

ISA



Linking  
Landscape Environment  
Agriculture and Food

LEAF  
Research Center

# LEAF EXPLORATORY PROJECTS 2020

## FUNDED PROPOSALS

### BOOK OF ABSTRACTS

2020

# LEAF EXPLORATORY PROJECTS

These are annual projects for the exploration of research ideas and the consolidation of knowledge that will be explored later, in more depth, in applications for National or International Project Calls. In each call, 30 projects are funded with 5k€.

The development of these projects must result in at least one publication, preferably Q1, as a way of evaluating the quality of the work produced.

There is no limit to the number of researchers that make up a project team, but at least two LEAF members must be involved, preferably from different groups, and may be integrated into a PhD plan. The promotion of synergies between researchers with a higher productivity rate and beginners, to create a dynamic of quality production in LEAF, is valued.

For the 2020 call, 30 proposals were funded, although one quitted. The abstracts of proposals that were approved and running are provided in this document.

## AfroBeans

# African beans diversity patterns: a multidisciplinary study with Angola and Mozambique flora

PI: Maria M. Romeiras

The Leguminosae family is the third largest family of flowering plants, with over 20,000 species. This is an economically important family that contains a large number of plants, namely of pulses which are the edible seed from a legume plant. The Portuguese-speaking African countries (PALOP) hold a great socio-cultural diversity, and particularly Angola and Mozambique are among the richest floristic regions of the world, with many native Leguminosae species. This exploratory project aims to characterize the pulses ethnocultural diversity and to evaluate the morphological, chemical and cytogenomic diversity of the native *Vigna* and the introduced *Phaseolus* species, occurring in Angola and Mozambique, providing new data to promote the conservation and the sustainable use of these unique resources.

## *AgriWWater2Fertilizer*

# Water circularity in industrial horticulture - treatment and reuse of agricultural wastewater and biofertilizer production

PI: Paula Alvarenga

Considering the increasing scarcity of water and natural resources, the treatment of agricultural drainage water (i.e. agricultural wastewater, AWW) is of paramount importance, allowing the protection of the receiving surface waters, and the recycling of nutrients and water. One possibility is the production of microalgae using the AWW as growing media, a win-win solution once it allows the lowering of the pollution load of the discarded AWW, the possibility of water reuse in irrigation, and the production of a biofertilizer (i.e. the microalgae biomass). In fact, microalgae offer the potential to recover nutrients from waste streams and, subsequently, the biomass produced can be used as a sustainable slow-release fertilizer, with additional benefits, relatively to the mineral fertilizers, regarding soil quality improvement. The use of microalgae as a multifunctional biofertilizer in modern agriculture is widely described and different waste streams have been evaluated as growing media, but, to our best knowledge, the use of AWW has not been experimented yet. In this context, the main aim of the study is to evaluate the possibility of using AWW from industrial horticulture, usually with intensive irrigated crops, as growing media to produce microalgae to be used as a biofertilizer. To achieve that aim: (i) agricultural drainage water will be collected, (ii) the water will be physiochemically and ecotoxicologically characterized, before and after the treatment (iii) best-adapted microalgae species will be selected and used to treat the AWW, and (iv) the microalgae biomass produced will be evaluated considering their fertilizing potential as liquid medium, or as dehydrated biomass, to lettuce production.

*AlgaeHealthyBread*

# Microalgae in bread for healthy food

PI: Maria Cristiana Nunes

Microalgae are recognized as a source of numerous phytochemicals with a positive impact on human health and represent a sustainable food ingredient with great importance in the context of climate changes and food shortage. The incorporation of microalgae in food matrices can lead to changes in the rheological and sensory properties, in addition to the nutritional value, as has been demonstrated for several products, including baked goods. Although green colour is currently a trend in the food sector, the incorporation of microalgae in bread has limitations in terms of sensory properties and consumer acceptance, mainly due to coloration and marine flavour. In this context, microalgae subjected to extraction with ethanol, with less pronounced colours and flavours, can represent a promising alternative. The impact of different incorporation levels of *Chlorella vulgaris* and *Tetraselmis chuii* in conventional wheat and gluten-free breads will be evaluated considering the different physico-chemical composition of the extracted microalgae biomasses. The technological functionality of these microalgae will be studied according to the mechanical properties of the doughs (rheology) and breads (texture and volume), nutritional composition, bioactivity and bioaccessibility as well as colour and sensory evaluation of breads by an untrained panel of consumers.

## AQUAporin

# Exploring the functional relevance of aquaporins for water and other crucial molecules transport in cork oak and grapevine

PI: Farzana Sabir

Cork oak and grapevine are two highly important economic plants of Portugal, providing high ecological, social and economic values. The cork oaks constitute a significant component of the unique ecosystem *montado* in Portugal. On the other hand, a wide range of Portuguese edaphoclimatic conditions offers remarkable regional specific wines. However, climatic factors such as drought stress with imbalanced nutrient uptake may drive significant impacts on the sustainability of *montado* ecosystem (1), and on viticulture. Therefore, it is essential to understand the molecular mechanism of water and nutrient transport in cork oak and grapevine, especially in the context of plant water status. Aquaporins are essential to transport water and small molecules in all living organisms. They have been associated to play crucial role in plants under various stresses, especially under water stress (2). Besides water, they transport various physiologically important small solutes (2). Limited information regarding cork oak and grapevine aquaporins obstructs the understanding of water transport and stress tolerance associated mechanisms in these plants. The present project intends to understand the role of aquaporins for water and nutrient homeostasis, especially under drought stress in the two important cultures, cork oak and grapevine. Specifically, in what concerns cork oak, two aquaporins (PIP2;4/TIP2;1) will be cloned and characterized in the *aqy-null Saccharomyces cerevisiae* (established from previous study (3,4)). Their relevance in water and glycerol transport will be evaluated by stopped-flow. Besides, in what concerns grapevine, the putative role of two grapevine aquaporins (TIP2;2/NIP2;1) in transport of boron will be analysed in the same yeast expression system and at whole plant level. Intracellular accumulation of boron will be estimated by coupled plasma mass spectrometry (ICP-MS). Differential expression of aquaporins in the presence of boron under drought stress will also be analysed. The obtained integrated knowledge will contribute to understanding the relevance of aquaporin for water and nutrient balance in plants.

## *ATLAS : Assessing Sustainable Agri-food Systems*

# **Optimizing a Fit-to-Purpose Tool for Impacts Assessment in Sustainable Agri-Food Systems: A Case Study Application of Social Life Cycle Assessment (S-LCA) in Mixed Crop-Livestock Farming Systems in Madagascar**

PI: David Fangueiro

The emergent Social Life Cycle Assessment approach is presented as a holistic tool for exploring production dynamics within agro-ecologically adapted farming systems but it has been scarcely applied to developmental agriculture contexts. Taking Madagascar as a case study, this project will focus on crop-livestock diversification as risk-averse, agro-ecological adaptation to climate change and resource scarcity. The project will subsequently employ a productivity model as the impacts assessment component of the S-LCA approach. The project's main goals are to investigate the adaptability of S-LCA to a new global agri-food context and to test the utility of a productivity model for socio-economic impacts assessment within agro-ecological mixed-farming systems.

## Bio4FoodRes

# Biocatalysis applied to the production of added-value compounds for Food and Pharmaceutical Industries from Agro-Industry residues and by-products

PI: Suzana Ferreira-Dias

The sustainable valorization of local agro-resources, agro-residues and by-products, which are usually unexploited and cause environmental pollution, must comply with environmentally friendly processes to produce high added-value products. Biomass wastes and agro-residues from Mediterranean crops (olive pomace and grape stems and seeds), will be used as sources of biomass and/or oil to obtain the following added-value products: (i) oligosaccharides with prebiotic activity; (ii) glucose, (iii) structured lipids and (iv) bioactive phenolic extracts for food/pharmaceutical applications. Biocatalysis is a green and sustainable technology. Therefore, enzyme-catalysed processes and/or green technologies will be used. Biomass will be submitted to autohydrolysis to obtain a liquid stream containing hemicellulose-derived compounds (e.g. xylans or mannans), and the residual solid fraction rich in cellulose. Oligosaccharides (xylo-oligosaccharides, XOS, or mannano-oligosaccharides, MOS) with prebiotic activity will be obtained by hydrolysis of xylans or mannans, catalysed by free or immobilized xylanases or beta-mannanases. Cellulases and beta-glucosidases will act on cellulose fraction to obtain glucose for fermentation or other industrial uses. Olive pomace and grape by-products are rich in bioactive compounds, such as polyphenols with antioxidant and anti-inflammatory properties. Polyphenols will be obtained by non-pollutant ultrasound-assisted extraction, as alternative to toxic solvent-extraction. Olive pomace and grapeseed oils will be extracted to produce structured lipids (SL), which are novel lipids with improved functional and bioactive properties, for food and pharmaceutical uses. Low-calorie dietetic SL will be produced by acidolysis of these crude oils with medium-chain fatty acids or interesterification with their ethyl esters, catalysed by immobilized *sn*-1,3 regioselective lipases, in solvent-free media. This project will meet the *Sustainable Development Goals*, defined in the *2030 Agenda for Sustainable Development*, adopted in 2015 by the United Nations.

## *Biowaste4Value*

# **Addition of fruit waste concentrated solutions obtained by membrane technology to non-conventional diet pig´s manure for improving AD process**

PI: Antónia Macedo

The need to mitigate the environmental impact of the wastes generated during animal production calls for recycling, reinventing their life cycle, assigning them to a new use, which contributes to the reduction of the waste, applying to a circular economy model. The production of biogas from pig manure receiving diet from algae (PMalgae) and sugar concentrated solutions obtained from fruit biowaste (SSFB) through membrane processes. In this proposal, different membranes processes (ultrafiltration, nanofiltration) and operating conditions (feed circulation rate, transmembrane pressure), for optimizing the separation/concentration of SSFB. The obtained SSFB will be used as co-substrate in the anaerobic co-digestion (AcoD) for process optimization. The following parameters: different ratios of PMalgae/SSFB, hydraulic retention time, organic load rate, specific methane yield and digestate quality will be monitored to define the best operational conditions to apply to real scale. It is expected that the methodologies used can give a good contribute for the recovery of value from biowaste and is one way to respond to the economic circularity.

## BugSnack

# Desenvolvimento de snacks inovadores utilizando farinha do inseto *Tenebrio molitor*

PI: Maria Otília de Almeida Carvalho

A procura de fontes proteicas mais sustentáveis e saudáveis, que sirvam de alternativa à proteína animal, é uma tendência de consumo muito relevante nos últimos tempos. Do mesmo modo, o desenvolvimento de snacks saudáveis, também se encontra nas prioridades das tendências do sector alimentar, tendo em conta a evolução dos estilos de vida e a procura de alimentos convenientes, saudáveis e de custo controlado. Com a realização deste projeto exploratório pretende-se desenvolver snacks com a adição de diferentes níveis de farinha de um inseto - *Tenebrio molitor* L. (Coleoptera, Tenebrionidae), utilizando um processamento tradicional e uma tecnologia inovadores: impressão 3D. Pretende-se deste modo criar diferentes texturas apelativas ao consumidor, que possam ser direcionadas para diferentes mercados alvo.

*CultIVAPortugal*

# Prospecção de plantas silvestres infrautilizadas e marginais com valor agroalimentar (espécies NUS), em Portugal

PI: Dalila Espírito-Santo

Pretende-se completar o inventário das NUS em Portugal com uma abordagem holística que complementa os dados botânicos e ecológicos com informação relativamente à contribuição de cada espécie para a segurança nutricional das populações que as utilizam/utilizavam, bem como ao seu potencial de exploração gastronómica, a fim de facilitar o estabelecimento de prioridades e a possibilidade de iniciar programas de cooperação, em particular ibero-americanos dentro da *Red Iberoamericana de Cultivos Infrautilizados y Marginados com Valor Agrolimentario (CultIVA)* que inclui investigadores do LEAF.

## EFYALGAS

# Efficiency of Algae in removing Arsenic for rice crop Amelioration

PI: Maria da Glória Esquível

Arsenic is a toxic contaminant of geogenic origin that is present along rivers and in environment with anthropogenic influences. As is a significant problem for one of the major global crop, rice, that accumulates As especially in the edible organ, the grain. The use of microalgae has emerged as a promising biotechnology for simultaneous absorb and metabolize metals or metalloids like arsenic. With our expertise to improve microalgae growth along with the experience in trace elements quantification, we aim to improve the reduction of As accumulation in rice using microalgae strategies that could be achieved in an exploratory project. We will evaluate the capability of the model microalgae *Chlamydomonas reinhardtii* to remove arsenate from water and compared it with two different species *Chlorella vulgaris* and *Scenedesmus* sp. The microalgae with the best response towards decreasing As concentration in solution and tolerance to the stress will be chosen for the amelioration of arsenic toxicity in rice plants. Inoculation of the algae into the hydroponics culture of rice with arsenic will be monitored and the element will be quantified in the solution and in the biomass of the microalgae and in rice plants. This project integrates experts in complementary fields and all members will profit from the synergism of this collaboration.

## *FishWaste2Bio*

# Fish Waste to Bioenergy and Biobased products

PI: Rita Fragoso

In recent years there has been a development of fish processing, increasing its waste production to over 20 Mt in 2018 (FAO, 2020). Fish waste constituents include 15-30% crude protein, up to 25% fat having high nutritive value for human consumption. However, inappropriate management of this waste stream can lead to negative environmental impacts and therefore it should be looked as a resource and a source of added-value compounds. **FishWaste2Bio** aims to develop exploratory work to assess the technical feasibility of a two-step valorisation route, firstly producing added-value compounds from recovered fish waste oil, followed by anaerobic co-digestion of the exhausted waste (that has a low content in fatty acids and therefore minimizes process's inhibition). This biorefinery approach will also contribute to the economic sustainability of the valorisation route as the biobased compounds obtained have high economic value.

*Heras.PT*

## Diversidade, conservação e utilização das heras nativas de Portugal

PI: Pedro Talhinhos

As heras são plantas com interesse botânico e paisagístico, sendo cultivadas desde a antiguidade clássica. De entre as 13 espécies reconhecidas, quatro (*H. iberica*, *H. hibernica*, *H. azorica* e *H. maderensis*) são autóctones em Portugal, duas das quais endémicas, fazendo do ocidente europeu e Macaronésia o maior centro de diversificação do género *Hedera*. Diversas espécies de heras ocorrem como cultivares, sendo difícil diferenciar espécies nativas de exóticas visto que esta distinção se baseia em caracteres morfológicos, por vezes de interpretação ambígua. Estudos preliminares mostram que o nível de ploidia e o tipo de tricomas são os caracteres que permitem a sua distinção de forma inequívoca. Neste projeto pretende-se determinar que espécies de hera ocorrem nos jardins e parques e na natureza, em Portugal. Sendo a hera utilizada como planta ornamental há mais de 2000 anos, muitas plantas aparentemente espontâneas poderão ter sido introduzidas pelo homem. Inclusive, dado a sua facilidade de cultivo e interesse ornamental, as espécies nativas parecem estar a ser substituídas por cultivares hortícolas de espécies exóticas. O conhecimento da ocorrência e distribuição de espécies nativas e introduzidas permitirá estabelecer níveis de risco para as espécies nativas e as exóticas. Por outro lado, a identificação inequívoca de espécies nativas permitirá o seu uso mais racional em espaços intervencionados pelo homem, nomeadamente em jardins e no recobrimento de paredes e edifícios. As espécies nativas em Portugal serão adequadas a estas utilizações? Quais são os principais desafios para a utilização destas espécies como ornamentais, contribuindo para a sua preservação e valorização? Para responder a estas questões juntamos uma equipa LEAF abrangendo as valências de Botânica, Citogenómica, Fitopatologia, Infraestruturas Verdes e Arquitetura Paisagista.

## *HotSpot*

# Land Use Planning in Headwaters System – Places of Opportunity

PI: Selma B. Pena

The Headwater System (HS) is a continuous structure of the landscape along the ridgelines that performs a high number of ecological functions, also gathering unique landscapes. This ecological corridor is a Green Infrastructure that, in 2019, had returned to the National Ecological Reserve framework, a land use planning instrument from Portugal. The hypothesis is that the HS can be a place of opportunity (Hotspots) where the restoration should be performed, with benefits in water and soil conservation, biodiversity increase, risk reduction, and provision of ecological connectivity. The first goal of this project is to deepen the knowledge of the HS in Scale and Time by understanding the potential natural vegetation, geomorphology, and the cultural components of landscape. The second goal is to research the solutions of land use planning and ecosystem restoration in the HS, providing a vision for the future of these landscapes.

*LAMB-MEaT*

# Metabolome and mineral profile in cooked lamb meat of tropical hair sheep

PI: André M. de Almeida

Ovine production is one of the most important agricultural activities worldwide, particularly in tropical countries where they provide meat, milk and wool, crucial for the livelihood of local populations. The sheep produced in the tropics are essentially of the hair type (e.g. Damara or Dorper). Little information is available on such breeds regarding the nutritional composition of their meats, particularly concerning the specific effects of cooking on proteins, metabolites and mineral profiles. The recent advent of the Omics techniques allows filling such knowledge gaps as they generate very complete, broad and high-throughput datasets. In this project, we will study the effect of boiling (cooking) on the metabolome and mineral profiling in the meat (muscle) in three breeds of sheep of different genetic background: the Damara and the Dorper (tropical hair sheep) and the Merino (wool breed of European origin) in a very novel approach. Samples were obtained from a trial previously conducted and we will use an approach based on NMR-Based metabolomics and Inductively Coupled Plasma – Optical Emission Spectrometry. Results will be integrated with productive performance, general meat traits and proteomics analysis currently being conducted in the framework of another project. With this project, we will determine important nutritional characteristics as affected by the cooking process and understand their significance to human nutrition.

*LeaSyBiome*

# Leaf symptoms in grapevines affected by esca: exploring the microbiome hypothesis

PI: Giovanni Del Frari

Esca is a grapevine trunk disease that poses major concerns to worldwide viticulture. Affected plants suffer from reduced longevity, productivity and quality of the yield, altogether causing enormous economic losses. The causal agents are a diverse array of fungi that infect and colonize grapevine woody tissue, inducing the appearance of both internal and external symptoms. While internal symptoms have been widely studied and –in most part– understood, the appearance and expression patterns of external symptoms (i.e. affecting leaves) remain unclear. To date, none of the leading theories concerning leaf symptoms etiology has been confirmed by empirical data, and the scientific community remains divided over the real causal agent (e.g., fungal toxins, byproducts of wood degradation, hydraulic failure). Surprisingly, no research has been conducted over the grapevine leaf microbiome, and its potential involvement in symptomatology appearance and/or differential patterns of symptomatology expression. In this study, we will use next-generation sequencing (DNA metabarcoding) to investigate the endophytic microbiome of grapevine leaves of cultivars Cabernet Sauvignon and Touriga Nacional. We will examine the ITS1 region for fungal and 16S region for bacterial communities, in leaves that manifested differential symptomatology patterns and control (healthy) leaves. Through this study, we will be the first group to (A) use DNA metabarcoding to examine the grapevine leaf endophytic microbiome, (B) explore the connection between leaf microbiome and symptomatology patterns in grapevine leaves. The positive outcomes of this study go beyond the pure academic understanding, offering insight on potential strategies to control symptoms expression, for example using synthetic microbiomes.

## *MicroBerry*

# Cluster microbiome of Moscatel Graúdo: the sun-exposed side of the canopy *versus* shaded clusters

PI: Luísa Carvalho

Grape berries hold a large variety of microorganisms (the microbiome) typical from the vineyard environment, many of which influence must fermentation, helping shape wine quality, and others with roles in plant stress defense. The abundance and type of microorganisms are more influenced by viticultural practices, such as row orientation, trellis system or irrigation than by grape variety. Understanding how canopy exposition to direct sun and heat during the warmest hours of the day influences microbial composition may have a significant influence on the wine industry as well as for vine growers. Ultimately, being able to alter these properties will enable the possibility of making a wine with specific chosen characteristics. With the current project, we intend to characterize the bacterial and fungal microbiome of the sun exposed and shaded sides of the canopy, using a metataxonomics approach, to better understand how the meso and micro climatic conditions can shape these populations and the characteristics of the musts produced.

## *MoringaFood*

# ***Moringa oleifera* seeds characterization and possible uses in food**

PI: Isabel Sousa

MO (*Moringa oleifera*) is a very important plant and a valuable resource as food for the diets of people in many developing countries, due to its diverse range of nutrients, but also in healthy supplementation of diets of mature people in developed countries, used as a nutraceutical. For the latter, mainly the leaves are available as a stable powder or in compressed pills. Seeds of moringa are left in great quantities after the collection of the leaves and they are an amazing source of nutrients, from PUFA rich edible oil (33%) to protein (33%) vitamins, minerals, antimicrobials and other bioactive compounds (polyphenols, glycosylates and more) with major impact on reduction and prevention of civilization common diseases (e.g. diabetes, high cholesterol, cancer). To study the possible uses of MO seeds as a source of food, the strategy will be to characterize the whole seed, the oil extracted from the seeds and the flour produced from the remainder defatted cake. Centesimal composition will be determined as well as antioxidative potential, presence of glycosylates (potentially toxic), vitamins C and A, and minerals, on the three different materials. These can be especially adequate to supplement deficient diets on protein and vitamins C and A, and on minerals like iron and calcium. This project aims at achieving chemical characterization of MO seeds to support its potential to enrich foods. Concerns with sensory properties and consumer's acceptability will also be taken into account and some model foods like cookies (solid), milk like beverages (liquid) and sauces (emulsions) will be produced and sensory tested. These MO seeds are actually a byproduct of the MO crop, since its main use is a powder from the leaves for food/ diet supplementation. They have a great potential as a source of bioactive ingredients with direct impact on health.

## MycoHaloph

# Unravelling the combined use of microbial and soil technologies to optimize edible halophyte cultivation in underused saline soil

PI: Ana Delaunay Caperta

Increasing soil salinization and decrease of water quality due to intensive agricultural practices is a recognized threat to food security in the future and an environmental problem affecting people and ecosystems throughout the planet. Still, saline soils might be used to advantage for the cultivation of underutilized, salt tolerant wild species (halophytes) for agricultural purposes. There are now clear evidences that the employment of microorganisms found in mutualistic association with plants as well as soil technologies help them to gain tolerance to abiotic stresses and to grow in saline conditions. The major question of this project is how to optimise cultivation of valued halophytes with the potential to generate novel crops. To this end, growth and reproduction, and physiological responses of halophyte *Limonium algarvense* to halo-tolerant microbial inoculation will be monitored in microcosm conditions using saline soils amended and not with a Technosol. This study will have two major outcomes: i) new insights into the relationship between microbes and halophytes; ii) scientific support to the combined use of microbes and soil technologies to allow the utilization of saline marginal soils for agriculture. The results of this project can be generalized to other edible *Limonium* species to be tested and/or other halophyte species and in other conditions of saline soils, in the future.

## MYCOVITIS-GBANK

# Recovery of arbuscular mycorrhizal fungal diversity in Portuguese vineyard soils threatened by climatic and edaphic challenges: germplasm bank creation and specialized case-adapted inoculum production

PI: Amaia Nogales

Plant inoculation with arbuscular mycorrhizal fungi (AMF) has become a novel approach to enhance plant productivity and stress tolerance while reducing environmental costs in agroecosystems. However, to make mycorrhizal applications economically and environmentally profitable, specially in vineyards, it is essential to conduct a previous selection of the most compatible AMF isolates/consortia for each target soil. Native AMF isolates are usually more infective and beneficial for their host plants than non-adapted commercial mycorrhizal inoculants [1], but there is currently no AMF germplasm bank in Portugal to support mycorrhizal use under the different local edaphic and climatic conditions. Thus, the present project aims to 1) to perform a survey of cultivable AMF of different Portuguese winegrowing regions, including AMF communities from vineyard soils and from surrounding non-disturbed soils, 2) to preserve native AMF communities in a collection using grapevine rootstocks as host plants, and 3) to characterize AMF consortia isolated from the different sites, in order to determine the most efficient and appropriate consortium for each soil type. The availability of an AMF collection specifically directed to viticultural use will enable the development of suitable strategies to recover vineyard soil mycorrhizal biodiversity, that may be crucial to improve grapevine growth and adaptation to stress conditions. In the project, the methodologies that will be used were already well-established at ISA during MYCOVITIS project, such as targeted metagenomic approach for AMF diversity study, as well as grapevine growth and physiology monitoring, nutrient analysis and mycorrhizal colonization estimation [2]. A multidisciplinary team of researchers from LEAF Research Groups I and II will be included for this purpose, with vast experience in soil-plant-microbe interactions and in the creation and curation of germplasm collections. Besides, it will count with the consultancy of Dr. Patricia Silva-Flores (Chile), an expert in AMF biodiversity studies, and with Dr. Felipe Gainza, from the Center for Research and Innovation of Viña Concha y Toro, the biggest winery in South America. This winery is using grapevine plants inoculated with native AMF in their new plantations and will conduct some parallel experiments in Chile to validate the strategies developed hereby.

*NEF-ISA*

## **Núcleo de Estudos Ficológicos - NEF-ISA - Recuperação de uma coleção histórica como ferramenta para a investigação ficológica e implementação de Núcleo de Estudos Ficológicos em LISI (ISA)**

PI: José Carlos Costa

A doação de um exemplar de Algário do século XIX de um colecionador privado ao Herbário João de Carvalho e Vasconcellos (LISI) trouxe a oportunidade para o seu estudo histórico, sistemático, e relevância para estudos ecológicos sobre a biodiversidade ficológica nele presente. Consideramos a sua utilização um ponto de partida para a implementação de um algário entre as coleções arquivadas no Herbário. Os herbários funcionam como bancos de conhecimento botânico, fornecendo importantes evidências físicas acerca da distribuição no espaço e tempo dos organismos na época em que foram colhidos. Com o aumento das alterações climáticas e pressões antropogénicas, principalmente nas zonas costeiras, é necessário aprofundar o conhecimento e reunir a máxima informação possível acerca das comunidades de macro e microalgas que lá se encontram. Este projeto ambiciona munir o LISI com uma coleção de macro e microalgas para que o LEAF possa também iniciar a investigação sobre a biodiversidade ficológica.

## Non-foodCropMine

# Cultivate non-food products in degraded mine areas with designed Technosols

PI: Erika Silva Santos

The recovery of mine areas and reuse of wastes from several industries are priorities at environmental and economic level. Moreover, conventional closure systems in mining sector have high cost of implementation and maintenance being ineffective in the improvement of the ecosystem quality. Also, decreasing of agricultural areas to cultivate non-food products need urgent options. The recovery of non-productive mine soils is a promising niche for agriculture leading to significant ecological and economic improvements. This project addresses the design of an integrated system in an active mine (Los Frailes, Spain) comprising the cultivation of autochthonous plants with aromatic and medicinal properties (*Lavandula pedunculata* and *Rosmarinus officinalis*) in Technosols, derived from wastes and based on specific soils characteristics, and the extraction (with greener methods) of high-value bioactive plant extracts. This project includes all crop value-chain and circular economy and sustainable approaches. A multidisciplinary team from LEAF (G1, G2), Universidade de Aveiro, an environmental consultant company and an active mine are included for this purpose.

## OLIVETHY

# Flavoured olive oils by co-extraction with thyme

PI: Fátima Peres

The innovation of new products in the olive oil sector has had a great development, namely in the gourmet area, with several products on the market, generally resulting from the addition of products of vegetable origin to olive oil, such as aromatic herbs, condiments, nuts and algae, among others. An innovative method is co-processing also designed by co-extraction, based on the addition of ingredients, for example fruits or aromatic plants, before the malaxation step or during milling. This technique allows to select both the type of olives, the ingredient with the greatest aromatic and bioactive potential, as well as the technological conditions of extraction. Moreover, this method does not need the filtration step, conversely to when aroma infusion is performed. For olive oils from organic agriculture, the production of co-processed oils can be an opportunity for differentiation, especially in the non-traditional consumer market, increasing the competitiveness in rural areas. This project proposes a study of olive co-processing with thyme on the yield and quality of the final product, a gourmet olive oil. The trials will be performed using overripe fruits with low aromatic potential (cvs. 'Galega Vulgar' and 'Cobrançosa' with a ripening index greater than 6), and two species of thyme (*Thymus x citriodora* and *Thymus mastichina* L.). A complete chemical characterization of all the raw material will be done as well as the complete analysis of the flavored oils produced (quality, sensory and bioactive compounds).

## ProBio

# Probiotic beverages from underexploited plants and co-products of minimally processed fruit and vegetables industries

PI: Vítor Alves

Co-products from minimally processed fruit and vegetables, discarded in large quantities, still have a high value due to their content of functional compounds (vitamins, minerals, antioxidants and fibers) whose intake is fundamental in the prevention of diseases and in consumer health and wellness. In addition, unexploited plants (e.g. *Rumex crispus*, common name “labaça crespá”), which is naturally growing in Portugal, and cultivated *Petroselinum crispum*, common name “salsa”, are also excellent sources of nutrients and can confer desirable sensory characteristics. All these raw materials present an excellent valorization potential through the production of functional beverages. This project intends to contribute to that valorization with an exploratory approach on the development of novel fermented beverages, presenting good sensory properties and valuable nutritional value, by fermentation using lactic acid bacteria (LAB) with probiotic properties.

## QUALEGG

# The effects of storage on the quality of brown-shelled vs white-shelled eggs

PI: Madalena Lordelo

The European consumer tends to gravitate towards brown-shelled eggs in comparison to white-shelled eggs due to a perceived notion that brown is more natural or healthy. Strains of hens that lay white eggs, however, tend to be more efficient in terms of egg production, and therefore could produce cheaper eggs which is a clear advantage for the producer and for the consumer. The objective of the current study is to compare a number of nutritional and physical characteristics, which are of interest to the consumer, between white and brown eggs. Furthermore, it is our goal to gain insight into egg consumer perceptions associated with shell color.

## *Roofood*

# The rare food rooftop – traditional food from rustic plants in a green roof/ Utilização de plantas alimentares tradicionais em coberturas verdes

PI: Teresa Afonso do Paço

O microclima da cobertura dos edifícios pode diferir daquele encontrado ao nível do solo, dado que existe uma maior exposição à radiação e ao vento. As plantas de ambientes hostis, cultivadas ou mesmo encontradas espontaneamente na natureza, poderão ser uma solução interessante para utilização em coberturas verdes destinadas à produção agrícola em ambientes urbanos. Pretende-se avaliar a adaptação de plantas comestíveis oriundas das zonas centro e sul do país, usadas tradicionalmente, a coberturas verdes na zona de Lisboa. Tem-se em vista contribuir para a sua preservação, ajudando a encontrar soluções mais sustentáveis de agricultura urbana em cobertura.

*RT\_NutMalus*

## Development of a quick tool for real-time assessment of the nutritional status of apple orchards

PI: Cristina Oliveira

The standard diagnostic method of nutrient status in fruit trees, leaf analysis, is laborious and costly but mainly, the time when it can be performed does not allow corrections in the year itself. Assessing the nutrient content in sap analysis, as opposed to the leaf analysis, would increase the opportunity of adjusting the fertilization, along the growing cycle. The possibility of using a quick test RQflex® with accuracy in in-situ sap analysis was previously proven in *Malus domestica* Borkh 'Gala' through regression analysis between RQflex® and laboratory methods. In this project, we aim to develop this tool monitoring the nutritional status of trees in real time in intensive Gala apple orchards, in different productive systems. Together with the sap analysis, analyses of mineral in various organs (buds, flowers, leaves and fruits) will be carried out at various stages of the cycle with the aim of correlating them with the sap analyses, and assess under what conditions the sap analyses reflect the values of laboratory tests. A real time in situ method is of outmost importance for decision support system to accomplish a rational fertilization with productivity and environmental benefits.

## SexType

# Grapevine redefines new frontiers in the sex-determining region

PI: Margarida Rocheta

*Vitis vinifera* can be divided into two subspecies: *Vitis vinifera vinifera*, one of the most important agricultural crops in the world, and its wild ancestor, *Vitis vinifera sylvestris*, considered a repository of genetic diversity for viticulture. Three flower types can be observed in *Vitis vinifera*: hermaphrodite in *V. v. vinifera*, and male or female flowers in different wild *V. v. sylvestris* individuals. An interesting aspect of these subspecies is that they possess a complex sexual system without heteromorphic sex chromosomes. Furthermore, it is assumed that the different flower types in the wild ancestor arose through specific floral patterns of organ abortion. Several grapevine breeding studies, through analysis of F1 offsprings led to the hypothesis that dioecy in *Vitis vinifera* is derived from a hermaphrodite ancestor and could be controlled by either, one or two linked genetic determinants following Mendelian inheritance. Experiments using molecular approaches suggested that these loci were located in a specific region of the chromosome 2 of *Vitis vinifera*. Recently, two studies on wild and domesticated grapevine shed light in the sex determining region (SDR) and point a set of genes that could be involved in SDR. Both studies agree on *INAPERTURE POLLEN1 (INP1)* being responsible for male sterility but disagree on the female sterility gene. However, there is some controversy on the boundary of the sex locus and the female sterility gene. Additionally, another work establishes relationships between wild and cultivated populations and uncovers that most of the cultivated grapevines result from wild introgressions. We can hypothesize that they play a critical role throughout the domestication of this agronomic species, from the Caucasus to the Iberian Peninsula. In Portugal we have unique grapevine diversity resources that deserve a closer look regarding sex determination and the development of grape clusters with commercial value. The phenotype of the above varieties raises important questions related to grapevine SDR that still need to be answered, namely: 1) where are locate the gene(s) responsible for male sterility; 2) where and what type of modification occurred in the SDR haplotypes that originated the three phenotypes mentioned above.

## TomBugBite

# Tomato defensive responses to mirids used in biological control

PI: Elisabete Figueiredo

Protected tomato is one of the most important horticultural crop in Portugal. Due to an increasing demand for products grown with lower use of pesticides, mirids become an important biological control (BC) agent, especially the Dicyphini species, to control whiteflies and *Tuta absoluta*. However, mirids are zoophytophagous, and when prey is scarce their plant feeding can cause damage, resulting in economic losses. This project aims to compare the phytophagous behaviour of three important Dicyphini species and to evaluate the type and degree of impact induced to tomato. We believe that understanding the tomato responses to the damage caused by Dicyphini will allow a better species choice decision to be used in BC. This choice could then, in addition to the voracity, take into account the balance between the positive and negative effects of mirids phytophagy.

## *VEGarum*

# Innovative, healthy and tasty fermented products from seaweeds

PI: Marisa V. Santos

The increasing interest and curiosity of consumers for innovative, sustainable, diverse, healthy and flavourful foods, along with the growth of specific market segments (such as vegan and vegetarian), and the rising awareness on the environmental impact of actual population eating habits are leading to changes in the food industry towards Functional Food. Among these, vegetal-based fermented foods are time-honoured products, combining nutrition, health benefits and sustainability, with enhanced relish tastes. Several fermented products can be associated to centuries of different ethnic and cultural traditions, such as garum and allec, two fermented fish products with countless descriptions and historic evidences of production and trade during the Roman occupancy of the current Portuguese territory. Seaweeds are marine resources with great nutritive potential and a source of umami-taste compounds, making them ideal for the production of new vegetal-based fermented foods in-line with the more recent food trends. This project aims to develop new sauce and pate-like products suitable for vegan and non-vegan consumers based on macroalgae, using the traditional knowledge of garum and allec, combining the benefits of these sea-vegetables with those from fermentation processes to produce two flavourful healthy products. Ultimately, this project can also contribute to the blue economy through sustainable transformation of marine resources into highly nutritional products for human consumption.

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