grazing pressure, considering simultaneously the pasture availability, livestock numbers, and the time spent grazing in a given paddock, has been devised as a useful indicator to monitor grazing.
Global Navigation Satellite System (GNSS) technology enables livestock tracking and monitoring of position and movements of animals which may be used to produce maps of real grazing activity across time. This information can be used to estimate possible number of grazing days based on real paddock utilization rather than potential paddock utilization. Furthermore the identification of preferred grazing areas within a paddock may be used to derive site specific management actions, such as positioning of water sources or use of electric fencing.
In the present preliminary study, we combine monitoring of spatial behaviour of livestock with monitoring of pasture availability. We used 4 GPS collars (Digit Animal) to monitor a herd (48 cattle) movement across a 38ha paddock over the pasture growing season (2018) in Herdade da Mitra, Universidade de Évora. Data on pasture biomass availability as well as nutritive value were obtained and the number of grazing days estimated using the protein and energy requirements of the cattle herd as well as its spatial distribution. Cattle behaviour changed in response to pasture decline of nutritive value. Monitoring these two variables may assist producers to better manage grazing intensity and paddock utilisation.

Keywords: Grazing monitoring; GPS collars; Extensive grazing systems; Livestock tracking

Sales-Baptista E, D'abreu MC, Ferraz-de-Oliveira MI. 2016. Overgrazing in the Montado? The need for monitoring grazing pressure at paddock scale. Agroforesty Systems 90(1):57-68. DOI:10.1007/s10457-014-9785-3

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POSTER NUMBER 19

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A utilização de colares GPS para gerir a pressão de pastoreio no Montado

AGRO-SILVO-PASTORAL SYSTEMS

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1. AS QUESTÕES

1.1. Como é que os animais usam o espaço em pastoreio extensivo?

1.2. Qual o impacto que o pastoreio tem sobre o ecossistema Montado?

2. Enquadramento

✓Os sistemas de pastoreio extensivo

- Os sistemas de pastoreio extensivo são uma combinação integrada de solos, plantas, animais e decisões humanas.
- Gerir estes sistemas para maximizar a produção animal num contexto de sustentabilidade ambiental é um desafio.
- Tanto o sobrepastoreio como o subpastoreio afetam negativamente o ecossistema.
- A pressão de pastoreio é a relação entre o nº de cabeças normais e a disponibilidade de pastagem por unidade de área por unidade de tempo.
- A pressão de pastoreio é um bom indicador para monitorizar o pastoreio

✓Tecnologia GNSS para monitorizar animais

- A monitorização dos animais com recurso a colares GPS permite a obtenção imediata e precisa de informação sobre a sua localização espacial e temporal .

- **Esta informação pode ser utilizada para:**
  identificar zonas preferenciais de pastoreio
  identificar zonas não pastadas

3. Metodologia

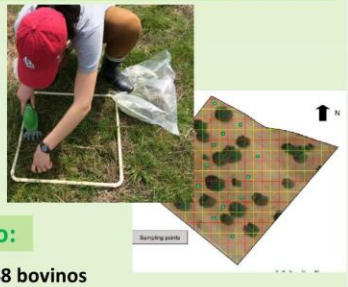
✓Monitorização da disponibilidade da pastagem:

Cerca com 38ha - (Herdade da Mitra)

Cortes de erva (Primavera 2018):

Biomassa (kg matéria seca/ha)

Valor nutritivo (proteina bruta, g/kgMS e fibra, NDF g/kgMS)



✓Monitorização do rebanho:

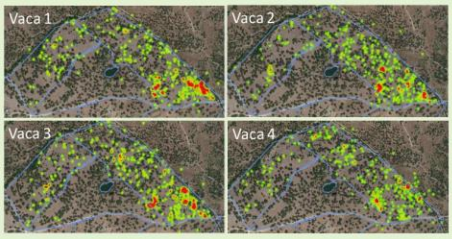
Rebanho de 48 bovinos
4 colares GPS
Colheita de posições (lat. e long. cada 30min)
Transmissão de dados via rede sigfox
Autonomia de cerca de 12 meses

✓Mapas de intensidade de ocupação

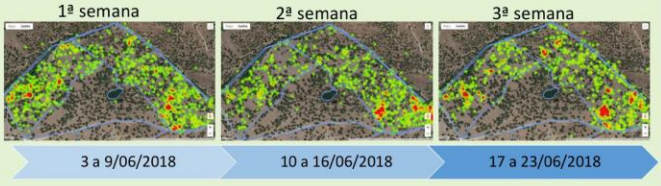
- Mapas construídos utilizando a função *heatmap* da API do Google Maps.
- A escala de cores indica o número de posições recolhidas numa vizinhança definida de cada ponto.

4. Resultados preliminares

Variação entre animais: Mapas de intensidade de ocupação individual- uma semana



Variação temporal: Mapas de intensidade de ocupação conjunta – três semanas consecutivas



Estimativa dos dias de pastoreio possível

- Disponibilidade de energia e proteina da pastagem
- Necessidades em energia e proteína dos animais

Energia							
Área (ha)	MS (kg/ha)	UFL (/kg MS)	UFL (/área total)	UFL Utilizáveis (50%)	Vacas (nº)	UFL Manutenção (/vaca/dia)	Pastoreio possível (dias)
38.2	1421.5	0.64	34 753	17 376	48	4.73	77

Proteína						
Area (ha)	MS (kg/ha)	PB (g/kg MS)	PB (kg/área total)	PB Utilizável (50%)	PB (nº)	PB (g/vaca/dia)	(dias)
38.2	1421.5	80.2	43548	21 774	48	444	102

5. Conclusões

A monitorização dos animais utilizando colares GPS permite fazer uma gestão mais informada do pastoreio

goBOV+ CYTED Precision Grazing

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POSTER NUMBER 20

Use of *In Vitro* Plant Cultures for the Production of High-value Phytochemicals

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Plants are very important sources of bioactive compounds with significance in many fields from medical to agrochemical. *In vitro* plant cultures under controlled conditions is a good option to produce valuable phytochemicals throughout the year with no seasonal restrictions. This method is particularly important in the case of rare or endangered species since it facilitates the mass production of plant material without the negative impact on natural ecosystems. Furthermore, several strategies can be applied to improve the production of bioactive compounds, namely changing medium composition and elicitation [1]. This work reports the effect of the addition of three different elicitors (yeast extract, salicylic acid, and silver nitrate) to the culture medium on phytochemical composition and antioxidant activity of extracts from *in vitro* regenerated shoots of *Lavandula viridis* L'Hér and *Thymus lotocephalus* G. Lopez & R. Morales (Lamiaceae), two wild species that produce bioactive phytochemicals [2, 3]. Total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively, and individual phenolics were analysed by liquid chromatography-photodiode array (HPLC-PDA). The antioxidant activity was measured by namely DPPH, ABTS, FRAP and ORAC assays. The valuable phenolic rosmarinic acid was the main compound found in the studied extracts and the three elicitors tested significantly stimulated the production of rosmarinic acid both in *L. viridis* (from 71.04 mg/$g_{extract}$ in medium without elicitors to 104.23 mg/$g_{extract}$ in medium with silver nitrate extract) and *T. lotocephalus* (from 48.61 mg/$g_{extract}$ in medium without elicitors to 78.57 mg/$g_{extract}$ in medium with yeast extract). On the other hand, the radical scavenging capacities as well as ferric reducing ability were improved by elicitation in *T. lotocephalus* but not in *L. viridis* extracts. The results of the present investigation perspectives plant *in vitro* culture as a good option to study secondary metabolism in Lamiaceace plants aiming the production of high-value phytochemicals.

Keywords antioxidants**;** elicitation; Lamiaceae; phenolics; rosmarinic acid

[1] T. Isah, S. Umar, A. Mujib et al., Plant Cell Tissue Organ Cult., 2018, 132, 239–265.
[2] P. Costa, S. Gonçalves, P. Valentão et al., Food Chem. Toxicol., 2013, 57, 69–74.
[3] P. Costa, S. Gonçalves, P. Valentão et al., Food Chem., 2012, 135, 1253–1260.

This work received financial support from the project INTERREG - MD.Net: When Brand Meets People. R. Rodríguez-Solana acknowledges the financial support from the Foundation for Science and Technology, Portugal (Grant SFRH/BPD/103086/2014).

POSTER NUMBER 20

Use of *in vitro* plant cultures for the production of high-value phytochemicals

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Introduction

Plants are very important sources of bioactive compounds with significance in many fields from medical to agrochemical. *In vitro* plant cultures under controlled conditions is a good option to produce valuable phytochemicals throughout the year with no seasonal restrictions. This method is particularly important in the case of rare or endangered species since it facilitates the mass production of plant material without the negative impact on natural ecosystems. Furthermore, several strategies can be applied to improve the production of bioactive compounds, namely changing medium composition and elicitation [1].

Figure 1. Aspect of the studied plants in the natural habitat.

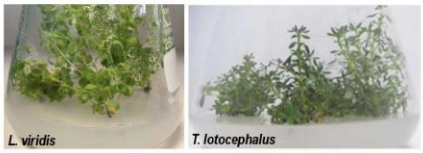
This work reports the effect of the addition of three different elicitors (yeast extract, salicylic acid, and silver nitrate) to the culture medium on phytochemical composition and antioxidant activity of extracts from *in vitro* regenerated shoots of *Lavandula viridis* L'Hér and *Thymus lotocephalus* G. Lopez & R. Morales (Lamiaceae) (Figure 1), two wild species that produce bioactive phytochemicals [2, 3].

Materials and Methods

In vitro culture treatments:

- Control without elicitor
- Yeast extract (500 mg/L)
- Salicylic acid (50 µM)
- Silver nitrate (50 µM)

Shoots were cultured in MS medium [4] with or without (control) elicitors under controlled conditions



Extraction of phenolics

Dried plant material was extracted with methanol by ultrasound-assisted extraction

Phytochemical analysis

- Total phenolic content (TPC, Folin-Ciocalteu assay)
- Total flavonoid content (TFC, aluminum chloride assay)
- Rosmarinic acid content (RAC, HPLC-PDA)

Antioxidant activity

- 2,2-Diphenyl-1-picrylhydrazyl (DPPH)
- 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)
- Ferric reducing antioxidant power (FRAP)
- Oxygen radical absorbance capacity (ORAC)

Results and Discussion

TPCs of extracts from shoot cultures regenerated *in vitro* in different culture media ranged between 195.27 ± 6.05 and 246.20 ± 10.65 $mg_{GAE}/g_{extract}$ in *L. viridis*, and 95.49 ± 3.34 and 118.34 ± 6.24 $mg_{GAE}/g_{extract}$ in *T. locephalus* (Table 1). This parameter was only significantly improved by yeast extract in *T. lotocephalus*. TFCs were not significantly affected by elicitation (Table 1).

Table 1. Total phenolic (TPC), flavonoid (TFC) and rosmarinic acid (RAC) contents in extracts from *L. viridis* and *T. lotocephalus* shoots cultured in different culture media.

Plant species/treatment	TPC ($mg_{GAE}/g_{extract}$)	TFC ($mg_{QE}/g_{extract}$)	RAC ($mg/g_{extract}$)
L. viridis			
Control	246.20 ± 10.65	68.27 ± 3.21	71.04 ± 0.06
Yeast extract	195.27 ± 6.05	65.00 ± 3.45	94.24 ± 0.48*
Salicylic acid	209.56 ± 21.73	63.41 ± 2.46	91.70 ± 0.78*
Silver nitrate	198.79 ± 11.77	73.45 ± 2.55	104.23 ± 0.59*
T. lotocephalus			
Control	95.49 ± 3.34	49.06 ± 1.08	48.61 ± 0.09
Yeast extract	118.34 ± 6.24*	54.92 ± 2.62	78.57 ± 0.99*
Salicylic acid	104.53 ± 1.66	54.99 ± 2.67	60.59 ± 0.60*
Silver nitrate	107.44 ± 4.75	50.96 ± 1.56	59.89 ± 0.40*

Values are expressed as mean ± SE (n = 3).
* Represents significantly higher in comparison with the control without elicitor (Dunnett's test, $P < 0.05$).

The HPLC analysis revealed the valuable phenolic compound rosmarinic acid (Figure 2) as the main compound of the studied extracts.

The three elicitors tested significantly stimulated the production of rosmarinic acid in both *L. viridis* (from 71.04 $mg/g_{extract}$ in medium without elicitors to 104.23 $mg/g_{extract}$ in medium with silver nitrate extract) and *T. lotocephalus* (from 48.61 $mg/g_{extract}$ in medium without elicitors to 78.57 $mg/g_{extract}$ in medium with yeast extract) (Table 1).

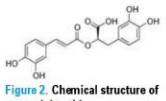


Figure 2. Chemical structure of rosmarinic acid.

Rosmarinic acid is a characteristic compound of Lamiaceae species, which possesses beneficial activities for human health, namely antioxidant, anti-inflammatory, antimicrobial and neuroprotective properties. The antioxidant activity of this compound is particularly recognized and associated to the presence of two ortho-dihydroxy groups in the molecule.

The DPPH and ABTS radicals scavenging capacities as well as ferric reducing ability was significantly improved by the three elicitors tested in *T. lotocephalus* (Figure 3). On the contrary, elicitation did no affect the antioxidant activity of *L. viridis*, nor the antioxidant capacity measured by ORAC assay in both studied species (Figure 3).

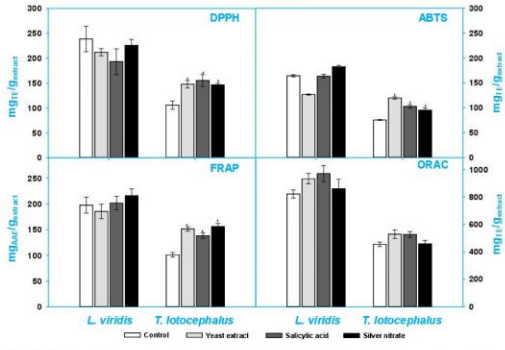


Figure 3. Antioxidant capacity determined by ABTS, DPPH, FRAP, and ORAC assays in extracts from *L. viridis* and *T. lotocephalus* shoots cultured in different culture media. Values are expressed as mean ± SE (n = 3). * Represents significantly higher in comparison with the control without elicitor (Dunnett's test, $P < 0.05$).

Conclusions

The results of the present investigation suggest *in vitro* culture as a good alternative to produce phenolic antioxidants from aromatic plants, although the results are species-dependent, and encourage more detailed studies including other elicitation treatments and other strategies.

Acknowledgements: This work is supported by National Funds from Fundação para a Ciência e a Tecnologia (FCT), through the project UID/BIA/4325/2013 and from the project INTERREG - MD.Net: When Brand Meets People. R. Rodríguez-Solana acknowledges the financial support from the FCT Portugal (Grant SFRH/BPD/103086/2014).

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[1] T. Isah, S. Umar, A. Mujib et al., Plant Cell Tissue Organ Cult., 2018, 132, 239–265.
[2] P. Costa, S. Gonçalves, P. Valentão et al., Food Chem. Toxicol., 2013, 57, 69–74.
[3] P. Costa, S. Gonçalves, P. Valentão et al., Food Chem., 2012, 135, 1293–1299.
[4] T. Murashige, F. Skoog, Physiol. Plant., 1962, 15, 473–497.

POSTER NUMBER 21

Using Sattelite Images to Estimate Pasture Quality

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Monitoring seasonal changes of pasture quality in wide areas of Montado is unrealistic by direct field measurements. Nevertheless, estimates of crude protein (CP) and fiber (NDF) content as indicators of nutritive value, are essential for an efficient grazing management. This information allow for a more precise use of supplementary feed, thus having a real impact on the farmer income. It will also allow to avoid overgrazing, highlighting trends within the grazing system. Remote sensing imagery offers distinctive advantages for monitoring pasture spatial and temporal patterns and can be a tool to identify the resilience of the Montado silvopastoral system to climatic changes. In this study, we investigated the relationship between CP, NDF and reflectance in the visible–near-infrared–shortwave infrared (VIS–NIR–SWIR) spectral range, using field, laboratory measurements, and satellite imagery (Sentinel-2).

Three plots of (20m x 20m) corresponding to 3 Sentinel-2 pixels, were demarcated in a Montado paddock (~0.4ha; 2.3 ha; 38◦32.20 N; 8◦01.10 W) with an understory of native pasture and holm-oaks. The experimental plots have been set outside the influence of the tree canopy. Field measurements were conducted from April 2018 to June 2019. Four samples were obtained in each plot, using a quadrant of 0.25 x 0.25 cm to clip aboveground grass, gathering twelve biomass samples per date. Pasture data (canopy height, pasture biomass (kg pasture/ha), and pasture quality (%CP and % NDF) were measured using AOAC methods (2005).

Modelling pasture power quality (PPQ) parameters were made using the algorithm Random Forest (RF) supervised classification. A variable selection approach was implemented to find fewer and uncorrelated bands resulting in the best model for each chemical parameter (CP and NDF). The best models found for predicting CP and NDF contents were based on reflectance measurements but they differ for CP and NDF (previous preliminary results were R2 = 0.76, RMSEP = 1.2% for CP; and R2 = 0.80, RMSEP = 2.8% for NDF). These models are better predictors of pasture quantity than quality (previous preliminary results of R2 = 0.69, RMSEP = 5544 kg/ha). Predictions will be further compared with different vegetation indices (e.g. Normalized Difference Vegetative Index-NDVI) calculated from the Sentinel-2 imagery to access indexes accuracy as proxies to CP and NDF. This study shows that among the normalized difference index (NDI) using hyperspectral data NDI 63 and 52 had the highest variable importance and that the best performing bands were on the red-edge.

Keywords: pasture quality; ecological quality; field spectroscopy; crude-protein (CP); neutral detergent fiber (NDF); Sentinel-2 time-series

This work was co-funded by National Funds through FCT—Foundation for Science and Technology under the Project UID/AGR/00115/2019

POSTER NUMBER 21

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Estimar a qualidade da **pastagem** com **Imagens de Satélite**

AGRO-SILVO-PASTORAL SYSTEMS

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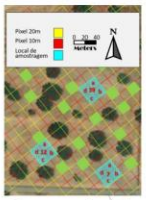
O problema: Grandes áreas

As pastagens são uma importante forma de uso da terra na Europa, cobrindo mais de um terço da produção agrícola europeia.

As pastagens são fundamentais na alimentação de herbívoros além de oferecerem serviços de ecossistemas, incluindo o controle de erosão, a gestão da água e o sequestro de carbono



Campo experimental

O maneio das pastagens, em conjunto com as condições climáticas locais, afecta a produtividade e a qualidade das pastagens e, portanto, a produção agrícola.

O objectivo:

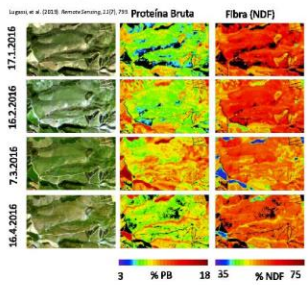
Investigar a utilidade do uso de dados obtidos com o Sentinel-2 (bandas originais e índices de vegetação) para prever a Proteína e Fibra usando medidas de campo e de laboratório para construir modelos estatísticos.

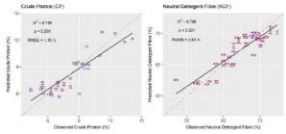
A solução: Detecção Remota

O programa Sentinel 2 da Agência Espacial Europeia compreende dois satélites que fornecem imagens com resolução espacial de 10m, a cada cinco dias.

Os dados oferecem a oportunidade de desenvolvimento de algoritmos robustos, que podem ser usados para monitorizar as pastagens de forma eficiente e rentável.

Os dados do Sentinel 2 serão capazes de capturar mudanças espaço-temporais na pastagem ?



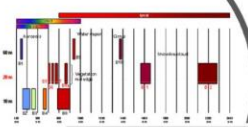


Precisão e exactidão

Processamento

Aquisição de Dados

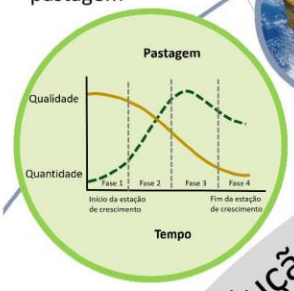
Resolução espectral



Sentinel-2 Bands	Central Wavelength (µm)	Resolution (m)
Band 1 - Coastal aerosol	0.443	60
Band 2 - Blue	0.490	10
Band 3 - Green	0.560	10
Band 4 - Red	0.665	10
Band 5 - Vegetation Red Edge	0.705	20
Band 6 - Vegetation Red Edge	0.740	20
Band 7 - Vegetation Red Edge	0.783	20
Band 8 - NIR	0.842	10
Band 8A - Vegetation Red Edge	0.865	20
Band 9 - Water vapour	0.945	60
Band 10 - SWIR - Cirrus	1.375	60
Band 11 - SWIR	1.610	20
Band 12 - SWIR	2.190	20

Cortes

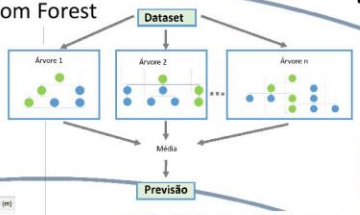
Variações na pastagem



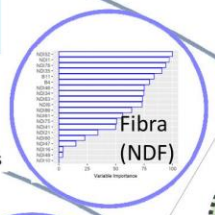
Sentinel 2 a/b

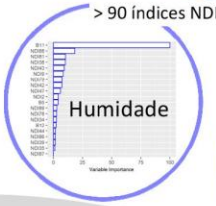
Avaliação Estimativa

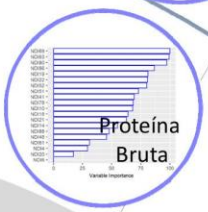
Random Forest



> 90 índices NDI testados







$r^2 = 0.80$
RMSEP = 2.8%

Fibra

Proteína Bruta

$r^2 = 0.76$
RMSEP = 1.2%

Introdução

É impossível monitorizar as alterações sazonais da qualidade de pastagens à escala regional, em vastas áreas do Montado, utilizando medições diretas no campo.

No entanto, utilizar estimativas do teor de proteína bruta (PB) e fibra (NDF) como indicadores de valor nutritivo é essencial para um eficiente maneio do pastoreio.

Material e Métodos

Período Experimental:
Abril de 2018 a Junho 2019.
12 amostras de biomassa por data

Amostragem da Pastagem:
Pastagem natural sob coberto.
3 parcelas de (20m x 20m) correspondentes a 3 pixeis do Sentinel-2

Séries Temporais:
5 em 5 dias. 90 bandas. Algoritmo Random Forest

Valor Alimentar:
Biomassa da pastagem.
Proteína e fibra analisados pelos métodos AOAC (2005).

Resultados

O algoritmo Random Forest (RF) foi utilizado para avaliar a relação entre PB e NDF e as variáveis preditoras derivadas dos dados do Sentinel-2.

Verificou-se que os dados do Sentinel-2 podem ser usados para prever com precisão os valores de PB e NDF num ecossistema complexo

Conclusões

De entre os mais de 90 índices de diferenças normalizadas (NDI) testados, os determinados usando bandas na zona do vermelho foram os que tiveram maior importância relativa dentro do modelo Random Forest.
A previsão da qualidade da pastagem a partir do modelo é superior comparada com a previsão da biomassa .

A reter:

As imagens de satélite são uma ferramenta promissora para identificar a **resiliência do sistema** face às mudanças espaço-temporais induzidas pelo homem e pelo ambiente.

Precision Grazing

CYTED

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POSTER NUMBER 22

What is In a Name? Solving Taxonomic Issues with Practical Relevance

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Desert truffles (mycorrhizal hypogeous *Ascomycota*) are found in arid and semi-arid areas of the globe and have great ecological and economic importance. *Terfezia* is undoubtedly the most diversified of all desert truffle genera, but its taxonomy is far from resolved. Specifically, the large number of newly described species [e.g. 1,2] plus the high intraspecific morphological variability observed within some *Terfezia* lineages as rendered the use of molecular techniques mandatory for specimen's discrimination. By compiling and using the public available ITS data on *Terfezia* spp. on the custom-curated UNITE database to reconstruct the genus phylogeny, at least 17 distinct lineages within the genus were found [3]. The work lead to the successful resolution of some of the more pressing taxonomic issues, namely the unveiling of several lineages hidden within the *T. leptoderma/olbiensis* complex and the misapplied synonymy between *T. trappei* and *T. cistophila*.
Although molecular techniques are valuable tools to discriminate species, they should be always complemented with specimen's morphological and ecological description. To the best of our knowledge, we proposed a first identification key to *Terfezia* genus, considering the new resolved phylogeny [3] and highlighting the importance of morphological and ecological characterization.
What is in a name? Accurate *Terfezia* species determination is important for our understanding of the ecological functioning of the system (e.g. essential role in soil conservation), and is crucial if we consider their economic significance for the rural populations on the Mediterranean basin. Desert truffles fruit bodies are a potentially important food source for animals and humans, rich in proteins and poor in carbohydrates and lipids. Plus, given the considerable prices they may reach in local markets, their cultivation has the potential to enhance the socio-economic development of rural and/or local populations around the Mediterranean basin.

Keywords *Terfezia*, taxonomy, UNITE, Mediterranean basin, ecology, desert truffles,

Bordallo JJ, Rodríguez A, Santos-Silva C, Louro R, Muñoz-Mohedano J, Morte A, *Terfezia lusitanica*, a new mycorrhizal species associated to *Tuberaria guttata* (*Cistaceae*). Phytotaxa, 2018. 357(2): 141–147.; Zitouni-Haouar F, Carlavilla JR, Moreno G, Manjón JL, Fortas Z, Genetic Diversity of the Genus *Terfezia* (*Pezizaceae, Pezizales*): New Species and New Record from North Africa. *Phytotaxa*, 2018. 334(2): 183–194; Louro R, Santos-Silva C, Nobre T, What is in a name? *Terfezia* classification revisited. Fungal biology, 2019. 123(4), 267–273.

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POSTER NUMBER 22

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WHAT IS IN A NAME?
Solving Taxonomic Issues With Practical Relevance

AGRO-SILVO-PASTORAL SYSTEMS

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Desert truffles (mycorrhizal hypogeous Ascomycota) are found in arid and semi-arid areas of the globe. They are not only a key component of the mycological flora on the Mediterranean basin but also economically significant for the local rural populations.

Desert truffles fruit bodies are a potentially important food source for animals and humans, rich in proteins and poor in carbohydrates and lipids. Plus, given the considerable prices they may reach in local markets, their cultivation has the potential to enhance the socio-economic development of rural and/or local populations around the Mediterranean basin.

Terfezia is undoubtedly the most diversified of all desert truffle genera, but its taxonomy is far from resolved.

Approach: We compiled and used the public available ITS data on Terfezia spp. on the custom-curated UNITE database to reconstruct the genus phylogeny and we confronted the results with putative plant host and soil parameters associated with the different specimens, whenever available. We proposed an identification key to *Terfezia* genus highlighting the importance of morphological and ecological characterization.

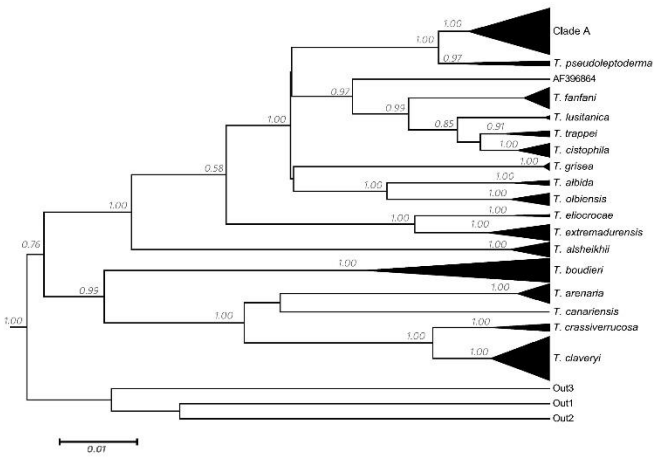
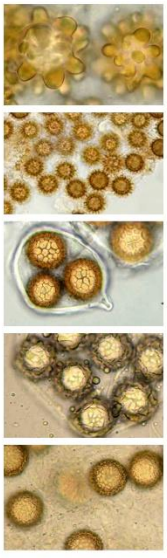


Fig. 1. Phylogenetic relationship between *Terfezia* species. The phylogeny corresponds to the majority rule consensus tree of trees sampled in a Bayesian analysis, and the posterior probability values are shown for main nodes.



Key to *Terfezia* species

(Ascocarps measurements do not include ornamentations)

1 Spiny spores 2
Warty or warty reticulated spores 11

2(1) In alkaline soils 3
In acid soils 5

3(2) With *Cistaceae*; spores ≥14 µm with blunt and/or truncated spines 4
With *Pinus* and/or *Quercus*; spores <14 µm with pointed spines ..... *olbiensis*

4(3) Peridium light colour; gleba with green colours ..... *albida*
Peridium dark colour; gleba without green colours ..... *grisea*

5(2) Ascocarp diameter >4.5 cm 6
Ascocarp diameter <4.5 cm 7

6(5) Ascocarp brown-reddish; spores with straight pointed spines ..... *fanfani*
Ascocarp brown; spores with conical (≈3-4 µm at base) blunt and truncated spines ..... *extremadurensis*

7(5) Gleba without green colours; with *Cistaceae* 8
Gleba with green colours; with *Cistaceae*, *Pinus* or *Quercus* 9

8(7) Spores ≥15 µm with flexuous blunt spines; with *Cistaceae* ..... *pseudoleptoderma*
Spores <15 µm with straight spines; with *Cistus* exclusively ..... *cistophila*

9(7) Peridium <1 mm 10
Peridium ≈1 mm ..... *trappei*

10(9) With *Pinus* and/or *Quercus*; spores with spines >4 µm long ..... *pini*
With *Tuberaria guttata*; spores with spines <4 µm long ..... *lusitanica*

11(1) In alkaline 12
In acid soils 16

12(11) Spores with warts making a complete and clear reticulum 13
Spores with warts sometimes forming an incomplete reticulum 14

13(12) Gleba strong pink; spores ≥18 µm ..... *canariensis*
Gleba whitish; spores <18 µm ..... *eliocrocae*

14(12) Spores <22 µm 15
Spores ≥22 µm ..... *boudieri*

15(14) Asci with 6-8 spores; spores with spines >1.5 µm long ..... *claveryi*
Asci with 4-6 spores; spores with spines <1.5 µm long ..... *crassiverrucosa*

16(11) Ascocarps >2 cm; spores warty without reticulum ..... *arenaria*
Ascocarps <2 cm; spores warty with a complete reticulum ..... *alsheikhii*

Research highlights

- *Terfezia* classification is updated and pressing taxonomic issues were solved
- applied synonymy between *T. trappei* and *T. cistophila* seems incorrect
- several lineages are hidden within *the T. leptoderma/olbiensis* complex
- an identification key to *Terfezia* genus is now available
- absence of geographic and ecological data greatly hinders identification

More in: Louro, R et al., What is in a name? Terfezia classification revisited, Fungal Biology, https://doi.org/10.1016/ j.funbio.2019.01.003

Instituto de Ciências Agrárias e Ambientais Mediterrânicas
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POSTER NUMBER 23

Km0 Alentejo - Short Circuits in extended partnership promoted by ICAAM

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Km0 is an initiative promoted by ICAAM within the framework of the SALSA project advisory group - small farms, small food businesses and sustainable food and nutrition security. The Regional Tourism Entity (ERT), the Commercial Association of Évora (ACDE), the Évora Regional Business Centre (NERE), intermunicipal waste management company (GESAMB), Évora City Council, are currently involved in the project, in addition to ICAAM, in a group of 8 entities. A certification company will guarantee these short supply chains where the producer and the sale to final consumer are no longer than 50 km, in a straight line, and in which we have no more than one intermediary between the two. With this initiative, the different entities intend to promote local production, to support small and medium farmers, local varieties and authenticity of the regional offer to tourism. The Km0 has also the goal of a reduction in the carbon footprint of our food at the same time that its nutritional content is preserved. Although this project fits more within the ambit of ICAAM's social responsibility towards society, it will also allow a series of studies on the dynamics provoked on food systems and on the impacts on regional environment and economy. It is still an extremely innovative process, with a strong replication potential to other regions.

Keywords: Short food supply chains, Km0, social innovation, social responsibility.

The authors are grateful for the generous involvement of all the partner entities, and the financial support from the Évora Regional Business Center, the Regional Tourism Entity and the Évora City Council.

POSTER NUMBER 23

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Mediterranean Institute for Agriculture, Environment and Development

Km0 Alentejo - Short supply chains in a broad partnership promoted by ICAAM

RURAL DEVELOPMENT

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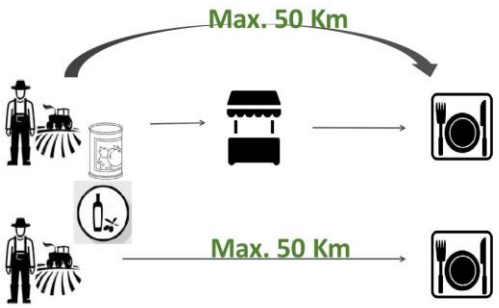
km.0

Km0 is an initiative promoted by ICAAM within the framework of the SALSA project advisory group - small farms, small food businesses and sustainable food and nutrition security.

Évora, a cultural heritage city in the heart of Southern Portugal, where the Mediterranean diet, linked to traditional recipes and local products, reveal the identity of its territory. The Km0 label ensures that recipes are made with local products, supporting local agriculture and economies and having lower impact in the environment.



MAIN LINES:

- A certification company will guarantee the short supply chains where the producer and the sale to final consumer are no longer than 50 km, in a straight line.
- There is no more than one intermediary between producer and final consumer.
- Contribution to the reduction of our food carbon footprint.
- This project fits more within the ambit of ICAAM's social responsibility.

With this initiative, the different entities intend to promote local production, to support small and medium farmers, local varieties and authenticity of the regional offer to tourism. The Km0 has also the goal of a reduction in the carbon footprint of our food at the same time that its nutritional content is preserved. Although this project fits more within the ambit of ICAAM's social responsibility towards society, it will also allow a series of studies on the dynamics provoked on food systems and on the impacts on regional environment and economy. It is still an extremely innovative process, with a strong replication potential to other regions.

Find Km0 establishments at www.km0alentejo.pt

POSTER NUMBER 24

PASTOR.I: A Smartphone Application to Facilitate Grazing Management

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Grazing in extensive beef farming systems is often manage in an empirical way based on past experience and on the visual appreciation of animal behavior and forage potential. Records of entrances and exits of the animals in the paddocks in a regular basis are rare. However, knowing the occupation period and the animal density, when coupled with biomass defines the grazing pressure and carry capacity. This knowledge is essential for planning and making informed decisions, that influence the profitability of the farm. Moreover, adequate grazing pressure is crucial for the sustainability of many SSPs where system maintenance is dependent on the balance between grazing pressure and regeneration or maintenance of trees and shrubs.

Pastor.i is a smartphone application (APP) designed to allow pasture data logging to be very simple. The application is synchronized with the website and allows the producer to have in his pocket all the farm, being possible to identify the paddock, calculate the area, record the movements of the animals and consult the occupation history of the paddock. The application calculates the actual stocking rate, that can be associated with the location of the animals, obtained if the animals are using collars with GPS, which allows to know the areas of the paddock that are most grazed, visualized through heat maps. The information enables localized actions, such as fertilizing or sowing, to improve areas that are not grazed. The application also allows you to save photos of the sward. This temporal photographic record provides information on the condition of trees, the botanical composition and on the tendency of grazing to improve or to worsen coverage. The APP is available for download, is compatible with Android and is being tested with focus groups.

Keywords: stocking rate, ruminants, silvopastoral systems, overgrazing

This work was co-funded by National Funds through FCT—Foundation for Science and Technology under the Project UID/AGR/00115/2019

POSTER NUMBER 24

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Mediterranean Institute for Agriculture, Environment and Development

Pastor.i

AGRO-SILVO-PASTORAL SYSTEMS

Uma Aplicação para Smartphone que Facilita a Gestão do Pastoreio

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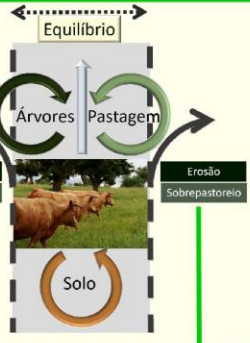
O Sistema Silvopastoril

A coabitação de pastagens e florestas num ecossistema silvopastoril não é fácil de preservar.



As actividades pecuárias são o principal factor que influencia a dinámica das comunidades de plantas forrageiras e de árvores.

Pressão de pastoreio

O problema

Gestão empírica do pastoreio baseada em:

- Experiência prévia
- Aprecisação visual do comportamento dos animais
- Observação do potencial forrageiro

Não é uma práctica usual registar as entradas e as saídas dos animais quando mudam de cerca

Registo

O objectivo

Uma Aplicação para Smartphone

Faz com que os dados sejam fáceis e rápidos de registar

Ter a capacidade de gerir o pastoreio na palma da mão.

- armazenar e aceder aos seus dados em qualquer lugar
- manter uma base de dados
- tomar decisões fundamentadas

APP + Web Site

Abordagem metodológica

Localização

Localização das cercas

A marcação dos limites das cercas pode fazer-se com um dedo, no smartphone, ou usando o rato, no computador e usando a página de internet, que está sincronizada com o smartphone.

Dados das cercas

Bebedouros e portas

1

Pastagem

Histórico das cercas

Cronologia dos acontecimentos passados
Pode saber-se os dias de ocupação da cerca e o encabeçamento

Arquivos de fotos

Guardar as fotos permite comprovar as tendências de mudança ao comparar os mesmos lugares da pastagem em diferentes datas e anos

2

Animais

Cálculo de Encabeçamento

Só se tem de atribuir uma nova cerca ao rebanho para que fique registrado a entrada e a saída das parcelas de pastoreio.

Mapas de calor

Se as vacas tiverem colares com GPS produzem-se mapas de uso

3

Como vamos testar?

Prova de funcionalidade:

Projeto I&D:
Grupos de foco
4 herdades
~ 1100 ha
~ 500 animais

Prova de utilização:

O sistema será avaliado, adaptado e melhorado, para possibilitar a futura expansão a mais produtores

Executa as tarefas para que foi projectada?
Como se comporta em diferentes contextos e circunstâncias?
A aplicação é rápida e intuitiva ou lenta e frustante?
A navegação é simples e clara?

Vantagens

Mais informação

Identifica zonas e cercas sobre e subpastoreadas

É um apoio informado às decisões que são necessárias para aumentar a eficiência sustentável do sistema: por exemplo nas zonas não utilizadas pelos animais pode recuperar-se pastagens degradadas

Riscos

Falta de fidelidade

Os utilizadores de telemóveis descarregam em média 8,8 aplicações por mês. Mas segundo o Google, 26% das aplicações são utilizadas só uma vez.

O objectivo é que os produtores disfrutem quando utilizam a aplicação e a mantenham como parte integrante da suas actividades diárias.

Versão preliminar compatível com Android

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UID/AGR/00115/2013; ALT20-03-0246-FEDER-000022; H2020 HNV-Link GA:696391; CYTED_Ecogram Red 517RT0530

POSTER NUMBER 1

Biocontrol Services Provided by Birds and Bats as Incentives for their Conservation in Olive Groves

Jiménez-Navarro, Gerardo[1], Javier Rodríguez-Pérez[1], José M. Herrera[2]

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Understanding the negative consequences of agricultural intensification on biodiversity is a major concern in ecology. In this way, agroecological research aims to enhance biodiversity conservation in agricultural ecosystems by providing alternative, environmentally sustainable, management practices [1]. Such is the case of pest control carried out by natural enemies (biocontrol services), that offers a sustainable solution to conventional pest management, opening new incentives up for biodiversity conservation [2].

In Portugal, and in the Alentejo region in particular, the conservation management of agroecosystems based on the role of biodiversity is specially urgent in olive (*Olea europaea*) groves because it represents an ever increasing share of land-surface and management intensification [3]. However, this crop expansion and intensification could be associated with a significant decline of bird and bat communities, in s/uch a way that most Iberian species are included as threatened in red lists. Moreover, despite their acknowledged role in pest regulation in other crops [e.g., 4], the potential biocontrol services that birds and bats could provide in olive groves remains actually unclear. Is the intensification of olive groves affecting bird and bat distribution patterns? Which species are more sensitive to management intensification? Could these species provide biocontrol services in olive groves? Does management intensification compromises the economic profitability of crops at the expense of biocontrol services provided by these groups? By gathering these key gaps, this thesis will deliver many insights of direct relevance for a more sustainable olive farming.

In this context, a basic draft of this doctoral thesis will be presented, whose overarching goal is the ecological and economic assessment of the biocontrol services provided by birds and bats in olive groves.

More specifically, the thesis will focus on the potential impact of these vertebrates on two major insect olive pests worldwide, the Olive fruit fly (*Bactrocera oleae*) and the Olive fruit moth (*Prays* oleae). I will explain the specific objectives of the thesis and some of the first preliminary results obtained until now.

Keywords: biodiversity conservation; birds and bats; biocontrol services; management intensification; olive groves

This work was funded by the Portuguese National Funding Agency for Science, Research and Technology (FCT) through the project PTDC/AAG-REC/6480/2014 and the predoctoral fellowship SFRH/BD/133017/2017. The project ALT20-03-0145-FEDER-000008 provided some support.

POSTER NUMBER 1

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Biocontrol services provided by birds and bats in olive groves as incentive for their conservation

Gerardo Jiménez Navarro[1], Javier Rodríguez Pérez[1], José M. Herrera[2]
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[2]ICAAM – Institute of Mediterranean Agricultural and Environmental Sciences, University of Évora

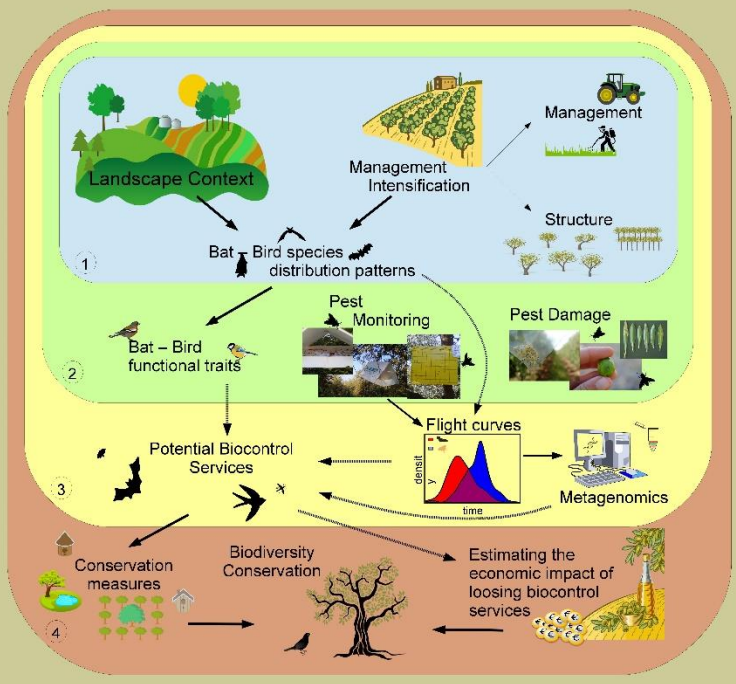
AGRO-SILVO-PASTORAL SYSTEMS

Olive (*Olea europaea*) is currently one of the most widespread crops along the Mediterranean basin, and current trends predict an ever increasing share of land-surface devoted to this crop, as well as an intensification of management practices [1,2]

This crop expansion and intensification could drive a significant impoverishment of bird and bat communities

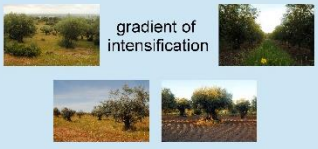
Overarching goal of the thesis
an ecological assessment of biocontrol services provided by birds and bats in olive groves along a gradient of management intensification. More specifically, to determine their impact on two major insect pests, the Olive fruit fly (*Bactrocera oleae*) and the Olive fruit moth (*Prays oleae*)

1. Estimation of the impact of management intensification (management practices and structural alterations within groves) in olive farms and landscape context on the community structure of birds and bats
2. Search for possible relationships between insect pest populations and the damage they cause, and the occurrence of bird and bat species that could act as potential biocontrol agents using functional traits
3. Evaluation of bird and bat species as potential biocontrol agents of *P. oleae* and *B. oleae* by developing flight curves of vertebrates and insects and by estimating pest presence in diet composition (using metagenomic techniques)
4. Estimation of the economic impact of biocontrol services loss through infestation rates
   Knowing the management practices affecting bird and bat species will help to provide effective strategies to promote biodiversity conservation in olive groves



1

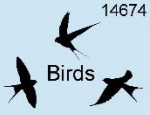
60 sampling points



Field surveys in Spring, Summer and Autumn

Audio-visual stations during 10 min (two rounds per season)

73 species
14674 individuals

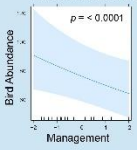


Landscape Context (1000 m radius)
- Naturalness Index: Natural > Subnatural > Agricultural > Urban
- % Water

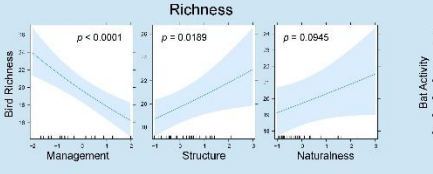
In Farm Context
- Management Index: chemicals inputs, machinery, tillage, pruning,...
- Structural Index: tree density, DBH, tree height, canopy area,...

Preliminary Results

Total Abundance



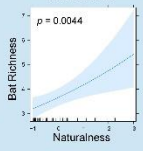
Richness



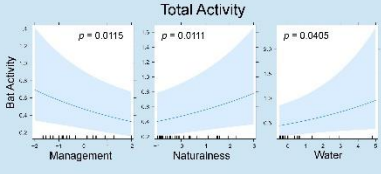
Automated acoustic monitoring during three consecutive nights

18 species / species groups
4252 bat calls (activity)

Richness



Total Activity



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1. Neves, B., Pires, I. M. (2018). The mediterranean diet and the increasing demand of the olive oil sector: shifts and environmental consequences. *REGION*, 5(1), 101-112.
2. Eurostat (2019). Agricultural Production - Orchards. Available online:https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_crops#Olives

Aknowledgements
This work was funded by the Portuguese National Funding Agency for Science, Research and Technology (FCT) through the project PTDC/AAG-REC/6480/2014 and the predoctoral fellowship SFRH/BD/133017/2017. The project ALT20-03-0145-FEDER-000008 provided some support. All pictures belong to Nereida Melguizo Ruiz.

POSTER NUMBER 2

Characterization of Extra Virgin Olive Oils Phenolic Profile: Impact of Different Portuguese Olive Cultivars

M. Ferro [1,2], MM. Castro[1], A. Cordeiro[3], MT. Carvalho[3], C. Inês[3]; A. Peixe[2], MF. Duarte [12]

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The phenolic profile characterization and quantification of Portuguese extra virgin olive oils (EVOO) is nowadays a differentiating factor, and an important valorization tool, mainly after the approval of the health claim by the European Food Safety Authority. Our research group has been working in the monovarietal EVOO phenolic profiles. OLEAVALOR project, led by University of Évora, aims the valorization of seven Portuguese olive cultivars: 'Galega Vulgar', 'Cobrançosa', 'Verdeal Alentejana', 'Cordovil de Serpa', 'Azeiteira', 'Blanqueta' and 'Carrasquenha de Elvas'. Each monovarietal EVOO was obtained by ABENCOR and analyzed by HPLC-UV with Kinetex Biphenyl column [1]. The phenolic profile of these EVOO were compared for three consecutive years. According to the results, in the first two years, Oleuropein concentrations were higher in 'Carrasquenha de Elvas' and 'Azeiteira', inversely proportional to the concentrations of Hidroxytyrosol and Tyrosol, with higher amounts found in 'Cordovil de Serpa' and 'Verdeal Alentejana'. In the third year, the results show a general decrease on Oleuropein concentrations, whereas for Tyrosol and Hidroxytyrosol both 'Azeiteira', 'Verdeal Alentejana' and 'Blanqueta' cultivars presented the higher concentrations. Simultaneously, for the second year of production, phenolic profile of these samples was also evaluated for the shelf time, in order to monitor the chemical stability over time, under controlled conditions. Other physical and chemical parameters were also evaluated. In sum, this work strongly suggest that the variability of the EVOO phenolic profiles contributes for the oxidative stabilization, and consequently, organoleptic stabilization.

Keywords Extra Virgin Olive oil; Portuguese olive cultivars; phenolic profiles; HPLC-UV Kinetex Biphenyl column

1. Ferro MD, Santos SAO, Silvestre AJD, Duarte MF. Chromatographic separation of phenolic compounds from extra virgin olive oil: development and validation of a new method based on a biphenyl HPLC column. International Journal of Molecular Sciences. 2019;20(1):201-14.

This project was funded by European Regional Development Fund (FEDER) under the Alentejo 2020 to OleaValor (ALT20-03-0145-FEDER-000014)—Valorização das Variedades de Oliveira Portuguesas. Authors also acknowledge FCT for PhD grant to Miguel Ferro (SFRH/BD/140083/2018).

POSTER NUMBER 2

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Characterization of extra virgin olive oils phenolic profile: Impact of different Portuguese olive cultivars

OLIVE GROVES
OLIVE OIL

M. Ferro [1,2], MM. Castro[1], A. Cordeiro[3], MT. Carvalho[3], C. Inês[3]; A. Peixe[2], MF. Duarte[1,2]

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[3]Instituto Nacional de Investigação Agrária e Veterinária, I.P., UEIS Biotecnologia e Recursos Genéticos, Estrada de Gil Vaz, Apartado 6, 7351-901 Elvas, Portugal

Introduction

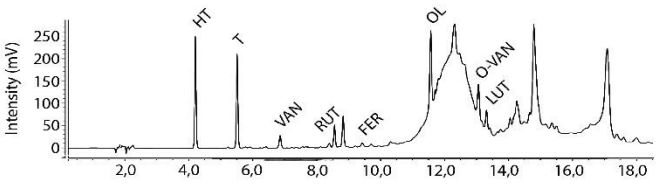
Portuguese extra virgin olive oil (EVOO) production has been increasing during the past decade being recognized by its high quality. As a differentiating factor, the characterization and quantification of the EVOO phenolic profile is nowadays a strong valorization tool, mainly after the approval of a health claim by the European Food Safety Authority[1] which associates virgin olive oil polyphenols consumption to the protection of blood lipids from oxidative stress.

The information regarding the phenolic profile of traditional Portuguese EVOO is not very vast,[2] within the present work we intend to compare EVOO phenolic profile (from the main traditional Portuguese cultivars, produced under the same weather-climate conditions for three consecutive years).

Aim: To valorize traditional Portuguese olive cultivars, by their chemical profile regarding the extra virgin olive oil phenolic composition

Results & Discussion

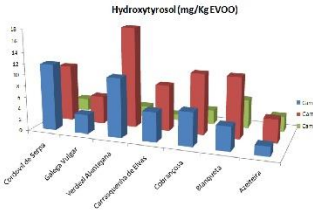
EVOO PHENOLIC PROFILE

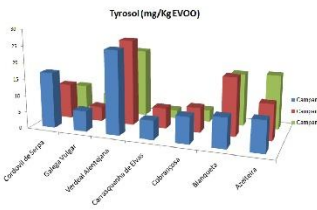


Phenolic compounds identified and quantified in monovarietal EVOO(Cordovil de Serpa) by HPLC.
HT: Hydroxytyrosol; **T**: Tyrosol; **VAN**: Vanillic Acid; **RUT**: Rutin; **FER**: Ferulic Acid; **OL**: Oleuropein; **O-VAN**: O-Vanillin; **LUT**: Luteolin.

- A total of 8 phenolic compounds were identified in all years, as well as after six months of storage under controlled conditions (22ºC and 16h/day/light and 8h/day/dark)

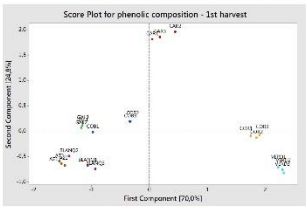
MAIN PHENOLIC COMPOUNDS QUANTIFICATION OVER THREE CONSECUTIVE YEARS





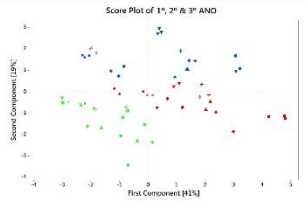
- The third year presented overall lower amounts of HT;
- For three years of production a similar pattern regarding T was observed;
- 'Azeiteira' and 'Galega Vulgar' showed to be the two cultivars with lower HT concentrations, while regarding T 'Galega Vulgar' and 'Carrasquenha de Elvas' presented the lower amounts;
- 'Cordovil de Serpa' and 'Verdeal Alentejana' presented the highest amounts of HT and T on the two first years. On the third year 'Verdeal Alentejana' presented also the higher amounts of T, but for HT 'Blanqueta' showed better results.

EVOO PCA ANALYSIS OVER THE THREE YEARS



PCA analysis of phenolic compounds quantified from monovarietal EVOO of 1st of harvests.

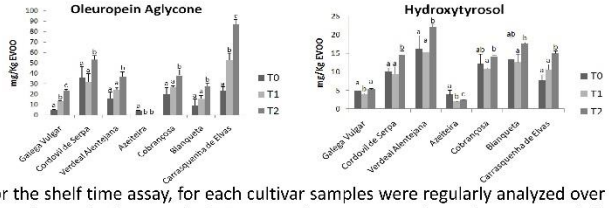
GAL: 'Galega'; **COR**: 'Cordovil de Serpa'; **CAR**: 'Carrasquenha de Elvas'; **COB**: 'Cobrançosa'; **VERD**: 'Verdeal Alentejana'; **BLANQ**: 'Blanqueta'; **AZ**: 'Azeiteira'.



PCA analysis of phenolic compounds and other chemical properties, such as acidity, K232 and K270, quantified from monovarietal EVOO over the three years.
BLUE: 1st year; **RED**: 2nd year; **GREEN**: 3rd year

- On the first year all varieties form separate clusters, only with "COB1" and "BLANQ2" located on a different region;
- From the three years analysis we may observe that overall three main clusters were formed, corresponding one for each harvest.

PHENOLIC PROFILE VARIATION OVER SIX MONTHS SHELF TIME



- For the shelf time assay, for each cultivar samples were regularly analyzed over a total period of 6 months (**T0**: 0 months; **T1**: 3 months and **T2**: 6 months), under controlled conditions: 22ºC and 16h/day/light and 8h/day/dark;
- For all cultivars a significant increase of Oleuropein Aglycone was observed, except for 'Azeiteira'.
- As for HT, the concentrations were maintained constant through time, apart from 'Cordovil de Serpa', 'Verdeal Alentejana' and 'Carrasquenha de Elvas' that presented slight increases of their HT concentration over time.

Conclusions

- ✓ For three consecutive harvests, some differences on EVOO phenolic profiles were observed, mainly regarding HT, showing general decrease on 3nd year;
- ✓ 'Verdeal Alentejana' presented the highest amounts of T for all three years;
- ✓ Over six months of shelf time, a general increase on Oleuropein Aglycone concentrations was observed, wile for HT a more stable pattern was observed;
- ✓ EVOO presented considerable variability over the three different harvests
- ✓ Exists a wide variability of the phenolic profile among the different EVOOs

References

[1] European Commission. Commission Regulation (EU) No 432/2012; 2012.
[2] Peres, F.; Martins, L. L.; Mourato, M.; Vitorino, C.; Antunes, P.; Ferreira-Dias, S. Food Chem. 2016, 211, 51–58.

Acknowledgments

This project was funded by European Regional Development Fund (FEDER) under the Alentejo 2020 to OleaValor (ALT20-03-0145-FEDER-000014)—Valorização das Variedades de Oliveira Portuguesas. Authors also acknowledge FCT for PhD grant to Miguel Ferro (SFRH/BD/140083/2018). Authors are also grateful to FCT–Fundação para a Ciência e a Tecnologia for the financial support to MED Research Unit (FCTUID/AGR/00115/2019).

POSTER NUMBER 3

Chemical Characterization of Olive Oil of four Traditional Portuguese Varieties from Two Consecutive Crops

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The olive branch is a strategic line in Portuguese agricultural and economic policy. Worldwide, production has been maintained and, as far as the European Union is concerned, olive oil production has grown significantly in recent years, with Spain first in the world ranking of producing countries followed by Greece, Italy and Portugal, which remains in fourth place in Europe (IOC, 2018). This trial is a part of the OLEAVALOR project, which aims at the valorisation of Portuguese olive tree varieties.
Chemical characteristics of olive oil from four traditional Portuguese olive varieties, “Azeiteira”, “Cobrançosa”, “Blanqueta” and “Galega” at harvest (Maturation Index - MI - between 2,3 and 4.7) were analysed.
Acidity (A), Peroxides Index (PI), Oxidative Stability (OS) and UV spectrophotometric indices (K232, K270 and ΔK) were evaluated.
Results for similar MI and varieties were higher in the 2017 crop for PI and lower for OS. Maturation date was identical in the two years for the same varieties, with “Blanqueta” being comparatively earlier than other varieties in 2018. With regard to spectrophotometric indices, there are similar values for all varieties at study.
When comparing results from 2017 and 2018, an influence of the crop year on the chemical characteristics and stability of olive oils of these varieties was found. Further research is necessary for the evaluation of the influence of climate conditions on the quality characteristics of olive oil from these varieties.
The experimental trial was financed by the EAFRD and the National Funds through the Regional Operational Program ALENTEJO 2020, Operation ALT20-03- 0145-FEDER- 000014 - "Valuation of Portuguese Olive Varieties (Oleavalor)".

Keywords: *Olea europaea* L.(1), olive oil (2), olive tree varieties (3), olive oil yield (4), quality criteria (5)

POSTER NUMBER 3

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Chemical characterization of olive oil of four traditional Portuguese varieties from two consecutive crops

Instituto Politécnico Portalegre

OLIVE GROVES OLIVE OIL

Graça Pacheco de Carvalho[1], Elsa Lopes[1], Francisco Mondragão-Rodrigues[1,2], Luis Alcino Conceição[1, 2], Augusto Peixe[2], Manuel A. Martínez-Cañas[3] & Jacinto Sánchez Casas[3]

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ABSTRACT

The main objective of this work is to analyze some chemical characteristics of olive oil produced from four varieties of Portuguese olive trees during two consecutive harvest periods.

Keywords: *Olea europaea L.*(1), olive oil (2), olive tree varieties (3), olive oil yield (4), quality criteria (5)

INTRODUCTION

The olive branch is a strategic line in Portuguese agricultural and economic policy. Olive oil in Portugal represents about 3 to 5% of world production, standing in fourth place in Europe, after Spain, Italy and Greece (IOC, 2018). This trial is a part of the OLEAVALOR project, which aims at the valorisation of Portuguese olive tree varieties. This study, which is part of the OLEAVALOR project "Valuation of Portuguese Olive Varieties", in the Alentejo region, aims to evaluate and improve the productive potential of the main olive varieties in the región. Some chemical characteristics of olive oil from four traditional Portuguese olive varieties, 'Azeiteira', 'Cobrançosa', 'Blanqueta' and 'Carrasquenha de Elvas' at harvest (Maturation Index - MI - between 2,3 and 4,7) were analysed.

METHODOLOGY

Olive samples of the varieties under study were collected in 20 olive trees/olive groves during two consecutive harvest period (2017/18 and 2018/19) with Maturation Index (IM) according to the methodology and formula applied by the IFAPA de Mengíbar, Jaén (COI, 2011)) between 2.3 and 4.7. Acidity (A), Peroxides Index (PI), Oxidative Stability (OS) and UV spectrophotometric indices (K232, K270 and ΔK) were evaluated according to European Commission Regulation (EU) nº 1348/2013 of 16 December 2013 amending Regulation (EEC) nº 2568/91.

RESULTS

Table 1 – Chemical results

Variety	Harvest date	MI	Acidity (%)	Peroxid Index (mEq/Kg)	Oxidative Stability (h)
Galega	08/11/2017	4	0,16	4,15	63,79
Galega	20/12/2018	4	0,32	1,33	93,31
Azeiteira	17/01/2018	3,98	0,25	5,21	46,13
Azeiteira	10/01/2019	4,71	0,30	1,67	111,44
Cobrançosa	13/12/2017	3,77	0,41	5,64	71,76
Cobrançosa	20/12/2018	3,77	0,25	1,34	145,16
Blanqueta	17/01/2018	3,86	0,19	2,59	53,61
Blanqueta	22/11/2018	2,34	0,27	2,83	76,765

Figure 1- Laboratory analysis

Results for similar MI and varieties in the 2017 crop were higher for PI and lower for OS. Higher OS values and lower PI values are typical of greater stability and storage duration olive oils, as those found in every sample in the 2018 crop. The "Azeiteira" and "Cobrançosa" varieties were the ones showing higher OS values (111,44 h and 145,16 h, respectively) (Table 1). Maturation date was identical in the two years for the same varieties, with "Blanqueta" being comparatively earlier than other varieties in 2018. With regard to spectrophotometric indices, there are similar values for all varieties at study.

CONCLUSIONS

When comparing results from 2017 and 2018, an influence of the crop year on the chemical characteristics and stability of olive oils of these varieties was found. Further research is necessary for the evaluation of the influence of climate conditions on the quality characteristics of olive oil from these varieties.

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POSTER NUMBER 4

Discussing more Innovative Governance Options to Tackle Complex Changes in Olive Groves and the Olive Oil Sector in Alentejo (Portugal).

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Olive groves and the olive oil sector in Alentejo (Portugal) are currently undergoing a rapid and arguably unsustainable processes of intensification, financialisation and globalisation which especially affect the most productive agricultural land with access to irrigation permits and infrastructure. In parallel, least productive land is either maintained for traditional and extensive olive-based farming systems that are generally less advantageous financially or even at times abandoned. This results in a complex mosaic of rural land-use systems with high degrees of social-ecological and territorial heterogeneity across scales. Such a complex mosaic can potentially provide with a number of ecosystem and landscape services that need to be enhanced, and also with disservices that need to be avoided. This demands devising more innovative governance structures and instruments. Problems detected in the current governance framework include the fragmentation and lack of coherence among the key land-use policy instruments affecting olive governance, the absence of an overarching strategic and operational framework for spatial coordination and planning of olive grove expansion across spatial-temporal scales and institutional levels, and the many conflicts arising between those actors at the extreme ends of the policy and market-power scales. This is then complicated even more by the prevalence of a productivist discourse that largely permeates media, policy and the general public and that largely ignores the importance for sustainability of maintaining a balance between extensive and intensive systems of production. Generally speaking, intensive olive oil producers are more open to adopt technological innovation, whilst they are not as eager as traditional olive grove farmers to embrace interactive or governance innovation. In this poster, we will present the overall picture of governance institutions and tools driving rural land-use change in olive sector in Alentejo. Then we will also identify the main gaps preventing this governance framework to help the olive sector contribute to achieve UN´s sustainable development goals 2 (zero hunger), 13 (climate action) and 15 (life on land). We will then close up by proposing a set of governance options for the future that can help tackle the gaps identified and that will be defined on the basis of 4 different scenarios: International Competition, Market Segmentation, Euorpeization and Ecologization.

Keywords: Sustainability; Farming Systems; Olive Groves; Olive Oil; Alentejo; Governance

This work was partly funded under the EU grant nr. 635577, H2020-SFS-2014-2 (SUFISA: Sustainable finance for sustainable agriculture and fisheries", and PRIMA program (SUSTAINOLIVE: "Novel approaches to promote the SUSTAInability of OLIVE groves in the Mediterranean"). This work was also funded by National Funds through the FCT - Foundation for Science and Technology under the Project UID/AGR/00115/2013.

POSTER NUMBER 5

Establishment of a Reliable Protocol for gDNA Extraction from Olive Oil

Andreia Dias[1&], Ana Catarina Marques[1&], Isabel Velada[1], Teresa Carvalho[2], Tânia Nobre[1], Hélia Cardoso[1*], Maria João Cabrita[3]

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With no possibility to reach in quantity the production of countries with large areas of olive orchards, as a small country, Portugal needs to define a strategy for valuing the high quality and specificities of its olive oil. No doubt the focus must be on the valorization of the Portuguese cultivars, the key factor in determining the singularity of the produced olive oils. Fraud detection, as the use of non-Portuguese varieties, is the main aim of varietal and DOP olive oil producers. In this sense, it is mandatory to have tools allowing the control of the varietie(s) giving rise to the olive oil, both in quality and in quantity. One objective of the project Por3O running at University of Évora is the establishment of a molecular tool that identifies the varieties used to produce a given olive oil. Ideally, this tool could be further proposed for screening for frauds and to support olive oil certification. However, as PCR-based tool, it requires the availability of genomic DNA (gDNA) with quality enough to be used on fragment amplification. The step related with extraction of high quality gDNA from olive oil samples, which considers gDNA integrity and absence of inhibitory compounds, is often a limiting step in the development of a PCR-based genotyping tools. The establishment of a robust and efficient extraction protocol is thus crucial. In theory, the use of DNA commercial kits has the advantage of a higher reproductivity greatly removing technician expertise biases. Several DNA commercial kits were tested on its capacity to extract gDNA from a commercial blend olive oil and they were compared with an in-house method based on CTAB-based protocol previously published [1]. The different methods were compared in terms of starting volume of oil sample required for extraction, average gDNA concentration, total gDNA extraction yield and efficiency in Single-Sequence Repeats (SSRs) markers amplification. Considering the results achieved, we propose a workflow regarding gDNA extraction to further molecular analysis.

Keywords: CTAB method, SSRs, olive genotyping, olive oil , traceability, fraud detection

1.Raieta K., Muccillo L. and V. Colantuoni, A novel reliable method of DNA extraction from olive oil suitable for molecular traceability. Food Chem., 2015. 172: 596-602.

This work was financially supported by FCT (Foundation for Science and Technology) under the Project Por3O – Portuguese Olive Oil Omics for traceability and authenticity (PTDC/AGRPRO/2003/2014) and by national funds through UID/AGR/00115/2019.

POSTER NUMBER 5

Jornadas Med 2019
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Establishment of a reliable protocol for gDNA extraction from olive oil

OLIVE GROVES OLIVE OIL

Andreia Dias[1&], Ana Catarina Marques[1&], Isabel Velada[1], Teresa Carvalho[2], Tânia Nobre[1], Hélia G. Cardoso[1*], Maria João Cabrita[3]

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*Corresponding author: hcardoso@uevora.pt

INTRODUCTION

With no possibility to reach in quantity the production of countries with large areas of olive orchards, as a small country, Portugal needs to define a strategy for valuing the high quality and specificities of its olive oil. No doubt the focus must be on the valorization of the Portuguese cultivars, the key factor in determining the singularity of the produced olive oils. Fraud detection, as the use of non-Portuguese varieties, is the main aim of varietal and DOP olive oil producers. In this sense, it is mandatory to have tools allowing the control of the varietie(s) giving rise to the olive oil, both in quality and in quantity.

One objective of the project *Por3O* is the establishment of a molecular tool that identifies the varieties used to produce a given olive oil. Ideally, this tool could be further proposed for screening for frauds and to support olive oil certification. However, as PCR-based tool, it requires the availability of genomic DNA (gDNA) with quality enough to be used on fragment amplification. Here we will describe a robust and efficient gDNA extraction protocol, which allow its further use for Single-Sequence Repeats (SSRs) markers amplification. Several DNA commercial kits will be here compared on its capacity to extract gDNA from a commercial blend olive oil and its applicability on SSRs amplification.

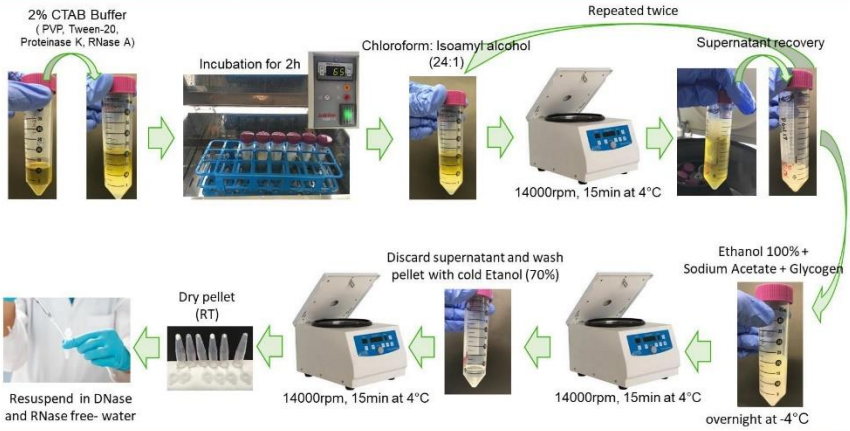
MATERIALS AND METHODS

Establishment

Ten different commercial kits (more information in Table below) were tested on its capacity to isolate genomic DNA (gDNA) from a commercial blend olive oil and they were compared with an in-house method based on CTAB-based protocol previously published [1] including some modifications (see procedure on the right).
The different methods were compared in terms of starting volume of oil sample required for extraction, average gDNA concentration, total gDNA extraction yield (see results in Table below) and efficiency in Single-Sequence Repeats (SSRs) markers amplification (SsrOeUA-DCA4, SsrOeUA-DCA9 and SsrOeUA-DCA11).

Validation

For procedure validation gDNA was isolated following the the in-house protocol, from two olive oils (Abencor system) per cultivar and a commercial monovarietal olive oil. Three cultivars were considered: 'Cobrançosa', 'Galega vulgar' and 'Arbequina'. In total, gDNA was isolated from 9 olive oils.
Same SSRs tested for gDNA extracted with kits were here amplified. gDNA isolated from leaves of the same cultivars was used as control.

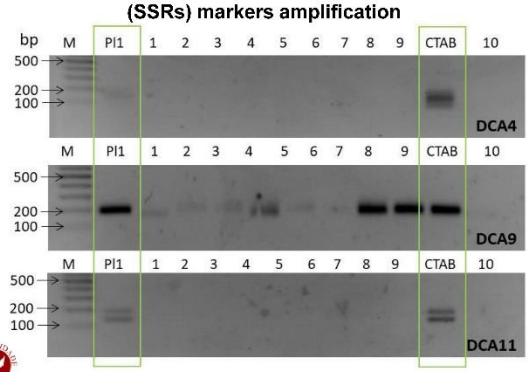


RESULTS AND DISCUSSION

Comparison of different methods for gDNA isolation

	Method	Starting vol. (µL)	[gDNA] (ng/µL)	Eluted (µL)	Yield (ng)	Purity ($A_{260/280}$)	Purity ($A_{260/230}$)
1	DNeasy Plant Mini Kit (QIAGEN)	200	2	250	575	2.57	0.16
2	NucleoSpin Plant II kit (MN)	200	28	100	2800	1.44	0.50
3	GeneJet Plant Genomic DNA Purification Mini kit (Thermo)	200	6	100	630	0.88	0.48
4	GeneJet Genomics DNA Purification kit (Fermentas)	200	25	250	6100	1.22	0.38
5	innuPREP Plant DNA kit (AG)	200	13	80	1024	0.79	0.08
6	Biomics DNA kit (Zymo)	200	17	40	672	0.75	0.32
7	Quick DNA (Zymo)	200	22	100	2150	1.08	0.40
8	LEV (Promega)	200	24	30	708	0.88	0.31
9	SEV (Promega)	50	26	30	768	0.86	0.29
10	QIAmp PowerFecal DNA kit (QIAGEN)	200	9	30	270	1.14	0.57
CTAB	CTAB	1000	24	20	478	1.08	0.13

Comparison on efficiency in Simple-Sequence Repeats (SSRs) markers amplification

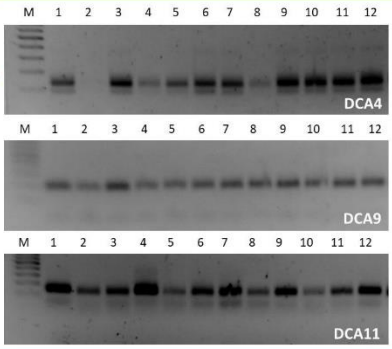


- ✓DNA commercial kits have the advantage of higher reproducibility, greatly removing technician expertise biases;
- ✓It requires small amounts of sample volume.

However...

gDNA isolated with commercial kit was not efficient in SSRs markers amplification

The in-house method, even though requiring more time for gDNA isolation and more volume to proceed with the isolation protocol, resulted in a much higher efficiency in SSRS markers amplification.



Legend:
1 - Plant 'Cobrançosa'
2 - Cob1 (Abencor)
3 - Cob5 (Abencor)
4 - 'Cobrançosa' commercial olive oil
5 - Plant 'Galega vulgar'
6 - Gal18 (Abencor)
7 - Gal22 (Abencor)
8 - 'Galega' commercial olive oil
9 - Plant 'Arbequina'
10 - Arb4 (Abencor)
11- Arb9 (Abencor)
12 - 'Arbequina' commercial olive oil

REFERENCES

[1]- Raieta K., Muccillo L. and V. Colantuoni, A novel reliable method of DNA extraction from olive oil suitable for molecular traceability. Food Chem., 2015. 172: 596-602.

ACKNOWLEDGMENTS

This work has been cofinanced by FEDER and Orçamento de Estado, through Fundação para a Ciência e Tecnologia, under the project PTDC/AGR- PRO/2003/2014 – Por3O – Portuguese Olive Oil Omics for traceability and authenticity, and FEDER Funds through the Operational Programme for Competitiveness Factors-COMPETE and National Funds through FCT – Foundation for Science and Technology under the Project UID/AGR/00115/2019).

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POSTER NUMBER 6

Genome-wide analysis and expression profile of PIN-formed auxin carrier genes during in vitro IBA-induced adventitious rooting in Olea europaea L.

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Olive ((*Olea europaea* subsp. *europaea* var. *europaea* L.) comprises several cultivars with reduced capacity to be propagated due its recalcitrant behaviour to form adventitious roots (AR). This prevents their propagation and consequently their availability in the nurseries. By overcoming the difficult-to-root behaviour of these cultivars it will be possible to use the full genetic diversity of the olive species and consequently to take advantage of the differentiating potential of its final products. There are many protocols used in vegetative propagation to induce AR formation based on auxins, a group of phytohormones largely known as involved in many processes of plant development, including root initiation and development. However, most of these protocols are still based on "trial/error" approaches, where several variables need to be tested. This happens because the genetic control underlying AR formation is not completely elucidated. Auxin is mainly synthesized in the young leaves and apical meristem of the shoot [**1**]. The major auxin distribution is regulated by transport from cell to cell, known as polar auxin transport (PAT) [**2**]. PAT is mediated by three main classes of membrane auxin transporters, the auxin resistant 1/like aux1 (AUX/LAX), the ATP binding cassette subfamily B (ABCB/MDR/PGP) and the pin-formed (PIN) carriers. The PIN gene family encodes a subgroup of auxin efflux carriers shown to be involved in various developmental processes, including lateral/adventitious root formation, in several plant species. To date, *PIN* genes have been identified in several plant species by genome-wide approaches [**3**], however, there is still no information regarding their identification in olive. The recent publication of *O. europaea* genome allowed us to perform the identification of all members belonging to *PIN* gene family in this species (*OePIN*). Our work aims to characterize *OePIN* family, as well as, to investigate the involvement of its members during AR, by studying their expression profiles in IBA-induced *in vitro* cultured microshoots of cv. 'Galega vulgar', , attempting to understand if the hard-rooting behaviour of this cultivar might be related with a disturbance in auxin transport. Additionally, reactive oxygen species (ROS), as key signaling molecules that regulate growth and development and coordinate responses to biotic and abiotic stresses in plants, are also being analysed in IBA-treated and non-treated explants. Also, cells from phloem, cortex and sub-epidermis are being isolated and gene expression will be performed on these cells in order to find out which cells are responsible for the formation of adventitious roots.

Key words: olive; difficult-to-root; vegetative propagation; polar auxin transport; gene expression; ROS.

This work is financially supported by FEDER and National Funds through the Programa Operacional Regional ALENTEJO 2020, Operação ALT20-03-0145-FEDER-000014– "Valorização das Variedades de Oliveira Portuguesas (OLEAVALOR)", and by National Funds through FCT - Foundation for Science and Technology under the Project UID/AGR/00115/2013. H. C. acknowledges FCT the PostDoc fellowship (Ref. SFRH/BPD/109849/2015).

POSTER NUMBER 6

Genome-wide analysis and expression profile of *PIN*-formed auxin carrier genes during *in vitro* IBA-induced adventitious rooting in *Olea europaea* L.

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OLIVE GROVES
OLIVE OIL

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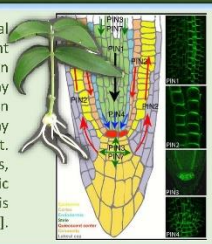
INTRODUCTION

Olive (*Olea europaea* subsp. *europaea* var. *europaea* L.) comprises several cultivars with reduced capacity to be propagated due its recalcitrant behaviour to form adventitious roots (AR). This prevents their propagation and consequently their availability in the nurseries. There are many protocols used in vegetative propagation to induce AR formation based on auxins, a group of phytohormones largely known as involved in many processes of plant development, including root initiation and development. However, most of these protocols are still based on "trial/error" approaches, where several variables need to be tested. This happens because the genetic control underlying AR formation is not completely elucidated. Auxin is mainly synthesized in the young leaves and apical meristem of the shoot [1].



The major auxin distribution is regulated by transport from cell to cell, known as polar auxin transport (PAT) [2]. PAT is mediated by three main classes of membrane auxin transporters, the auxin resistant 1/like aux1 (AUX/LAX), the ATP binding cassette subfamily B (ABCB/MDR/PGP) and the pin-formed (PIN) carriers. The PIN gene family encodes a subgroup of auxin efflux carriers shown to be involved in various developmental processes, including lateral/adventitious root formation. To date, *PIN* genes have been identified in several plant species by genome-wide approaches [3], however, no information exists regarding their identification in olive. Our work aims to characterize *OePIN* family, as well as, to investigate the involvement of its members during AR, by studying their expression profiles in IBA-induced *in vitro* cultured microshoots of cv. 'Galega vulgar', attempting to understand whether the hard-rooting behaviour of this cultivar might be related with a disturbance in auxin transport.

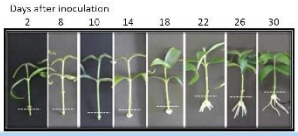
MATERIALS AND METHODS

Adventitious Rooting Induction

Stem segments (microcuttings) with four-to-five nodes were prepared from the upper part of *in vitro* grown plantlets of cv. 'Galega vulgar' and all leaves were removed with the exception of the upper four. The base (approx. 1.0 cm) of each microcutting was immersed in a sterile solution of IBA (indole-3-butyric acid) for 10 s [4,5,6] .

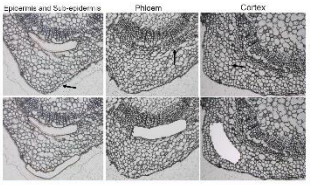
Microcuttings were inoculated in semi-solid olive culture medium (OM) and placed in growth chambers.

After several time points, microcuttings were collected and basal segments were cut.



Laser Microdissection (LMD)

Stem basal segments were placed in cryomolds containing optimal cutting temperature (OCT) compound and then freezed with isopentane and liquid nitrogen. Cryosections were placed in PEN membrane glass slides and OCT was removed with xilol, etanol 70% and 100%.



Microdissections were performed with a LMD6500 microscope (Leica).

Reactive Oxygen Species (ROS) Detection

Hydrogen peroxide (H_2O_2) and superoxide (O^{2-}) anion were detected by using 3,3'-Diaminobenzidine (DAB) and Nitrotetrazolium blue chloride (NBT), respectively.

In silico identification of OePIN gene family members

To search for *PIN* members in *Olea europaea* subsp. *europaea*, a blast search was made at the olive genome databases (http://denovo.cnag.cat/genomes/olive/ and https://www.ncbi.nlm.nih.gov/genome/?term=Olea+europaea+var.+sylvestris+genome). For classification of retrieved sequences a phylogenetic tree was constructed using PIN sequences from 13 eudicot plant species. Sequences were aligned in MUSCLE software and phylogenetic tree was constructed with MEGA 7 software [7] using the Neighbor-Joining (NJ) method. The inferred tree was tested by bootstrap analysis using 1000 replicates.

Gene Expression Analysis

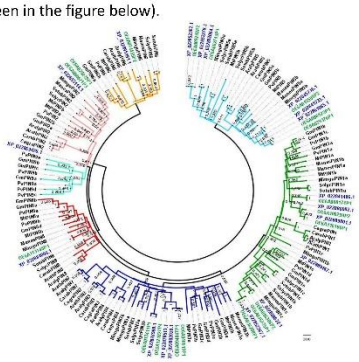
Total RNA was isolated from whole tissue (Maxwell SimplyRNA, Promega) and from microdissected tissue sections (RNeasy Micro kit, Qiagen) from 20 basal segments. Real-time PCR reactions were carried out with SYBR green chemistry and quantification cycle (Cq) values were acquired with the Applied Biosystems 7500 software.

RESULTS AND DISCUSSION

In silico identification of OePIN gene family members

✓ 21 *PIN*-homologous sequences were retrieved at the var. *europaea* whole genome databases. From those, 2 truncated loci (not considered for phylogenetic studies) and 2 duplicated loci (OE6A036288P1/OE6A046725P1; OE6A040519P1/OE6A094847P1) were identified .

✓ 17 genes were considered as the composition of *PIN* gene family in *Olea europaea* var. *europaea* (*OePIN*) (accessions shown in green in the figure below).

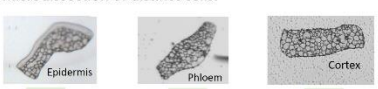


✓ The newly identified genes were named according to the cluster of PIN subfamily where they grouped. This analysis revealed that *OePIN* family is composed by members belonging to six subfamilies, named as *OePIN1*, *OePIN2*, *OePIN3*, *OePIN5*, *OePIN6* and *OePIN8*.

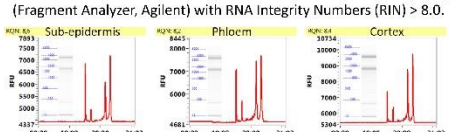
✓ Differences in the pattern of gene radiation could be seen among the different subfamilies (clusters with different colors in the phylogenetic tree).

Laser Microdissection

Olive tissue cryosections from epidermis (plus sub-epidermis), cortex and phloem showed high integrity allowing an efficient laser microdissection of distinct cells.



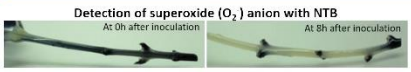
Total RNA isolated from distinct tissues showed high quality (Fragment Analyzer, Agilent) with RNA Integrity Numbers (RIN) > 8.0.



The implemented laser microdissection protocol revealed to be extremely efficient to obtain RNA of high quality to perform gene expression analysis from distinct olive cell types.

ROS levels are regulated in olive stems

Detection of superoxide (O_2^-) anion with NTB

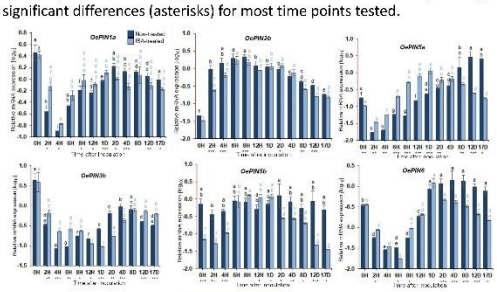


Detection of hydrogen peroxide (H_2O_2) with DAB

High levels of ROS were observed soon after explant preparation but they decreased 8 h after inoculation in both non-treated and IBA-treated explants, probably due to increased AOX enzyme [8].

OePIN genes are differentially expressed in olive stems

The *OePIN* mRNA levels were changed throughout time within each condition (non-treated and IBA-treated explants) (different letters), however, with different expression profiles between conditions, leading to significant differences (asterisks) for most time points tested.



A disturbance in auxin transport, demonstrated by altered *OePIN* genes expression levels in the first time points, may occur after explant preparation for AR induction. When IBA is applied, *OePIN* genes are differentially regulated comparing to non-induced microcuttings.

The disturbance in auxin transport may be promoted by high levels of ROS, which are an indicator of plant response to stress conditions (cutting/mechanical damage), associated to explant preparation.

In conclusion, OePIN family members deserve to be further investigated to better understand the molecular mechanisms underlying olive adventitious rooting so that, in the future, vegetative propagation capacity will be no longer an obstacle to make any olive variety available to olive producers.

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POSTER NUMBER 7

Influence of Weather Conditions on Timing of Olive Flowering in Traditional Portuguese Varieties in Alentejo Region

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This study, included in the OLEAVALOR project ("*Valorização das variedades de oliveira portuguesas*"), was carried out to collect information on the flowering phenology according the BBCH scale, during two consecutive years (2017 and 2018) in traditional olives varieties 'Galega Vulgar', 'Azeiteira' and 'Cobrançosa', in intensive olive groves (7m x 5m), with irrigation, on a farm located in Monforte (Alentejo region, Portugal). The observations were made on 20 trees per olive grove, using an experimental design consisting of 5 blocks with 4 trees, for each variety.

Comparing both years, the phenological stage of start of flowering (BBCH 60, observed for the first time) occurred much later in 2018 than in 2017 (142 vs 132 DOY for 'Cobrançosa', 140 vs 114 DOY for 'Galega Vulgar' and 138 vs 112 DOY for 'Azeiteira'), having maintained, however, the flowering sequence of the varieties. The flowering period (start of flowering to end of flowering) was shorter in 2018 when compared to 2017 (between 11 to 17 days in 2018 vs 16 and 21 days in 2017). The first months of the year (Jan - Apr) until the start of flowering were cooler in 2018 compared to 2017, which may explain these differences. Also, the average temperature in the periods (1jan - SF) and (1jan - FF) seems to have influenced the occurrence of the start of flowering (SF) and full flowering (FF) dates. Lower values of average temperature (11,6 ºC and 11,9ºC in 2018 against 12,6 ºC and 13,2 ºC in 2017, for 'Cobrançosa' variety for example) are corresponding to a later SF and FF dates in 2018. These results clearly show a strong interannual variability resulting from environmental conditions for the three varieties under study.

These results are crucial for the phenological characterization of these varieties of olive trees and allow their possible recommendation for the planting of new olive groves adapted to a warmer and drier climate. However, to confirm their differentiated behaviour, it is necessary to repeat this phenological evaluation in the next olive growing season.

Key words: *Olea europaea*, Phenology, environmental conditions, Climate change

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OLIVE GROVES
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This work was carried out with the main objective to study the flowering phenology according the BBCH scale, during two consecutive years (2017 and 2018) in traditional olives varieties 'Galega Vulgar', 'Azeiteira' and 'Cobrançosa', in order to be able to predict the changes of the phenological behavior caused by climate changes, in particular during flowering.

Keywords: *Olea europaea*, Phenology, environmental conditions, Climate change

INTRODUCTION

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

This study, which is part of the OLEAVALOR project "*Valorização das variedades de oliveira portuguesas*", in the Alentejo region, aims to evaluate and improve the productive potential of the main olive varieties of the region ('Galega vulgar', 'Cobrançosa', 'Azeiteira',' Blanqueta', 'Carrasquenha de Elvas' and 'Cordovil de Serpa').

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

METHODOLOGY

Flowering phenology conditions were recorded according to the BBCH scale adapted to olive tree by Sanz-Cortés *et al.* (2002). Observations of the flowering period (BBCH 60 to BBCH 69) were carried out on 20 trees of each variety ('Galega vulgar', 'Cobrançosa' and 'Azeiteira') and took place between April and May of 2017 and May and June of 2018, with a frequency of two observations per week. In each tree, we followed the Fleckinger method, described by Fernández-Escobar & Rallo (1981), in which the dominant phenological state is recorded.

RESULTS AND DISCUSSION

Comparing both years (Figure 1), the phenological stage of start of flowering (BBCH 60, observed for the first time) occurred much later in 2018 than in 2017 (142 vs 132 DOY for 'Cobrançosa', 140 vs 114 DOY for 'Galega Vulgar' and 138 vs 112 DOY for 'Azeiteira'), having maintained, however, the flowering sequence of the varieties. The flowering period (start of flowering to end of flowering) was shorter in 2018 when compared to 2017 (between 11 to 17 days in 2018 vs 16 and 21 days in 2017). The first months of the year (Jan - Apr) until the start of flowering were cooler in 2018 compared to 2017, which may explain these differences. Also, the average temperature in the periods (1jan - SF) and (1jan - FF) seems to have influenced the occurrence of the start flowering (SF) and full flowering (FF) dates. Similar results were obtained by Bonofiglio *et al.* (2009). Lower values of average temperature in these periods (SF and FF) (11,6 ºC and 11,9ºC in 2018 against 12,6 ºC and 13,2 ºC in 2017, for 'Cobrançosa' variety for example) are corresponding to a later SF and FF dates in 2018. Another variable showing to have a strong influence in flowering dates reported by Oteros *et al.* (2013) and Aguilera *et al.* (2015), is the cumulative rainfall during pre-flowering months (March and April), higher values delays the onset of the olive flowering period and increased the rate of phenological development (Figure 2) as occurred in 2018. These results clearly show a strong interannual variability resulting from environmental conditions for the three varieties under study.

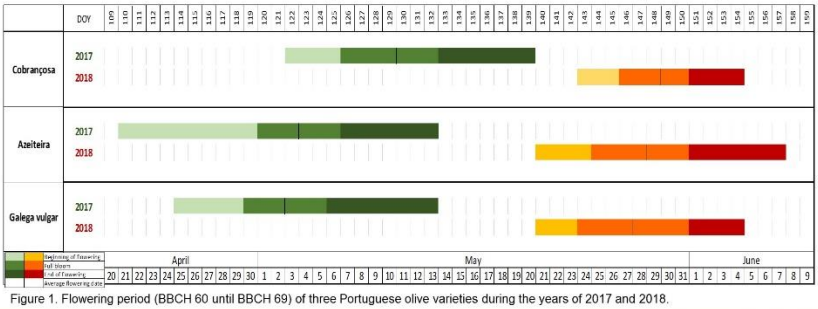

Figure 1. Flowering period (BBCH 60 until BBCH 69) of three Portuguese olive varieties during the years of 2017 and 2018.

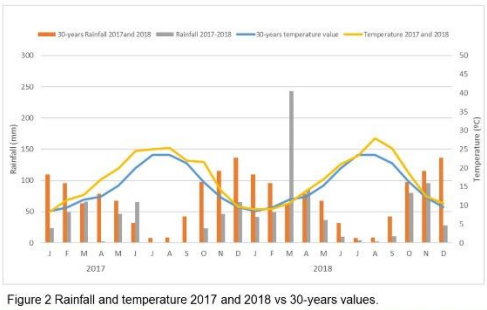

Figure 2 Rainfall and temperature 2017 and 2018 vs 30-years values.

CONCLUSION

The existence of variability among the varieties studied is an important factor to deal with climate change, considering that this phenomenon may alter the olive tree's phenological behavior in the incoming decades. These results are crucial for the phenological characterization of these varieties of olive trees and allow their possible recommendation for the planting of new olive groves adapted to a warmer and drier climate. However, to confirm their differentiated behaviour, it is necessary to repeat this phenological evaluation in the next olive growing season.

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POSTER NUMBER 8

Multivariate Analysis of Stable Isotope to Discriminate Geographically the Extra Virgin Olive Oils from three Mediterranean Regions

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The knowledge of the isotopic composition of extra virgin olive oil (EVOO) allows evaluating both the authenticity and geographical origin, being a huge tool against the fraud. The carbon and oxygen isotope content reflect the geo-climatic conditions of the production area, the local agricultural practices, the water uptake, the addition of lower quality oils, etc.
This study aimed to assess if EVOOs produced in three of the main Mediterranean regions producers of olive oil could be discriminated on the basis of multivariate statistical analysis of geoclimatic and isotopic data. To that end, a total of 138 EVOO samples from 3 Mediterranean regions, Portugal, France and Turkey (67, 50 and 21, respectively) and in 2 different cultivation years (2016 and 2017) were sampled. Each sample was geo-referenced, obtaining data of altitude (m.a.s.l), longitude (UTM), latitude (UTM), mean annual precipitation (mm), mean annual temperature (oC) and oceanic distance (km). The isotopic composition (δ13C and δ18O) of EVOOs was obtained using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS).
One-way analysis of variance (one-way ANOVA) using δ13C as independent variable (IV) indicated that of Portuguese and Turkish EVOOs are significantly ($P < 0.05$) different among them but not among its cultivation years. Nevertheless, French samples were significantly different ($P < 0.05$) between cultivation years. On the other hand, using δ18O as IV showed that there were significant ($P < 0.05$) differences between cultivation years for Portuguese and French samples. Further, the 18O composition of EVOO samples cultivated in 2017 was significantly different between the three analyzed countries.
Principal component analysis (PCA), using both the geoclimatic information and the isotopic composition of the 138 EVOO samples analyzed, clearly sorted the samples in three clusters, which correspond to each of three Mediterranean regions studied. In light of the results, the multivariate isotopic analysis of EVOO samples may discriminate not only between geographical regions but also among cultivation years.

Keywords: Stable isotopes, EVOO, Statistical analysis, Geographic origin, One-way ANOVA.

PTDC/AGRPRO/2003/2014 – Por3O - Portuguese Olive Oil Omics for traceability and authenticity and ARIMNET2/0001/2015 – MedOOmics –Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting

POSTER NUMBER 8

Multivariate analysis of stable isotope to discriminate geographically the extra virgin olive oils from three Mediterranean regions

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INTRODUCTION

The knowledge of the isotopic composition of extra virgin olive oil (EVOO) allows evaluating both the authenticity and geographical origin, being a huge tool against the fraud. The carbon and oxygen isotope content reflect the geo-climatic conditions of the production area, the local agricultural practices, the water uptake, the addition of lower quality oils, etc. This study aimed to assess if EVOOs produced in three of the main Mediterranean regions producers of olive oil could be discriminated on the basis of multivariate statistical analysis of geoclimatic and isotopic data.

MATERIAL AND METHODS

To that end, a total of 138 EVOO samples from 3 Mediterranean regions, Portugal, France and Turkey (67, 50 and 21, respectively) and in 2 different cultivation years (2016 and 2017) were sampled. Each sample was geo-referenced, obtaining data of altitude (m.a.s.l), longitude (UTM), latitude (UTM), mean annual precipitation (mm), mean annual temperature (°C) and oceanic distance (km). The isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of EVOOs was obtained using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS).

Fig. 1. Geographic location of EVOO samples

RESULTS

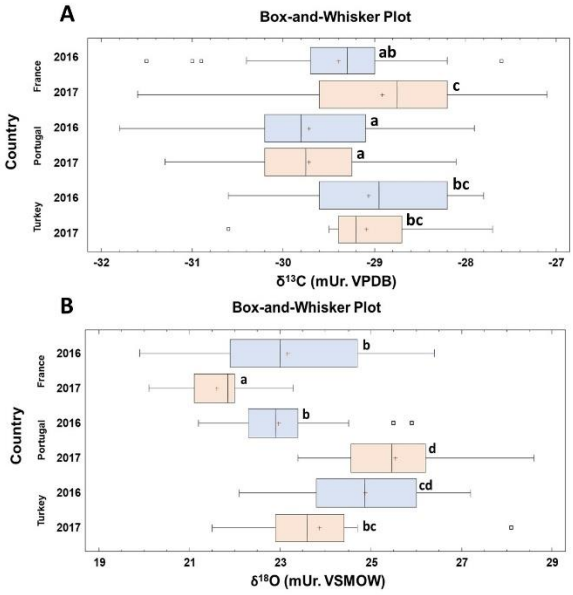

Fig. 2. Boxplots of the bulk carbon ($\delta^{13}C_{bulk}$ values, A) and oxygen ($\delta^{18}O_{bulk}$ values, B) of EVOO samples. Boxplots display the ranges, lower and upper quartiles (Q1, Q3), and the median (Q2). Box plots with different letters indicate significant difference . (ANOVA; Means compared using Tukey test, $P = 0.05$)

One-way analysis of variance (one-way ANOVA) using $\delta^{13}C$ as independent variable (IV) indicated that of Portuguese and Turkish EVOOs are significantly ($P < 0.05$) different among them but not among its cultivation years (**Fig. 2A**). Nevertheless, French samples were significantly different ($P < 0.05$) between cultivation years (**Fig. 2A**). On the other hand, using $\delta^{18}O$ as IV showed that there were significant ($P < 0.05$) differences between cultivation years for Portuguese and French samples (**Fig. 2B**). Further, the ^{18}O composition of EVOO samples cultivated in 2017 was significantly different between the three analyzed countries (**Fig. 2B**).

Principal component analysis (PCA), using both the geoclimatic information and the isotopic composition of the 138 EVOO samples analyzed, clearly sorted the samples in three clusters, which correspond to each of three Mediterranean regions studied (**Fig. 3**). In light of the results, the multivariate isotopic analysis of EVOO samples may discriminate not only between geographical regions but also among cultivation years.

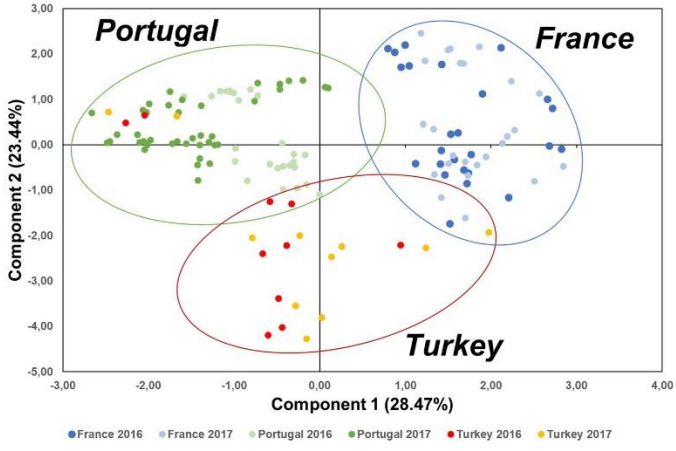

Fig. 3. Principal Component Analysis (PCA) scores of EVOO samples from 3 Mediterranean regions (Portugal, France and Turkey) and in 2 different cultivation years (2016 and 2017)

Acknowledgments:

PTDC/AGRPRO/2003/2014 – Por3O - Portuguese Olive Oil Omics for traceability and authenticity and ARIMNET2/0001/2015 – MedOOmics – Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting. N JM is funded by scholarship PosDoc/Por3O/OU117. Raquel Garcia acknowledge the FCT for post-doctoral research fellowship- SFRH/BPD/109912/2015.

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POSTER NUMBER 1

Amino Acid Content of Aragonez Grapes: The Influence of Soil Fertilization

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Grape soil fertilization has been used to enhance the quality of grapes and therefore wines. Vineyard fertilization practices aim to ameliorate the supply of available soil nutrients to the levels required for optimum grapevine growth and yield. Most soils will contain adequate amounts of micronutrients. However, nitrogen, phosphorus and potassium (principal macro-nutrients) as well as magnesium, calcium and Sulphur (secondary macro-nutrients) are the ones that usually can limit grape production.

Magnesium tends to be deficient in vineyards if the soil pH is too low, and Alentejo soils usually have low pH. Magnesium is required as a component of chlorophyll molecules and for metabolic processes and influences fruit formation and berry ripening. The aim of this work was to understand the influence of several nutrients' application on soil vineyards on the amino acid content of the must from Aragonez grapes. The experiment was conducted in a randomized block design, with three replications, in a splitplot arrangement. Two different doses of Mg were applied (D1 and D2). For each one there was six different treatments: 1) with N, P, Ca, S, K; 2) with P, Ca, S, K ; 3 with N, Ca, S, K; 4) with N, P, S, K; 5) with N, P, Ca, K; 6) with N, P, Ca, S. A control plot with N, P, Ca, S, K but without Mg addition was also considered, in a total of 39 plots. Grapes were picked up at harvest ripening and must was kept at -80°C until analysis. The amino acid content of the must was quantified using a HPLC Waters Alliance System 2695-series Separation Module equipped with Alliance Series Column Heater and the detection was carried out using a photodiode array detector (2998 PDA Detector) (Waters, USA). The column used was an ACE HPLC column (5 C18-HL) particle size 5 µm (250 mm x 4.6 mm). Prior to injection, samples were derivatized. Results shows that some differences among the amino acid content of the grape must samples can be observed.

Keywords: Amino acids, must, Aragonez, fertilization

[1] Bell, s. J. and Henschke, P. A., Aust. J. Grape Wine Res., 11 (2015) 242-295
[2] Jan Hendrik Swiegers *et al.*, Australian Journal of Grape and Wine Research, 11 (2005) 139–173
[3] Sergio Gómez-Alonso *et al.*, J. Agric. Food Chem., 55 (2007) 608-613
[4] Teresa Garde-Cerdán *et al.,* Food Research International., 102 (2017) 451-457

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POSTER NUMBER 1

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Amino Acid content of Aragonez grapes: the influence of soil fertilization

VINEYARDS AND WINE

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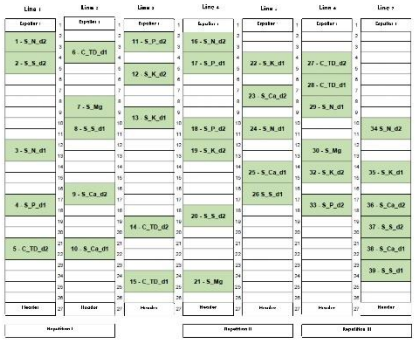
Introduction

Grape soil fertilization has been used to enhance the quality of grapes and therefore wines. Vineyard fertilization practices aim to improve the supply of available soil nutrients to the levels required for optimum grapevine growth and yield. Most soils will contain adequate amounts of micronutrients. However, nitrogen, phosphorus and potassium (principal macro-nutrients) as well as magnesium, calcium and sulphur (secondary macro-nutrients) are the ones that usually can limit grape production. Magnesium tends to be deficient in vineyards if the soil pH is too low, and Alentejo soils usually have low pH. Magnesium is required as a component of chlorophyll molecules and for metabolic processes and influences fruit formation and berry ripening. The experiment was conducted in a randomized block design, with three replications, in a split-plot arrangement. Two different doses of Mg were applied (D1 and D2). For each one there was six different treatments: 1) with N, P, Ca, S, K; 2) with P, Ca, S, K ; 3) with N, Ca, S, K; 4) with N, P, S, K; 5) with N, P, Ca, K; 6) with N, P, Ca, S. A control plot with N, P, Ca, S, K but without Mg addition was also considered, in a total of 39 plots. Grapes were picked up at harvest ripening and must was kept at -80°C until analysis.
The aim of this work was to understand the influence of several nutrients application on soil vineyards on the amino acid content of the must from Aragonez grapes. The amino acid content of the must was quantified by HPLC-DAD and statistical tools were used to evaluate the results.

Material and methods

Grape samples



Sample preparation

1) Must preparation
- Collect 200 grapes
- Mecanically smash the grapes and filter the juice
- Store at -20°C

2) Reaction of Derivatization

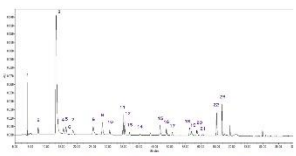
1º Step
To 1 mL of must add:
+ 1.75 mL Phosphate buffer
+ 750 µL MeOH
+ 30 µL DEEMM
+ 20 µL L-2-aminoadipic acid (IS)

2º Step
1) 30 min. ultrassound
2) 2h at 70°C

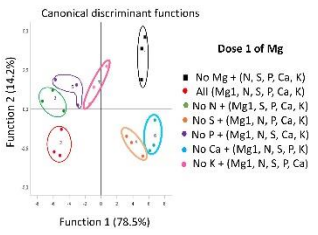
HPLC analysis

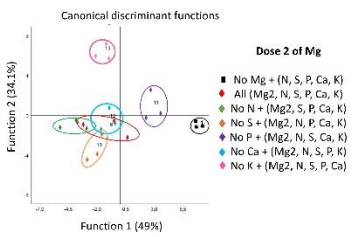
- HPLC Waters Alliance System 2695 series Separation Module equipped with Alliance Series Column Heater.
- Detection: Photodiode array detector (2998 PDA Detector) (Waters, USA) at λ 269, 280 and 300 nm.
- Column: ACE HPLC column (5 C18-HL) particle size 5 µm (250 mm x 4.6 mm) heated at 20°C.
- Volume of injection: 10 µL
- Flow rate: 0.9 mL/min.
- Mobile phases: (A) 25 mM acetate buffer pH=5.8 with 0.02% sodium azide, (B) ACN/MeOH (80:20 (v/v)).
- Gradient of elution: 90% of A until 20 min, 90% to 83% of A in 10 min, 81% of A for 1 min, 81% to 80.5% of A in 1.01 min, 80.5% to 77% of A in 9.5 min, 77% to 70.6% of A in 20.6 min, 70.6% to 18% of A in 13 min, 18% to 0% of A in 4 min.

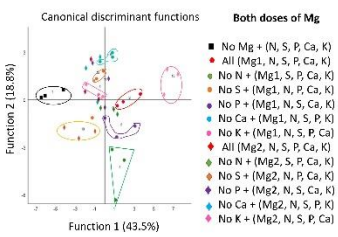
Results



(1) Asp, (2) Gln, (3) IS, (4) Asn, (5) Ser, (6) His, (7) Glu, (8) Gly, (9) Thr, (10) Arg, (11) Ala, (12) GABA, (13) Pro, (14) Tyr, (15) Val, (16) Met, (17) Cys, (18) Ile, (19) Trp, (20) Leu, (21) Phe, (22) Orn, (23) Lys







Conclusions

- The results seem to indicate that the different soil fertilization regimes affect the amino acid composition of the grapes, which is visible through the discriminant canonical analysis performed.
- The applied Mg dose also appears to influence amino acid synthesis, since the total amino acid content in the All Mg2 treatment is lower than that found for the All Mg1 treatment.

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[1] Bell, s. J. and Henschke, P. A., Aust. J. Grape Wine Res., 11 (2015) 242-295
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POSTER NUMBER 3

Identification of Fungi Responsible for Grapevine Trunk Disease in Alentejo Region

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Grapevine trunk diseases (GTDs) are among the most important fungal diseases affecting grapevines in all the major growing regions of the world [1], with up to 133 fungal species belonging to 34 genera associated to these diseases [2]. Management of GTDs is difficult and their complete eradication is not possible. For these reasons, GTDs control is mainly focused on disease prevention and mitigation [3]. The work here presented had the aim to identify molecularly the endophytic fungi present in different tissues of grapevines, and among them the fungi responsible for GTDs. Pure fungal cultures were isolated from symptomatic grapevines belonging to two cultivars present in vineyards from Alentejo region ('Trincadeira' and 'Alicante Bouchet'). Mycelium was then harvested, gDNA was extracted [4], and for fungal genetic classification the ribosomal internal transcribed spacer (ITS) region of nuclear rDNA was amplified through PCR by using ITS1 and ITS4 primers [5]. All fungal sequences considered were at least 98 % identical to the best hit in the NCBI database. The identified fungi associated with GTDs were *Hormonema viticola, Stereum armeniaccum, Philophorafastigiata, Diaporthe, Truncatella angustata, Cytospora acaciae, Diplodia pseudoseriata, Neofusicocum parvum,* and *Pestalotiopsis sp.* This study allowed a deeper knowledge of the fungi present in vineyards from Alentejo region associated to GTDs, and will contribute to further studies on fungi molecular identification in order to monitor the behavior of the disease in the vineyards.
Key words: Grapevine trunk disease**,** *Vitis vinifera*; fungal genetic classification, internal transcribed spacer (ITS).

1. Pintos C., Redondo V., Costas D., Aguín O., Mansilla P. Phytopathology Mediterranea, 2018. 57: (3) 407−424; 2. Gramaje, D., U´rbez-Torres, J. R, Sosnowski M. R. Plant Disease, 2018. 102:12-39. ; 3. U´rbez-Torres, J. R. Phytopathology Mediterranea, 2011. 50:S5-S45. ; 4. Doyle J and Doyle J. Phytochemical Bulletin, 1987. 19:11-15; 5. White, T.J.; Bruns, S.; Lee, S.; Taylor, J. In PCR Protocols: A Guide to Methods and Applications; Academic Press: Cambridge, M A, USA, 1990; pp. 315–322.

MDC was supported by the project "Development of a new virus-based vector to control TSWV in tomato plants", reference ALT20-03-0145-FEDER-028266 and PM was supported by the project "Control of olive anthracnose through gene silencing and gene expression using a plant virus vector", reference ALT20-03-0145-FEDER-028263 both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo), ALGARVE 2020 (Regional Operational Program of the Algarve) and through the Foundation for Science and Technology, in its national component.

POSTER NUMBER 3

Jornadas Med 2019
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Identification of fungi responsible for grapevine trunk disease in Alentejo region

A.B. de Almeida[1], J. Concas[2], M.D. Campos[3], C.M.R Varanda[3], P. Materatski[3], S. Murolo[2], G. Romanazzi[2], M.R. Félix[4]

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VINEYARDS AND WINE

INTRODUCTION: Grapevine trunk diseases (GTDs) are among the most important fungal diseases affecting grapevine plants in all the major growing regions of the world [1] with up to 133 fungal species belonging to 34 genera associated to this disease [2]. Management of GTDs is difficult and its complete eradication is not possible. For these reasons, its control is mainly focused on disease prevention and mitigation [3].

OBJECTIVE: Molecular identification of the endophytic fungi present in different tissues of grapevines, and among them the fungi responsible for GTDs.

MATERIALS AND METHODS

SAMPLING AND SAMPLES TREATMENT

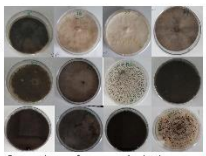
Grapevine varieties: 'Trincadeira' and 'Alicante Bouchet'

Plant tissues: Roots, petiole and shoots

Location: Alentejo region, Portugal

Sample plants: 25 symptomatic and 25 asymptomatic plants

Isolates: 111 fungi were growth in PDA media, separated by morphological characteristics

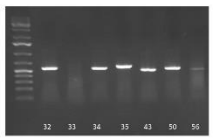


Pure culture of some endophytic fungi found in the tested tissues of the grapevine plants

MOLECULAR ANALYSIS

gDNA extraction: CTAB protocol

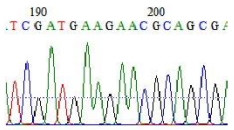
PCR amplification: ITS region was amplified by PCR using ITS1 and ITS4 primers [5] and it was verified through a 1% agarose gel



Amplified samples in a 1% agarose gel.

Sequencing: The PCR amplification products were purified using a NZYTech kit and sent to be sequenced in Macrogen Inc., Madrid

Sequence's analysis: The sequences were analysed using the program "BioEdit Sequence Alignment Editor v.7.2.3." [6] and the homologous sequences were searched by the data base on NCBI website through "BLAST"



Fungal senquence by the program "BioEdit Sequence Alignment Editor v.7.2.3."

All the fungi sequences that showed resemblance of 90% were used to classified the analysed fungi.

RESULTS

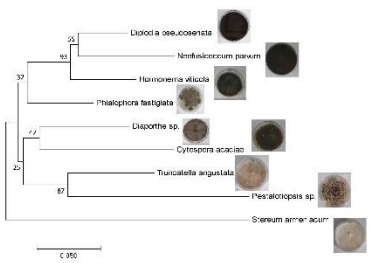
IDENTIFICATION OF FUNGI

Endophytc fungi identified by their % of homology based on ITS region and the vegetal material where they were detected.

FUNGUS	% homology based on ITS region	Vegetal material in culture		
		roots	petioles	shoots
Acrocalymma vagum	100%	+		
Alternaria alternata	100%	+	+	
Alternaria sp.	100%	+	+	
Clonostachys sp	99,47%	+		+
Aspergillus niger	100%	+		
Aspergillus terreus	98,75%		+	
Beauveria bassiana	100%	+	+	
Bjerkandera adusta	98,65%	+	+	+
Botrytis cinerea	99,14%	+	+	+
Phialophora fastigiata	99,50%	+		
Chaetomium succineum	99,71%	+		
Clonostachys rosea	100%	+		+
Cytospora acecicae	100%		+	+
Diaporthe sp.	100%	+	+	+
Diplodia teriata	97,89%			+
Epicoccum nigrum	99,66%	+	+	+
Fusarium sp.	100%			+
Fusarium oxysporum	100%	+		
Fusarium verticillioides	97,89%	+		+
Hormonema viticola	99,71%	+	+	+
Trichoderma harzianum	95,25%	+	+	+
Rhizoctonia botaticola	99,72%	+		
Neofusicoccum parvum	92,05%	+	+	+
Penicillium chrysogenum	100%	+		
Penicillium glabrum	98,02%	+	+	+
Penicillium sp.	100%	+	+	+
Pestalotiopsis	91,09%			+
Phlebia setulosa	99,27%	+		
Phlebiopsis gigantea	100%	+		
Rutstroemiaceae sp.	99,72%	+		+
Stereum armeniaccum	99,75%	+		
Talaromyces sp.	99,50%	+		
Trichoderma sp.	96,60%	+	+	+
Truncatella angustata	99,08%	+		

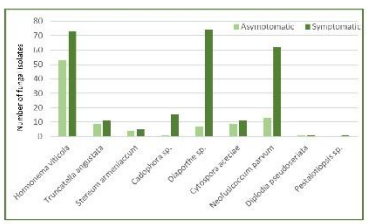
- Most fungi were detected in the roots tissues.
- 9 fungal isolated species occurred in all the plant tissues.
- 9 fungal isolates cause GTDs

PHYLOGENETIC ANALYSIS OF FUNGI ASSOCIATED TO GTDs



- *Diplodia pseudoseriata* and *Neofusicoccum parvum* are more closely related because belongs to the *Botryosphariaceae* family.
- *Pestalotiopsis* sp. and *Phialophora fastigiate* are phylogenetically distant but both are associated to Petri disease complex.
- *Diaporthe* sp. and *Cytospora acacia* belong to the *Diaporthales* order

GTDs FUNGI ANALYSIS



Number of isolates detected for each GTDs fungus specie.

- *Hormonema viticola* was the most prevalent fungus in both, symptomatic and asymptomatic, plants.
- *Diaporthe* sp. and *Neofusicoccum parvum* are manly present in symptomatic plants.

FUNGUS	Cultivar		Vineyard		
	T	A.B.	F.E.A.	V.R.H.	C.M.
Hormonema viticola	+	+	+	+	+
Truncatella angustata	+	+	+	+	+
Stereum armeniaccum	+	+	+		+
Cadophora sp.	+	+	+		+
Diarporthe sp.	+	+	+	+	+
Cytospora aceciae	+	+	+	+	+
Neofusicoccum parvum	+	+	+	+	+
Diplodia pseudoseriata	+		+	+	
Pestalotiopsis sp.	+			+	

T : 'Trincadeira'
A.B. : 'Alicante Bouchet'
F.E.A : Fundação Eugenio de Almeida
V.R.H: Vale do Rico Homen
C.M.: Campo Major

- *Diplodia pseudoseriata* and *Pestalotiopsis* sp. are presents only in 'Trincadeira' variety.

CONCLUSIONS AND FUTURE PERSPECTIVES

This study allowed a deeper knowledge of the fungi present in vineyards from Alentejo region associated to GTDs, and will contribute to further studies on fungi molecular identification in order to monitor the behavior of the disease in the vineyards.

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1 Pintos C., Redondo V., Costas D., Aguín O., Mansilla P. Phytopathology Mediterranea, 2018. 57: (3) 407–424
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POSTER NUMBER 4

Physiological evolution and phenolic assessment of Vitis vinífera Trincadeira variety during two consecutive years in Herdade da Mitra, Évora, Portugal

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Grapes are non-climacteric fruits with skin, flesh, seeds and their growth has a double sigmoidal curve, divided in an initial quick growth, followed by lag phase and then a second ripening process, also known as vérasion, in red grape varieties [1]. Authors claim that climate has an anthropogenic influence in grapes, therefore extreme events of high temperatures have implication in grape berries development and consequently in wine quality [2]. Cultivars present many differences between themselves, although terroir affects grape quality, their genetic inheritance is still the main responsible for the production and accumulation of compounds related with wine quality (namely polyphenols) [2].
In this work we selected a common variety, Trincadeira, produced in Herdade da Mitra vineyard (Alentejo) for two consecutive years (2017 and 2018). From vérasion to maturation, the development of grape berries was studied (along with berry constitution) and the content of bioactive compounds (total phenols, flavonoids, anthocyanins and tannins) were determined in grape skins. Grape berries were heavier in the year 2018, although maturation was earlier in 2017, possibly due to the great weather differences. Despite of berries were smaller, the amounts of all compounds studied were higher in 2017 in the generality of dates. However, total phenolic and anthocyanin contents didn't show statistical significative differences during maturation but at harvest, phenol content was higher, and anthocyanins were about two times higher in 2017 than in 2018. Even tough flavonoids and tannins showed a higher content in 2017 grapes with statistical significative differences during all dates studied. Results point out that monitorization of bioactive compounds is important to estimate the harvest date.

Keywords *Vitis vinífera*, Trincadeira, Vérasion, Phenolic compounds, Anthocyanins

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Introduction

Grapes are non-climacteric fruits with skin, flesh, seeds and their growth has a double sigmoidal curve, divided in an initial quick growth, followed by lag phase and then a second ripening process, also known as véraison, in red grape varieties [1]. Authors claim that climate has an anthropogenic influence in grape development and consequently in wine quality hence terroir might affect the production and accumulation of compounds related with wine quality (namely polyphenols) [2].

In this work we collected samples of a common variety, Trincadeira, for two consecutive years during maturation, the development of grape berries was studied and the content of bioactive compounds were determined in grape skins.

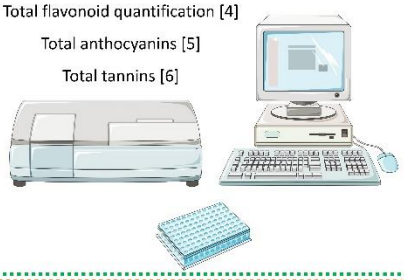
Methodology

Samples collected from Véraison (day 0) to harvest (day 28) in Herdade da Mitra, Évora, Alentejo

Skins extracted mannually

Extraction with Acidified Ethanol (50/50, v/v)

Folin Ciocalteau method [3]
Total flavonoid quantification [4]
Total anthocyanins [5]
Total tannins [6]



Results and discussion

Berry development

Grape berries were heavier in the year 2018, although maturation was earlier in 2017, possibly due to the great weather differences. Skin and pulp content was higher in 2018 at harvest.

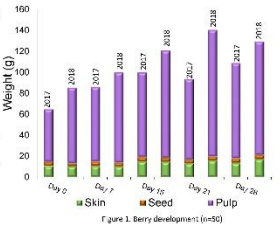


Figure 1. Berry development (n=50)

Total phenolic content

Total phenolic content didn't change during maturation (p>0,05) but at harvest, 2017 phenol content was higher than in 2018.

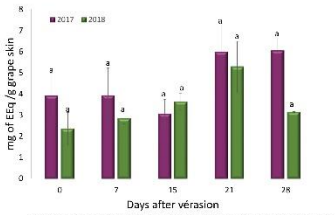


Figure 2. Total phenolic content expressed in mg of epicatechin equivalents in g of grape skin (n=3±std. deviation)

Total flavonoid content

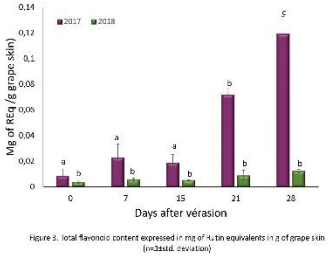


Figure 3. Total flavonoid content expressed in mg of Rutin equivalents in g of grape skin (n=3±std. deviation)

Flavonoids showed a higher content in 2017 grapes (p>0,05) during all dates studied.

Total anthocyanin content

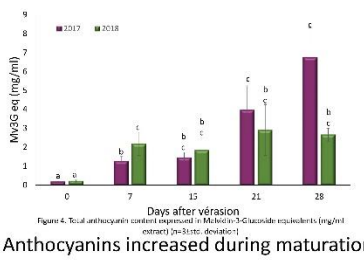


Figure 4. Total anthocyanin content expressed in Malvidin-3-Glucoside equivalents (mg/ml extract) (n=3±std. deviation)

Anthocyanins increased during maturation (p<0,05) at most dates in both years studied. Their content was about two times higher in 2017 than in 2018 at harvest.

Total tannins content

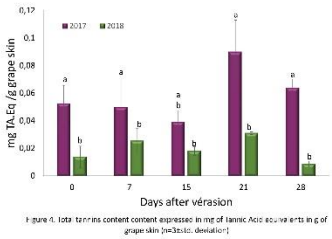


Figure 4. Total tannins content content expressed in mg of tannic Acid equivalents in g of grape skin (n=3±std. deviation)

Tannins content was higher in 2017 in all dates studied (p<0,05).

Despite of berries were smaller, the amounts of all compounds studied were higher in 2017 in the generality of dates
Results point out that monitorization of bioactive compounds is important to estimate the harvest date.

Acknowledgments : This work was financed by national funding from FCT – Fundação para a Ciência e a Tecnologia within the scope of the project UID/AGR/00115/2013

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Instituto de Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

POSTER NUMBER 1

Different Irrigation Strategies on Pomegranate Production and Quality

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Pomegranate (*Punica granatum* L.) is very appreciated by its nutritional and sensorial attributes. In last years, pomegranate orchards have emerged in the new irrigation areas of Alentejo, Portugal. However, water has become a progressively more limited resource, especially in arid and semi-arid regions, such as Mediterranean countries, which has led to an increasing demands for more efficient water saving strategies. Regulated deficit irrigation (RDI) is a water saving strategy, which consist in irrigation below the maximum crop evapotranspiration (ETc) during a specific stage of crop cycle [1]. The aim of this work was to evaluate the effect of different irrigation strategies on fruit production and quality, in a drip irrigated pomegranate orchard (cv. Wonderful) located in Baixo Alentejo region. Three irrigation strategies were tested, along 2018 campaign: Control (C) – farmer irrigation (100% ETc); RDI1 – applied at flowering stage - initial stage of fruit formation; RDI2 – applied at ripening stage. Nine trees per treatment, distributed by 3 blocks were monitored. At harvest (Oct/Nov 2018) the yield parameters were quantified, and five fruits (85-100 mm) per tree were analyzed for physicochemical and nutritional properties. Irrigation strategies didn't affect (P<0.05) the total yield, number of produced fruits and weight of fruit size less than 85 mm. However, RDI1 reduced the yield of fruits greater than 85 mm and increased the number of cracked fruits comparatively to C, while RDI2 did not differ significantly from the C and RDI1 (P<0.05). Both RDI strategies resulted in fruits with higher percentage of arils (P=0.023), and higher soluble solids content was found in fruits from RDI2 comparatively to other treatments (P<0.001). Preliminary results showed that RDI strategies, such as RDI2 may be a good water saving approach to use in pomegranate orchards of the Alentejo.

Keywords regulated deficit irrigation; fruit; production; physicochemical attributes; nutritional value

1.Y. Selahvarzi, et al., Effect of deficit irrigation on flowering and fruit properties of pomegranate (*Punica granatum* cv. Shahvar). Agric. Water Manag., 192. 189–197, 2017.

This work was supported by Program INTERREG V-A Spain-Portugal (POCTEP) 2014-2020 through FEDER under scope of INNOACE (049_INNOACE_4_E). The authors would like to thank FCT/MCTES for the financial support to ICAAM (UID/AGR/00115/2019).

POSTER NUMBER 1

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Different irrigation strategies on pomegranate production and quality

IRRIGATED AGRICULTURE

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Introduction

→ In last years, pomegranate orchards have emerged in the new irrigation areas of Alentejo.

→ Water has become a progressively more limited resource, especially in arid and semi-arid regions, such as Mediterranean countries, which has led to an increasing demands for more efficient water saving strategies.

→ **Regulated deficit irrigation** (RDI) is a water saving strategy, which consist in irrigation below the maximum crop evapotranspiration (ETc) during a specific stage of crop cycle.

Aim

✓ **Evaluation of the effect of different irrigation strategies on fruit production and quality in a drip irrigated pomegranate orchard (cv. Wonderful) located in Baixo Alentejo region.**

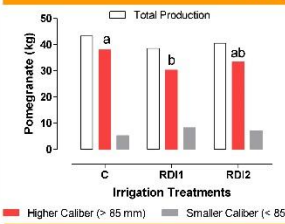
Conclusions

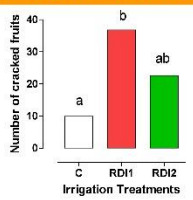
✓ Both RDI strategies resulted in fruits with higher percentage of arils and higher soluble solids content was found in fruits from RDI2 comparatively to other treatments.

✓ Preliminary results showed that RDI strategies, such as RDI2 may be a good water saving approach to use in pomegranate orchards of the Alentejo.

Results

Pomegranate Production Parameters





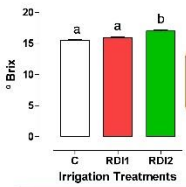
Irrigation treatments did not affect the total yield, number of produced fruits and weight fruit size < 85 mm

RDI1 ↓ yield of fruits size > 85 mm and ↑ number of cracked fruits

RDI2 led to similar prodution results to Control

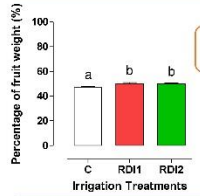
Pomegranate Quality Parameters

Total Soluble Solids



RDI2 ↑ total soluble solids contente *P < 0.001*

Aril Percentage



RDI ↑ aril proportion *P = 0.023*

Antioxidant activity

Arils	Mean	SEM
FRAP (mg Fe/100 mL juice)*	537.3	129.60
TEAC (mg Trolox /100 mL juice)*	386.9	72.13

*FRAP - Ferric reducing antioxidant power equivalents ; TEAC – Trolox Equivalent Antioxidant Capacity

Pomegranate physicochemical properties

Fruit		Mean	SEM
Weight (g)		376.5	59.93
Diameter (mm)		92.5	5.58
Height (mm)		92.6	5.73
Peel colour parameters	*L**	48.7	4.27
	*a**	39.5	5.00
	*b**	24.5	2.24
	C (Chroma)	46.9	3.95
	H (Hue angle)	32.7	5.06
Arils		**Mean**	**SEM**
Colour parameters	*L**	25.9	1.70
	*a**	9.8	1.41
	*b**	4.5	0.94
	C (Chroma)	10.8	1.57
	H (Hue angle)	24.8	3.66
pH		2.9	0.13
Titratable Acidity (% Citric Acid)		2.0	0.41
Total Phenols (mg GAE/100 mL juice)*		158.5	35.53

*GAE – gallic acid equivalents

Irrigation system did not affect most of the physicochemical properties and antioxidant activity of pomegranate

Material and Methods

Experimental Design

✓ Monte das Carvoeiras, Beja, Portugal
✓ Pomegranate Variety: cv. Wonderful
✓ 3 blocks per treatment - 9 trees monitored by treatment - 3 per block
✓ Sampling: october/november 2018

Irrigation Treatments

Control (C)	Farmer Irrigation (100% ETc)
RDI1*	25 % ETc applied at flowering and initial fruit growth
RDI2*	25 % ETc applied at fruit maturation phase

ETc – Crop Evapotranspiration; * - 100 % ETc applied at other phases of plant cycle

Pomegranate Production

- Total Yield
- Weight of fruit size: < and > 85 mm (diameter)
- Number of cracked fruits
- Number of produced fruits

Pomegranate Quality

5 fruits per tree

Whole fruit
- Weight (g)
- Diameter (mm)
- Height (mm)

Peel and Aril
- Weight (g)
- Colour

Juice
- Total Soluble Solids
- Titratable Acidity
- pH
- Total Phenolic Content (Mena et al. 2013, *J. Sci. Food Agric.*, 93: 1922–1927)

Antioxidant Activity
- FRAP method (Benzie et al 1999, *Methods in Enzymology*, 299:15-27)
- TEAC method (Re et al. 1999, *Free Radic. Biol. Med.*, 26: 1231-1237)

Acknowledgements:

Project: INNOACE (049_INNOACE_4_E) : Task 6 - Proposals for an efficient management of fertilization in irrigated fruit plantations in the Alentejo-Extremadura zone.

Financial support: ICAAM (UID/AGR/00115/2019)

POSTER NUMBER 2

Establishment of a Taqman-based Approach to Monitor *Fusarium* spp. airbone spores

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Aerobiological studies provide important information about the biological particles present in the air. Monitoring the presence of airborne fungal spores can help farmers to prevent the onset of fungal diseases that may affect both quantity and quality of crops. The fungi *Fusarium* spp. are among the most important phytopathogenic fungal communities with high impact at regional level by affecting important cultures such as almond, tomato, maize and cereals. The establishment of an approach that would enable farmers to early react upon the possibility of a *Fusarium* spp. infection will lead to a better control of the diseases associated to those fungi. Currently, to monitor *Fusarium* spp. airborne spores it is followed the *Hirst*-type methodology, which is based on spore's identification and quantification by optical microscope; a hard and time consuming process due to the spore's small size and colorless wall. In this context, the development of an alternative methodology that enable to get accurate and reliable results in a faster way will be of high interest. A Taqman specific assay for *Fusarium* spp. detection and quantification was previously established for a different purpose [1] and was here applied as a molecular-based tool to detect airbone *Fusarium* spp. spores. To collect the biological particles from the atmosphere a Burkard 7-Day Volumetric Spore Trap, and the *Hirst* associated methodology, was used as the methodology recommended by the European Aerobiology Society (EAS) and International Association for Aerobiology (IAA) [2]. As proof-of-concept, the analysis was focused on samples weekly collect, from 1st October - 31st December 2018 (14 weeks) at the station of Portuguese Aerobiology Network (RPA – SPAIC) (38º34'N; 7º54'W). Genomic DNA (gDNA) was extracted from collected biological particles adhered to the melinex tape following the CTAB protocol [3] with some modifications. Considering the results achieved, we consider the Taqman-specific assay as an alternative methodology for monitoring *Fusarium* spp. airbone spores.

Keywords: *Hirst* methodology, pathogenic fungi control, Taqman assay, airbone spores monitoring

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INTRODUCTION

Aerobiological studies provide important information about the biological particles present in the air. Monitoring the presence of airborne fungal spores can help farmers to prevent the onset of fungal diseases that may affect both quantity and quality of crops. The fungi *Fusarium* spp. are among the most important phytopathogenic fungal communities with high impact at regional level by affecting important cultures such as almond, tomato, maize and cereals. The establishment of an approach that would enable farmers to early react upon the possibility of a *Fusarium* spp. infection will for sure lead to a better control of the diseases associated to those fungi. Currently, to monitor *Fusarium* spp. airborne spores it is followed the Hirst-type methodology, which is based on spore's identification and quantification by optical microscope; a hard and time consuming process due to the spore's small size and colorless wall (Fig. 1). In this context, the development of an alternative methodology that enable to get accurate and reliable results in a faster way will be for sure of high interest. A Taqman specific assay for *Fusarium* spp. detection and quantification was previously established for a different purpose [1] and was here applied as a molecular-based tool to detect *Fusarium* spp. spores in the airborn.

Fig. 1: *Fusarium* sp. spores observed at optical microscope.

METHODOLOGY

SAMPLE COLLECTION

Biological particles were collected from the atmosphere using a Burkard 7-Day Volumetric Spore Trap (Fig. 2). As proof-of-concept, the analysis was focused on samples weekly collect, from 1st October - 31st December 2018 (14 weeks) at the station of Portuguese Aerobiology Network (RPA – SPAIC) (38°34'N; 7°54'W).

Fig. 2: *Burkard 7-Day Volumetric Spore-trap®* of Évora monitoring station RPA – SPAIC.

AEROBIOLOGICAL METHOD

Hirst associated methodology, was used as the methodology recommended by the European Aerobiology Society (EAS) and International Association for Aerobiology (IAA) [2].
Fusarium spp. spores were identified by appearance and morphological characteristics (colour, size and shape) using an optical microscope at a magnification of 400x along one longitudinal line at the center of the slide, and by comparison with bibliographic material [3, 4]. Spore counts were expressed as daily average number of spores per cubic meter of air (spores/m³/day).

MOLECULAR APPROACH

GENOMIC DNA (gDNA) was extracted from collected biological particles, adhered to the melinex tape containing a silicon solution, by following the CTAB protocol [5] including some modifications (Fig. 3). The concentration and purity of the solutions were obtained through the NanoDrop 2000c Spectrophotometer (Thermo).

Fig. 3: Images showing several steps of the gDNA extraction procedure, going from removal of biological particles from the milenex tape till DNA precipitation.

SYBRGreen TECHNOLOGY was used. qPCR reactions were carried out using 2 µL of gDNA following the procedure previously described [1]. gDNA from *Fusarium* spp. was included in the analysis as positive control.
As a measure of **SENSITIVITY** and the **QUANTITATIVE RANGE** of the developed qPCR procedure, the limit of detection was determined. Standards were prepared by a two-fold serial dilution of the gDNA from *Fusarium* spp.

RESULTS

AEROBIOLOGICAL ANALYSIS BY TRADITIONAL METHODOLOGY

- ✓ The maximum daily concentration was recorded on 13th November 2018, with 177 spores/m³ of air (Fig. 4).
- ✓ A total of 3449 spores of *Fusarium* spp. were recorded (identified and quantified) in the atmosphere samples of autumn of 2018 of Évora, and they were identified throughout all the period of study (Fig. 5).

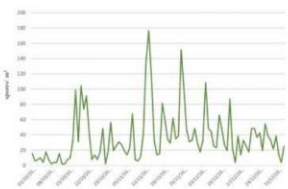

Fig. 4: *Fusarium* spp. spores concentration in the atmosphere.

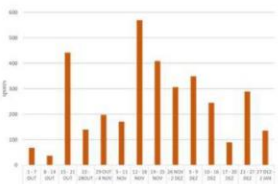

Fig. 5 *Fusarium* spp. spores index per week during the period of study.

AEROBIOLOGICAL ANALYSIS BY MOLECULAR APPROACH

Establishment of the method

- ✓ Detection limit of the *Fusarium* spp. qPCR assay was determined from the quantification cycle (Cq) of the lowest plasmid dilution that fell within the linear standard curve. qPCR assay presented an efficiency of 106 % (slope=-3.181 and R^2=0.99), with all parameters felling within the acceptance criteria, allowing the detection of 7.60 x 10^{-4} ng of *Fusarium* spp. (Table 1).

Table 1 Sensitivity of the TaqMan qPCR assay

Dilution	gDNA in PCR (ng)	*Fusarium* spp. Cq value (±SD)
P.C.	100.00	20.86 (± 0.23)
2^{-1}	50.00	21.76 (± 0.15)
2^{-2}	25.00	23.06 (±0.10)
2^{-3}	12.50	23.46 (± 0.20)
2^{-4}	6.25	24.68 (± 0.14)
2^{-5}	3.13	25.92 (± 0.38)
2^{-6}	1.56	27.00 (± 0.09)
2^{-7}	7.81×10^{-1}	27.68 (± 0.23)
2^{-8}	3.91×10^{-1}	28.60 (± 0.11)
2^{-9}	1.95×10^{-1}	29.72 (± 0.11)
2^{-10}	9.77×10^{-2}	30.81 (± 0.20)
2^{-11}	4.88×10^{-2}	31.28 (± 0.10)
2^{-12}	2.44×10^{-2}	32.50 (± 0.03)
2^{-13}	1.22×10^{-2}	33.46 (± 0.15)
2^{-14}	6.10×10^{-3}	34.24 (± 0.34)
2^{-15}	3.05×10^{-3}	35.12 (± 0.10)
2^{-16}	1.53×10^{-3}	36.20 (± 0.27)
2^{-17}	7.60×10^{-4}	37.27 (± 0.54)

P.C.: *Fusarium* spp. positive control

Evaluation of samples during the period of study

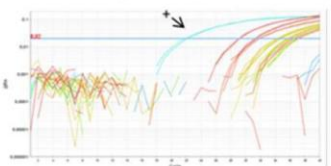

Fig. 6: Amplification plot of the samples collected during the 14 weeks for detection of Fusarium spp. by qPCR. +: positive control.

- ✓ Amplification plot (Fig. 6) reveled amplification of the samples throughout all the period of study. There were observed Cq values that vary from 29,10 to 37,02, all in the range detectable by the method.

CONCLUSIONS

- ✓ Both approaches allow the identification of *Fusarium* spp. in the samples collected from 1st October - 31st December 2018.
- ✓ Given the morphological characteristics of this spore type (mostly colorless, very small in size and the different forms it presents), its monitoring by the standardized methodology (*Hirst* methodology) is a process that consume very time. Therefore, the use qPCR combined with TaqMan technology arises as a alternative methodology that enable to get accurate and reliable results in a faster way.
- ✓ Although with results still preliminary, we consider the Taqman-specific assay as a promising methodology for monitoring *Fusarium* spp airbone biological spores.

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ACKNOWLEDGEMENTS

This work was financially supported by national funds through UID/AGR/00115/2019. MDC was supported by the project "Development of a new virus-based vector to control TSWV in tomato plants" with the reference ALT20-03-0145-FEDER-028266, co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo), ALGARVE 2020 (Regional Operational Program of the Algarve) and through the Foundation for Science and Technology, in its national component.

POSTER NUMBER 3

Climate Change Mitigation and Adaptation in Public Collective Irrigated Systems

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Irrigated agriculture is the largest consumer of water (in the country, in Europe and in the world). The need to increase crop production led to the transfer of a rainfed agriculture to irrigated agriculture, improving at about 10 times the productivity of cultures. However, also created the trend for the election of certain specific cultures.

Rehabilitation of collective irrigation systems with many years of operation and modernization of the existing perimeters, predominantly in channel flow, to transport in pressure increasing energy consumption, claims to adapt to climate change of this water supply service.

For this reason, the Collective Irrigated Systems are great consumers of water and energy and it's necessary to develop and implement plans for optimize water and energy management and also a climate change mitigation and adaptation plan.

In this work a Performance System to the Climate Change Mitigation and Adaptation Plan is presented, evaluating success of mitigate and adaptation measures, the satisfaction of agricultural irrigation users and the preservation of the sustainability of the service.

Keywords

Collective Irrigated Systems, water use efficiency, energy use efficiency, climate change, adaptation, mitigation.

A. Lambert and W. Hirner, Losses from Water Supply Systems: Standard Terminology and Performance Measures*, IWSA Blue Pages*, 2000.; H. Alegre, J.M. Baptista, E. Cabrera Jr., F. Cubillo, P. Duarte, W. Hirner, W. Merkel, R. Parena, *Performance indicators for water supply services*, second edition ed.: IWA Publishing, 2006.; A. Mamade, D. Loureiro, D. Covas, H. Alegre, Energy Auditing as a Tool for Improving Service Efficiency of Water Supply Systems, *Procedia Engineering*, 89 (2014) 557 – 564.; N. Carriço et. al, Eficiência Hídrica e Energética em Aproveitamentos, Hidroagrícolas, *14º Congresso da Água*, 2018, Évora.

AGIR Project - Avaliação da Eficiência do Uso da Água e da Energia em Aproveitamentos Hidroagrícolas, coordinated by FENAREG and supported by National Irrigation Authority and National Rural Development Program (2014-2020).

POSTER NUMBER 3

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Climate Change Mitigation and Adaptation in Public Collective Irrigated Systems

IRRIGATED AGRICULTURE

M. Moreira[1], R. C. Guimarães[1], M. Rijo[1], C. Arranja[2], D. Cordeiro[2], D. Loureiro[3], H. Cunha[3], P. Luz[4], N. Carriço[5], B. Ferreira[5], G. Rodrigues[6], C. Chibeles[7], G. Sousa[8], M. Matos[9]

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1. Introduction

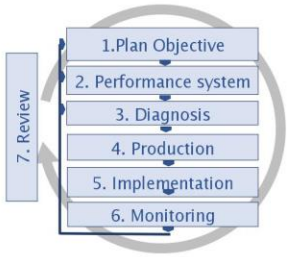
Collective Irrigated Systems are a great consumers of water and energy → Optimize water and energy efficiency → develop a climate change mitigation and adaptation Plan

2. Objective of the study

- Collective Irrigation Systems on the way to **Nearly Zero Energy**.
- A Performance System to the **Climate Change Mitigation** and **Adaptation** Plan, ensuring the water supplying and satisfaction of the agricultural irrigation users and preserving the sustainability of the service.

3. Methodology of the Climate Change Mitigation and Adaptation Plan

Step–process to create the plan
PDCA technique



4. Water Balance

Water balance of Collective Irrigation Systems (m³)

System input volume	Authorised consumption	Billed authorized consumption	... metered	Revenue water
			... unmetered	
		Unbilled authorized consumption	... metered	Non-revenue water
			... unmetered	
	Water losses	Evaporation losses	...in channels	
			...in reservoirs	
		Apparent losses	Unauthorized consumption	
			Metering inaccuracies	
		Real losses	Leakages in pipes	
			Infiltration in channels	
			Infiltration in reservoirs	
			Overflows in channels	
			Overflows in reservoirs	

Adapted from IWA water balance

Water balance at farm scale (m³)

			Comments
Water application	Crop consumption		Data - climatic variables
	Water losses	Evaporation	Data - climatic variables
		Runoff	Result - Risk Classes
		Percolation	Classification of soil texture, water amounts and application rates
		Wind drift	Data - soil characterization
			Data- climatic variables

5. Energy Balance

Energy balance (kWh)

System total energy = potential gravitic energy + Pumping energy	Energy associated with authorized consumption	Energy delivered to farmers	Minimum energy required
			Superfluous energy
		Energy dissipated associated with consumption	in channels and pipes
			in gates and valves
			in pumps
			in turbines
		Energy recovered	from authorized consumption
			from water losses
	Energy associated with water losses	Energy dissipated associated with water losses	in nodes where water losses occur
			in channels and pipes
			in gates and valves
			in pumps
			in turbines

Adapted from LNEC/IST energy balance

Energy/GHG emissions balance (kWh/ t CO_2e)

System total energy	Renewable source energy	Own renewable sources	GHG'ers
	Nonrenewable source energy	Electrical grid source	GHG eqs
		Electrical grid source	GHG eqs

6. Performance System

Water and energy efficiency performance assessment system of the collective irrigation network

Climate change mitigation and adaptation performance assessment system of the collective irrigation network

Goals	Criteria	Performance indicator	Description	Comments
Sustainability of irrigation water services	Economic and financial sustainability			These performance assessment indicators evaluate irrigation water services sustainability, despite the application of climate change mitigation and adaptation actions.
	Infrastructural Sustainability			
	Operational and maintenance sustainability			
Nearly zero energy collective irrigation systems (NZE–CIS)	Energy efficiency	CIS15–Energy efficiency of the pumping facilities	Rate between useful energy and energy supplied to pumping facilities.	Measures of improving energy efficiency in the largest energy consumers in collective irrigation systems
		CIS16– Total energy supplied to the system.	Rate between total energy supplied to the system (gravity and pumping) and minimum energy required.	Measures of improving energy efficiency at other infrastructures in collective irrigation systems.
	Reducing energy consumption	CIS17–Energy associated with water losses inefficiency	Rate between energy associated with physical water losses and total energy supplied to the system (gravity and pumping).	Measures the reduction of energy consumption based on the reduction of physical water losses.
	Reducing GHG emissions	CIS22– Renewable energy rate	Percentage of total energy consumed from renewable sources.	Measures on own renewable energy and electrical grid renewable energy.
		CIS23– Own renewable energy sources	Percentage of total energy consumed from own renewable sources.	Measures on renewable energy autonomy (recovered when there are conditions for installing microturbines or other sources)
		CIS24– GHG emissions balance	Rate between total GHG emissions and GHG emissions planned fitting current targets.	Identify collective irrigation systems capacity to meet national and European targets.
Adequacy of service provided to irrigation water users	Accessibility of service			These performance assessment indicators evaluate continuity of the economic accessibility of the irrigation water users and service quality, despite the application of climate change mitigation and adaptation actions
	Quality of service			

7. Case Studies

ODIVELAS – VALE DO SORRAIA – VIGIA

8. Conclusions

Climate Change Mitigation and Adaptation Plan will allow the preparation, prevention and establishment of the Collective Irrigation Systems contingency plan.
This is a good example of – **Research on the strategic use of water and their impacts, and adaptation to climate change as well as mitigation measures together to** develop a tool to measure how collective irrigation systems are **prepared for** climate **change** today and tomorrow.

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A. Lambert and W. Hirner, Losses from Water Supply Systems: Standard Terminology and Performance Measures, IWSA Blue Pages, 2000.

H. Alegre, J.M. Baptista, E. Cabrera Jr., F. Cubillo, P. Duarte, W. Hirner, W. Merkel, R. Parena, Performance indicators for water supply services, second edition ed.: IWA Publishing, 2006.

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10. Acknowledgements

AGIR | Avaliação da Eficiência do Uso da Água e da Energia em Aproveitamentos Hidroagrícolas

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POSTER NUMBER 4

Precision Conservation Technologies as Tools to Fodder Production,

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Given the climate change scenario and spatial heterogeneity of agricultural landscapes in Mediterranean regions, the need for sustainable intensification of fodder production to support extensive animal production systems requires new technical itineraries based on new forms of mechanization and the adoption of georeferenced sensory technologies that allow spatial and temporal analysis to mapping field and crop variations. The term *precision conservation* has emerged as a way of describing approaches that aim to conserve soil and water in agricultural and natural lands, based on a combination of spatial technologies and procedures to improve the efficiency and effectiveness of inputs [1] [2]. In this three years (2017 – 2019) study, we used precision agriculture to delineate management zones and cost effective crop of an annual biodiverse mixture fodder under no-till. The experiment took place in an area of 31 ha at the coordinate's 38° 53' 39''N 7° 03' 03''W. The field was mapped for soil apparent electrical conductivity with a Veris 2000 XA sensor combined with the use of a Global Navigation Satellite System, soil and crop samples were monitored in 16 different georeferenced locations and normalized difference vegetation index (NDVI) evaluated by remote sensing using Sentinel -2. Data were processed by linear model and geospatial ArcGIS software. Results showed spatial auto correlation for soil apparent electrical conductivity (EC_a) and topography as well as two different areas were identified considering soil EC_a, soil texture and the need of herbicide at the sowing moment. Along the crop cycle statistical analysis resulted in significant correlation coefficient values between fresh and dry matter yield and the higher vegetation index values (0.79) reached between the months of March and April. Although with a predomination of grasses families the average yield value of 8120 kg ha^{-1} of dry matter is according to the seed manufacturer's technical catalogue and comparable to that on Italian ryegrass under no tillage systems [3] and so demonstrating the contribution that precision conservation tools can afford to sustainable intensification of fodder production.

Keywords: no till, on-the-go sensors, remote sensing, yield grass, mechanization

Basso, B., Sartori, L., Bertocco, M., Cammarano, D., Martin, E., & Grace, P. (2011). Economic and environmental evaluation of site-specific tillage in a maize crop in NE Italy. European Journal of Agronomy, 35, 82–83; Fraser, A., 2018. Land grab/data grab: precision agriculture and its new horizons. J. Peasant Stud. 1–20.; Bueno, J., Amiama, C., Hernanz, J.L., 2007. No-tillage drilling of Italian ryegrass (Lolium multiflorum L.): Crop residue effects, yields and economic benefits. Soil and Tillage Research, Volume 95, Issues 1-2: 61-68, doi: 10.1016/j.still.2006.11.002.

Work carried out under the MechSmart Forages project http://mechsmartforages.ipportalegre.pt/

POSTER NUMBER 5

Project China Europe Platform for Water - Horizontal Activities

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The University of Évora (UEvora) participates in the project to support the China Europe Platform for Water (CEWP) in partnership with the Ministry of Environment and Energy Transition. CEWP is a governmental platform (represented by the Member States of the European Union at the ministerial level) and UEvora has proposed a working group on Restoration, Ecosystem Services and Biodiversity which is part of the thematic area "Water Management and Ecological Security". This project aims to contribute to the development and reinforcement of activities that are promoted by CEWP in the following areas: "Water and Rural Areas"; "Water and Energy"; "Water and Urbanization" and "Water Management and Ecological Safety". Being transversal objectives the development of Policies, Research and Business. Portugal will ensure the Secretariat of CEWP in the years 2020-2021, organizing this year the high level meeting in Portugal. In this project the UEvora participates in subjects as diverse as: Circular Economy; Open Innovation; Matchmaking activities (Companies); Alignment of Horizon Europe Funds with Chinese funds dedicated to cooperation and research in the water sector as well as emerging issues. For additional information and involvement in future activities contact: aimendes@uevora.pt

Keywords: China Europe Water Platform, Open Innovation, Horizon Europe

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POSTER NUMBER 6

Transcriptome Changes Induced by Soil Disturbance and Preceding Plant Species in Wheat (*Triticum aestivum* L.) Roots

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In the last years it has been shown that the growth of wheat (*Triticum aestivum* L.) in a soil conveying Mn toxicity improved if the species *Ornithopus compressus* rather than *Lolium rigidum* was grown prior to wheat, provided that soil was undisturbed and thus an intact extraradical mycelial network of arbuscular mycorrhizal fungi (AMF) was present when wheat was planted [1]. These preceding plant species were also found to induce different AMF communities in wheat roots [2].
To understand the response mechanisms of wheat to these preceding plant species we conducted a transcriptomic analysis in roots of 5 weeks old wheat, planted after *L. rigidum* or after *O. compressus*, with and without soil disturbance prior to planting.
The results showed that the soil disturbance induced an over-expression of genes related to stress responses and phosphate starvation in wheat roots. Considering the undisturbed regime alone, we found that previous colonization of *O. compressus* had induced the expression of wheat genes related to growth (for example related to translation or regulation of gene expression), whereas the *L. rigidum* treatment led to the up-regulation of genes related to stress responses, such as oxidative stress, cellular detoxification or Mn ion binding.
These results are the first to show that the preceding plant species - *O. compressus* or *L. rigidum* - clearly induced changes in the transcriptomic responses of wheat, probably associated to the different mycorrhizal communities present in the roots. These results are of major importance to understand the mechanisms of protection triggered by different AMF communities.

Keywords: *Triticum aestivum*; Mn toxicity; transcriptome; arbuscular mycorrhizal fungi; stress

1. Brito I, Carvalho M, Alho L, Goss MJ. Managing arbuscular mycorrhizal fungi for bioprotection: Mn toxicity. Soil Biol Biochem. Elsevier Ltd; 2014;68: 78–84. doi:10.1016/j.soilbio.2013.09.018
2. Campos C, Carvalho M, Brígido C, Goss MJ, Nobre T. Symbiosis Specificity of the Preceding Host Plant Can Dominate but Not Obliterate the Association Between Wheat and Its Arbuscular Mycorrhizal Fungal Partners. Front Microbiol. 2018;9: 2920. doi:10.3389/fmicb.2018.02920

This work was funded by National Funds through FCT – Foundation for Science and Technology, under the Project UID/AGR/00115/2013, and co-financed by the European Union through the European Regional Development Fund, under ALENTEJO 2020 (Alentejo Regional Operational Program) through the project “Improving pasture production in acid soils in montado: chemical and biological approach,” Reference No. ALT20-03 -0145- FEDER-000039. Catarina Campos acknowledges the postdoctoral fellowship from the Portuguese Foundation for Science and Technology (Ref SFRH/BPD/108734/2015).

POSTER NUMBER 6

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Transcriptome changes induced by soil disturbance and preceding plant species in wheat (*Triticum aestivum* L.) roots

IRRIGATED AGRICULTURE

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INTRODUCTION

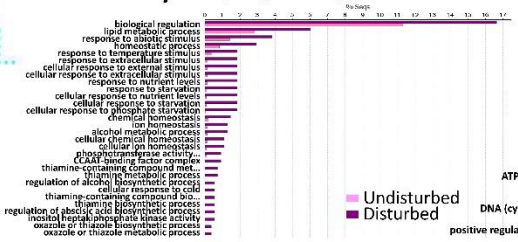
Our earlier work has shown that the growth of wheat (*Triticum aestivum* L.) in soil inducing manganese (Mn) toxicity improved if the species *Ornithopus compressus* rather than *Lolium rigidum* was grown prior to wheat, provided that the soil was undisturbed and thus an intact extraradical mycelial network of arbuscular mycorrhizal fungi (AMF) was present when the wheat was planted [1]. These preceding plant species were also found to induce different AMF communities in wheat roots [2].

To understand the molecular mechanisms of response of wheat to these preceding plant species, a transcriptomic analysis using Illumina sequencing platform was conducted in roots of 5 weeks old wheat, planted after *L. rigidum* or after *O. compressus*, with and without soil disturbance prior to planting.

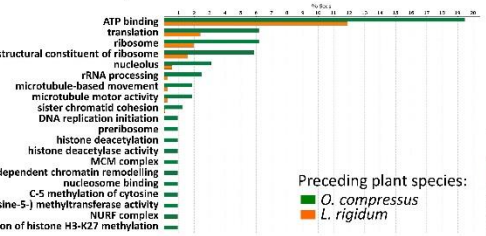
RESULTS

- Soil disturbance induced an over-expression of genes related to stress responses and phosphate starvation in wheat roots (**a**). In undisturbed soil, the expression of genes related to metabolism of organic substances was increased (**b**).
- Within the undisturbed soil, the previous colonization of *O. compressus* induced the expression of wheat genes related to growth (for example related to translation or regulation of gene expression) (**c**), whereas the *L. rigidum* treatment led to the up-regulation of genes related to stress responses, such as oxidative stress, cellular detoxification or Mn ion binding (**d**).
- The analysis of activated metabolic pathways also showed that wheat from the undisturbed *L. rigidum* treatment increased the production of secondary metabolites (**e**).

a) Up-regulated genes disturbed



c) Up-regulated genes after *O. compressus*

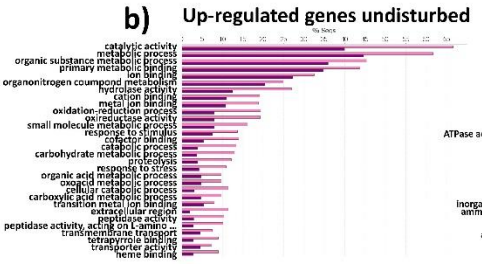


e)

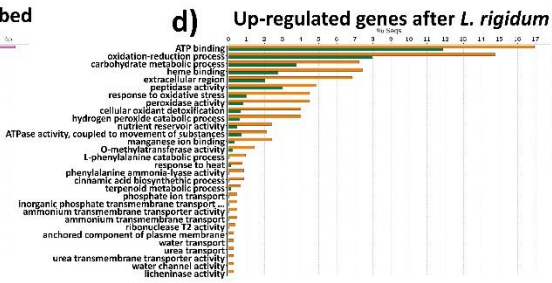
Number of activated pathways

Metabolic activities	Und *vs* Dis		Undisturbed *O. compressus vs L. rigidum*	
Biosynthesis of antibiotics	1	1	1	1
Carbohydrate metabolism	14	10	10	10
Nucleotide metabolism	2	2	2	1
Amino acid metabolism	10	5	11	10
Metabolism of other amino acids	3	4	3	4
Lipid metabolism	9	6	2	10
Metabolism of cofactors and vitamins	8	3	4	5
Biosynthesis of other secondary metabolites	7	3	4	6
Xenobiotics biodegradation and metabolism	4	5	4	4
Energy metabolism	6	4	2	4
Metabolism of terpenoids and polyketides	1	2	1	5
Glycan biosynthesis and metabolism	5	4	1	4
Chemical structure transformation maps	0	0	0	1
Signal transduction	1	1	1	1
Translation	1	1	1	1
Immune system	2	0	2	2

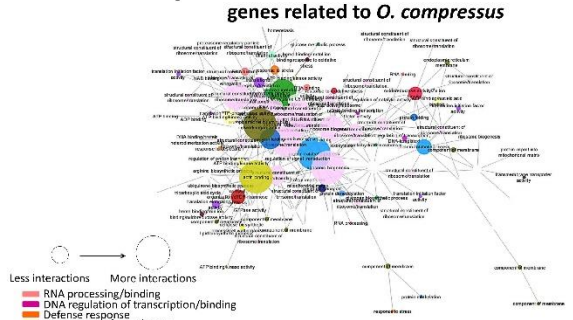
b) Up-regulated genes undisturbed



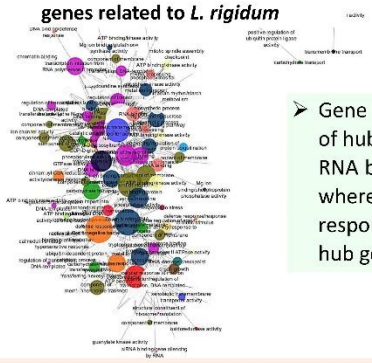
d) Up-regulated genes after *L. rigidum*



f) Network of interactions between wheat genes related to *O. compressus*



g) Network of interactions between wheat genes related to *L. rigidum*



- Gene network analysis showed a high number of hub genes related to translation, ribosome or RNA binding in the *O. compressus* treatment (**f**), whereas in the *L. rigidum* treatment the defense responses or metal ion binding were among the hub genes (**g**).

CONCLUSIONS

These results are the first to show that the preceding plant species clearly induce changes in the transcriptomic responses of wheat, probably associated to the different mycorrhizal communities present in the roots. These results are of major importance to understand the mechanisms of protection triggered by different AMF communities.

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This work was funded by National Funds through FCT – Foundation for Science and Technology, under the Project UID/AGR/00115/2019, and co-financed by the European Union through the European Regional Development Fund, under ALENTEJO 2020 (Alentejo Regional Operational Program) through the project "Improving pasture production in acid soils in montado: chemical and biological approach," Reference No. ALT20-03 -0145- FEDER-000039. Catarina Campos acknowledges the postdoctoral fellowship from the Portuguese Foundation for Science and Technology (Ref SFRH/BPD/108734/2015).

POSTER NUMBER 7

Treatment and Reuse of Agroindustrial Wastewaters

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The agro-industry is the main activity of the Alentejo region. However, it leads to the production of various types of wastewater with high environmental impact. These wastewaters can cause serious problems into the environment, such as, eutrophication phenomena, oxygen rapid depletion, soil salinity and groundwater contamination, as well as potential risks for the human health. Thus, appropriate mechanisms of management were required. Moreover, the agroindustrial wastewaters may have significant economic benefits owing to their fertilizer value [1]. Under the HYDROREUSE project (http://hydroreuse.pt/) is ongoing the characterization of four types of agroindustrial wastewaters produced in the Alentejo region (slaughterhouse, winery, olive-oil and cheese production wastewaters) and the evaluation of the performance of precipitation and oxidation processes to treat these wastewaters. Additionally, the treated wastewater is being tested in hydroponic system with tomato plants.
Slaughterhouse, winery, olive-oil and cheese production wastewaters presented an organic matter content evaluated by chemical oxygen demand (COD) between 2000 and 8000 mg L-1, being rich in nutrients such as phosphorus, calcium and magnesium. The application of physicochemical processes to raw slaughterhouse wastewater, namely acid precipitation with H2SO4 addition, led to the reduction of COD (97%), phosphorus (37%), turbidity (98%), total phenols (74%) and absorbances (77-97%). Olive-oil wastewater has been treated by means of oxidation with calcium peroxide, reducing COD (58%), phosphorus (95%), turbidity (100%), total phenols (55%) and absorbances (43-99%). Similar results were obtained when winery and cheese wastewater have been treated by basic calcic precipitation.

Keywords agroindustrial wastewater; contamination; physicochemical properties; physicochemical processes; reuse; hydroponic system

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This work was supported by the Program Alentejo 2020, through the FEDER under the scope of “HYDROREUSE” project (ALT20-03-0145-FEDER-000021). S. Luz also thanks to the FCT/MCTES under research grant (SFRH/BD/129849/2017).

POSTER NUMBER 7

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Treatment and Reuse of Agroindustrial Wastewaters

IRRIGATED AGRICULTURE

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OBJECTIVE

- ✓ **Characterization of four types of agroindustrial wastewaters produced in the Alentejo region.**
- ✓ **Evaluation of the performance of precipitation and oxidation processes to treat these wastewaters.**

CONCLUSIONS

Wastewaters presented an organic matter content evaluated by chemical oxygen demand (COD) between 2000 and 8000 mg L^{-1}, being rich in nutrients (P, Ca and Mg). The application of physicochemical processes to raw wastewater led to the reduction of COD, phosphorus, turbidity, total phenols and absorbances.

RESULTS & DISCUSSION

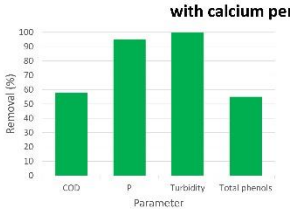
1. Physicochemical characteristics of wastewaters

Parameter	Units	Cheese	Olive-oil	Slaughterhouse	Winery
pH	Sorensen scale	4.52±0.205	4.63±0.142	6.89±0.237	6.80±0.056
Conductivity	dS m^{-1}	5.23±0.277	2.69±0.133	2.96±0.109	1.62±0.064
Turbidity	NTU	595±49.5	280±2.6	798±156.2	321±145.1
COD	mg L^{-1}	4917±353.6	7910±330.7	4660±1040.3	2003±423.3
Phosphorus	mg L^{-1}	3412±1255.6	1050±84.7	677±90.7	210±24.3
Calcium	mg L^{-1}	236±28.9	211±42.6	145±21.3	110±12.9
Magnesium	mg L^{-1}	110±36.8	73.6±15.86	54.7±19.61	55±14.8
Total alkalinity	mg L^{-1} $CaCO_3$	1340±251.1	2039±344.8	885±96.1	701±48.1
Bicarbonates	mg L^{-1} $CaCO_3$	1340±251.1	2039±344.8	885±96.1	701±48.1
Total hardness	mg L^{-1} $CaCO_3$	1040±168.7	971±470.9	586±120.6	504±49.9
Calcium hardness	mg L^{-1} $CaCO_3$	589±72.2	526±106.2	362±53.2	277±32.1
Magnesium hardness	mg L^{-1} $CaCO_3$	450±151.3	445±435.5	225±80.7	228±60.8
Total phenols	mg L^{-1}	75.9±6.76	540±50.4	34.8±10.19	27.8±3.01
Absorbances					
220 nm	cm^{-1}	0.639 ± 0.0383*	1.027±0.0994*	0.184±0.0473*	0.143±0.0397*
254 nm	cm^{-1}	0.180 ± 0.0147*	0.366±0.0637*	0.093±0.0315*	0.093±0.031*
410 nm	cm^{-1}	0.092 ± 0.0065*	0.076±0.0140*	0.047±0.0152*	0.033±0.0161*
600 nm	cm^{-1}	0.060 ± 0.0072*	0.031±0.0054*	0.054±0.0198**	0.067±0.0059***

COD - chemical oxygen demand; * (1:50); ** (1:25); *** (1:20)

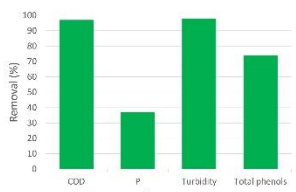
2. Application of physicochemical processes

Treatment of olive-oil wastewater by means of oxidation with calcium peroxide



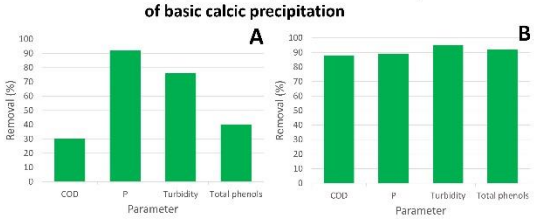
Removal: COD (58%) phosphorus (95%) turbidity (100%) total phenols (55%)

Treatment of slaughterhouse wastewater by means of acid precipitation with H_2SO_4 addition



Removal: COD (97%) phosphorus (37%) turbidity (98%) total phenols (74%)

Treatment of cheese (A) and winery (B) wastewaters by means of basic calcic precipitation



Removal: COD (30-88%) phosphorus (89-92%) turbidity (76-95%) total phenols (40-92%)

3. Reuse of treated wastewater for irrigation purposes (hydroponic system)

MATERIALS & METHODS

1. Physicochemical characteristics of wastewaters

- ✓ The raw wastewaters were taken from companies located in the Alentejo region, South of Portugal.
- ✓ Standard methods were used to determine the properties of raw and treated wastewaters.

2. Application of physicochemical processes

- ✓ Olive-oil wastewater – CaO_2 addition (concentration = 1-9.0 g L^{-1})
- ✓ Slaughterhouse wastewater – H_2SO_4 addition (pH = 1.0-6.0)
- ✓ Cheese wastewater – $Ca(OH)_2$ addition (pH = 6.0-13.0)
- ✓ Winery wastewater – $CaCO_3$ addition (concentration = 12.5-125 g L^{-1})

Acknowledgements:

"HYDROREUSE" project - Treatment and reuse of agro-industrial wastewaters using an innovative hydroponic system with tomato plants (ALT20-03-0145-FEDER-000021)

S. Luz research grant (SFRH/BD/129849/2017)
Projet UID/AGR/00115/2019

POSTER NUMBER 8

Reservoir Evaporation Estimation in a Mediterranean Climate. Comparing Direct and Indirect Methods

C. Miranda Rodrigues[1], R. Salgado[2], M. Potes[2], R. C. Guimarães[1], M. Moreira[1]

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Reservoir evaporation in semi-arid region facing scarcity, namely Southern Portugal, is one of the most important contribution to the losses of water in collective irrigation systems and water resources management. Irrigation crops depend on total water availability and its year variability.
Evaporation is the component of the reservoir water balance less known by technical and scientific community. So, it is a major research subject.
The objective of this research is to compare the results of the indirect methods (Standard class-A pan measurements, mass transfer, energy balance, combination approach) with the direct methods measures (Eddy Covariance).
Atmospheric, water (water temperature was measured at several depths) and EC variables were continuous measured on a floating platform over water at Alqueva reservoir, from Jun to Oct 2014.
The results of this case study could be applied to other reservoirs in Mediterranean climate region.

Keywords: Reservoir evaporation, Eddy covariance, turbulent fluxes of latent heat, energy balance, Mediterranean climate.

M. Potes, R. Salgado, M. J. Costa, M. Morais, D. Bortoli, I. Kostadinov & I. Mammarella (2017) Lake–atmosphere interactions at Alqueva reservoir: a case study in the summer of 2014, Tellus A: Dynamic Meteorology and Oceanography, 69:1, 1272787

ALEX Project – Alqueva hydro-meteorological Experiment (2014-2020) supported by FCT: project EXPL/GEO-MET/1422/2013 and FEDER - COMPETE: FCOMP-01-0124-FEDER-041840

POSTER NUMBER 8

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Reservoir evaporation estimation in a Mediterranean climate. Comparing direct and indirect methods

IRRIGATED AGRICULTURE

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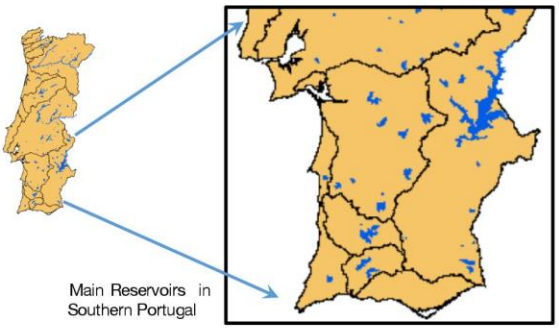
1. Introduction and objectives

Reservoir evaporation in semi-arid region facing scarcity, namely Southern Portugal, is one of the most important contribution to the losses of water in collective irrigation systems and water resources management. Irrigation crops depend on water availability, total and in year variability.

Evaporation is the component of the reservoir water balance less known by technical and scientific community. So, it is a major research subject.

The objective of this research is to compare the results of the indirect methods with the direct methods measures.



Main Reservoirs in Southern Portugal

2. Eddy Covariance

The Eddy Covariance Method (EC) is a non-invasive technique and the most common technique used to assess turbulent fluxes over all types of surfaces. To estimate water reservoir evaporation by EC, a fast-response instrumentation (20 Hz) is setup above water surface, usually on a floating platform, and variables such as u, v and w components of wind speed, sonic temperature and absolute humidity (H_2O), are measured. Turbulent fluxes of latent heat (evaporation) (LE) are calculated as covariances between fluctuation of vertical wind (w') and H_2O concentration expressed as water vapour density (ρ_w').

$$LE = \lambda \overline{w' \rho_w'}$$

λ is the latent heat of evaporation

EC system (IRGASON; Campbell Scientific) installed on a floating platform at Alqueva reservoir, within the framework of the ALEX project (www.alex2014.cge.uevora.pt)

3. Indirect Methods

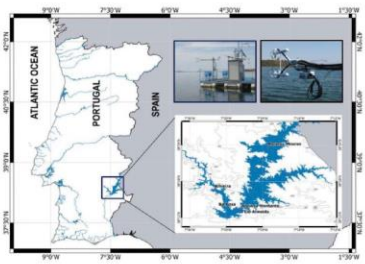
- standard class-A pan measurements;
- mass transfer ; $E = NU_2 (e_s - e_a)$
- energy balance; $E = \frac{R_n - \Delta Q}{\rho_w \left[\lambda (1+\beta) + c_{pw} (T_s - T_b)\right]} \times 86.4 \times 10^6$
- combination approach.

$$E = (2\alpha - 1)\left(\frac{s}{s+\gamma}\right)\left(\frac{R_n - \Delta Q}{\lambda \rho_w}\right) \times 86.4 \times 10^5$$

$$- \frac{\gamma}{s+\gamma}\left[0.26(0.5 + 0.54U_2)(e_a^* - e_a) \times 10^{-2}\right]$$

4. Case study

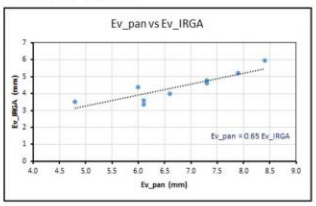
Atmospheric, water and EC variables were continuous measured on a floating platform over water, Jun to Oct 2014. Water temperature was measured at several depths.

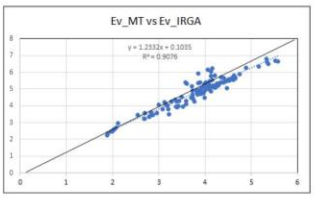


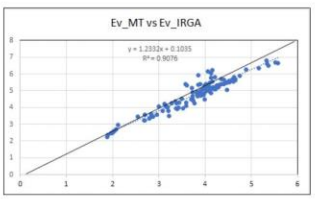
Alqueva reservoir: geographic location, floating platforms and meteorological stations

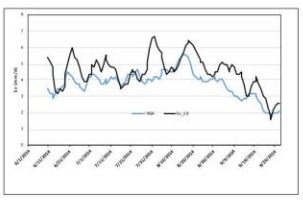
Potes *et* al. 2017

5. Results









6. References

M. Potes, R. Salgado, M. J. Costa, M. Morais, D. Bortoli, I. Kostadinov & I. Mammarella (2017) Lake–atmosphere interactions at Alqueva reservoir: a case study in the summer of 2014, Tellus A: Dynamic Meteorology and Oceanography, 69:1, 1272787

7. Acknowledgements

ALEX2014 Alqueva hydro-meteorological Experiment

Instituto de Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

POSTER NUMBER 1

Genetic, Morphological and Biochemical Characterization of *Cynara cardunculus* from Alentejo Region

MM. Castro[1], D. Rosa1, A. Paulino1, AM. Ferro1, T. Brás[1,2], D. Mendonça[3], F. Simões[3], E. Machado[4], Almeida. E[4], AF. Belo[45],, C. Pinto-Cruz[4,5], L. Marum[1,4], MF. Duarte[1,4]

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Cynara cardunculus (*Cc*) is a Mediterranean species used as a multipurpose crop, not only in food but also as solid biofuel, as a source of paper pulp, while the seeds are extracted for oil and the flowers employed in cheese production. Furthermore, *Cc* leaves have been used in folk medicine given to their hepatoprotective and choleretic actions due to their phenolic and terpenic composition. The main goal of this work was to address the natural cardoon genetic, morphological and biochemical variabilities within different natural occurring geographic populations. It was studied a collection of 4 *Cc* geographic populations, comprising 23 individuals distributed across Alentejo region, South of Portugal. Genetic diversity of the population was determined using 10 microsatellite markers, previously identified. To the study of plants morphological profile were evaluated 32 morphological descriptors. Previously, our research group reported cynaropicrin (Cyn) as the main compound detected in *Cc* leaves-lipophilic extracts (CLE) [1], which belong to sesquiterpene lactones family well recognized by several biological activities. Biochemical profiles, specifically Cyn quantification, was assess by HPLC. Results of morphological characterization, genetic diversity and biochemical profiles will be presented. ValBioTecCynara represents a combined strategy to address the natural cardoon genetic, molecular, morphologic, and biochemical variabilities, to identify individuals with certain and specific required profiles.

Keywords *Cynara cardunculus*; biochemical profiles; genetic diversity; cynaropicrin.

1. Ramos PAB., et al. Lipophilic extracts of *C. cardunculus* L. var. altilis (DC): a source of valuable bioactive terpenic compounds. Journal of Agriculture and Food Chemistry, 2013. 61, 8420–8429.

The present work was supported by ValBioTecCynara-ALT20-03-0145-FEDER-000038 project. cofinanced by FEDER under the Alentejo 2020 Program. FCT for UID/AGR/00115/2019 to ICAAM, and SFRH/BD/110969/2015 (Teresa Brás).

POSTER NUMBER 1

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Genetic, Morphological and Biochemical Characterization of *Cynara cardunculus* from Alentejo Region

ANIMAL PRODUCTION AND HEALTH

MM. Castro[1], D. Rosa[1], A. Paulino[1], AM. Ferro[1], T. Brás[1,2], D. Mendonça[3], F. Simões[3], E. Machado[4], E. Almeida [4], AF. Belo[4,5], C. Pinto-Cruz[4,5], L. Marum[1,4], MF. Duarte[1,4]

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Introduction

- ✓ *Cynara cardunculus* (*Cc*) is a Mediterranean species used as a multipurpose crop.
- ✓ *Cc* leaves have been used in folk medicine given to their hepatoprotective and choleretic actions due to their phenolic and terpenic composition. Cynaropicrin (Cyn) was reported as the main compound detected in *Cynara cardunculus* leaves-lipophilic extracts[1].
- ✓ In South part of Portugal, within Alentejo region, there is a great Cc phenotipic variability, which urges to be explored as a source of morphological, genetic, and chemical variability, for further crop economic valorization.

Aim: To address the natural cardoon genetic, morphological and biochemical variabilities within different natural occurring geographic populations.

Material and Methods

Cynara cardunculus geographic populations

From a total of 18 *Cc* geographic populations, within the presented study 4 *Cc* populations were assessed, comprising 23 individuals distributed across Alentejo region, South of Portugal.

4 geographic populations

Herdade da Abóboda, Serpa (HA)
Juromenha A and B (JUR A/B)
Évora (EV)

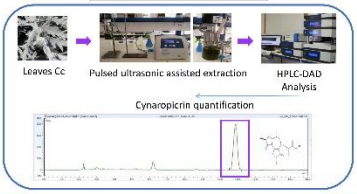
Morphological characterization

44 morphological and biomass measurements were obtained from each individual including:
- ✓ All plant
- ✓ Stem
- ✓ Leaves
- ✓ Major and secondary Inflorescence
- ✓ Seeds

Genetic characterization

Genetic and structural analysis by GenAlEx and STRUCTURE softwares

Chemical characterization



Results and Discussion

Morphological characterization

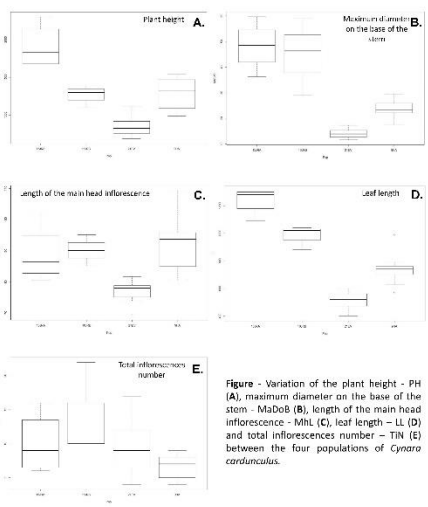


Figure - Variation of the plant height - PH (**A**), maximum diameter on the base of the stem - MaDoB (**B**), length of the main head inflorescence - MhL (**C**), leaf length – LL (**D**) and total inflorescences number – TIN (**E**) between the four populations of *Cynara cardunculus*.

Genetic characterization

- **Genetic diversity**

Table - Genetic diversity of 4 studied geographic populations of *Cynara Cardunculus*

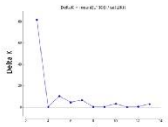
Geographic population	Na	Ne	I	Ho	He	F
HA	5.500	3.713	1.388	0.733	0.689	0.031
JUR A/B	4.000	3.017	1.201	0.586	0.654	0.097
EV	3.100	2.434	0.844	0.443	0.465	0.039

Na- Number of different alleles; Ne- Number of effective alleles; I-Shannon's information Index; Ho-observed Heterozygosity; He-Expected Heterozygosity; F-Fixation Index.

HA geographic population showed an increase of the number of alleles (observed and effective) and heterozygosity (observed and expected), when compared with **EV** and **JUR A/B.**

- **Population structure**

The optimum number of populations (K) was estimated by STRUTURE and identified by STRUTURE HARVESTER software, through comparing log probabilities of data for each value of K.



K=3 (Δk =81.76) is the optimum number of populations

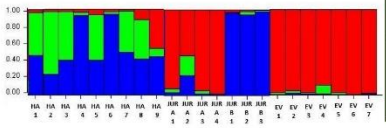
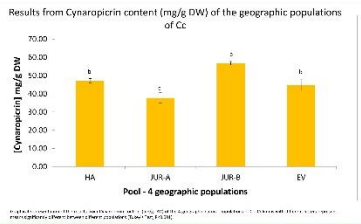
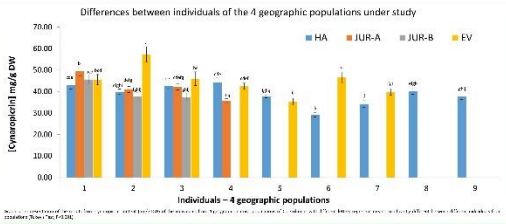


Figure - Structure analysis of 4 *Cynara Cardunculus* geographic populations. Clusters inferred from the population structure analysis at K=3.

- **HA** geographic population was included in the three groups **1**, **2** and **3** (red, blue and green);
- The genotypes from **EV** were clustered with genotypes from **JUR A**, while the **JUR B** shared some genetic similarity with **HA.**

Chemical characterization





- There is a great variability in the Cyn content among individuals of the different populations;
- There is no significant difference between individuals **2** and **3** of **JUR B**, but there is difference in individual **1**, in the genetic characterization the 3 individuals of JUR B are genetically the same.

Conclusions

- ✓ The extensive morphologic characterization of *Cynara cardunculus* thistle allowed us to confirm that there are significant differences among all populations and for all morphological descriptors.
- ✓ Genetic diversity of the 3 geographic populations (HA, JUR and EV) showed that HA has an excessive heterozygosity while the EV geographic population presented a low heterozygosity, which it is possible to confirm in the results of population structure. Considering the genetic similarities, three subgroups were found, in which EV and JUR A can be clustered in the same group.
- ✓ The results presented in the graphics show the cynaropicrin content (mg/g DW) from Cc leaves populations pool and individuals, we can observe that the populations present very different levels in Cyn what it suggests a great natural variability. This natural variability carries an enormous chemical potential, which ultimately needs a selection of the best producers.

New analyses are being conducted and extended to other geographic populations of Portugal. *Cynara cardunculus* natural variability exploitation, and the relationship between genotypes, with desired morphological and chemical profiles, will be essential for generating a molecular database useful for new biotech strategies, and future breeding programs, contributing to the economic valorization of *Cynara cardunculus* at regional and national levels.

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[1] Ramos PAB., et al., 2013. Lipophilic extracts of *C. cardunculus* L. var. altilis (DC): a source of valuable bioactive terpenic compounds. *Journal of Agriculture and Food Chemistry*, 61, 8420–8429.

Acknowledgements
This work was supported by the Program Alentejo 2020, through the European Fund for Regional Development under the scope of ValBioTecCynara – *Economic valorization of Cardoon (Cynara cardunculus): study of natural variability and biotechnological applications* (**ALT20-03-0145-FEDER-000038**)
The authors acknowledge FCT for UID/AGR/00115/2019 to ICAAM, and for SFRH/BD/110969/2015 (Teresa Brás)

POSTER NUMBER 2

Lipid Oxidation in Lamb Meat Enriched in Polyunsaturated Fatty Acids

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The dietary recommendations appoint as favorable to human health the increased consumption of polyunsaturated fatty acids (PUFA), which has motivated an extensive research in order to enhance PUFA levels in ruminant products. Supplementation of diets with PUFA-rich lipid sources are used as a strategy to increase PUFA levels in ruminant meat, particularly in conjugated linoleic acid isomers (CLA) and n-3 PUFA. However, PUFA are highly prone to oxidation and the susceptibility to oxidation increases with the degree of unsaturation. So, meat enrichment in PUFA may compromise its quality.
However, meat oxidative stability depends on the balance between prooxidant and antioxidant components and adequate levels of antioxidants in diet may prevent excessive lipid oxidation. The impact of dietary supplementation with PUFA-rich lipid sources with different degrees of unsaturation on lipid oxidation of meat and the efficacy of α-tocopherol supplementation was evaluated in six productive experiments with lambs. Lambs were fed diets with forage: concentrate ratio ranging from 60:40 to 20:80, supplemented with lipid sources rich in PUFA (vegetable and fish oils and algae extract). All diets contained α-tocopherol (22.5 mg/kg). Meat samples were cold stored during 11 days. Meat lipid oxidation ranged from 1.08 to 1.94 mg malondialdehyde (MDA)/kg meat in diets supplemented with vegetable oils rich in linoleic acid (18:2n-6). Dietary enrichment in n-3 PUFA, resulted in higher lipid oxidation values averaging 2.58 mg MDA/kg meat in diets enriched in linseed oil rich in linolenic acid (18:3n-3) and 2.48 mg MDA/kg meat when fish oils or algae rich in long chain n-3 PUFA were used. The level of α-tocopherol used, was not enough to prevent the quality loss of meats enriched in n-3 PUFA, as the lipid oxidation values were above the threshold value of 2 mg MDA/kg for consumer acceptability of beef. So, particularly in n-3 PUFA enriched diets, higher α- tocopherol levels or combination with other antioxidants should be considered.

Keywords Lamb; meat; polyunsaturated fatty acids; α-tocopherol; lipid oxidation

This work was supported by the Program Alentejo 2020, through the FEDER under the scope of "ValRuMeat" (ALT20-03-0145-FEDER-000040). The authors would like to thank FCT/MCTES through the PTDC/CVT/ 103934/2008, UID/AGR/00115/2019 and UID/CVT/00276/2019 projects.

POSTER NUMBER 2

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Lipid oxidation in lamb meat enriched in polyunsaturated fatty acids

ANIMAL PRODUCTION AND HEALTH

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Introduction

→ Consumption of polyunsaturated fatty acids (PUFA) has been considered as favorable to human health, which has motivated an extensive research in order to enhance PUFA levels in ruminant products.

→ Supplementation of diets with PUFA-rich lipid sources is used to increase PUFA levels in ruminant meat. However, PUFA are highly prone to oxidation, which may compromise the meat quality.

Aim

Evaluate the impact of dietary supplementation with PUFA-rich lipid sources with different degrees of unsaturation on lipid oxidation of meat and the efficacy of α-tocopherol supplementation, in six productive experiments with lambs

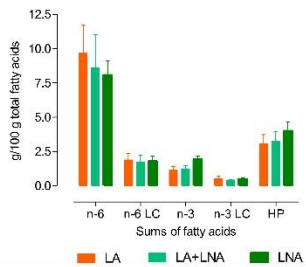
Conclusions

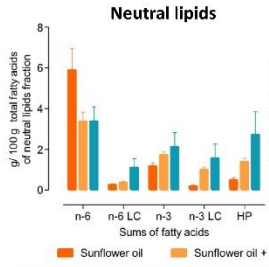
- ✓ Dietary supplementation with lipid sources rich in n-3 PUFA increase the lipid oxidation in meat
- ✓ α-tocopherol level was not enough to prevent the lipid oxidation in meats enriched in n-3 PUFA
- ✓ Higher levels of α-tocopherol or combination with other antioxidants should be considered in n-3 PUFA enriched diets

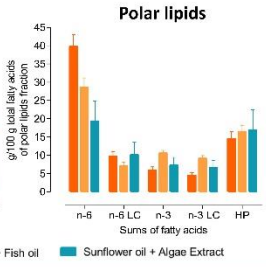


Results

Fatty acid composition of intramuscular fat







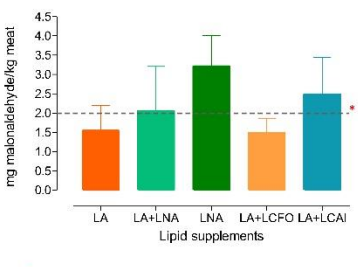
Lipid supplements rich in linoleic acid (18:2n-6, LA)
- ↑ total n-6 PUFA

Lipid supplements rich in linolenic acid (18:3n-3, LNA)
- ↑ total n-3 PUFA
- ↑ highly peroxidable (HP) PUFA

Fish oil and algae extract
- ↓ total n-6 PUFA
- ↑ total n-3 PUFA, n-3 LC-PUFA and HP-PUFA

Algae extract
- ↑ total n-6 LC-PUFA in neutral lipids
- Highest levels of total n-3 PUFA, n-3 LC-PUFA and HP-PUFA in neutral lipids

Lipid oxidation



* threshold value of 2 mg MDA/kg for consumer acceptability of beef

Lipid supplements rich in linolenic acid (18:3n-3, LNA)
- ↑ lipid oxidation

Lipid supplements rich in n-3 LC-PUFA from Algae Extract
- ↑ lipid oxidation

n-6 – n-6 polyunsaturated fatty acids; **n-6 LC** – n-6 long chain polyunsaturated fatty acids; **n-3** – n-3 polyunsaturated fatty acids; **n-3 LC** – n-3 long chain polyunsaturated fatty acids; **HP** – Highly peroxidizable polyunsaturated fatty acids, sum of polyunsaturated fatty acids with 3 or more unsaturated bonds

Material and Methods

Animals

- 6 productive trials
- 145 Merino Branco ram lambs
- 6 weeks of trial

Diets

- Forage:concentrate (60:40 – 20:80)
- α-tocopherol (22.5 mg/Kg)

Supplemented with:

→ 6% Soybean oil → 6% Sunflower oil → 3.20% Sunflower + 1.81% peanut + 0.18% olive oils	LA
→ 4.20% Sunflower + 1.80% Linseed oils	LA+LNA
→ 5.04% Linseed + 0.72% Olive + 0.24% Sunflower oils	LNA
→ 4% Sunflower oil + 2% fish oil	LA+LCFO
→ 4% Sunflower oil + 3.53% Algae extract	LA+LCAI

Diets fatty acid composition

Lipid supplements	Linoleic acid (18:3n-6, LA)	Linolenic acid (18:3n-3, LNA)	n-6 Long Chain (n-6 LC)	n-3 Long Chain (n-3 LC)
	g/100g of total fatty acids			
LA	39.6-51.7	3.60-8.00	-	-
LA+LNA	50.1	13.8	-	-
LNA	29.2	32.2	-	-
LA+LCFO	16.1	2.11	0.33	1.47
LA+LCAI	40.6	7.56	3.46	7.67

Sampling

- ***Longissimus thoracis muscle*** (72 h after slaughter)

Vacuum packed, stored at –80°C → Fatty acid quantification in intramuscular fat (Folch *et al.* 1957, *J Biol Chem*, 226:497-509; Oliveira *et al.* 2016, *Anim. Feed Sci. Techn.*, 213: 64-73)

Individually placed on polystyrene trays, over-wrapped with O_2 permeable film, stored at 2°C for 7 days → Lipid oxidation determination (*Grau et al. 2000, J. Agric Food Chem, 48:1155-1159*)

Aknowledgements:
This work was supported by the Program Alentejo 2020, through the FEDER under the scope of "ValRuMeat" (ALT20-03-0145-FEDER-000040). The authors would like to thank FCT/MCTES through the PTDC/CVT/ 103934/2008, UID/AGR/00115/2019 and UID/CVT/00276/2019 projects.

POSTER NUMBER 3

Mediterranean Shrubs: Natural Anthelmintics in the Diet Selected by Grazing Goats

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The project VegMedCabras aims to promote ecological and sustainable grazing of Mediterranean shrub vegetation by goats. These animals with very selective feeding behavior, tend to actively seek out the most nutritive parts of woody plants, showing adaptations to the chemical defenses of this kind of vegetation. At the same time, goats have a higher sensitivity to gastrointestinal strongyles (GIS) infection compared to other ruminants. In order to control parasitic infection, the use of synthetic anthelmintics is recurrent, resulting in increased resistance to treatments associated with environmental contamination and residues in the final product. Several authors [1,2] have verified that bioactive compounds present in the shrub vegetation are a promising solution in the control of the gastrointestinal strongylosis.
The objectives of the project are to document the species selected by browsing goats, evaluate their nutraceutical value (nutritional value and anthelmintic potential), to promote the animals' productive efficiency, the sustainability of the farms, the biodiversity of the environment as well as the control of combustible vegetation.
Twenty-six non-producing Charnequeira breed goats were used, having access to a shrub area and a dryland pasture. Goats were grouped according to the length of exposure to the shrub area. Natural GIS infections were evaluated individually for egg elimination levels prior to the start of the test and monthly thereafter. Health status of the animals was evaluated through the microhematocrit and total plasma protein. Browsing/grazing goats were observed on separate occasions and shrubs were sampled to evaluate the nutraceutical value of the selected diets.
The preference of the goats for *Olea europaea* var. *sylvestris* and *Rhamnus lycioides* was evident. With these shrubs decreased availability, goats increased the demand for other species, namely *Pistacia lentiscus* and *Quercus coccifera*. The results obtained in the evaluation of the parasitic infection indicate a relationship between the decrease on GIS elimination levels and broesing the shrub vegetation. Also, after a grazing period of about 6 months, the biomass reduction was striking.

Keywords: goats; mediterranean shrubs; natural anthelmintics; gastrointestinal strongyles; sustainable production; nutraceitical plants

This work is funded by the project VegMedCabras - Mediterranean shrubs: natural anthelmintics in the diet selected by grazing goats (ALT20-03-0145-FEDER-000009) co-financed by ALENTEJO2020 program-European Regional Development Fund.

POSTER NUMBER 3

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Mediterranean Shrubs: Natural Anthelmintics in the diet selected by grazing goats

ANIMAL PRODUCTION AND HEALTH

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VegMedCabras

Introduction

- Goats have very selective feeding behaviour
- Bioactive compounds present in the shrubs vegetation

- High sensitivity to GIS infection
- Increased resistance to synthetic anthelmintics
- Enviromental contamination and residues in final product

Promote ecological and sustainable grazing of Mediterranean shrub by goats

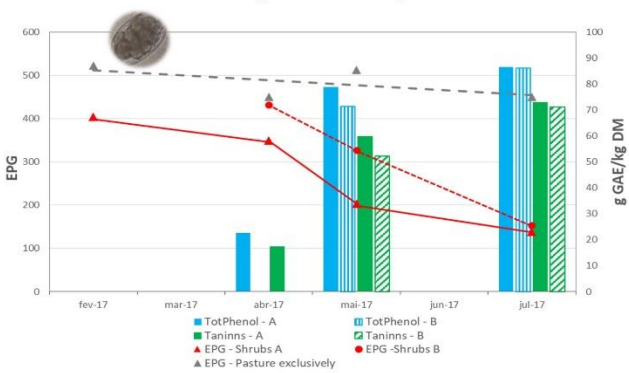
- Collect and document the species selected
- Evaluate nutraceutical value
- Control combustible vegetation

- Evaluate goats individually for: egg elimination and health status (EPG, microhematocrit and total plasma protein)

Material and Methods

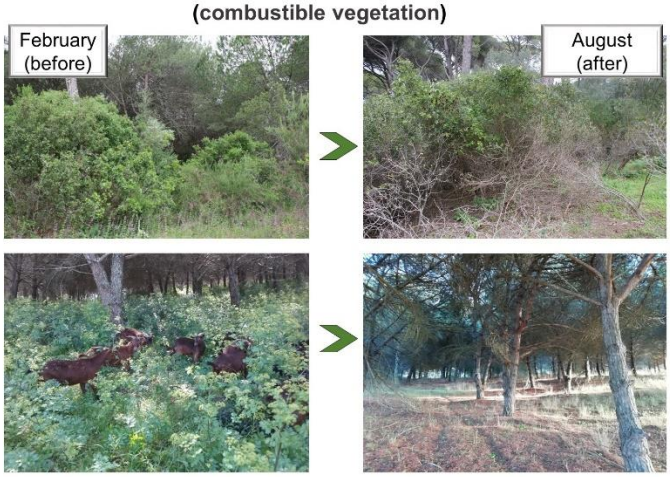
RESULTS

Relationship between the elimination of EPG and the composition of the selected diets in phenolic compounds and tannins

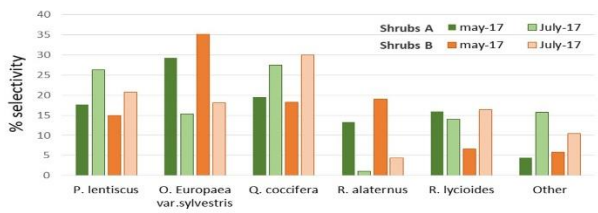


Shrubs A : Goats starting browsing in February
Shrubs B : Goats starting browsing in April
Pasture : Goats grazing exclusively on pasture

Biomass reduction (combustible vegetation)



Goats` selectivity in relation to shrub vegetation available over the study period



CONCLUSIONS

- Preference for *Olea e. sylvestris* and *Rhamnus,* and when the availability of these shrubs decrease, goats turn to *Pistacia lentiscus and Quercus coccifera;*
- *Clear relationship between decrease on GIS elimination levels and browsing the shrub vegetation;*
- *Striking reduction combustible vegetation.*

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Instituto de Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

POSTER NUMBER 4

Nutritional Strategies to Modulate the Ruminal Biohydrogenation and Improve the Fatty Acid Profile of Ruminant Fat – Use of *Cistus ladanifer*

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The consumption of ruminant fat has been associated to detrimental effect on human health, due to their high levels of saturated fatty acids (SFA) and low content of polyunsaturated fatty acids. Which results from lipid metabolism in the rumen, where the dietary lipids are biohydrogenated, producing high amounts of SFA. Ruminal biohydrogenation (RBH) modulation has been target on numerous works to increase the healthy PUFA, such as conjugated linoleic acid isomers (CLA) and vaccenic acid (*t*11-18:1) in ruminant fat. Vaccenic acid is the precursor for endogenous synthesis of the major CLA isomer found in ruminant fat, the rumenic acid (*c*9,*t*11-18:2). Diet supplementation with plants or plant extracts rich in secondary compounds as condensed tannins (CT) has been explored as modulators of RBH. *Cistus ladanifer* L. (rockrose) is a Mediterranean shrub that contain high levels of CT. To explore the *C. ladanifer* use as a way to modify the RBH, two trials were developed. In order to clarify which *C. ladanifer* fraction might modulate the RBH an *in vitro* study was performed, where five *C. ladanifer* fractions – essential oil, dichloromethane extract, total phenolics, non-tannin phenols and CT, were incubated with ruminal fluid for 6 h. Fraction of CT was the most effective on RBH modulation, leading to highest *t*11-18:1 accumulation. The second trial was designed to explore the effect the levels of *C. ladanifer* CT (0, 1.25 and 2.5%) and two ways of CT supply (aerial part *vs*. CT extract from *C. ladanifer*) on fatty acid profile of lamb meat. Basal diet was composed of dehydrated lucerne with soybean oil (6%). Inclusion of *C. ladanifer* CT extract in diet at level of 1.25% CT led to the highest *t*11-18:1 content in intramuscular fat. However, the increase of *c*9,*t*11-18:2 content in intramuscular fat failed in this diet, which may be due to downregulation of stearoyl-CoA desaturase activity, the enzyme that convert *t*11-18:1 to *c*9,*t*11-18:2. *Cistus ladanifer* CT extract seems to be a promising approach to enhance the nutritional value of ruminant fat, but is essential to ensure the endogenous synthesis of *c*9,*t*11-18:2.

Keywords Ruminal biohydrogenation, *Cistus ladanifer*, condensed tannins, ruminants, fatty acids.

Financial support from the Alentejo2020 program through the FEDER to project CistusRumen (ALT20-03-0145-FEDER-000023), and from FCT/MCTES to UID/AGR/00115/2019 project.

POSTER NUMBER 4

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Nutritional strategies to modulate the ruminal biohydrogenation and improve the fatty acid profile of ruminant meat – Use of *Cistus ladanifer*

ANIMAL PRODUCTION AND HEALTH

O. Guerreiro[1,2], S.P. Alves[3,4], D. Soldado[1], L. Cachucho[1], A. Francisco[3,5], M.F. Duarte[1,2], J. Santos-Silva[3,5], R.J.B. Bessa[3,4], E. Jerónimo[1,2]

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olinda.guerreiro@cebal.pt | **www.cistusrumen.pt**

Cistus | Rumen

Introduction

→ The consumption of ruminant fat has been associated to detrimental effect on human health, due to their high levels of saturated fatty acid and low content of polyunsaturated fatty acids, as result of lipid metabolism in rumen

→ Modulation of ruminal biohydrogenation is a good approach to improve ruminant fat healthiness, allowing to increase the conjugated linoleic acid isomers (CLA)

→ *Cistus ladanifer* (Esteva), a shrub quite abundant in Mediterranean region, is able to modulate the ruminal biohydrogenation, increasing the ruminal production of vaccenic acid (*t*11-18:1), the precursor for the endogenous synthesis of CLA - rumenic acid (*c*9,*t*11-18:2)

Aim

In order to explore the *C. ladanifer* use as a way to modify the ruminal biohydrogenation and improve the fatty acid profile of ruminant fats, were developed two trials with the objective to:

✓ I) Clarify which *C. ladanifer* fraction might modulate the ruminal biohydrogenation → *In vitro* study

✓ II) Evaluate if a specific *C. ladanifer* fraction is able to induce the same effect of *C. ladanifer* plant on fat fatty acids profile → Productive trial with lambs

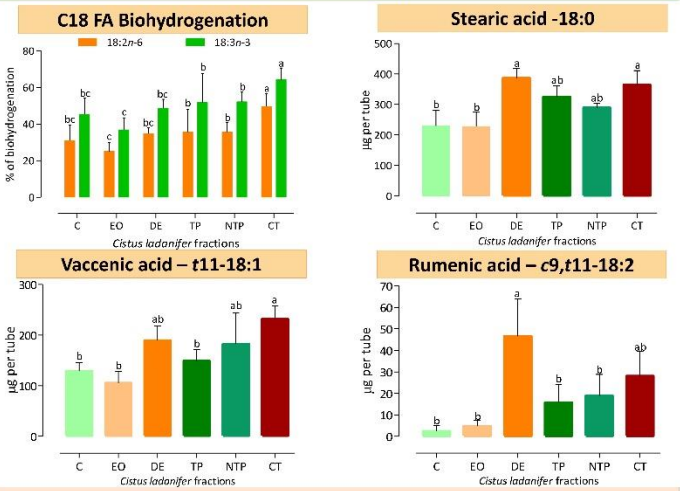
Conclusion

***Cistus ladanifer* CT extract might be a good approach to enhance the nutritional value of ruminant fat**

- ✓ Condensed tannins fraction was the most active on modulation of the ruminal biohydrogenation, leading to highest *t*11-18:1 accumulation
- ✓ Dietary supplementation with 1.25% of *Cistus ladanifer* CT extract increased *t*11-18:1 content in intramuscular fat

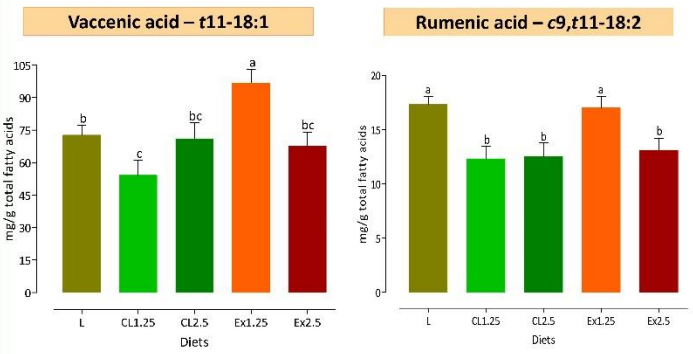
Results

I - *In vitro* study



Condensed tannins fraction (CT) was **the most effective** on the biohydrogenation modulation → **highest accumulation of *t*11-18:1** in ruminal fluid

II – Productive trial with lambs



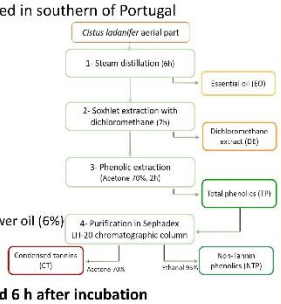
Cistus ladanifer condensed tannins extract at level of 1.25% (**Ex1.25**) resulted in the **highest *t*11-18:1** content in fat, but not increased the ***c*9,*t*11-18:2** content; which may be due to downregulation of Stearoyl-CoA desaturase activity associated with the low fat deposition observed in present work.

Material and Methods

I - *In vitro* study

- *Cistus ladanifer* aerial part (leaves and soft stems) harvested in southern of Portugal
- ***Cistus ladanifer* fractions**

  - Essential oil (**EO**)
  - Dichloromethane extract (**DE**)
  - Total phenolics (**TP**)
  - Non-tannin phenolic (**NTP**)
  - Condensed tannins (**CT**)



- **Incubation with ruminal fluid:**
  - Control (C) - forage:concentrate (70:30) supplemented with sunflower oil (6%)
  - Incubation of substrate + 10% of each *C. ladanifer* fraction
  - Strained ruminal fluid + McDougall's buffer solution (1:2)
- **C18 fatty acid profile determined in ruminal fluid at 0 and 6 h after incubation**

(Alves *et al.* 2013, *PLoS One* 8:e58386)

II – Productive trial with lambs

- 36 ram lambs – crossbred Merino Branco with 19.9 ⊥ 1.86 kg
- 5 weeks of trial, after 1 week of adaptation.
- Randomly assigned to individual pens – six pens to each diet
- **Diets**
  - **L** – Dehydrated Lucerne (DL) + 60 g/kg soybean oil
  - **CL1.25** – DL + 60 g/kg soybean oil + 125 g/kg *C. ladanifer*
  - **CL2.5** – DL + 60 g/kg soybean oil + 250 g/kg *C. ladanifer*
  - **Ex1.25** – DL + 60 g/kg soybean oil + 20.5 g/kg *C. ladanifer* CT extract
  - **Ex2.5** – DL + 60 g/kg soybean oil + 41 g/kg *C. ladanifer* CT extract
- *Cistus ladanifer* aerial part (leaves and soft stems) harvested in southern of Portugal
- Condensed tannins (CT) content: aerial part – 100.4 g CT/kg DM ; extract – 616.3 g CT/kg DM
- **Fatty acid analysis in intramuscular fat**

(Folch *et al.* 1957, *J Biol Chem*, 226:497-509; Oliveira *et al.* 2016, *Anim. Feed Sci. Techn.*, 213: 64-73)

Acknowledgements: FCT Tecnologia para a Ciência e a Tecnologia
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ALENTEJO 2020 PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional
Project: CistusRumen - ALT20-03-0145-FEDER-000023
Sustainable use of Rockrose (*Cistus ladanifer* L.) in small ruminants - Increase of the competitiveness and reduction of the environmental impact

POSTER NUMBER 5

RESPIRA Project: Understanding the Role of Environmental Contaminants in Respiratory Disease

Sousa, ACA[1-4]; Miranda, S[5]; Marques, A[5]; Valente, C[6]; Amaro, R[7,8]; Pereira, CC[7,8]; Teixeira, JP[7,8]; Silva, T[19]; Henriques, I[19]; Kunisue, T[4]; Tanabe, S[4]; Taborda-Barata, L[1,2]; Pastorinho, MR[1,2,10]

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A shift from an outdoor to an indoor lifestyle took place in the last century, and presently we spend nearly 90% of our lives in constructed environments. These artificial habitats possess unique features (e.g., insulation, humidity) that promote the accumulation of, not only biological agents (e.g. bacteria, fungi), but also chemical contaminants, acting as a key interface between such agents and humans. However, humans did not have a large enough evolution period so as to be prepared to deal with these new, chemically rich, artificial habitats. Scientific evidence suggests that the increasing incidence of Non Communicable Diseases (NCDs) in western societies might have a connection to the ubiquitous exposure to indoor environmental contaminants. Respiratory diseases present a paradigmatic case study, as in large part their aetiology resides with environmental contaminants, to which a high responsiveness to these agents is added. Furthermore, the economic burden of respiratory diseases is overwhelming, exceeding €380 billion in Europe, with asthma and chronic obstructive pulmonary disease (COPD) representing the greatest portion with over €200 billion. The study of indoor environmental quality as well as the development and progression of chronic respiratory diseases have received a great deal of attention in the past few years. However, the majority of the conducted surveys focus on single contaminants exposure. RESPIRA project aims to contribute towards a better understanding of the role of multiple stressors in respiratory diseases by providing a multidisciplinary approach to the environmental determinants of respiratory health. The project includes the evaluation of indoor contamination by metals and organometals in COPD patients' houses (and matched controls) by analysing their levels in house dust, as well as the determination of these elements in human samples. Furthermore, the microbial indoor community will also be evaluated in air and dust samples collected from these houses. This case control study is being developed in collaboration with the Pulmonology Department of Estarreja Public Hospital, which is responsible for patients' recruitment. The goal of RESPIRA project is to highlight potential associations between the indoor contaminants and disease exacerbations experienced by patients. Recommendations regarding the minimization of exposure will be proposed in order to reduce the number/severity of exacerbations and prevent new cases, which ultimately will be translated into better respiratory health status of patients with chronic respiratory disease and of the general populations.

Keywords: Indoor; Environmental contaminants; Non Communicable Diseases (NCDs); Exposure reduction.

POSTER NUMBER 5

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

The RESPIRA Project: Understanding the role of environmental contaminants in respiratory disease

ANIMAL PRODUCTION AND HEALTH

Sousa, ACA[1-4]; Miranda, S[5]; Marques, A[5]; Valente, C[6]; Amaro, R[7,8]; Pereira, CC[7,8]; Teixeira, JP[7,8]; Silva, T[9]; Henriques, I[9]; Kunisue, T[4]; Tanabe, S[4]; Taborda-Barata, L[1,2]; Pastorinho, MR[1,2,10]

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Introduction

- **600 000 people die every year in the EU from respiratory disease**[1]
- **The total cost of respiratory disease in the EU exceeds 380 billion Euro (highest contributors - €200 billion – COPD* and Asthma).**[1]
- **Environmental contaminants play an important role in respiratory disease, particularly in chronic exposure scenarios.**[2]

*chronic obstructive pulmonary disease

Rationale

- **People spend most of their time in the Built Environment (>90% of a day).**[1,3]
- **The indoor environment is a source and concentrator of chemical contaminants and harbors a specific microbiome.**[2,3]
- **Exposures to chemical and biological contaminants deleteriously affect human respiratory health → Model = COPD.** [3,4]

1 in 8 deaths in the EU are from respiratory diseases

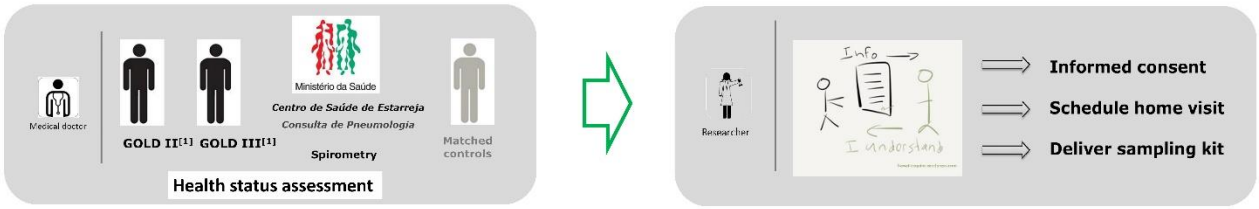
www.erswhitebook.org

RESPIRA
ENVIRONMENTAL RISK FACTORS FOR THE PROGRESSION OF PULMONARY DISEASES

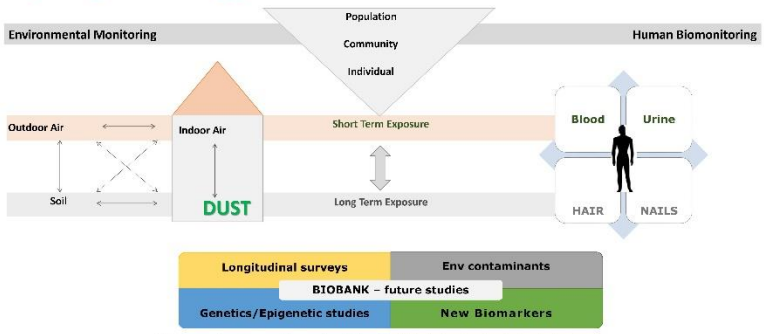
Objectives

- **To assess the levels of environmental contaminants in organic samples of COPD patients and their households.**
- **To characterize the indoor microbiome (fungal and bacterial communities).**
- **To highlight potential associations between the indoor contaminants and disease exacerbations experienced by patients.**
- **To identify indoor contamination sources in order to propose preventative strategies to reduce exposure.**

Recruitment



Sampling strategy



All human and environmental samples will be collected and stored under standardized protocols.

CONNECTIONS TO OTHER RESEARCH

ONE HEALTH - Pets as sentinels of Human Exposure

Toxicity pre-screening using alternative models, e.g., *Daphnia magna*

***Un*Conventional sources** of exposure to environmental chemicals

Ethics

Project approved by: Ethics Committee of the Faculty of Health Sciences, University of Beira Interior (CE-FCS-2016-008); Ethics Committee of Centro Hospitalar do Baixo Vouga (ref. 1208070066); *Comissão Nacional de Proteção de Dados* (Proc.No. 8678/2016).

Instituto ce Ciências Agrárias e Ambientais Mediterrânicas
Knowledge connecting land, food and people

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Funding

ESTARREJA

POSTER NUMBER 6

Selecting Portuguese Cattle Breeds for Feed Efficiency Using Residual Feed Intake

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The use of the average daily gain as the main selection criterion in cattle breeds, has led to a continuous increase in the live weight of the animals, with a corresponding increase in the maintenance requirements of the herds and a greater susceptibility to adverse feeding conditions. The animals with the highest growth potential are not always the most efficient in the use of feed, contributing to an increase in feed cost and, consequently, to a decrease in the profitability of beef production. Less efficient animals are also those that have higher greenhouse gas production, such as methane, having a more pronounced effect on the environment.

Residual feed intake (RFI) is a measure of feed efficiency, and is defined as the difference between an animal's actual feed intake and its expected feed intake based on its size and growth. The use of RFI as a selection criterion will enable producers to attain more food-efficient animals and can contribute to a lower production of greenhouse gases. In the medium / long term, the implementation of RFI will reduce the maintenance requirements of beef herds by about 10%, reduce voluntary food intake by about 12%, reduce methane emissions by 25% to 30% as well as excretion of nitrogen, phosphorus and potassium by about 17%.

The acquisition of feeding crates with electronic recognition of the animal and permanent control of feed intake through the GO BovMais project, enabled, for the first time, an accurate determination of the RFI in Mertolengo and Alentejano steers. The on-going collection of feed intake and growth data of animals under performance test will contribute to the selection program of those breeds.

Keywords: residual feed intake, beef cattle, selection, portuguese breeds

This work is funded by PDR 2020 under the Project GO BovMais – 101-031130

POSTER NUMBER 6

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

SELEÇÃO DAS RAÇAS DE BOVINOS PORTUGUESAS PARA A EFICIÊNCIA ALIMENTAR USANDO O *CONSUMO ALIMENTAR RESIDUAL*

ANIMAL PRODUCTION AND HEALTH

Manuel Cancela d'Abreu [1,2], Elvira Sales-Baptista [1,2], Daniela Paulo[3], Marco Pinho[3], Nuno Carolino[4]

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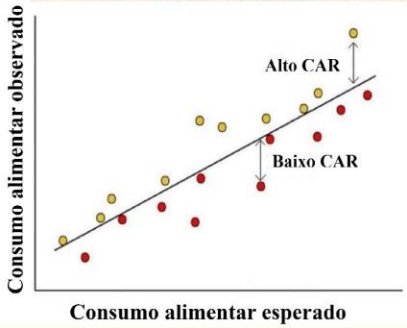
As medidas atuais de eficiência alimentar estão adequadas aos desafios que a produção extensiva de bovinos enfrenta?

Enquadramento:

O **ganho médio diário** e o **índice de conversão** $\frac{Kg_{alimento}}{Kg_{pesovivo}}$, ao ser utilizado como principal critério de seleção, tem conduzido a um aumento continuado do peso vivo dos reprodutores , com o aumento das necessidades de manutenção e uma maior suscetibilidade a condições alimentares adversas.

Consumo alimentar residual

(CAR) é uma medida de eficiência alimentar definida como a diferença entre a ingestão alimentar real de um animal observada num determinado período e o consumo estimado para esse mesmo período com base no peso corporal e no crescimento observado.



Porquê:

O consumo alimentar é uma característica genética com heritabilidade média e considerado um excelente indicador da eficiência com que os bovinos utilizam os alimentos

Objetivo:

Determinar a eficiência biológica e alimentar de bovinos machos de raças portuguesas a partir da medição da Consumo Alimentar Residual (CAR)

Animais que produzem menos gases com efeito de estufa

Como fazer:

Determinação do CAR em novilhos das raças Mertolenga e Alentejana em teste performance

Comedouros automáticos

Ensaio em curso

- Período Experimental: 30 de Maio a 1 de Outubro
- Número de Animais: 27 (3 grupos)
- Alimentação *ad libitum*
- Amostra alimento: 3 em 3 semanas (alimento + sobras)
- Registo automático do consumo diário
- Pesagens: 21 em 21 dias, com os animais em jejum

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GO BOVT

POSTER NUMBER 7

Use of Agro-industrial By-products in Animal Feed: Chemical Composition and Nutritional Value

L. Cachucho[1], K. Paulos[2], D. Soldado[1], O. Guerreiro[1,3], J. Santos-Silva[2], E. Jerónimo[1,3], M. T. Dentinho[2]

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Animal feed represents one of the main costs of livestock systems. The animal feed production industry consumes large quantities of imported raw materials, including cereals and oilseed cake, leading to a high dependence on products originated abroad, with great price and composition variation. Moreover, feed production, transportation and use contribute largely to environmental impact of livestock production. The use of by-products in animal diets, instead of conventional raw materials (cereals and oil seeds), is a strategy which, besides strong product valorization, can considerably reduce costs in the use of feed compound, allow recycle agro-industrial waste and reduce the environmental impact of livestock production. In Portugal the utilization of agro-industrial by-products in animal feed is still limited and essential information on availability, nutritional value and more favorable condition of agro-industrial by-products management for application in animal feed is scarce. Within SubProMais project (http://www.subpromais.pt/) is ongoing the evaluation of the availability, chemical composition and nutritional value of agro-industrial by-products. After initial survey of information on availability of agro-industrial by-products in Ribatejo and Alentejo regions, several by-products from cereals, fruit, legumes and tubers production and processing and dairy industry were collected and analyzed. Some of these agro-industrial by-products are sources of energy and nutrients, being suitable for animal nutrition particularly for ruminants, which has ability to use effectively many of these feed resources. Moreover, agro-industrial by-products showed to be good sources of bioactive compounds, such as phenolic compounds and vitamins, which can be useful for added-value applications in animal diets, promoting the animal health and the quality of products.

Keywords: by-products, bioactive compounds, animal nutrition, livestock production, sustainability

Work performed under the project “SubProMais – Uso de subprodutos agroindustriais na alimentação animal” funded by PDR2020 though European Regional Development Fund. The authors would like to thank FCT/MCTES for the financial support to UID/AGR/00115/2019 project.

POSTER NUMBER 7

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Use of agro-industrial by-products in animal feed: chemical composition and nutritional value

ANIMAL PRODUCTION AND HEALTH

L. Cachucho[1], K. Paulos[2], D. Soldado[1], O. Guerreiro[1,3], J. Santos-Silva[2], E. Jerónimo[1,3], M. T. Dentinho[2]

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Introduction & Aim

- ✓ Animal feed represents one of the main costs of livestock systems. The use of agroindustrial by-products in animal diets instead of conventional raw materials allow recycle the agro-industrial waste and can contribute to reduce the dependence on conventional raw materials, the feed costs and the environmental impact of livestock production.
- ✓ In Portugal the utilization of agro-industrial by-products in animal feed is still limited and essential information on availability, nutritional value and more favorable condition of agro-industrial by-products management for application in animal feed is scarce.

❑ **The objective of this work was to evaluate the chemical composition and nutritional value of agro-industrial by-products, with particular focus on by-products from fruit, legumes and tuber production.**

Conclusion

By-products from fruit, legumes and tuber production are suitable for animal nutrition.

- ✓ Sources of energy and nutrients
- ✓ High *in vitro* organic matter digestibility
- ✓ Low or moderate levels of cell-wall content
- ✓ Crude protein level higher than 15% of DM in some by-products
- ✓ Source of bioactive compounds

- ✓ Low dry matter
- ✓ High seasonality of some byproducts
- ✓ Conservation difficulty

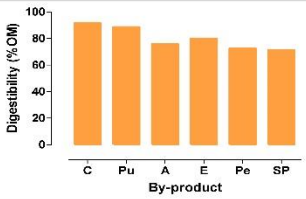
Results

Chemical Composition

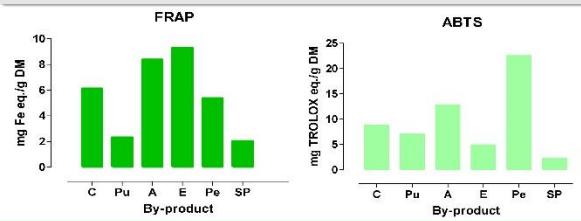
	Cabbage (C)	Pumpkin (Pu)	Asparagus (A)	Eggplant (E)	Pepper (Pe)	Sweet potato (SP)
Dry matter (%)	9.37	5.30	7.73	5.90	6.80	24.90
Crude protein (% DM)	15.56	17.10	32.19	13.90	23.56	3.02
Ether extract (% DM)	1.96	7.17	1.19	2.61	1.15	0.66
Crude fiber (% DM)	11.01	16.31	21.02	25.45	24.28	4.94
NDF (% DM)	15.41	20.55	31.12	41.45	30.57	15.00
ADF (% DM)	13.50	19.03	22.72	36.19	25.41	6.28
ADL (% DM)	0.96	2.55	3.84	5.88	6.24	0.57
Total ashes (% DM)	9.93	13.90	8.4	5.80	-	2.50
Phenolic compounds (mg GAE/g DM)	3.52	2.51	5.65	3.14	7.59	1.07

GAE: Gallic Acid Equivalents. OM: Organic matter. FRAP: Ferric reducing antioxidant power. ABTS: Trolox Equivalent Antioxidant Capacity

in vitro Digestibility



Antioxidant activity



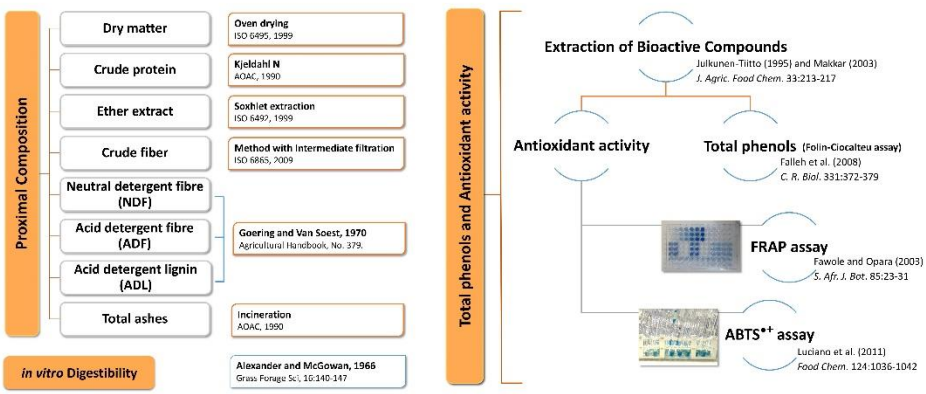
Material and Methods

Sample collection

- By-products from fruit, legumes and tubers production
  - Cabbage (C)
  - Pumpkin (Pu)
  - Asparagus (A)
  - Eggplant (E)
  - Pepper (Pe)
  - Sweet potato (SP)

- Alentejo and Ribatejo regions
- 2018-2019

Analytical determinations



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Project: SubProMais- PDR2020-101-030993
Use of agro-industrial by-products in animal feed
FCT Fundação para a Ciência e a Tecnologia
Project: UID/AGR/00115/2019

POSTER NUMBER 1

Effects of Aliphatic Acids, Furans, and Phenolic Compounds on E.*COLI* Physiology

Moniz, P [1,2]; Silva-Fernandes, T [1,2].; Torrado, I.[1,3,4]; Alves-Ferreira, J.[1,3,4] Carvalheiro, F[3]; Duarte, L.C.[3] Fernandes, M. C.1,[2*]

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The process of developing robust microbial cell factories has been receiving an increased attention in the last years, as these microorganisms are a requirement to achieve efficient fermentation performances when using industrial relevant media, namely (hemi)cellulosic hydrolysates. In fact, although extensive research is being carried out for the optimization of hydrolysis processes to selectively obtain fermentable monosaccharides, several potential microbial inhibitors will always be present, namely acetic acid and phenolic compounds (as they are constituents of the biomass), together with sugar degradation products, such as, formic acid, or furan derivatives (furfural and 5-hydroxymethyl furfural).
E. coli is one of the most relevant and useful bacterial biocatalysts and it was used in this work as a model microorganism. A minimum nutrient medium was optimized and the performance of *E. coli* strain TG1 was studied in the presence of acids, furans and phenolic compounds that are characteristic of biomass hydrolysates.
The efficient utilization of biomass requires the fractionation of its components. One possible option to achieve this, is to hydrolyse biomass into is soluble sugars, followed by biotechnological production of bioproducts using microorganisms.
Although these processes may be an advantageous strategy for providing environmentally friendly products, is still a process under intensive development, presenting further difficulties as typically, only a few organisms are available to be used industrially due to techno-economic, legal/safety constrains. Besides, most of the non-sugars components that are present in the hydrolysates may inhibit fermentation processes.

Keywords: *E. coli*, inhibitors, lignocellulosic hydrolysates

SelecTEcoli project (Ref. ALT20-03-0145-FEDER-000034) from Alentejo2020 through the European Regional Development Fund (ERDF) supported this work. ICAAM and CEF are a research unit funded

POSTER NUMBER 1

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

EFFECTS OF ALIPHATIC ACIDS, FURANS, AND PHENOLIC COMPOUNDS ON *E.COLI* PHYSIOLOGY

RURAL DEVELOPMENT

P. Moniz [1,2], T. Silva-Fernandes [1,2], I. Torrado [1,3,4], J. Alves-Ferreira[1,3,4] , F. Carvalheiro [3], L. C. Duarte[3] , M. C. Fernandes[1,2*]

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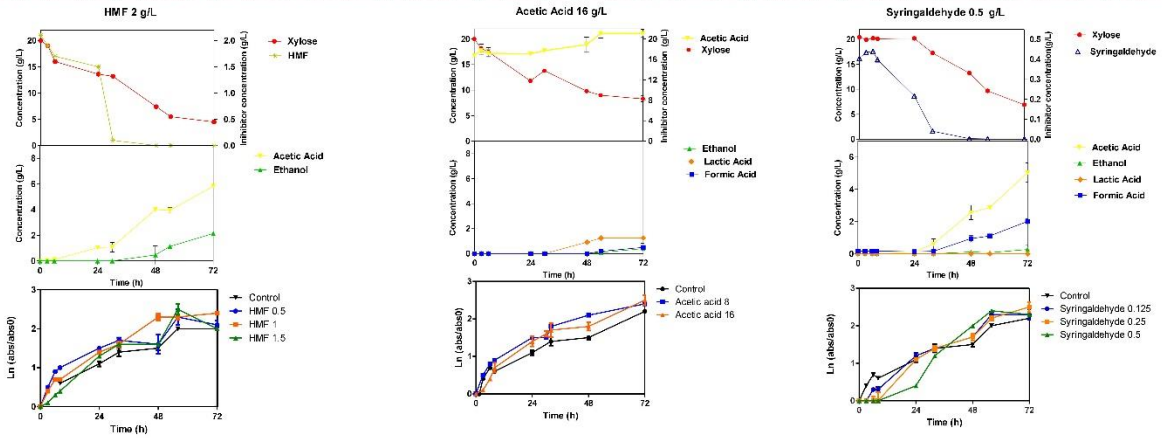
SelecTEcoli

Objectives and Methodology

E. coli is one of the most relevant and useful bacterial biocatalysts and it is used in this work as a model microorganism. A minimum nutrient medium was optimized and the performance of *E. coli* strain TG1, previously selected due to its efficient xylose assimilation, was studied in the presence of several potential inhibitors that are characteristic of biomass hydrolysates.

Strain: *Escherichia coli* TG1 (K-12 *supE thi-1 Δ(lac-proAB), Δ(mcrB-hsdSM)5, ($r_K^- m_K^-$)*).
Inoculum: Stock cultures are produced in a minimum nutrient medium containing xylose as sole carbon and energy source (20 g/L), and maintained at -80 ºC with glycerol. When needed, the cultures are spin-down and used to reproducibly inoculate the fermentation assays.
Culture Conditions: Temp.: 37ºC. Semi-aerobic conditions. Magnetic stirring. pH controlled automatically at 7 by addition of 2M KOH.
Analytical: Xylose, acetic and formic acid, HMF, furfural, syringaldehyde and vanillin were analysed by HPLC using an Aminex HPX-87H column (Bio-Rad, USA).

Results



- Except for Furfural, that inhibited cell growth for concentrations higher than 1.2 g/L (data not shown), *E. coli* TG1 presented a significant tolerance to the other potential metabolic inhibitors
- Acetic acid induced a slight decrease on initial growth rate for an initial concentration of 8 g/L, but it was then totally assimilated, yielding a higher final cell concentration
- as compared to the control fermentation. A similar behavior as also found formic acid up to 6 g/L (data not shown)
- Syringaldehyde, tested under lower concentrations, did not induce any significant change on cell growth rate or yield. It was metabolized, but apparently not assimilated, a similar trend was also found for vanillin

Conclusions

The *E. coli* strain tested, TG1, seemed to be robust in the presence of the inhibitors studied, and it was only significantly inhibited by high concentrations of furfural

Acknowledgements

This work was supported by the SelecTEcoli project (Ref. ALT20-03-0145-FEDER-000034) from Alentejo2020. I. Torrado gratefully acknowledges the PhD grant from FCT SUSFOR doctoral programme PD/BD/114175/2016. CEF and ICAAM are research units funded by FCT (UID/AGR/00239/2019 and UID/AGR/00115/2019, respectively).

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ALENTEJO 2020 PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional

POSTER NUMBER 2

Ecological Restoration of Highly Degraded Habitats in a Mediterranean Context: Insights From a 12 year Project in SECIL Quarries

A. Sampaio[1,6], P.A. Salgueiro[1,2,6], C. Silva[1,2,6], S. Eufrázio[1,2,6], A. Oliveira[1,2,6], D. Medinas[1,2,6], O. Miralto[1,6], P.F. Pereira[4,5,6], V. Salgueiro[1,2,6], A. Silva[3], A. Mira[1,2,5,6]

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Extractive activities are considered one of the most harmful industries, being responsible for losses of more than 80% of biodiversity. At the dawn of the Decade on Ecosystem Restoration declared by the United Nations for 2021-2030, habitat restoration rises as an unparalleled opportunity to achieve Sustainable Development Goals standards. The SECIL project – ongoing since 2007 – bases on the premises of ecological restoration and applied ecology, aiming to minimize impacts of quarry exploitation over fauna communities, while developing conditions for the natural recolonization of the restored areas. The project extends the SECIL's quarry rehabilitation plan, which aims to recreate the original Mediterranean sclerophyllous scrubland occurring at the surrounding Arrábida Natural Park. The project is structured on five major types of actions: (1) Net Impact Assessment of extractive activities on biodiversity; (2) development of a Management Plan based on adaptive management; (3) long-term fauna monitoring; (4) scientific research through the development of pilot studies; and (5) scientific dissemination and communication. An investment of more than 1,000,000€ over the 12 years has allowed the fulfillment of several milestones. In this presentation, we aim to uncover the impact of quarry exploitation on fauna community in the natural park. We will outline the most successful management practices to mitigate impacts and enrich habitat conditions. We will unveil the results of long-term monitoring which point out to a slackening in the convergence of community similarity between restored and natural areas over the years. We will also show how pilot studies have demonstrated that landscape connectivity for small mammals is sustained; and how animal mediated seed dispersal reveals exchanges between restored and natural areas, but can be utterly improved. Going beyond a purely aesthetic recovery and aiming the rehabilitation of the ecological processes and ecosystem services is the motto that has been compelling us to outreach further innovative and effective approaches to reduce the impact of the extractive industry over biodiversity.

Keywords: fauna community; biodiversity action plan; adaptive management; long-term monitoring; net impact assessment; ecological processes

SECIL— Companhia Geral de Cal e Cimento, SA.

POSTER NUMBER 2

RURAL DEVELOPMENT
JORNADAS MED 2019

ECOLOGICAL RESTORATION

OF HIGHLY DEGRADED HABITATS IN A MEDITERRANEAN CONTEXT

Insights from a 12 year project in SECIL quarries

A. D. Sampaio[1], P.A. Salgueiro[1,2], C. Silva[1,3], S. Eabrázio[1,3], A. Oliveira[1,3], D. Medinas[1,3], O. Miralto[1], P.F. Pereira[1,3], V. Salgueiro[1,3], A. Silva[1], A. Mira[1,3]

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Introduction

Net Impact Assessment

Action plan

Long-term monitoring

FAUNA ABUNDANCE

FAUNA RICHNESS

COMMUNITY

Project outputs and achievements

Quarries alive

Case studies

CONNECTIVITY

SEED DISPERSAL

NO!

YES!

NO!

Conclusion remarks

Acknowledgements

POSTER NUMBER 3

Microwave Pre-treatment- an Alternative to Upgrade Mediterranean Biomass

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Mediterranean region is home to large industrial activities in the primary sectors agri-food, forestry, aquaculture and fisheries industries. Together with the manufacturing of cork products these sectors are significant contributors to the national economy. These activities also result in abundant quantities of residues, all being potential feedstock for the biorefinery. Among agro-industrial wastes olive pomace is one of the most abundant in the Mediterranean Basin.

The extracted olive pomace (EOP) is the main by-product of the olive-pomace oil extraction industry but there are no effective upgrade technologies for its valorization, being currently used as a low added-value energy source. Nevertheless, its significant polysaccharide content (close to 50%), make it an interesting material for biotechnological upgrade in the biorefinery framework.

The efficient utilization of lignocellulosic materials like EOP requires a selective fractionation of their structural macromolecular components to enable their selective utilization.

Amongst the most promising biomass pre-treatment options available, autohydrolysis presents several technological advantages that turn it into one of the most favorable choices. However, it requires high temperature, which is a significant constraint due to its energy requirements. Therefore, there is a need for alternative heating techniques that not only reduce the energy input, but that can also increase the overall process efficiency[1].

Microwave (MW) is being widely studied in many chemical areas due of its high heating efficiency, easy operation and also because it satisfies many requirements of green chemistry.

In this work, aqueous mixtures of EOP were pre-treated under MW heating using a magnetron frequency of 2450 MHz. Oligosaccharides (OS), compounds with high market potential were always the main product found in the liquid fraction. The process kinetics are discussed based on the severity factor and compared to standard autohydrolysis experiments, discussing the potential application of microwave technology at industrial level.

Keywords: extracted olive pomace, pre-treatment, microwave.

[1] Torrado, I., P. Moniz, H. Pereira, M. C. Fernandes, F. Carvalheiro and L. C. Duarte (2019). Microwave Assisted Biomass Deconstruction Processes: Scientometric evaluation. Proceedings of 27Th European Biomass Conference & Exhibition, May 2019 Lisboa, Portugal.

I. Torrado, ICAAM and CEF thanks FCT for grants (PD/BD/114175/2016, UID/AGR/00115/2019 and UID/AGR/00239/ 2019, respectively).

POSTER NUMBER 3

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Microwave pre-treatment - An alternative to upgrade Mediterranean biomass

RURAL DEVELOPMENT

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1. Introduction

Amongst the most promising biomass pre-treatment options, autohydrolysis [1] presents several technological advantages that turn it into one of the most favorable choices. However, it has a very significant constraint due to its high energy requirements. Therefore, there is a need for alternative heating techniques that not only reduce the energy input, but that can also increase the overall process efficiency.

Microwave (MW) is being widely studied in many chemical areas due of its high heating efficiency and easy operation, and is studied here as a potential alternative process using extracted olive pomace as a model feedstock, as the later is a very attractive biorefineries' feedstock due to its widespread abundance.

2. Methodology

Raw material: Extracted olive pomace (EOP) was kindly supplied by UCASUL (Alentejo, Portugal)

Microwave assisted Autohydrolysis

Ethos Easy bench-top microwave digester (Milestone, Srl, Italy)
Magnetron frequency of 2450 MHz; LSR 5(w/w);
170-230ºC (non-isothermal),

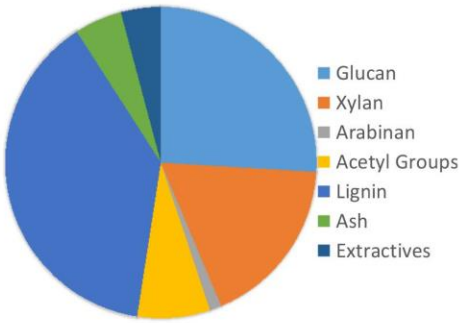
Analytical assays

Raw material and processed solids were chemically characterized after quantitative acid hydrolysis (NREL LAPs). Oligosaccharides concentrations were calculated from the increase in sugar monomers, concentrations after liquor hydrolysis.

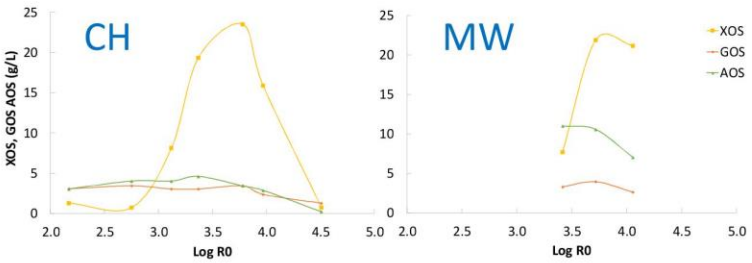
3. Main Results

3.1 Chemical composition of the feedstock

- EOP has a **high polysaccharide content, 46%** (w/w), of which:
  - 50% is cellulose (as estimated from glucan content)
  - 50% is hemicellulose (as estimated from xylan, arabinan and acetyl groups content)
- Significant high content of Klason Lignin (33.9%) compared with other materials
- This composition opens a wide range of strategies for the EOP valorization



3.2 Conventional heating(CH) *vs* Microwave (MW)



- Maximum XOS concentration was similar in both processes.
- Lower severity is needed for MW process to obtain the maximum XOS concentration.
- Autohydrolysis pre-treatment on non-isothermal regime using Microwave is a good alternative to achieve hemicellulose solubilisation on liquor and enriched cellulose solid

- Microwave raises the temperature of the whole volume simultaneously whereas for others the reaction mixture in contact with the walls is heated first.
- The business perspective and scalability of microwave technology is still poorly understood and it requires further studies, specially for continuous reactors

4. Conclusion

Microwave-based pretreatment holds a great promise for industrial application under the biorefinery framework

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Ivone Torrado gratefully acknowledges the PhD grant PD/BD/114175/2016 (FCT/SUSFOR doctoral Program). CEF and ICAAM are research units funded by FCT (PEst-OE/AGR/UI0239/2011 and UID/AGR/00115/2013, respectively).

POSTER NUMBER 4

SelecTEcoli - Selection and Characterization of *E. coli* Strains Presenting High Tolerance To Multi-inhibitors Derived from Lignocellulosic Biomass Pre-treatments

Fernandes, M. C.[1,2]*; Moniz, P [1,2]; Silva-Fernandes, T. [1,2]; Alves-Ferreira, J[1,3,4]; Torrado I.[1,3,4]; Carvalheiro, F[3]; Duarte, L.C.[3]

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The biochemical platform development is in a more advanced stage as compared to other biorefinery platforms, nevertheless, many improvements are still needed to further advance the concept. Specifically, research must be direct to the most challenging steps in the process: i) Improving pre-treatment technology, optimizing the fragmentation and fractionation processes to really make use of the whole plant; ii) improvement of pentose assimilating microbial biocatalysts; and iii) developing more cost-effective cellulases.
This project, is focused on the second topic as it aims to characterise and quantify the physiological response of the most relevant bacterial cell factory, *Escherichia coli*, to inhibitory compounds usually present in lignocellulosic hydrolysates in order to identify the mechanisms that determine these responses.
The understanding of inhibitory effects and the elucidation of potential resistance mechanisms in relevant cell factories, such as *E. coli*, may have a very significant technological impact. Actually, the development of more tolerant strains is believed to be a key feature to increase biorefinery deployment.

Keywords: Biorefinery Novel microorganisms, Inhibitor tolerance mechanisms, Omics

SelecTEcoli project (Ref.ALT20-03-0145-FEDER-000034) from Alentejo2020 - European Regional Development Fund supported this work. I. Torrado, ICAAM and CEF thanks FCT for grants (PD/BD/114175/2016, UID/AGR/00115/2019 and UID/AGR/00239/ 2019, respectively).

POSTER NUMBER 4

Jornadas Med 2019
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SelecTEcoli - Selection and characterization of *E. coli* strains presenting high tolerance to multi-inhibitors derived from lignocellulosic biomass pre-treatments

RURAL DEVELOPMENT

M. C. Fernandes[1,2*], P. Moniz[1,2], T. Silva-Fernandes[1,2], J. Alves-Ferreira[1,3,4], I. Torrado [1,3,4],F. Carvalheiro[3], L. C. Duarte[3]

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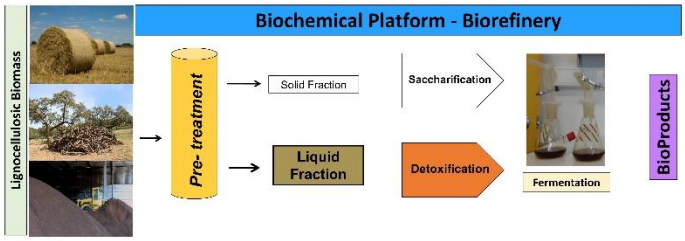

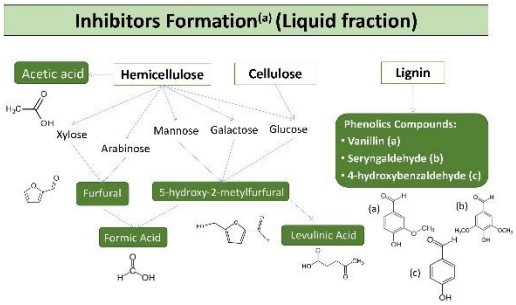

i) Improvement pre-treatment technology, optimizing the fragmentation and fractionation processes to really make use of the whole plant
ii) Improvement of pentose assimilating microbial biocatalysts
iii) Development more cost-effective cellulases

SelecTEcoli

ii) Improvement of pentose assimilating microbial biocatalysts

GOALS

- To characterize and quantify the physiological response of the most relevant bacterial cell factory, *Escherichia coli*, to inhibitory compounds usually present in lignocellulosic hydrolysates
- Identify the mechanisms that determine these responses
- Modify the strains to achieve a multi-tolerant strain of *E.coli* to the referred inhibitors

TASKS

Task 1 - Characterization of the differential tolerance among *E. coli* strains
Characterization of the differential tolerance among *E. coli* strains

Task 2 - Chemostat cultures under the presence of inhibitory compounds
Quantifying kinetic and stoichiometric parameters of selected strains growth, metabolic tolerance and product formation under chemostat cultivation

Task 3 – Omic studies
To compare transcriptomic, proteomic and metabolomics differences between standard and inhibited metabolic states

Task 4 - Metabolic Modelling
Development of an integrated metabolic model

Task 5 - Genetic transformation and validation studies
Development of novel, more inhibitor tolerant, *E. coli* strains

Acknowledgements

This work was supported by the SelecTEcoli project (Ref. ALT20-03-0145-FEDER-000034) from Alentejo2020 - European Regional Development Fund. I. Torrado, CEF and ICAAM thanks FCT for financial support (PD/BD/114175/2016, UID/AGR/00239/2019 and UID/AGR/00115/2019, respectively).

References: (a) E. Palmqvist, B. Hahn-Hägerdal (2000). Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25-33.

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POSTER NUMBER 5

Technology Transfer Exploiting a New Approach for Innovation-based Rural Development in Alentejo: The Case Study of Ferreira do Alentejo Municipality

R. Martins [1,2] and F. Duarte[1,2]

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CEBAL is a private, non-profit Research and Development (R&D) Unit located in Beja, Alentejo, south of Portugal. This R&D Unit develops scientific and technological knowledge in biotechnology field applied to agriculture-related areas and combines it with a strong strategy based on technology transfer, providing tailored made solutions for farmers and agro-food industries, as well as for municipalities and other public and non-public stakeholders.
Technology Transfer is a concept that seeks boosting R&D results generated by scientific sector as an economic growth strategy based on knowledge, central to innovation and to sustainable and intelligent empowerment of companies. However, technology transfer in rural areas, as Alentejo, is still in its infancy, mostly due to high number of smallholder farmers, the lack of agro-industrial transformation and a faint culture of interaction between actors (scientists, farmers and industrials, regulatory entities and political power), necessary for technology maturation and adaptation to the users' contexts and needs.
The work presented here introduces a new approach for management and development of technology transfer and innovation in Alentejo, based on a holistic, endogenous and territorial perspective, which elevates geographical and human proximity on fostering technological and rural development processes. The proposed approach is materialized with a decentralization action by creating Technology Transfer Centers from CEBAL, spread on Alentejo region, to explore a concept of knowledge and technology interfacing and sharing. Those intends narrowing liaisons between regional actors and providing more focused, adapt and collaborative activities of knowledge and technology transfer. The Municipality of Ferreira do Alentejo embraced this model and integrated it in its governance strategy, assuming a position of facilitator in the interaction between local agents and scientific units, thus promoting technology transfer and creating conditions for the growing attraction of differentiated and innovative companies for the county. Hence, the proposed technology transfer approach initiated its testing with the creation of the first Technology Transfer Unit from CEBAL at Ferreira do Alentejo in the end of 2018.

Keywords: Governance; innovation; rural development; technology transfer.

CEBAL's Technology Tranfer Center – Ferreira do Alentejo Unit is co-financed by Ferreira do Alentejo Municipality under the framework of a cooperation protocol stablish between both entities on October 2018.

POSTER NUMBER 5

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

TECHNOLOGY TRANSFER EXPLOITING A NEW APPROACH FOR INNOVATION-BASED RURAL DEVELOPMENT IN ALENTEJO: the case study of Ferreira do Alentejo Municipality

RURAL DEVELOPMENT

R. Martins[1,2,3] and M. F. Duarte[1,2,3]

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WHAT IS TECHNOLOGY TRANSFER?

A concept that seeks boosting Research & Development results generated by scientific sector as an economic growth strategy based on knowledge, central to innovation and to sustainable and intelligent empowerment of companies.

CONCERN:

In Portuguese rural areas, as Alentejo, Technology Transfer stills in its infancy, mostly due to:

- High number of smallholder farmers;
- Lack of agro-industrial transformation;
- Faint culture of interaction between actors (scientists, farmers and industrials, regulatory entities and political power);

Those are necessary for technology maturation and adaptation to the users' contexts and needs.

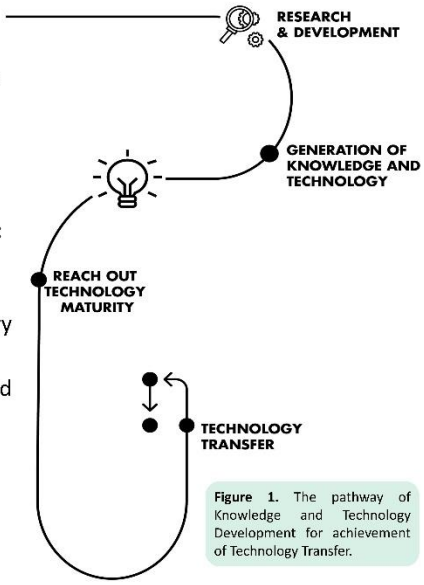

Figure 1. The pathway of Knowledge and Technology Development for achievement of Technology Transfer.

A NEW APPROACH FOR MANAGEMENT AND DEVELOPMENT OF TECHNOLOGY TRANSFER AND INNOVATION IN ALENTEJO

HIPOTHESIS:

✓ Fostering technological and rural development processes using geographical and proximity relations.

APPROACH:

➢ Decentralization action by creating Technology Transfer Centers from CEBAL, scattered through Alentejo region;

➢ Using a concept of knowledge and technology interfacing and sharing.

GOVERNANCE

CENTRO DE TRANSFERÊNCIA DE TECNOLOGIA
UNIDADE DE FERREIRA DO ALENTEJO

Cooperation protocol between Ferreira do Alentejo Municipality and CEBAL

MISSION:

- Strengthen transfer of technology and scientific knowledge into enterprises;
- Provide innovative and sustainable solutions in response to territory challenges;
- Improve the level of regional technology intensity.

OUTPUT: Narrowing liaisons between regional actors and providing more focused, adapt and collaborative activities of Knowledge and Technology Transfer

SUPPORTING LITERATURE:

- Garcia, R. *et al.* 2011. *Effects of geographical proximity to foster university-industry linkages*. Revista de Economia, v. 37, n. especial, p. 307-330, Editora UFPR;
- Roux, D. J., *et al.* 2006. *Bridging the science–management divide: moving from unidirectional knowledge transfer to knowledge interfacing and sharing*. Ecology and Society. 11(1): 4;
- Tomás Rea Becerra, R. & González Pérez, C. 2016. *Territorial development: between innovation and technological change*. RICSH Revista Iberoamericana de las Ciencias Sociales y Humanísticas;
- Torre, A. & Wallet, F. 2014. *Regional development and proximity relations*. Edward Elgar Pub. ISBN: 9781781002889.

ACKNOWLEDGES:

CEBAL's Technology Transfer Center – Ferreira do Alentejo Unit is financed by Ferreira do Alentejo Municipality under the framework of a cooperation protocol stablish between both entities on October 2018. ICAAM unit is funded by National Funds through FCT - Foundation for Science and Technology (UID/CTM/50011/20132019).

POSTER NUMBER 6

Upgrade of Mediterranean Biomass Residues to Sustain the Regional Bioeconomy Development

Torrado, I.[1,3,4]; Creto, A.R[3]; Carvalheiro, F[3]; Duarte, L.C.[3]; Pereira, H.[4]; Fernandes, M. C.[1,2]*

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The full economic deployment of the bioeconomy is still depending on the overcome of several hurdles, namely feedstock availability and seasonality, as well as technological problems. Among these, constrains related to feedstock availability are especially relevant, and it is envisage that the biorefinery will have to be able to process a large range of feedstocks from many diverse origins.
In order to evaluate the prospective biomass availability in Alentejo, a literature search was conducted to identify the major resources coming from forests, agriculture, and agro-food industries and their potential available quantities on a yearly basis.
The Biotechnological Valorization Potential Indicator concept (BVPI) that considers the impact of the main biological, physico-chemical, economical, geographical and politico-legal factors was then applied to rank the suitability of the identified resources, to be used as feedstock for the future biorefineries [1].
This analysis enabled to identify several potential feedstocks, and seven were selected as model materials: cork and holm oak prunings, almond shells, extracted olive pomace, almond hull, pine nut shell and corn cobs.
These materials were chemically characterized using reference protocols, showing a significant chemical diversity, consequence of their inherent biological and anatomical diversity. This can be explored for the production of different classes of added-value products, in addition to biofuels.

Keywords: lignocellulosic biomass, pre-treatment, microwave, bioeconomy.

[1] Duarte, L. C., Esteves, M. P., Carvalheiro, F. and Gírio, F. M. (2007), Biotechnological valorization potential indicator for lignocellulosic materials. Biotechnology Journal, 2: 1556-1563.

I. Torrado, ICAAM and CEF thanks FCT for grants (PD/BD/114175/2016, UID/AGR/00115/2019 and UID/AGR/00239/ 2019, respectively).

POSTER NUMBER 6

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Upgrade of Mediterranean biomass residues to sustain the regional Bioeconomy development

RURAL DEVELOPMENT

I. Torrado[1,2,3], A. R. Creto[3], F. Carvalheiro[3], L. C. Duarte[3], H. Pereira[2], M. C. Fernandes[1,4]

[1]Centro de Biotecnologia Agrícola e Agro-alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja)
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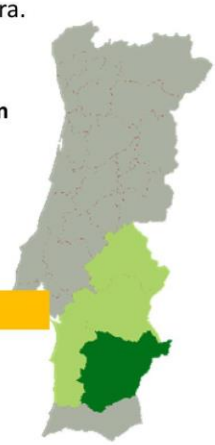
Aims

Bring value to, unexplored, natural resources, by-products and wastes through the development of **Green and sustainable technologies** to produce **Added-value (bio)products**

"Greater use of renewable resources is no longer just an option, it is a necessity"

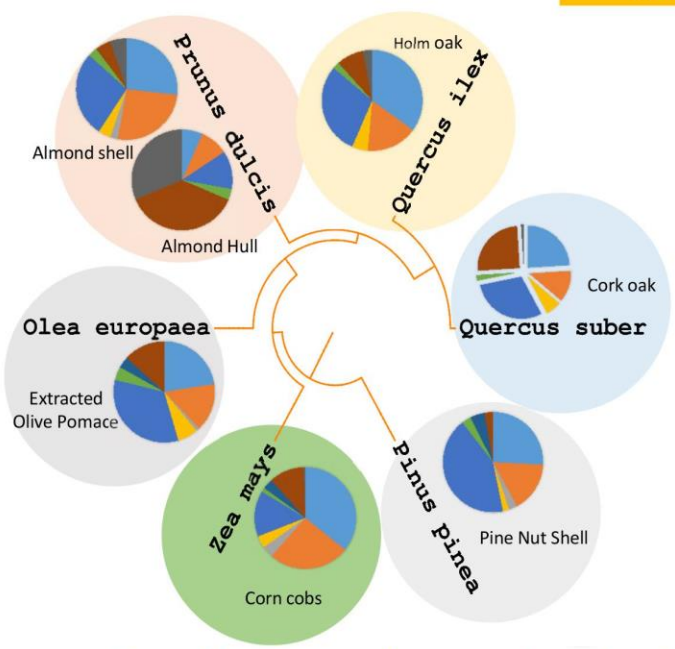
(EC, 2012, Europe's Bioeconomy Strategy)

Alentejo Region



Selected Biomasses

- Cork oak
- Almond shell
- Holm oak
- Extracted Olive Pomace
- Almond Hull
- Pine Nut Shell
- Corn cobs



✓**The composition of the selected materials reflects both their diverse biological origin and plant tissue source**

✓**This diversity can be explored for the production of different classes of added-value products, in addition to biofuels**

Biotechnological Valorisation Potential Indicator (BVPI) [1]

Material	Biological nature	Macromolecular composition	Water content	Physical characteristics	Seasonality	Economic value	Market dependency	Current technology / destination	Development stage of upgrade technology	Available quantities (actual)	Geographical concentration	Political or legal constraints	BVPI
Extracted Olive Pomace	1	1	3	1	3	2	3	2	2	3	3	1	25
Pine Nut Shell	2	2	3	0	3	2	3	2	1	1	2	1	22
Corn cobs	3	1	3	2	0	3	3	2	2	1	1	1	22
Cork oak	1	1	2	3	0	3	3	3	1	1	1	1	20
Almond shell	1	1	3	2	3	2	3	2	2	0	0	1	20
Holm oak	1	1	2	3	0	2	2	2	1	1	1	1	17
Almond Hull	1	3	2	3	1	1	1	0	0	0	0	1	13

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✓**The selected materials have a high potential to be used as biorefinery feedstocks**

Reference
[1] Duarte, LC, Esteves, MP, Carvalheiro, F, Girio, F 2007. Biotechnol. J. 2:1556-1563

I. Torrado, ICAAM and CEF thanks FCT for grants (PD/BD/114175/2016, UID/AGR/00115/2019 and UID/AGR/00239/ 2019, respectively)

POSTER NUMBER 1

Água-mel in Algarve

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Since ancient times that bee products have been used not only as food but also for alleviating diverse ailments. Nowadays the interest for these products keeps growing and constitutes an important income for beekeepers. In addition to bee products, which are broadly known and used for a wide range of uses (honey, propolis, royal jelly, bee pollen), there are others which are less well-known even in scientific terms, such as beebread, mead*, água-mel*, among other products. *Água-mel* is produced in the Southern Portugal, mainly Algarve and some regions of Alentejo, and it has also been reported in Italy (*abbamele*), particularly in Sardinia. The physico-chemical characterization of *água-mel* and some of their biological properties [1-5], and *abbamele* [6] have been studied and have revealed their potentialities not only in market niches as ingredient in "gourmet" cooking but also as nutraceutical.

The research on *água-mel* developed by our team was developed under the project Programa Apícola Nacional 2011–2013, Medida 6A.

Keywords: Bee products, chemical characterization, production, biological properties.

[1] Figueira, A. C., and T. Cavaco, Changes in physical and chemical parameters of the traditional Portuguese product água-mel during the production process. J. Food Process. Preserv., 2012. 36, 285–290.
[2] Miguel, M. G., M. D. Antunes, S. Aazza, J. Duarte, and M.L. Faleiro, Honey-based "água-mel" chemical characterization and microbiological quality. Ital. J. Food Sci., 2013. 25, 275–282.
[3] Miguel, M. G., L. Faleiro, M.D. Antunes, S. Aazza, J. Duarte, and A. R. Silvério, Antimicrobial, antiviral and antioxidant activities of "água-mel" from Portugal. Food Chem. Toxicol., 2013. 56, 136–144.
[4] Miguel, M. G., S. Aazza, M. D. Antunes, M. L. Faleiro, J. G. Barroso, L.G. Pedro, and A.C. Figueiredo, Mineral and volatile composition of água-mel from Portugal. European Food Research and Technology, 2016. 242, 171–178.
[5] Miguel, M.G., S. Aazza, M.D. Antunes, and M.L. Faleiro, Changes in the chemical parameters during the production of água-mel from Portugal. *Cyta - Journal of Food*, 2018. 16, 972–979.
[6] Spano, N., M. Ciulu, I. Floris, A. Panzanelli, M.I. Pilo, P.C. Piu, R. Scanu, and G. Sanna, Chemical characterization of a traditional honey-based Sardinian product: Abbamele. Food Chem. 2008. 108, 81–85.

POSTER NUMBER 1

Atividades Biológicas de Água-mel do Algarve

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QUALIDADE MICROBIOLÓGICA

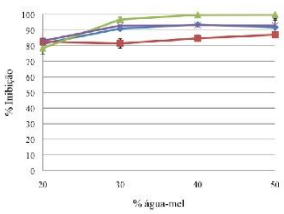
- Contagem de microrganismos mesofilos (NP-4405:2002),
- Bolores e leveduras (ISO 21527-2:2008),
- Esporos de clostrideos sulfito-redutores (ISO 15213:2003),
- Bactérias da família *Enterobacteriaceae* (ISO 21528-2:2004),
- Presença de *Salmonella* spp. (ISO 6579:2002)

Todas amostras foram negativas para todos os parâmetros analisados, excepto 1 que apresentou 3,41±0,09 Log_{10} UFC/g de microrganismos aeróbios e 4,05±0,11 Log_{10} UFC/g de esporos de clostrideos sulfito-redutores .

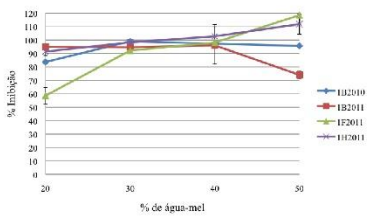
Durante a produção foram detetados microrganismos aeróbios e bolores e leveduras em números muito baixos, cerca de 30-300 UFC/g no início da produção (T0) e nos restantes tempos de amostragem (T2; T4; T6; T8; T10) todos os parâmetros foram negativos.

ATIVIDADE ANTIMICROBIANA

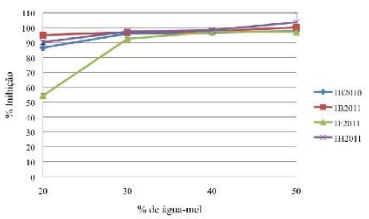
- As amostras selecionadas apresentaram atividade contra bactérias Gram negativas, Gram positivas e estirpes resistentes a antibióticos e ainda a leveduras



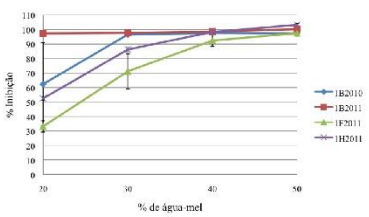
Inibição do crescimento de *Salmonella enterica* subspecie enterica serovar *Typhimurium* ATCC 14028



Inibição do crescimento de *Listeria monocytogenes* EGD



Inibição do crescimento de *S. aureus* CFSA 2



Inibição do crescimento de MRSA 6

Changes in the chemical parameters during the production of *água-mel* from Portugal

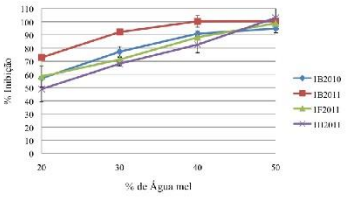
Maria Graça Miguel, Smail Aazza, Maria Dulce Antunes & Maria Leonor Faleiro

HONEY-BASED "ÁGUA-MEL" CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL QUALITY

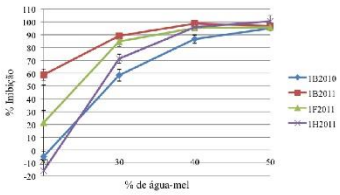
M. GRAÇA MIGUEL[a], M. DULCE ANTUNES[b], SMAIL AAZZA[b], JOANA DUARTE[c] and M. LEONOR FALEIRO[a]

Int. J. Food Sci., vol. 25 - 2015

ATIVIDADE ANTIMICROBIANA

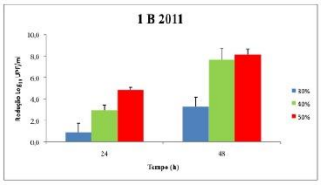


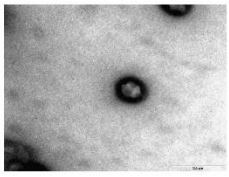
Inibição do crescimento de *Candida albicans* ATCC 90028

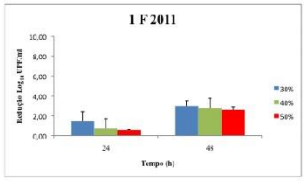


Inibição do crescimento de *Saccharomyces cereviseae* DSM 70449

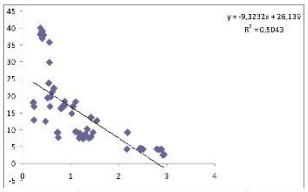
ATIVIDADE ANTIVIRAL



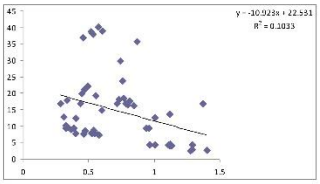




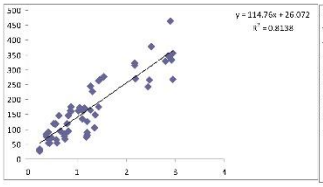
ACTIVIDADE ANTIOXIDANTE



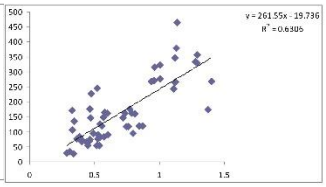
Relação pigmentos / capacidade captar NO



Relação fenol/capacidade captar NO



Relação pigmentos /capacidade captar peroxilo



Relação pigmentos/capacidade captar peroxilo

Jornadas Med 2019

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POSTER NUMBER 2

Potato cyst Nematodes in Portugal: Incidence and Phylogenetic Relationship

M.J. Camacho[1,2,3], M.L. Inácio[3], E. de Andrade[3], M. Mota[1,2,4]

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[2] NemaLab - Dept. de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal Portugal
[3] INIAV, I.P. - Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, 2780-159 Oeiras, Portugal.
[4] Dept. Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, C. Grande 376, 1749-024 Lisboa, Portugal.

The occurrence of potato cyst nematodes (PCN) in Portugal has been recorded for decades. *Globodera rostochiensis* was first reported in 1956 [1] and Globodera pallida in 1988 [2], both in the northern part of Portugal. To establish the status of PCN in the country, a field survey was conducted since 2010. PCN presence was confirmed in 183 samples through morphological and molecular analyses in a total of 679 soil samples examined (from 2013 to 2018). Our results showed that *Globodera* cysts were detected in all potato growing regions. The incidence of PCN in Portugal is quite high and there is no a dominant species. In order to study Portuguese *Globodera* isolates the ITS-rDNA region was amplified using the primers 5'-CGT AAC AAG GTA GCT GTA G-3' and 5'-TCC TCC GCT AAA TGA TAT G-3' [3] and sequenced. The length of PCR fragments in our study ranged from 736 bp which corresponds to the region between 3’ end of 18S and 5’ end of 28S rDNA. The GeneBank-BLAST homology search using seven obtained sequences of *G. pallida* and nine sequences of *G. rostochiensis* suggests that our ITS-rDNA sequences display high homology with these species and no significant intraspecific variation was observed. For *G. pallida*, variation was slightly smaller (99–100%) than for *G. rostochiensis* (97–99%) which can be due to its later introduction in the country. The increasing incidence of *G. pallida* may be a consequence of the phytosanitary measures that have been taken to prevent further spread of *Globodera* spp. in recent years (non-host crops rotation or growing of resistant potato cultivars). Thus, there is a need for a new approach to managing potato cyst nematodes, mainly *G. pallida*.

Keywords: PCN; *Globodera rostochiensis*; *Globodera pallida*

Macara, A. Aspectos sobre a importância dos nemátodos de interesse agrícola em Portugal e no Ultramar Português. Agros, 1963. 46: 367-384.
Santos, S. and Fernandes, M. The occurrence of Globodera rostochiensis and G. pallida in Portugal. Nematologia Mediterranea, 1988. 16:145.
Ferris, V. *et al*. Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. Fundamental & Applied Nematology, 1993. 16:177.

This work was developed within the scope of PhD Studentship SFRH/BD/138724/2018, supported by national funds through FCT (Fundação para a Ciência e a Tecnologia).

POSTER NUMBER 2

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

Potato cyst nematodes in Portugal: Incidence and phylogenetic relationship

HORTICULTURE

M.J. Camacho[1,2], M.L. Inácio[2], E. de Andrade[2], M. Mota[1]

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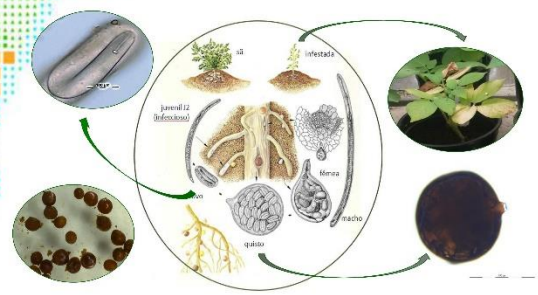
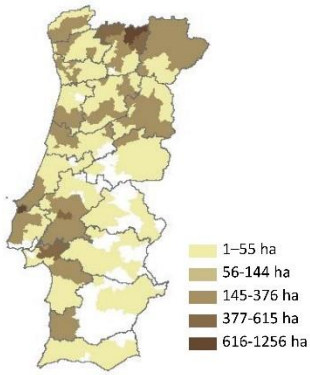
[2] INIAV, I.P. - Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras.

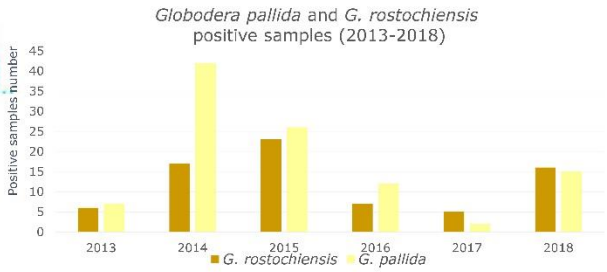


Potato Cyst Nematode life cycle (adapted from Paap, 2013).

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 many countries. The occurrence of PCN in Portugal has been recorded for decades. To establish the status of PCN in the country, a field survey was conducted since 2010.



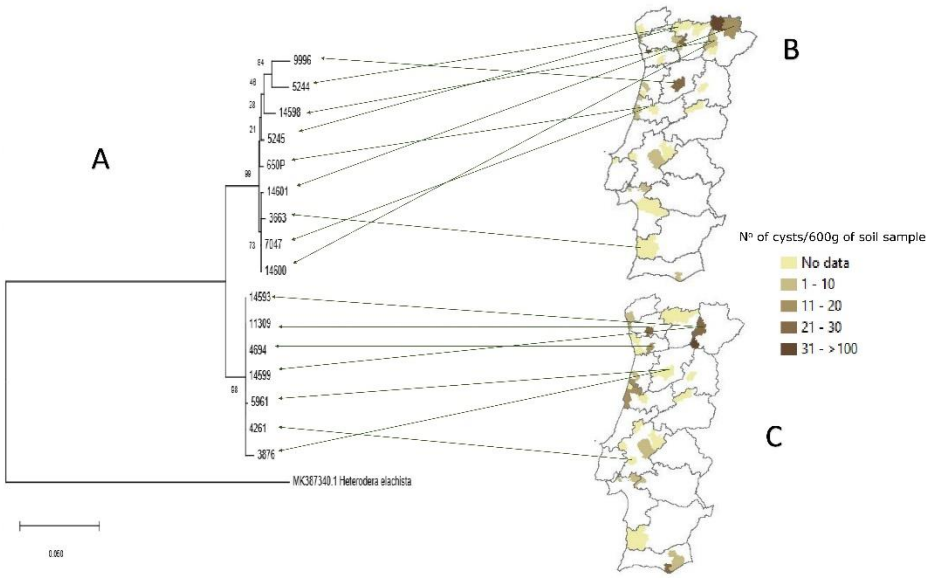
Potato production area (ha) by County (data from INE, 2011)



PCN presence was confirmed in 183 samples through morphological and molecular analyses in a total of 679 soil samples examined (from 2013 to 2018). Our results showed that *Globodera* cysts were detected in all potato growing regions. The incidence of PCN in Portugal is quite high and there is no dominant species, although *G. pallida* seems to have a tendency to predominate.

In order to study Portuguese *Globodera* isolates the ITS-rDNA region was amplified using the primers 5'-CGT AAC AAG GTA GCT GTA G-3' and 5'-TCC TCC GCT AAA TGA TAT G-3' and sequenced. The length of PCR fragments in our study ranged from 736 bp which corresponds to the region between 3' end of 18S and 5' end of 28S rDNA. The GeneBank-BLAST homology search using seven obtained sequences of *G. pallida* and nine sequences of *G. rostochiensis* suggests that our ITS-rDNA sequences display high homology with these species and no significant intraspecific variation was observed. For *G. pallida*, variation was slightly smaller (99–100%) than for *G. rostochiensis* (97–99%) which can be due to its later introduction in the country.

The increasing incidence of *G. pallida* may be a consequence of the phytosanitary measures that have been taken to prevent further spread of *Globodera* spp. in recent years (non-host crops rotation or growing of resistant potato cultivars). Thus, there is a need for a new approach to manage potato cyst nematodes, mainly *G. pallida*.



Portuguese *Globodera* spp. phylogenetic tree* (A) and Counties with positive detections of *Globodera rostochiensis* (B) and *Globodera pallida* (C) in Portugal between 2013 and 2018.

* Evolutionary relationships of Portuguese *Globodera* spp. collected in Portugal between 2013 and 2018. Phylogenetic tree was generated by the Neighbor-Joining method using the Kimura 2-parameter method and 1000 bootstrap replicates.

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REPÚBLICA PORTUGUESA

This work was developed within the scope of PhD Studentship SFRH/BD/138724/2018, supported by national funds through FCT (Fundação para a Ciência e a Tecnologia).

POSTER NUMBER 3

Studies on Citrus Responses to Citrus *tristeza virus* and Drought Stress

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Citrus plants can be severely affected by stressful conditions such as diseases and drought, which have a negative impact on citrus yield. The virology lab at the Universidade do Algarve studies *Citrus tristeza virus* (CTV), the causal agent of Tristeza that has already caused devastating epidemics. The disease severity depends on the CTV isolate and the variety of the infected citrus scion and rootstock, resulting generally in three distinct symptoms, seedling yellowing, quick decline and stem pitting. *Citrus aurantium*, a CTV susceptible rootstock, is generally considered as a good rootstock, compared to CTV resistant rootstocks. Current investigation is directed to the study at a molecular and proteomic level of the interaction between the rootstock *Citrus aurantium* and the scion in the presence of a CTV quick decline isolate.

Under a changing climate, drought is a critical problem that severely reduces crop production. *Citrus macrophylla* Wester, a rootstock used in the Mediterranean region, is known to confer enhanced tolerance to drought when compared to other citrus rootstocks. Transcriptional changes under drought stress were studied by Illumina RNA-seq technology and responsive genes were identified.

It is expected that associated with climate change, there will be a change in the dynamics of citrus diseases. In fact, *Trioza erytreae* is already present in Portugal. To face this problem, we are now devoted too to implementing protection measures in the Algarve region against this insect and to studying *Candidatus* Liberibacter spp., the causal agent of huanglongbing.

Keywords *Citrus tristeza virus*; *Citrus aurantium*; *C. macrophylla*; Illumina RNA-seq; drought stress

This work was supported by SourUnion - Analysis of rootstock-scion interaction that causes citrus decline in response to *Citrus tristeza virus*", funded by the Foundation for Science and Technology (FCT) and by the Investment and Structural European Funds (FEEI), by Portugal 2020 – Regional Operation Programme for the Algarve (CRESC 2020) (Ref. PTDC/BAA-AGR/30957/2017) and by UID/Multi/00631/2013/CEOT.

POSTER NUMBER 3

Studies on citrus responses to Citrus tristeza virus and drought stress

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

P.I.S. Pinto[1], A. Duarte[2,3], S.A. Dandlen[2,3], D.M. Power [1,3], N.T. Marques [3,4*]

[1]Centre of Marine Sciences (CCMAR) ; [2]Centre for Mediterranean Bioresources and Food (MeditBio); [3]Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. [4]Center of Electronics Optoelectronics and Telecommunications (CEOT)
* e-mail : nmarques@ualg.pt

HORTICULTURE

Current research at University of Algarve, lab of Virology, is focused on three main areas: **A**. Studies of the interaction between *Citrus aurantium* and scion varieties in the presence of *Citrus tristeza virus*; **B**. Preventing Huanglongbing epidemics for ensuring citrus survival; **C**. Studies of citrus responses to drought stress.

A. Citrus plants can be severely affected by the *Citrus tristeza virus* (CTV), the causal agent of Tristeza. The disease severity depends on the CTV isolate and the variety of the infected citrus scion and rootstock.
Mild CTV isolates that may not cause a specific symptomatology, are present in the Algarve region. Molecular typing of CTV isolates is under study to understand the impact of a possible entry of *Toxoptera citricida* in the Algarve region.

Citrus aurantium, considered an excellent rootstock due to its vigour, disease resistance to soil-borne pathogens, and adaptation to calcareous soils, is susceptible to CTV. Current studies have the objective of unveiling the factors that explain the increased susceptibility of *C. aurantium* to CTV, using molecular and proteomics approaches.

Fig. 1 - CTV viral particle photographed under a transmission electron microscope (Image from King, A.M.Q. et al., 2011. *Closteroviridae*, In: Virus Taxonomy. Elsevier Inc. ISBN 978-0-12-384684-6)

B. *Trioza erytreae* is present along the north and center coastal region in Portugal. This insect transmits the causal agent of the African form of citrus huanglongbing (HLB) disease, the bacteria *Candidatus* Liberibacter africanus. Fortunately, the bacteria has not yet been introduced in Europe.

The University of Algarve (UAlg), together with other European institutions, is committed to developing measures to halt the spread of *Trioza erytreae* and the introduction of HLB.

Fig. 2 – Blister damage in citrus leaves, surrounded by leaves that have not been attacked by the bacteria.

C. Under a changing climate, drought is a critical problem that severely reduces crop production. *Citrus macrophylla* Wester, a rootstock used in the Mediterranean region, is known to confer enhanced tolerance to drought when compared to other citrus rootstocks.

Transcriptional changes under drought stress were studied in *C. macrophylla* using Illumina RNA-seq technology. Responsive genes were identified and the main pathways affected were elucidated. The change in the expression of identified responsive genes to the combined stress of drought and CTV infection was investigated by qRT-PCR.

Fig. 3 - The phenotype of *Citrus macrophylla* plants under drought stress. (a) Control *C. macrophylla* plants; (b) *C. macrophylla* under drought stress for 15 days with wilted leaves.

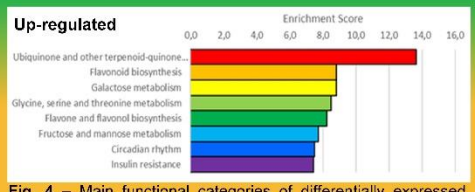

Fig. 4 – Main functional categories of differentially expressed genes (DEGs) up regulated in *C. macrophylla* in response to drought stress. DEGs were identified through homology with *C. clementina* orthologs. Significant enrichment ($p<0.05$) is shown for groups of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways.

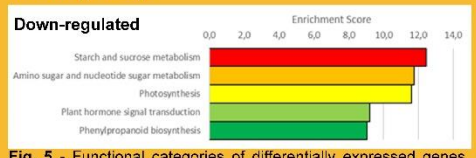

Fig. 5 - Functional categories of differentially expressed genes (DEGs), down regulated in *C. macrophylla*, in response to drought stress. DEGs were identified through homology with *C. clementina* orthologs.

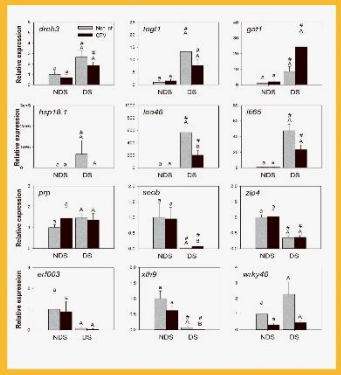

Fig. 6 – Quantitative RT-PCR (qRT-PCR) validation of the relative expression levels of transcripts selected from the DEG analysis.
The signal intensity of each transcript was normalized using the geometric mean of the expression levels of *citrus cytochrome oxidase* (*cox*) and *glyceraldehyde-3-phosphate dehydrogenase* C2 (*gapc2*) genes. The y-axis shows the normalized expression levels of each transcript. The x-axis indicates the result of two groups of comparisons: NDS = plants without drought stress and infected or not with CTV; DS = plants under drought stress and infected or not with CTV.

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POSTER NUMBER 4

The enigma of non-rhizobial endophytic bacteria in the rhizobia-legume symbiosis: clues from fundamental and applied research

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The ecological importance of legumes is unparalleled due to their ability to obtain nitrogen from a symbiotic interaction with a group of soil bacteria commonly known as rhizobia. Besides the typical nitrogen-fixing endosymbionts, legumes harbor other endophytic bacteria within their inner tissues [1] that also contribute to plant growth and health [2]. However, little is known about the molecular aspects of these interactions and most of our knowledge comes from molecular and evolutionary studies of binary legume-rhizobia model systems in gnotobiotic systems [3]. In addition, how plant-associated endophytic bacterial community interacts and which are the key effectors of these interactions that improve the symbiotic rhizobia-legume association remain unknown. A better understanding of these open questions will help to better predict how endophytic bacterial communities interact with plants in natural ecosystems, and this will contribute to improve the efficiency and reliability of inoculant strains.

In this project, we are investigating 1) how non-rhizobial bacterial endophytes (NREB) contribute to legume growth and how this association benefits from the presence of rhizobia and *vice versa*; 2) the hypothesis that NREB may help rhizobia to nodulate non-specific hosts and to unveil the mode of entry and colonization process of NREB in legumes; and 3) the key mechanisms involved in these interactions under control and different stress conditions. Our work shows that Mediterranean native legumes are a reservoir of plant growth-promoting endophytic bacteria and that the diversity and/or functionality of NREB communities are influenced by common agricultural practices, such as pH soil correction and rhizobial inoculation [4]. Plant growth assays revealed that the symbiotic performance of distinct rhizobia is improved when the legume host was co-inoculated with NREB, particularly under stress conditions. Genomic and functional studies have contributed to unveil key genes involved in the tripartite NREB-legume-rhizobia interaction. High throughput proteomic and confocal microscopy analyses disclose novel insights regarding the genetic factors involved in the endophytic lifestyle and in plant-endophytic bacteria interaction. Overall, our work will help to contextualize the molecular mechanisms involved in i) the promotion of plant growth under different stressful conditions, ii) the NREB lifestyle, and iii) the interaction of legumes with multiple endophytes.

Keywords: plant-bacteria interaction; endophytic lifestyle; plant growth promotion; stress; entry mode
This work was funded by Fundação para a Ciência e a Tecnologia (FCT) co-funded by FEDER Funds through the COMPETE 2020 Program in the framework of the project POCI-01-0145-FEDER-016810 (PTDC/AGR-PRO/2978/2014) and the Strategic Project UID/AGR/00115/2013 and UID/AGR/00115/2019. CB acknowledges a Post-doctoral fellowship from FCT (SFRH/BPD/94751/2013).

POSTER NUMBER 4

Jornadas Med 2019
Mediterranean Institute for Agriculture, Environment and Development

The enigma of non-rhizobial endophytic bacteria in the rhizobia-legume symbiosis: clues from fundamental and applied research

HORTICULTURE

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Background

The ecological importance of legumes is unparalleled due to their ability to obtain nitrogen from a symbiotic interaction with a group of soil bacteria commonly known as rhizobia. Besides the typical nitrogen-fixing endosymbionts, legumes harbor other endophytic bacteria within their inner tissues [1] that also contribute to plant growth and health [2]. However, little is known about the molecular aspects of these interactions and most of our knowledge comes from molecular and evolutionary studies of binary legume-rhizobia model systems in gnotobiotic systems [3]. In addition, how plant-associated endophytic bacterial community interacts and which are the key effectors of these interactions that improve the symbiotic rhizobia-legume association remain unknown. A better understanding of these open questions will help to better predict how endophytic bacterial communities interact with plants in natural ecosystems, and this will contribute to improve the efficiency and reliability of inoculant strains.

Questions addressed?

How non-rhizobial endophytic bacteria (NREB) contribute to legume growth and how this association benefits from the presence of rhizobia and *vice versa*?

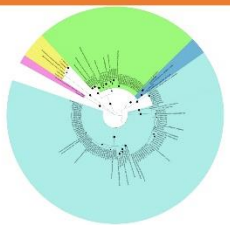
Which is the mode of entry and the process of colonization of NREB in legumes?

Can NREB help rhizobia to nodulate non-specific hosts?

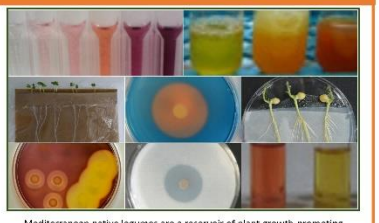
Which are the key mechanisms involved in NREB-legume-rhizobia interactions under control and different stress conditions?

Isolation, identification and characterization of endophytic bacteria

> 180 endophytic bacteria were isolated from chickpea and 12 Mediterranean native legume species.

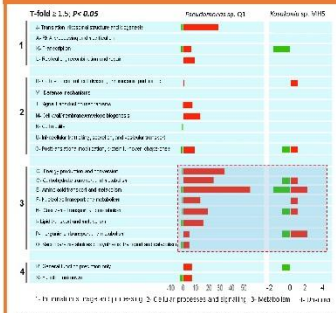


Based on 16S rRNA gene sequences the isolates were assigned to 4 different phyla.

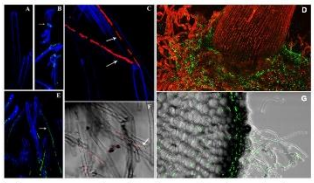


Mediterranean native legumes are a reservoir of plant growth-promoting endophytic bacteria.

High throughput proteomic and confocal microscopy analyses

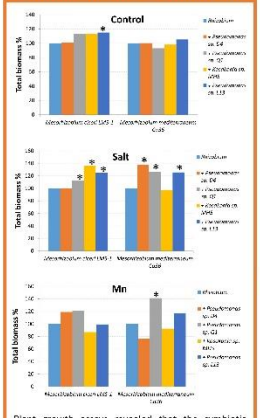


Analyses of the cellular and exoproteome of two endophytic bacteria exposed to chickpea root exudates contributed to unveil genetic factors involved in the endophytic lifestyle and plant-endophytic bacteria interaction.



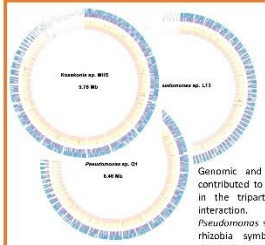
Confocal microscopy analyses allowed to visualize the mode of entry and colonization of NREB as well as the influence of the presence of a rhizobial strain in its mode of entry.

Plant growth assays under control and stress conditions

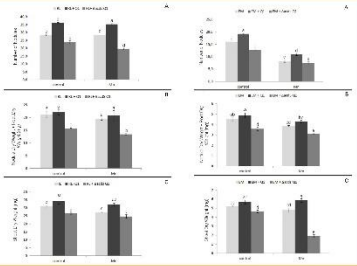


Plant growth assays revealed that the symbiotic performance of distinct rhizobia is improved when the legume host was co-inoculated with NREB, particularly under stress conditions.

Genomic and functional studies



Genomic and functional studies have contributed to unveil key genes involved in the tripartite NREB-legume-rhizobia interaction. For instance, the *Pseudomonas* sp. Q1 promotes legume - rhizobia symbioses by boosting the rhizobial symbiotic performance *via* ACC deamination.



Overall, our work will help to contextualize the molecular mechanisms involved in i) the promotion of plant growth under different stressful conditions, ii) the non-rhizobial endophytic bacteria lifestyle, and iii) the interaction of legumes with multiple endophytes.

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Instituto de Ciências Agrárias e Ambientais Mediterrânicas
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3- Oldroyd G.E. Nature Rev. Microbiol. 2013. 11, 252-263.

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