



**SPA2022**

# **SUSTAINABLE AND PRECISION AGRICULTURE SYMPOSIUM 2022**



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**Aula Magna, Sciences Faculty (S. Physics and  
Mathematics)**

**University of La Laguna**

**San Cristóbal de La Laguna, Tenerife (Spain), 18-20 July 2022**





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# Simposio Internacional **SUSTAINABLE AND PRECISION AGRICULTURE**

18-20 de julio 2022  
Aula Magna de Físicas, Universidad de la Laguna  
[www.spa2022.com](http://www.spa2022.com)



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**REGISTRATION DESK/VENUE**



**Aula Magna, Sciences Faculty, S. Physics and Mathematics, University of La Laguna**  
 Facultad de Ciencias, Sección Física y Matemáticas  
 Avda. Astrofísico Francisco Sánchez, s/n  
 38206-La Laguna, Tenerife, Spain

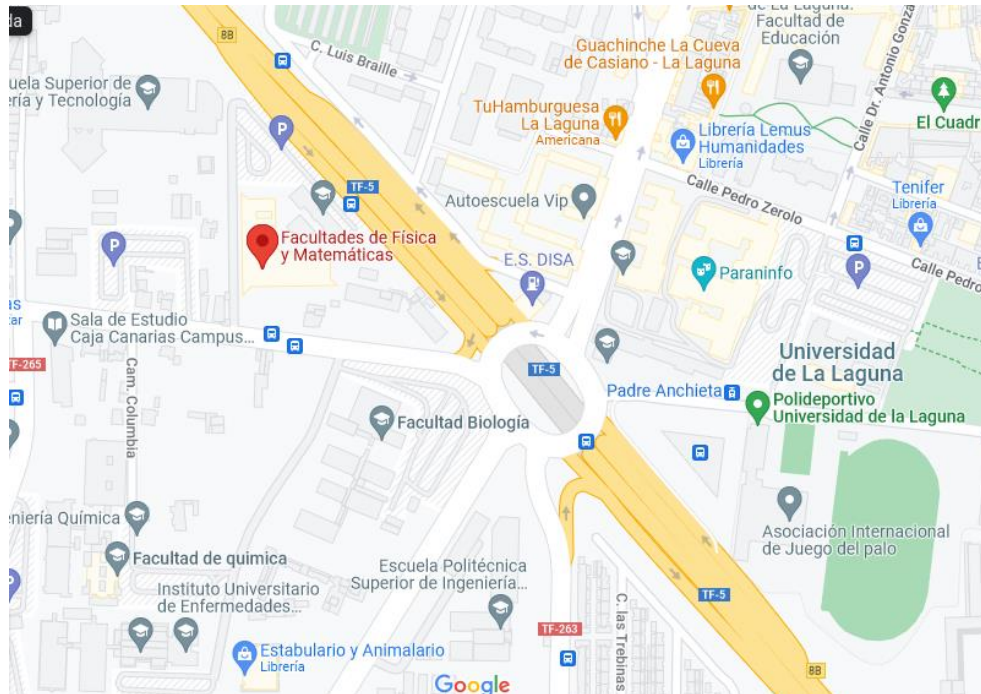
**CONTACT**

**Organizing Committee, E-mail address:**

**E-mail:** [webmaster@spa2022.com](mailto:webmaster@spa2022.com)

**Telephone:** +34 922 260112

**HOW TO ARRIVE**



The sessions will be held in the Aula Magna (Lecture Hall) of the Science Faculty, Section Physics and Mathematics ([www.ull.es](http://www.ull.es)), located in the town of [San Cristóbal de La Laguna](http://www.aytolalaguna.com/) (Tenerife, Canary Islands, <http://www.aytolalaguna.com/>), declared a World Heritage Site by the UNESCO in 1999). The center is located inside the Anchieta Science Campus of the University of La Laguna, near the Instituto Universitario de Bioorgánica “Antonio González”. **The venue is 5 min away from TENERIFE NORTH AIRPORT (TFN).**

**TRANSPORT**

From the Tenerife North Airport (Los Rodeos): take a taxi to the F, Física y Matemáticas (La Laguna, Anchieta Campus). An express airport bus is also available to the nearby Central Bus Station (Intercambiador de Guaguas de La Laguna), quite close to the venue.

From the Tenerife South Airport (Reina Sofía): take a 111 or 341 **TITSA** bus, known locally as a “guagua”, to the bus terminus in the capital, Santa Cruz de Tenerife, or a 340 TITSA bus to Tenerife North Airport. Then take a taxi to the IPNA and give the above campus directions.

Once in the Island, you can **move around with the efficient BUS SERVICE (TITSA, <http://www.titsa.com/index.php>)** which reaches all spots in the Island, even the Teide National Park (Cabildo de Tenerife: <http://www.tenerife.es/portalcabtte/en/discover-tenerife>). You can buy a discount ticket for a certain amount and use it for any route. The bus ticket can also be used in the city tram (**METROBUS**) that links Santa Cruz de Tenerife and La Laguna every 5 minutes.



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## Sustainable and Precision Agriculture Symposium 2022

### ORGANIZERS AND CONTACT DATA:

#### Local Organizing Committee

**SPA2022 Coordinators:** Isabel López Bazocchi (ULL) and Alicia Boto (IPNA-CSIC)

**Co-director of seminars:** José Francisco López Feliciano (ULPGC)

**Secretary and webmaster:** Fernando Lobo Palacios (ULL)

**Institutional Coordinators:** Mercedes Alonso (DGA-Gobierno de Canarias), Gloria Lobo (ICIA), Carmen Rubio (ULL), Andrés Borges (IPNA-CSIC) and Concepción González (IPNA-CSIC).

#### Other Members:

Arturo Hardisson de la Torre (ULL)

Vanesa Raya Ramallo (ICIA)

M<sup>a</sup> Carmen Jaizme Vega (ICIA)

María Ángeles Llaría López (Cabildo de Tenerife, UO Biodiversidad)

Fernando Lobo Palacios (ULL)

Paula Cristina Machado Ferreira Castilho (UMA)

Miguel Angelo Pinheiro de Carvalho (UMA)

Carolina Pérez Reyes (ULL)

Nereida Rancel Rodríguez (ULL)

#### Scientific Committee

José Manuel Pérez de la Lastra (IPNA-CSIC)

M<sup>a</sup> Carmo Barreto (UAZ)

Raimundo Cabrera Pérez (ULL)

Gilbert Duarte Silva (INIDA)

Dácil Hernández Mesa (IPNA-CSIC)

Fabricio Lopes de Macedo (UMA)

Carla Ragonezi Lopes (UMA)

Ana Seca (UAZ)

Erik Sequeira (UCV)

Duarte Manuel da Silva Mendonça (UAZ)

Carla Susana Silva Gouveia (UMA)

Laurent Torregrossa (LIA, UMONT, Francia)

Lukáš Spíchal (U. Placky, Czech R.)

José E. Fernández Luque (IRNAS-CSIC)

Carlos Saavedra Fernández (ULL)

## Presential Programme

The on-Line sessions (see On-line Program tab) will hold extra short lectures and oral communications. Participants will be able to interact via the web.

### Monday, July 18<sup>th</sup>

- 8:00-9:00 Registration
- 9:00-9:30 Opening ceremony.
- 9:30-10:30 [Opening session: Sustainable Agriculture](#)  
**Opening (plenary) lecture**  
**Dr Alexander Schätz**, R&D Leader (Formulations) in Syngenta SA (Switzerland)  
 Sustainability in Agrochemical Product Design - beyond active ingredients
- 10:30-11:00 **MAC Projects Presentation Session**

### 11:00-11:30 Coffee Break

- 11:30-12:10 [Second session: Precision Agriculture](#)  
**Invited lecture**  
**Prof. José Enrique Fernández Luque**, IRNAS-CSIC (Sevilla, Spain)  
 Approaches to meet the coming challenges in irrigation
- 12:10-12:45 **Invited lecture**  
**Prof. José Francisco López Feliciano**, IUMA-ULPGC (Gran Canaria, Spain)  
 Technology carriers for Precision Agriculture: a practical view with pros and cons
- 12:45-13:20 **Invited lecture**  
**Dr. Nuria De Diego**, University Palacky, Olomuc (Czech Republic)  
 Accelerating stress tolerance studies using biological translation from model plants to crops

### 13:20-15:00 Lunch



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15:30-15:45

### Third session: Sustainable and Precision Agriculture

#### Round Table: Agro and Food Sustainability

Moderator: Gloria Lobo, Ponents: Alicia Boto (IPNA-CSIC), Miguel Angelo Carvalho (Univ. Madeira), Duarte Mendonça (Univ. Açores), Gilbert Duarte (INIDA, Cabo Verde), Lukas Spíchal (Univ. Placky, Czech Rep.)

15:45-16:20

#### Invited lecture

**Prof. Manuel Pérez Ruiz**, Universidad de Sevilla (Spain)

Engineering technology for crop disease identification and management

16:20-17:00

#### Invited lecture

**Dr. Marino Expósito**, Sainsbury Laboratory, Cambridge University, UK

Visualizing ROS metabolism at subcellular level under fluctuating light conditions

17:00-18:30

#### Coffee Break and Meetings with Agrocompanies

19:00-20:30

#### Welcome Cocktail at MUNA (Museo de la Naturaleza y Arqueología) Santa Cruz de Tenerife. Bus departure from La Laguna at 18:45

## Tuesday, July 19<sup>th</sup>

09:00-09:40

### 4th Session: Biostimulants as active compounds to face abiotic stresses

#### Invited lecture

**Prof. Pedro L. Rodríguez**, IBMP-CSIC-UPV (Valencia, Spain)

Biotechnological approaches to increase drought tolerance based on abscisic acid receptor

09:40-10:20

#### Invited lecture

**Prof. Lukas Spíchal**, Univ Palacky (Olomuc, Czech Republic)

Plant phenotyping approaches in biostimulant research and development

10:20-11:00

#### Invited lecture

**Prof. Jorge M.L. Marques da Silva**, Universidade de Lisboa (Portugal)

Assessing plant vitality with optical techniques

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## Sustainable and Precision Agriculture Symposium 2022

11:00-11:30 **Coffee Break**

11:30-13:20 **5th session: Food Safety**

**Round Table on Food Safety.** Each participant will make a 15-min oral introduction to the subject

Moderator: **Carmen Rubio Armendáriz** (Research Group in Food and Environmental Toxicology, Universidad de La Laguna-ULL, Spain)

Participants:

**Isabel Peña-Rey** Executive Director of Agencia Española de Seguridad Alimentaria y Nutrición (AESAN)

**José Asterio Guerra García**, Responsable Área Agricultura GMR Canarias SAU

**Antonio V Herrera Herrera**, Department of Chemistry, Faculty of Sciences, ULL

**Ricardo Díaz Díaz**, Head of the Department of Environmental Analysis, Research and Technological Development Section, Instituto Tecnológico de Canarias

13:20-15:00 **Lunch**

15:30-15:45 **6th session: Agroecology, a tool for the future**

**Invited lecture**

**Dr. Jana Alonso and Dr. Mercedes Hernández** IPNA-CSIC / Laboratorio de Agrobiología-Cabildo de La Palma (Canary Islands, Spain)

How agroecological crops managing results in soil regeneration: Will be possible in the presence of volcanic ashes?

15:45-16:00 **Invited short lecture**

**Angie Marcela Pedraza Torres**, Instituto de Ciencias Ambientales, Universidad de Castilla-la Mancha (Toledo, Spain)

Biochar-earthworm interaction in the improvement of soil quality and potential use in bioremediation

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16:00-16:20

### 6th session: Agroecology, a tool for the future (cont.)

#### Invited short lecture

**María José Grajal Martín**, Instituto Canario de Investigaciones Agrarias (Tenerife, Spain)

Phylogenetic resources as a cornerstone of sustainable tropical fruit growing in the Macaronesia; the FRUTTMAC Project

16:20-16:40

#### Invited short lecture

**Mercedes Alonso and Albert Mora**, Dirección General de Agricultura-Gobierno de Canarias (Canary Islands, Spain)

Predatory insects as weapons to fight pests

16:40-17:00

#### Oral and Flash Communications

17:00-18:30

#### Coffe Break and Meetings with Agrocompanies

19:00-22:00

#### Gala Dinner at Casino de Tenerife

Santa Cruz de Tenerife. Bus departure from La Laguna at 18:45

## Wednesday, July 20<sup>th</sup>

09:00-09:40

### 7th Session: Biopesticides

#### Invited lecture

**Prof. Paula Cristina Castilho**, Universidad da Madeira (Portugal)

Waste from food industry as source of (bio)pesticidal substances

09:40-10:20

#### Invited lecture

**Fernando Pinacho Crisóstomo**, Universidad de la Laguna, Founder of Ecoberture (Tenerife, Spain)

New remote device for early detection and population monitoring of lepidopteran specie

10:20-10:35

#### Invited Short Lecture

**M<sup>a</sup> Carmo Barreto**, Universidade das Açores (Portugal)

From invasive plant to biopesticide: *Hedychium gardnerianum* against *Ceratitis capitata*



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10:35-10:50 **7th Session: [Biopesticides \(cont\)](#)**

### Invited Short Lecture

**Dr. Carolina Perez Reyes**, Universidad de La Laguna (Tenerife, Spain)

New insight into Canarian's Flora as a new source of biopesticides

10:50-11:00 **Flash Communications**

**Samuel Rodriguez-Sabina**, Universidad de La Laguna (Tenerife, Spain)

*Argyranthemum frutescens*, an endemic canarian plant as a promising biopesticide

**Nereida M. Rancel**, Universidad de La Laguna (Tenerife, Spain)

Subaerophytic Canarian microalgae: a promising source for new biopesticides

11:00-11:30 **Coffee Break**

11:30-12:00 **[Closing Session and Awards](#)**

### Short Presentation of E-Posters selected for Awards

12:00-13:00 **Closing (Plenary) Lecture**

**Prof. Laurent Torregrosa**, L'Institut Agro, France

The Microvine, a dwarf plant model providing innovative experimental insights for studies in grapevine physiology and genetics

13:00-13:30 **AWARDS and Symposium Closure**



# Abstract book

Texts of abstracts have not undergone any linguistic correction.  
Talks are presented by time order.

# Plenary and Invited Lectures

Talks are presented by time order.



**Dr Alexander Schätz**  
 Syngenta SA (Switzerland),  
<https://www.syngenta.com/en>

Born and raised in Southern Germany, Bavaria. PhD in Organic Chemistry at University of Regensburg and Technical University of Munich (Germany), with an emphasis on enantioselective catalysis. Postdoc and lecturer at ETH Zurich (Switzerland) with a focus on nanosystems for catalysis. Then he joined Syngenta’s R&D in Switzerland, where he is currently Group Leader Formulation Design (Fungicides & Biologicals).

## Sustainability in Agrochemical Product Design – beyond active ingredients

Syngenta’s Good Growth Plan supports the United Nations’ Sustainable Development Goals (SDGs). It directly contributes to SDG2, Zero hunger, and significantly supports many of the other goals. Sustainability is an integral part from developing innovative products that help farmers grow more from less to controlling the impact of our operations. During this lecture, concepts for sustainable products are explored with examples from R&D, Formulation development, Packaging, Application and supply chain.



**Prof. José Enrique Fernández Luque**  
IRNAE CSIC Sevilla (Spain)

José Enrique Fernández got his Ph.D. in Agronomy in 1989. Latter he spent two years at Silsoe Research Institute (UK), and in 1993 he won a permanent position as Tenured Scientist in CSIC, joining the IRNAS center. Dr Fernández is specialized on plant water relationships of crops typical of arid and semi-arid areas. He has worked with both herbaceous and woody crops, particularly with olive trees. In the last years he has focused into the design of irrigation strategies for deficit irrigation, and in methods to schedule precise irrigation from plant-based measurements. He developed most of this work for olive orchards of different management and plant densities, including hedgerow orchards with high plant densities of super-high-density orchards. Currently, he is interested on applying these approaches for a sustainable intensive agriculture in which the grower profit is ensured, the biodiversity is enriched, the efficiency of the use of water in agriculture is enhanced and the socioeconomic aspects of the rural areas are improved. Dr. Fernández has contributed, through his collaborations with other research groups and private companies, to the development of several methods for the assessment of water stress and irrigation scheduling in fruit tree orchards, from automatic and continuous measurements related to sap flow, trunk diameter variations and leaf turgor pressure. His latest publications include aspects on irrigation economy.

In 2006 he founded the *Grupo de Riegos y Ecofisiología de cultivos* (Grupo REC), leading he group since then. From April 2015 to June 2018, Dr. Fernández worked as Coordinator of Agriculture for the Spanish Agency of Evaluation and Prospective (ANEP). Since 2013 is Editor in Chief of the scientific journal *Agricultural Water Management* (IF= 6,6). In 2012 and 2013 he was deputy director of the IRNAS and, since November 2014, he is the director of the institute. Dr. Fernández has supervised 14 research projects and has participated in another 14 projects. He has also supervised 8 contracts with private companies, and has participated in another 4. In addition, he has supervised 9 Ph.D. Thesis (two of them with an award on the best Thesis of the year on the topic), plus 17 “Trabajos Fin de Carrera”, TFM’s y TFGs. He has 78 SCI papers, 45 non SCI, 22 monographs and book chapters and 18 divulgative papers.

## Approaches to meet the coming challenges in irrigation

We address main responses from the scientific and technical communities to the challenges that irrigators meet for ensuring food security in a context of growing population and climatic threat. Basically, more food, fibre and biofuel must be produced at the same time than the environment is preserved and fair profits for the growers are guaranteed. We focus on precision irrigation as a way to achieve such purpose, and analyse different scientific and technological approaches contributing to the success of precision irrigation. In addition to giving an overview of state-of-the-art sensors and related systems, we describe current trends to improve both their performance and appeal to growers. We, actually, analyse the reasons behind the poor level of adoption of these technologies by growers, and how that situation can be reverted. Finally, we consider different types of agriculture and to what extent they may allow us to meet the identified challenges. More precisely, we compare industrial agriculture with integrated agriculture, and consider the so called sustainable intensive agriculture as a way to find a suitable balance between main requirements impose to irrigated agriculture



**Prof. José Francisco López Feliciano**

Institute for Applied Microelectronics

Univ. Las Plamas de Gran Canaria (Gran Canaria, Spain)

Prof José Francisco López has supervised or participated in more than 30 research and development projects, some of them for companies such as Vitesse Semiconductor Corporation (California), Intelligent Pixels Inc. (California), Ensilica (Great Britain) and Thales Alenia Space (Spain). He has participated in program committees of various international congresses, and specifically, in 2003 he created the series of biannual congresses called "SPIE International Symposium on Microtechnologies for the New Millennium", and in 2010 he organized the "XXV Conference on Design of Circuits and Integrated Systems (DCIS)" in Lanzarote. In May 2014 he organized the "SPIE Satellite Data Compression, Communications and Processing" in Baltimore, USA. Additionally, he has been a reviewer for Elsevier's "Microprocessors and Microsystems" magazine, and is a reviewer for the National Agency for Evaluation and Prospective (ANEP) since 2011. He has written more than 40 articles in international journals and more than 80 articles published in congresses, and has participated in the creation of 3 technology companies, one of them international.

**Technology carriers for Precision Agriculture: A practical view with pros and cons**

Precision agriculture has gained adepts in the last years due to its ability to detect crop’s health and predict the yield adapted to climate change. It is mainly based on the use of GPS, sensors (mainly multi- and hyperspectral) and the use of algorithms based on artificial intelligence to manage big amounts of data. As result, there is a more efficient use of resources in crop production and a reduced input use (water, fertilizer, seeds, herbicides, insecticides, etc) with a clear economic and environmental impact. But these sensors need to be incorporated into devices, or “carriers”, able to generate a different aerial perspective and facilitate the capturing of data. The goal is to give and “easy-to-read” information map extracted from large amounts of data.

In this talk the author will talk about these sensors and will introduce some of the technology “carriers” mainly categorized depending on their altitude, cost and performance. Pros and cons will be discussed and new artifacts will be presented guiding the audience to a visionary scenario in which technology and agriculture will “fly” together.





**Dr. Nuria De Diego**  
University of Palacky  
Olomuc, Czech Republic

Nuria De Diego Sanchez performed her Ph.D. studies on plant physiology at Neiker and Basque Country University in 2012. She moved to Palacky University in 2013, joining the Department of Chemical Biology and Genetics as a Junior Researcher to study plant response to stress from the physiological and metabolic points of view. In 2015, she collaborated with the same department’s phenotyping group and developed different screening approaches for studying stress responses in different plant species. In 2021, Nuria De Diego moved to the Czech Advanced Technology and Research Institute, also at Palacky University, and became the Chief Scientist of the phenotyping group. She published more than 45 publications in journals with impact factors and has an h-index of 19.

### Accelerating stress tolerance studies using biological translation from model plants to crops

Plants are sessile organisms exposed to several environmental stimuli, biotic and abiotic stresses. To deal with the different stress situations, plants have developed different mechanisms responses (molecular, metabolic, and physiological changes). However, these responses are not always powerful enough to ensure the plant’s survival and production. Much effort is thus invested into researching and developing technologies that may help plants cope with stressful situations, e.g., biostimulants. Testing biostimulant effects in field conditions are highly relevant and unreplaceable; however, some of the claims, such as abiotic stress tolerance, can hardly be justified in the same field trial. In this regard, modern plant phenotyping using non-invasive digital technologies can speed up the development and characterization of new biostimulants using high-throughput and high-precision approaches in controlled and semi-controlled conditions.

Herein is an example of how a phenotype-based high-throughput screening approach using the model plant *Arabidopsis* was used, before an open field study of biostimulants as alternatives to improve plant production and nutrient quality in *Vitis vinifera* was carried out.

Another remarkable example is the translation of the outcomes from biostimulant testing in an indoor non-invasive large-scale bioassay using crops at very early developmental stages to maize grown in lean field conditions to the production phase under water restriction.



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Finally, preliminary results of our high-throughput screening method for selecting compounds that improve plant resistance to biotic stress based on the *Arabidopsis-Pseudomas* pathosystem will be shown. The mentioned examples should demonstrate that our indoor screening methods using model plants have the potential to accelerate the selection of compounds and/or genotypes that can then be successfully validated for their efficiency in increasing crop resilience in field conditions.



**Prof. Manuel Pérez Ruiz**  
University of Sevilla  
Sevilla, Spain

Prof. Manuel Pérez began his research activity in 2001 in the framework of Precision Agriculture activities as part of the research group led by Dr. Josse De Baerdemaeker (KULeuven-Dept. of Biosystems) in Belgium. For more than 20 years, he has been working continuously on topics related to sensors and instrumentation for agricultural machinery, GNSS, variable application techniques, analysis of spectral and thermal information, and intelligent systems for the control of weeds. He maintains a close collaboration with the Univ. of California, Davis (USA), where he carried out six stays with a total duration of two years between 2008 and 2014. Here, together with Dr. D. Slaughter, he continues training and participating in various research projects and publications. Also, in a European context, he has extended effective collaborations with stays at the Univ. of Florence (Italy-2015) and the Univ. of Ghent (Belgium-2019). He is currently in charge of the “Smart Biosystems Laboratory” research group composed of 15 members.

He has participated in 20 research projects (3 European, 11 national, and 6 regionals), in four of which he was the principal investigator. He has been a researcher in the European project, “Robot Fleet for High and Effective Agriculture and Forestry Management” with a budget of 6.5 M€. At the national level, Prof. Pérez has been the principal investigator of the projects AGL2013-46343-R and AGL2016-78964-R. While at the regional level, he has been the principal investigator of the projects of excellence P12-AGR-1227 and US-1263678, both focused on precision agriculture and phenotyping techniques.

Among his research results can be found more than 90 papers, whereas 42 are articles indexed in the JCR, Science Edition (75% in Q1; and two of them were recognized by the “EurAgEng Outstanding Paper Awards” in 2014 and 2016) more than 70 conference papers and delivered ten lectures where I was invited as a special guest. Other indicators of the quality of scientific productions include an h-index of 17, 2 positive research evaluations (2006-11; 2012-17), 1 positive transfer evaluation (2004-2014), 4 completed theses supervised, and 4 in progress.

## Engineering Technology for crop disease identification and management

In recent years there has been great progress in the use of remotely sensed data obtained from sensors mounted on terrestrial (tractor, robot, or rovers), aerial (unmanned aerial vehicles (UAVs), airplane flights or balloons), and satellite platforms for the early detection of crop diseases and thus carry out specific applications of pesticide products. Among the most promising technologies that provide high-resolution data are hyperspectral (higher number of wavebands) and multispectral sensors (a smaller number of wavebands). These sensors provide the radiation reflectance in the visible (VIS) and near-infrared (NIR) region for each pixel, generating three-dimensional datasets containing spatial and spectral information.

The platforms mentioned above have been well-studied by many research groups and their advantages and disadvantages are well-known. However, detection of biotic stresses at the early stages of incidence from high-resolution imagery is still challenging. Detection of biotic stresses with hyperspectral imaging has proven much more challenging than detection of abiotic stresses, as biotic stress symptoms may not be expressed at the canopy scale until the severity of infestation increases beyond threshold levels.

Currently, research on weed, insect, and disease detection is among the leading-edge applications. Much more effort is needed by the agricultural sector and technicians to develop commercially viable approaches for identification of insect infestation and diseases using spectral information from multispectral or hyperspectral crop sensing. Under field conditions, the main issues that must be addressed include accuracy improvements to counteract the disturbing factors that influence the recorded optical information (background, illumination, resolution etc.), isolating stressors' effects when multiple stressors coexist, rapid diagnosis, transferability of algorithms and the development of user-friendly and cost-effective applications. To solve these issues, a multi-actor approach with agricultural engineers and computer scientists, who are well versed in machine learning algorithms and approaches, is the way forward, like in other precision agriculture applications.



**Dr. Marino Expósito**

Sainsbury Laboratory, University of Cambridge

United Kingdom

Dr Exposito-Rodriguez is Research Associate at Saninsbury Laboaratory-University of Cambridge. His research is focused on visualising reactive oxygen species (ROS) metabolism at cellular and subcellular level in vivo and at real time. Using genetically encoded biosensors, Dr Exposito-Rodriguez proposed a model where the photosynthetic hydrogen peroxide-dependent retrograde signalling is transferred from chloroplasts to nuclei. Currently, his aim is integrate both, ROS and ABA metabolism developing of plant cell atlas under fluctuating environments. His has published a number of high impact papers on biosensor engineer and ROS metabolism.

## Visualizing ROS metabolism at subcellular level under fluctuating light conditions

Hydrogen peroxide ( $H_2O_2$ ) and lipid hydroperoxides (LOOH) are initiators and transducer of inter- and intra-cellular signaling in response to diverse environmental, pathological and developmental cues. The accumulation of both  $H_2O_2$  and LOOH is often coincident in tissues, but it is unknown if this happens in subcellular compartments. If it were to occur, then it would be a factor needing to be considered in determining peroxide signaling specificity. The fluorescent protein biosensor roGFP2-ORP1 provided a basis to address this issue. We show that recombinant roGFP2-ORP1 is not only oxidized by  $H_2O_2$  as often reported, but also by the fatty acid peroxides of lecithin-derived liposomes. We constructed a modified biosensor called roGFP2-synORP1, which has greatly diminished specificity towards LOOH and increased reactivity with lower  $H_2O_2$  concentrations than roGFP2-ORP1. These two roGFP2-based biosensors, targeted to chloroplasts, cytosol and the nucleus, were quantitatively imaged in parallel in *Nicotiana benthamiana* abaxial epidermal cells experiencing high light- and herbicide-induced photo-oxidative stress. From differential patterns of oxidation of these probes, it was inferred that the chloroplasts accumulated both peroxide types. In contrast, LOOH and  $H_2O_2$  accumulated exclusively in the cytosol and nucleus respectively, which would provide for spatially distinct peroxide signaling pathways.



**Prof. Pedro Rodríguez**  
IBMCP-CSIC-UPV (Valencia-Spain)

Prof. Rodríguez’s research focuses on the role played by receptors of the phytohormone abscisic acid (ABA), type 2C protein phosphatases (PP2Cs), and SnRK2-type kinases as regulators of ABA signaling, with particular emphasis on the biotechnological potential of the hormone to improve the tolerance of plants to drought and salinity, and thus favor their adaptation to climate change. His group has played a key role in discovering and characterizing the PYR/PYL/RCAR family of ABA receptors and their connection with PP2Cs and SnRK2s. He has led pioneering works on ABA signaling and its contribution to the plant response to environmental stress. His group has contributed several genetic strategies that enhance ABA signaling as a valuable tool for improving plant water use efficiency. Among them, are the constitutive inactivation of PP2Cs, the overexpression of ABA receptors, and the generation of synthetic versions of the receptors that enhance the effect of the hormone. His group has also played a pioneering role in studies on regulating the half-life of ABA receptors and PP2Cs.

## **Biotechnological approaches to increase drought tolerance based on abscisic acid receptors**

Fresh water availability is compromised by the effect of global warming and climate change. As agriculture represents about 70% of total freshwater consumption, enhanced water use efficiency of crops is required to optimize yield. Major water loss occurs through transpiration, which is regulated by the phytohormone ABA. The interaction of ABA with the family of pyrabactin resistance 1 (PYR1)/PYR1-like (PYL)/regulatory components of ABA receptors (RCAR) ABA receptors activates the signaling pathway that controls stomatal closure and is also required for root hydrotropism. Thus, strategies to activate PYR/PYL/RCARs and enhance ABA signaling are promising biotechnological tools to regulate transpiration and foster water foraging by roots. Through structure-based targeted design, we have combined chemical and genetic approaches to generate a new sulfonamide-based ABA agonist molecule (iSB09) and engineer a PYL1 ABA receptor, named PYL1<sup>5m</sup>, which efficiently binds iSB09. Spraying of iSB09 over Arabidopsis plants overexpressing PYL1<sup>5m</sup> leads to activation of ABA signaling and marked drought tolerance, including a strong antitranspirant effect. Additionally, genome-wide transcriptional analysis reveals a powerful induction of ABA response in PYL1<sup>5m</sup> plants



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by iSB09. No constitutive activation of ABA signaling and growth penalty was found in transformed plants, given that PYL15m displays lower ABA affinity than its wild-type version. Therefore, conditional and efficient activation of ABA signaling was achieved through a chemical-genetic approach based on iterative cycles for ligand and receptor optimization driven by the structure of ternary receptor-ligand-phosphatase complexes.





**Prof. Lukas Spíchal**  
University of Palacky  
Olomuc, Czech Republic

Prof. Spíchal obtained his MSc. in Biology and Chemistry at the Palacký University in Olomouc in 2002, followed by his Ph.D. in Biology in 2005. He spent several research stays at Free University, Berlin, Germany (2002-2012) for a total of 18 months. After his Ph.D. studies, he worked at Laboratory of Growth regulators, Faculty of Science UP Olomouc and Institute of Experimental Botany AS CR (2005-2019).

From 2010 he worked as a senior researcher at the Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University. From 2021 on he serves as leader of the research group “Phenotyping” in the Czech Advanced Technology and Research Institute (CATRIN).

His scientific interest covers chemical biology of plant hormones, development of agrochemicals and technologies for plant growth regulation, high-throughput bioassaying and automated plant phenotyping (WoS: 88 publications, > 2400 citations, h-index 28, > 30 granted patents). In 2012 he was awarded by prize “The best R&D team competition” at international Bioforum, Brno, Czech Republic.

Lukáš Spíchal has been the main applicant and co-applicant of 4 national grants for basic and applied research. In 2017 he founded the Czech Plant Phenotyping network (CzPPN), he is its coordinator and Czech representative in the Support Group of ESFRI project EMPHASIS. He is also a member of the Technical commission CEN/TC 455 “Plant Biostimulants” at European Standardization Committee (CEN).

In 2011 he co-founded and is CEO of spin-off company AgroBioChem, s.r.o., closely collaborating with Palacký University on research and development of new technologies and products for regulation of plant growth and development.



## **Plant phenotyping approaches in biostimulant research and development**

Commercial interest in biostimulants as a tool for sustainable green economics and agriculture concepts is on a steep rise, being followed by increasing demand to employ efficient scientific methods to develop new products and understand their mechanisms of action.

Biostimulants represent a highly diverse group of agents derived from various natural sources. A critical point nowadays is the evolution of the biostimulant-based products, so they are more focused on complex substances (i.e. seaweed extracts, humic and fulvic acids, animal- and plant-based protein hydrolysates, or formulations that includes microorganisms such as mycorrhizal fungi and rhizospheric bacteria) than on simple natural molecules (i.e. plant hormones or specific amino acids). Thus, the complexity of the new biostimulants due to their natural origin (i.e. seaweeds), the raw material (i.e. animal- and plant-based protein hydrolysates), and/or the preparation procedure needs an indepth study to understand not only their mode of action but also the stability of the batches and viability of the final products.

Plant phenotyping has been identified as a beneficial technology for simultaneously testing different batches, extraction processes, and final products, thanks to the high-throughput screening (HTS) approaches. Insight into the possible use of phenotyping approaches for HTS in biostimulant development and a view of how such approaches can be used to describe the effect of plant biostimulant application on traits of interest, pointing to a potential mechanism of action, will be presented.



**Prof. Jorge M.L. Marques da Silva**  
 Universidade de Lisboa  
 Portugal

Prof. Jorge Marques da Silva is a plant physiologist (Ph.D. in Biology – Physiology and Biochemistry) interested on the mechanisms of response to stress, chiefly on the effects of drought stress on the photosynthetic metabolism. As Associate Professor at the Faculty of Science, University of Lisbon, his main scientific interest is on understanding how the interactions of abiotic stresses with the photosynthetic metabolism impairs primary productivity. Currently working in high-throughput plant phenotyping, aiming to unravel environment-genetic interactions in phenotype development, with the ultimate goal of improving crop responses to climate change.

### Assessing plant vitality with optical techniques

The way radiation interacts with matter provides relevant information about its composition and structure. In the case of radiation interaction with living beings, it can also provide information about their physiological functionality. Humans have always obtained information about the composition and physiological functioning of plants by simple visual assessment, but the developments in physics in the field of spectroscopy and imaging have provided a set of optical techniques that have increased our capacity for analysis, either by making it quantitative or by extending it to wavelength range to which our eyes are not sensitive. This paper presents the non-invasive optical techniques used in plant biology, shows examples of their application to obtain structural and functional information in various contexts, including the assessment of biostimulants, and discusses the future of these techniques in the framework of high-throughput plant phenotyping and the new generation of Earth observation satellites.

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**Dr. Jana Alonso and Dr. Mercedes Hernández**

IPNA-CSIC / Laboratorio de Agrobiología de La Palma (Canary Islands, Spain)

Dr. Jana Alonso is Expert in proteomics and mass spectrometry (MS). She obtained her Biology degree and PhD in Molecular Biology and Biochemistry at the Autonomous University of Madrid. Her thesis in “Proteomics in *Drosophila melanogaster*” was supervised by Prof. Juan Fernández Santarén at the Molecular Biology Center “Severo Ochoa” (CSIC-UAM).

In 2008, Dr. Alonso was appointed head of the Proteomics Unit at the Health Research Institute of Santiago (IDIS), where she was involved in several research projects in basic and translational research. After five years, she joined Professor Daan van Aalten’s group (University of Dundee, UK), where she had the opportunity of applying her experience in MS to map the O-GlcNAc post-translational modification in proteins. In fact, she was able to decipher the “peptide sequon” of the O-GlcNAc transferase. In 2014, she joined the group of Dr. Alberto Baena-Lopez (University of Oxford), to explore the role of caspases in non-apoptotic situations. Applying CRISPR/Cas-9 and proteomics, she identified potential substrates and regulators of these “molecular scissors”.

Since 2018, Dr. Alonso is part of the Soil Fertility and Plant Nutrition group, being her goal to apply biochemistry, proteomics and mass spectrometry to the field of agrobiolgy. Currently her efforts are focused on the study of subtropical fruit crops from the physicochemical and proteomic points of view; and on the genetic typification and biochemical characterisation of the local species of hot and sweet pepper. She has recently passed the exams for promotion to Tenured Scientist in the Spanish Research Council.

Dr. Mercedes Hernández got her PhD in the University of La Laguna and carried out postdoctoral reserach in the Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (CSIC). On her return to IPNA-CSIC, she got research contracts with Cabildo de La Palma. She currently supervises the IPNA-CSIC Agrobiology Service that analyzes water, soils and foods for the administration and farmers.

## How agroecological crops managing results in soil regeneration: Will be possible in the presence of volcanic ashes?

Over recent years, the move to more sustainable agricultural forms has gained momentum. Agroecological management offers rewards in different areas: soil regeneration, favourable microbiology and plant physiology, improved crop yields and -perhaps most importantly- a reduction of the carbon and water footprints. Our research group is devoted to decipher the underlying mechanisms leading to these improvements of a particular biofertilizer that is currently being widely applied on the island of La Palma. This biofertilizer is based on a compost tea using animal farm by-products which reduce the use of external inputs. Empirically, this technique has shown a remarkable track record of success. Apart from its high returns, the attractiveness of this model is that it boosts the circular economy where the by-products are converted into a ready to use high quality biofertilizer. From the scientific point of view, our analysis demonstrates improved soil composition, an increase in population of beneficial microorganisms, greater crop performance, and resiliency to plagues and climate change. The recent volcano eruption happened in La Palma is challenging the well-established crops (such as banana, avocado, pitahaya, among others) to coexist with the presence of volcanic ashes. Will be able the agroecological model established in La Palma cope with it?



**Prof. Paula Cristina Castilho**  
 Universidade de Madeira  
 Portugal

Prof. Paula Cristina Castilho graduated in Chemistry by the Faculty of Sciences of Lisboa University, Portugal, and got a PhD in Chemistry from Durham University, UK.

Originally from Lisboa, in 1992 she was invited to build a Chemistry Department in the recently launched University of Madeira and spent a few years designing curricula for new degrees, hiring staff, buying lab equipment and designing laboratories. And teaching and trying to put up a decent research group.

Since Madeira has the largest remains of the original Laurisilva forest, it made sense to switch from Physical Organic Chemistry to Natural Products chemistry and the main goals of research have been centered on the chemical profiling and bioactivity evaluation of endemic species with traditional use in folk medicine.

Since 2019, the INTERREG project MACBIOPEST aiming at the production of biochemical pesticides from local plants is one of the main projects of the research group NatLab, of which Dr. Castilho is leader, and recently the focus shifted towards food residues as raw materials.

Paula Castilho is presently Head of the Chemistry department as well as Degree director the PhD program in Chemistry at Madeira University.

## Waste from food industry as source of (bio)pesticidal substances

Conventional pesticides used in crop protection may pose long-term threats and risks to living organism and are often perceived as having harmful effects on human health. Therefore, the development of greener and environmentally safe pesticides has been the aim of large research output for several years. To search for natural products based biopesticides, different sources can be used including plants, microorganisms, endophytes or agricultural and food industry wastes.

This talk will focus mainly on the later, in a circular economy perspective, and on molecular agents, often referred to as Biochemical pesticides: pesticides based on naturally occurring substances that control pests by non-toxic mechanisms, in contrast to those containing synthetic molecules that directly kill the pest. Biochemical pesticides fall into different biologically



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functional classes, including pheromones and other semiochemicals, plant extracts, and natural insect growth regulators. They are often described as easily biodegradable, with fewer residues, low toxicity to mammalian and non-target organisms. In addition, it is difficult to develop pesticide resistance since they contain a mixture of many substances, the effects of which can be attributed to different activities on pests, included influence on behavioral and physiological processes.

Aspects such as types of biopesticides, pest targets, classes of bioactive substances and mechanisms of action will be discussed.



**Dr. Fernando Pinacho Crisóstomo**  
ULL and Founder Ecobertura  
Canary Islands, Spain

Dr. Fernando Pinacho Crisóstomo is Doctor in Chemistry by the University of La Laguna. After his PhD, he and his wife founded the Company Ecobertura to develop insect pheromones; the Company has evolved and expanded its product offer. Dr. Pinacho currently enjoys an Agustin de Betancourt research and transfer contract in the University of La Laguna.

### **New remote device for early detection and population monitoring of lepidopteran species**

There is an estimation of approximately 160.000 insect species belonging to lepidopteran order. Some species can cause important economic losses in several types of crops, to mention few of them, the codling moth (*Cydia pomonella*) and diamond back moth (*Plutella xylostella*) cause significant damages at worldwide level on apple orchards and cruciferous vegetables respectively. Recently, the fall armyworm (FAW-*Spodoptera frugiperda*) has been detected in the African continent and according to an article of CABI, in Kenia the FAW can cause annual production losses between US\$159-177 millions. In the integrated pest management approach, pheromones traps are useful tools to detect and quantify the presence of a specific pest and help the decision make about a potential crop treatment. However, the conventional delta traps loaded with sticky papers demand constant maintenance which consume time and human resources. Although new devices based on IoT (internet of things) have been developed to address this problem, the maintenance is still an important issue. In this lecture a new device is presented, designed to improve the monitoring and maintenance time.



**Prof. Laurent Torregrosa**  
L' Institute Agro  
Montpellier, France

Prof. Laurent Torregrosa is professor of plant biology & genetics at L' Institute Agro in Montpellier (France). He started his researches about the genetic engineering of the grapevine resistance to grapevine chrome mosaic virus & powdery mildew in 1990. From 2000 to 2010, Dr. Torregrosa studied the genetic regulation of grapevine reproductive development (inflorescence to fruit) by reverse genetics and functional genomics. Since 2011, he has been leading programs on the impact of temperature and water deficit on grapevine vegetative and reproductive developments to identify traits of adaptation to heat and drought.

He has published more than 250 papers, books and communications, and is member of the department of Biology & Ecology of Institut Agro Montpellier, the Steering Committee of the International Grape Genome Program (IGGP), Vice-president of the International Viticulture & Enology Society (IVES), President of GIESCO ([www.giesco.org](http://www.giesco.org)) and expert of the International Office of Vine and Wine (OIV).

Prof. Torregrosa is currently director of the Institute of Higher Education in Vine and Wine Sciences (300 students) which offers a range of training programs from BSc to MSc in Viticulture-Enology, performed either in French or English, including the French degree of Enologist.

## **The Microvine, a dwarf plant model providing innovative experimental insights for studies in grapevine physiology and genetics**

Like most other perennial crops, the grapevine needs to undergo a juvenile period before fruiting. Thus, the development of reproductive organs from seedlings is only possible after the second or third vegetative cycle. Each proleptic axis then displays only one to three inflorescences per growing cycle. These biological features and the size of the adult vine are major hindrances to the design of experiments on fruit and plant physiology, and complicate and lengthen the time required for studies in grapevine breeding and genetics. The microvine is a dwarf phenotype resulting from a mutation in the VviGAI1 gene, which induces the miniaturization of all vegetative organs and conversion of tendrils into inflorescences without affecting berry development.





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The small size of the microvine allows tight control of environmental conditions. Spatial developmental gradients fit well with temporal series of each phytomer position. Thus, kinetic profiles can be inferred from spatial information. In the first part of the lecture, the molecular and genetic mechanisms determining microvine phenotypes will be described, reviewing the main biological properties of the microvine model. Subsequently, the results of recent studies in which the model was used for research in grapevine physiology and genetics will be summarized. The review focuses on experiments investigating the effects of temperature on vegetative and reproductive organogenesis, berry development, and biomass allocation at the whole-plant level. Furthermore, we discuss and illustrate how the model can be used to identify (QTL) quantitative trait loci in fruit development and adaptive traits that could be useful when selecting genotypes in anticipation of the effects of global warming.



## Sustainable and Precision Agriculture Symposium 2022

# Invited Short Lectures

Talks are presented by time order in presential sessions, then on-line sessions



## **An opportunity for sustainable agriculture**

José Asterio Guerra García, *GMR Canarias SAU*

Gestión del Medio Rural de Canarias (hereinafter GMR Canarias SAU), as a resource of the Government of the Canary Islands, carries out different technical assistance work commissioned by the Department of Agriculture, Livestock and Fisheries. Likewise, it participates in different cooperation projects co-funded by the European Regional Development Funds.

In one way or another, the projects carried out by the projects department of GMR Canarias SAU, impact on the foodsafety of consumers. They notably include the checks carried out within the National Programme for Official Control of Hygiene of primary agricultural production and of the use of phytosanitary products; control work, monitoring and tracking of different pests in the plant health sphere in the Canaries; other Cooperation projects within the sphere of Macaronesia, aimed at research into the benefits and risks of the consumption of plant products for the health of consumers and the development of minimisation strategies (PERVEMAC2 project) or the research, identification, risk analysis, training and awareness-raising about potential quarantine pests and non-quarantine regulated pests in the main crops of the Macaronesian regions (CUARENTAGRI project).

Lastly, our sales department's actions notably include selling a wide range of farming and agri-food products of small regional producers, offering continuity in supply, the guarantee of product origins, quality and food security.

### **Brief Biography**

José Asterio Guerra García holds a Doctorate in Biological Sciences from the University of La Laguna (2009) and a Masters in Geographic Information Systems from the University of Girona (2007). His research work has mainly focused on recognising the potential and soil degradation processes in the Canary Islands. He has published several articles in journals of international importance, chapters in books and made several contributions to conferences, both nationally and internationally. From September 2011 until the present, he has been head of the Agriculture Department of the public company GMR Canarias, S.A.U.

## Assessment of pesticides residues in food products from the Macaronesian region

Antonio V. Herrera Herrera, *Universidad de La Laguna*

The external dependence of the agri-food industry of the Macaronesia, as an ultraperipheral region, makes this area particularly vulnerable. Thus, contamination of foodstuffs from products on the international market can entail significant dangers for the population of this region. Among the diverse contaminants frequently found in food, pesticides are among the most harmful because of their negative effects on health. However, world agricultural production could not be maintained at the required levels without the intensive use of these phytosanitary compounds to prevent, mitigate or eliminate pests during harvest and food storage. In this regard, national and international organizations have set themselves the goal of controlling and monitoring these high-risk substances in order to protect the health of consumers and the quality of agricultural products. In this regard, cereals (due to their high nutritional value) and wine (due to their high quality in Macaronesia) have been evaluated within the framework of PERVEMAC II.

In this work, 676 samples (243 wines and 433 cereals) from the Macaronesian region (Canary Islands and Cape Verde) collected from 2017 to 2020 were analyzed using both ultra-high performance liquid chromatography (UHPLC) and gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS). For this purpose, a QuEChERS extraction protocol validated according to SANTE European Guidelines [2] was used. Residues of the selected pesticides were frequently detected in red, rosé and white wines, maize, wheat, rice, and barley. A non-accumulative risk assessment following the margin of safety approach [3] was carried out. In general terms, without considering the cumulative effect, the consumption of these commodities does not entail a severe health risk.

[1] R. Romero-González, Food safety: how analytical chemists ensure it, *Anal. Methods* 7 (2015) 7193-7201.

[2] Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Document No SANTE/12682/2019.

[3] FAO and WHO, A Risk-Based Decision Tree Approach for the Safety Evaluation of Residues of Veterinary Drugs, 2009.

### Brief Biography

Antonio V. Herrera-Herrera (Ph.D.) is a current researcher at the Instituto de Bio-Organica Antonio González of the University of La Laguna (ULL) in Tenerife (Canary Islands, Spain) and temporary part-time lecturer at the Chemistry Department of the same University His research has been primarily oriented to the development of new methodologies for the extraction and preconcentration of organic compounds in environmental and food samples by chromatographic and electromigration techniques.

## Phylogenetic resources as a cornerstone of sustainable tropical fruit growing in the Macaronesia. FRUTTMAC Project

María José Grajal-Martín, *Instituto Canario de Investigaciones Agrarias (ICIA)*

The phylogenetic resources represent the main source of variability for the development of materials of the species of interest. Materials with good adaptive characteristics to the increasingly changing environmental pressures are needed. The project Transfer of R+D+i for the sustainable development of tropical fruit trees in the Macaronesian región –FRUTTMAC- offers the possibility of knowing and sharing the information of the different tropical and subtropical fruit germplasm collections that are present in the Macaronesian region. The compiled information will be of public Access through the database RERFRUTTMAC.

In this way, and with the umbrella and financing of the European project -FRUTTMAC- an important effort is being made in the collection and harmonization of information of tropical and subtropical fruits by the responsible agents of the different participating countries. The description of each accession is being made, when available, using internationally recognized descriptors and, also characteristics of agronomic nature that have been considered to be of interest in the different species. The ultimate goal being a greater development of tropical and subtropical fruit growing in Macaronesia.

### Brief Biography

Dr. María José Grajal Martín, (Tenerife, Spain) obtained her PhD in Agronomy (Plant Breeding) in Washington State University in 1992. She is specialist in Plant breeding and Germplasm Resources in the Canarian Institute of Agricultural Research –ICIA- in the Department of Plant Production in Tropical and Subtropical Areas. Her work focuses, mainly, on plant breeding, selection and evaluation of different tropical fruit crops.

She is in charge of the morphological and molecular characterization as well as the agronomic evaluation of the banana and mango national germplasm collections., and has conducted the tests of distinctiveness, uniformity and stability (DUS) in banana and pineapple for the European Community.

In addition, she leads the ICIA breeding programs for mango and more recently, passion fruit. Some new mango cultivars with different characteristics of interest will be released shortly.

## Biochar-earthworm interaction in the improvement of soil quality and potential use in bioremediation

Angie Marcela Pedraza Torres, *Universidad de Castilla-La Mancha*

Agricultural soil fertility is under permanent threat of degradation. Erosion, decline of organic matter, salinization, or chemical contamination are major threats for soil quality. All these degrading processes seem to be exacerbated in the Mediterranean countries, where models predict a higher incidence of those environmental processes derived from the climate change such as desertification. Moreover, some types of agriculture such as intensive horticulture demands high water consumption, organic matter, and nutrients. It is necessary to develop mitigation and adaptation strategies, for this we need to act at two functionally linked levels: directly on the below-ground system (improving soil quality and nutrient burden) and, indirectly, on the above-ground system stimulating plant growth and promoting plant-pollinator interaction. The biodynamic platform is a good strategy, consist in the joint application of biochar and earthworms, this procedure takes advantages of the biological processes naturally occurring in soil (in situ) and biochar (the platform), retaining and stabilizing soil extracellular enzymes that are produced by microorganisms. Earthworms have the role of microbial-stimulating vectors facilitating the retention of extracellular enzymes on the surface of biochar (enzymatic bioactivation) and will disperse this activated biochar in the bulk soil (dynamic). Results of various investigations, including studies by our group, suggest that the joint inoculation of biochar and earthworms increases the detoxification capacity of agricultural soils, contributing to the resilience of soils against chemical stress factors such as insecticides, which make this platform in an active bioremediation strategy.

### Brief Biography

Born in Colombia, Environmental Engineer from the Central University of Colombia with a laureate degree for research in water bioremediation. Master's degree in environmental sustainability in local and territorial development, specialist in environmental quality from the University of Castilla-La Mancha in Spain. 5 years of related work experience in the private sector regarding corporate environmental management, additionally, former official of the Colombian agricultural institute in animal analysis and diagnosis and 3 years in the research field. Currently, PhD student in agricultural and environmental sciences in topics of ecotoxicology, soil pollution, soil enzymes, biochar, earthworms, biomarkers, and bioremediation.

## From invasive plant to biopesticide: *Hedychium gardnerianum* against *Ceratitits capitata*

Maria do Carmo Barreto, *Universidade das Açores*

*Hedychium gardnerianum* is a Zinziberaceae from the Himalayas which was introduced in the Azores as an ornamental plant, and which has escaped from gardens, spread across most of the islands and is now one of the worst invasive alien species [1]. This plant extremely resistant to predation, which was taken as a good indicator of its potential as a source of bioactive compounds [2], namely as a biopesticide source.

Taking this into account, ethanol extracts from this plant and fractions thereof (hexane, ethyl acetate and water) were tested against *Ceratitits capitata*, also known as Mediterranean fruit fly, which is responsible for significant economic losses in fruit production. Mortality and oviposition assays were carried out using a method adapted from Furtado et al [3], and it was found that both the ethanol extract and its fractions were effective when compared with the control, although at different levels. The water fraction was the most effective, causing 57.7% mortality at 72h, compared to 4.2% of the solvent-treated control, and it reduced oviposition to approximately 1/5<sup>th</sup> when compared to the same control.

Considering the excellent results obtained, *H. gardnerianum* aqueous extracts were prepared and tested against *C. capitata* using the same methodology, to propose a simple preparation which may be carried out directly by farmers. The aqueous extracts also had an interesting effect on both parameters assayed, causing 47.0% mortality at 72h compared with 17.9% by the corresponding solvent control, whereas oviposition was reduced from 2.39 eggs/ female in the solvent control to 1.08 in the females treated with *H. gardnerianum* aqueous extract.

These results corroborate *H. gardnerianum* as a good candidate to be used in simple aqueous preparations by farmers in the protection of fruits and justify the ongoing studies on the identification of the compounds responsible for the activities reported herein.

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[2] Tavares W.R., Barreto, M.C., Seca, A.M.L. 2020. Uncharted Source of Medicinal Products: The Case of the *Hedychium* Genus. *Medicines*, 7, 23, doi:10.3390/medicines7050023.

[3] Furtado, R., Baptista, J., Lima, E., Paiva, L., Barroso, J.G., Rosa, J.S., Oliveira, L. 2014. Chemical composition and biological activities of *Laurus* essential oils from different Macaronesian Islands. *Biochemical Systematics and Ecology* 55, 333e341, doi: 10.1016/j.bse.2014.04.004



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### Brief Biography

Maria do Carmo Barreto, PhD in Biochemistry, is auxiliary professor at the Faculty of Sciences and Technology, University of the Azores, and researcher at cE3c- Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute. Her main research interests are the biological activities of terrestrial and marine natural products, in search of pharmacological, cosmeceutical and biotechnological applications. She is the author of international peer-reviewed papers, book chapters, and patents. She teaches in PhD, MSc and BSc courses, supervises PhD and MSc students.





## New insight into Canarian's Flora as a new source of biopesticides

Carolina Pérez Reyes, *Universidad de La Laguna*

The Canary archipelago has a surprising ecological diversity due to their special geographical and climatic conditions, which has risen as a rich and varied flora with a high percent of endemic species. Thus, these endemic plants could be potential sources of plant-derived biopesticides and a eco-friendly and sustainable alternative to synthetic pesticides. This lecture will be focus on the evaluation of the antifungal and insecticidal potential of ethanolic extracts from plant species from the Canary Islands. The tested plants were selected based on ethnobotanical studies, as well as scientific reports on their potential in pest control.

### Brief Biography

Carolina P. Reyes is currently a Profesor Ayudante Doctor of Biochemistry at Universidad de La Laguna. After completing her PhD. in natural product research in 2007, she earned a postdoctoral fellowship on enzymatic biosynthesis natural product studies in Michael Burkart's laboratory (UCSD, USA). In 2010 Carolina accepted a position as senior scientist at Universidad de Las Palmas de Gran Canaria where she has developed from the beginning the Molecular Biology for molecular DNA taxonomy and phylogeny study of microalgae cultures and Natural Products research. Currently she is involves in different national and international projects on natural products combining biology and chemistry knowledge and techniques.

## Assessing the expected climate impacts in Madeiran agriculture by monitoring a network of agrosystem case studies.

Miguel Angelo Pinheiro de Carvalho, *Universidade da Madeira (On-line Sessions-Agroecology)*

Two climate scenarios for the Madeira Region starting in 2050 and 2070, have been proposed by the Climate-Madeira Strategy (CMS). These anticipate an average of 1.4 to 3.7°C rise in temperature and a 30 to 40% decrease in precipitation, by 2070. Winter and summer events were used to stress out these extreme changes in temperature and precipitation. Agriculture is a key sector of the Madeiran economy, providing the support and provisional functions, and is important to its sustainability and food security. According to the Strategy, agriculture will suffer severe impacts of climate changes and need for further climatic adaptation. Although there is a forecast of the climate changes that will occur, we do not know how these affect the agrosystems functions, nor even exists a referential that allows us to monitor and understand the changes underway. So, the evaluation and monitoring of agrosystems are fundamental to expand our knowledge and answer the questions that arise and to identify the adaptation needs of local food production systems to face climate change.

The CASBio project has been configured to initiate a research program on the influence of climate on a network of agrosystems and their elements, and based on these data, it proposes a referential to start the regular monitoring of the impacts of climate change on local food production systems. Six agrosystems were displayed in different climatic and agroecological conditions, including a vineyard, banana plantation, temperate fruit orchards, horticulture farms, and a semi-arid agrosystem in Porto Santo Island. All of them were evaluated and monitored, including their climate and soil edaphic conditions. The soil microbiology, plant and insects' diversity, and cultures were also evaluated as agrosystem functioning and conditions' indicators. A total of 37 parameters were assessed, including 8 independent and 29 dependent variables, among them the agrosystems' annual and extreme events variation of temperature, precipitation, water field capacity, soil conditions, microbiological communities, plant and insect diversity, and crop production. The annual temperature and precipitation of the meteorological data series of 1961-1991 and 2010-2020 were analyzed and compared with the CMS summer and winter extreme events, to understand the trend of climate conditions that affect the studied agrosystems. The results of the agrosystems monitoring were used to develop an abstract standard agrosystem (ASA) used as a referential in the comparison of the agrosystem case studies and to determine a baseline for the evaluated variables. In addition, the ASA baseline parameters allow us to identify the case study variables drift that could be determined by climate and to estimate a trend of climate change impact on agrosystems function. During this talk, the ASA will be presented and used to analyze the actual condition of the *Quinta das Vinhas* case study. The ASA and agrosystems case study baselines are seen as a starting point or long-term monitoring and allows further evaluation of the influence of climate change on agrosystems functions and sustainability.

### Acknowledgements:

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### Brief Biography

Miguel A.A. Pinheiro de Carvalho, Associate Professor at Life Sciences Faculty and Coordinator of ISOPlexis, Centre for Sustainable Agriculture and Food Technology, University of Madeira, Portugal. ORCID: 0000-0002-5084-870X He obtained a M.Sc. in Biochemistry and a Ph.D. in Plant Biochemistry and Physiology from the Voronezh State University, Russia Federation, and Aggregation Degree in Biochemistry and Biotechnology form University of Madeira, Portugal.

During the last 28 years he has teaching the courses of biochemistry, enzymology, environmental biochemistry, economic botany and agrodiversity at the Life Sciences Faculty and School of Management and Technology. In the meantime, coordinate the ISOPlexis, Centre for sustainable Agriculture and Food Technology and lead a multidisciplinary research program in agrodiversity monitoring and agriculture sustainability, using local genetic and other bioresources to promote sustainability and resilience of agrosystems and food production systems. His research has been presented in a form of over 13 books, 148 papers and book chapters and 143 conference oral or panel communications.

## IgY technology for the control of plant fungal diseases

José Manuel Pérez de la Lastra, *Instituto de Productos Naturales y Agrobiología del CSIC (On-line Sessions-Precision Agriculture)*

About one-tenth of all described fungal species can cause disease in plants. A common feature of this process is the need to break through the plant cell wall, an important barrier against pathogen attack. To this end, fungi possess a series of secreted enzymes that depolymerize the major structural polysaccharide components of the plant cell wall, i.e., cellulose, hemicellulose, and pectin. These enzymes are particularly important for plant pathogenic fungi that do not have specialized penetration structures.

The future of conventional chemical fungicides is at risk, so alternatives for disease control must be explored. In the context of environmentally friendly integrated control, priority should be given to combining different alternative methods: physical, biological and chemical methods with low toxicity. Our hypothesis is that we can design specific IgYs antibodies designed with the help of in silico tools to inhibit enzymatic activity secreted by fungi and that these antibodies are a good candidate for the control of fungal plant diseases.

Through peptide immunization, we can target IgY antibodies to the catalytic sites of these enzymes, or to the sites of interaction of these enzymes with plant tissue, and thus prevent them from binding properly to the tissue or prevent them from catalyzing the reactions necessary to digest the plant tissue.

As part of our strategy to produce antibodies and direct them against the fungal experiments, we must first analyze the critical sites of the proteins. We will explain the inhibition of enzymes secreted by *Botrytis cinerea* as an example of this approach.

### Acknowledgements:

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### Brief Biography

Dr. Pérez de la Lastra is a senior scientist of the CSIC in the IPNA, Head of the biotechnology group of Macromolecules, of the Department of Agrobiología. Dr. Pérez de la Lastra is author of more than 80 SCI articles (H index of 28) and several book chapters, which make a total of



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more than 100 publications. In addition, he has filled two patents and six contracts of transfer of monoclonal antibody technology. He has directed a total of five doctoral theses and several TFGs and TFMs, some of them international under the Erasmus+ program.

Dr. Pérez de la Lastra raised several monoclonal antibodies against porcine leukocytes and platelets, some of them are currently being commercialized by Bio-Rad. He was also one of the founder of the first spin-off company of the University of Córdoba: BioVet-UCO. He has participated in several international scientific committees, ie. as chairman of two international veterinary immunology symposiums, and as Spanish delegate of other international events.

In addition to numerous articles published in high-impact journals, Dr. Pérez de la Lastra is the inventor of a patent based on the combination of linear and conformational epitopes commons to mosquito and ticks, and has participated in numerous national and international projects on this topic.

## Libraries of host-defense peptides in the search of new phytosanitaries with low induction of resistances

Alicia Boto, *Instituto de Productos Naturales y Agrobiología del CSIC, (On-line Sessions-Precision Agriculture)*

Host-defense peptides (HDP) have elicited much interest as antimicrobials against plant and animal pathogens because of their potency, selectivity, low toxicity, low environmental impact, and particularly, because of their low induction of antimicrobial resistance. These peptides have been produced by plants and animals during millions of years to fight pathogens, but only recently have been seriously considered for use in agriculture due to some problems, such as stability and production costs. However, this scenario changed with the discovery that truncated natural peptides or short synthetic derivatives could retain a high antimicrobial activity. Moreover, analogues with D-residues or unnatural aminoacids or cyclic structures were more stable to degradation and their potency was often increased.

Inspired by these results, our group has prepared libraries of short peptides from one or a few “parent peptides” using the selective modification of “customizable units” such as hydroxyproline, serine, threonine or glutamic acid. The preliminary results as phytosanitary candidates are commented in this talk.

**Acknowledgements:** This work was financed by project APOGEO (Cooperation Program INTERREG-MAC 2014–2020, with European Funds for Regional Development-FEDER) and project ProID2020010134 (Programa de Subvenciones a la Realización I+D “M. Carmen Betancourt y Molina, from ACIISI-Gobierno de Canarias with FEDER funds). We also acknowledge project (TRANSALUDAGRO) financed by Cabildo de Tenerife, Program TF INNOVA 2016-21 (with MEDI & FDCAN Funds).

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### Brief Biography

Dr. Alicia Boto is a Senior Scientist at the Spanish Research Council (CSIC) in IPNA-CSIC (Tenerife), and is currently Head of the Group “Synthesis of Drugs and Bioactive Compounds” and Head of Department (Chemistry of Bioactive Natural and Synthetic Products). She has also been Coordinator of Scientific Outreach (Nov. 2007-Dec. 2019) and Deputy Director of IPNA-CSIC (Apr. 2014-Sept. 2020). She has recently participated as an Expert in the preparation of CSIC White Report-Strategic Area 4 (*Challenges in Biomedicine and Health*) in two



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Challenges: Drug Resistance in Infectious Diseases and Nanomedicine and currently participates in the Nanomedicine Platform.

Her current lines explore the selective modification of peptides, the development of new antimicrobials and quorum sensing inhibitors in a One-Health approach, the synthesis of phytosanitarios and bioestimulantes, and the development of fluorescent drugs that have displayed important antitumor and laser emission activity (phototherapy). She has published more than eighty articles in international journals ( $Q1 > 85\%$ ), and has delivered plenary and invited lectures in national and international symposiums. She has been Chair of the Organizing Committee of the international symposium Peptide Meeting 2018 (march 2018), the Pharmaceutical Weeks ULL-CSIC 2019 and 2021, and has participated in the Scientific Committee of the Agriculture and Food Sustainability (2021).

Her publications include several Featured Articles, Q1 Magazine Covers or Frontispieces, and Most Read Articles. She has also delivered invited or plenary conferences on these topics in several national and international congresses. Part of the results have been transferred to pharmaceutical, chemical and agrotech companies, and several Research and Technology Support Agreements have been signed. In recent years, patents with international extensions have been filed (two in 2016/1017), of which 70% have been licensed to companies (the last one to 20 countries including USA, EU). Recently awarded a 'sexenio' (six-year positive evaluation) on research transfer (2020).





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# Oral Communications





## Greywater quality control for reuse in gardening using optical sensors

Lahoz, F.,<sup>1\*</sup> de Armas-Rillo, S.,<sup>1</sup> Hernández-Rodríguez, C.,<sup>1</sup> Gil-Rostra, J.,<sup>2</sup> Yubero, F.<sup>2</sup>  
<sup>1</sup>Departamento de Física, IUdEA, INM, Universidad de La Laguna, 38206 Santa Cruz de Tenerife, Spain;

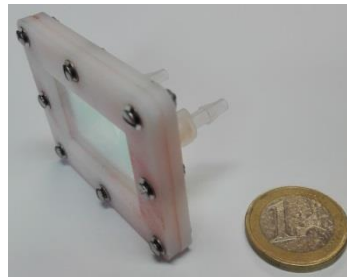
<sup>2</sup>Nanotechnology on Surfaces Laboratory. Instituto de Ciencia de Materiales de Sevilla (CSIC-Univ. Sevilla). Sevilla. Spain;

\*Corresponding author: [flahoz@ull.edu.es](mailto:flahoz@ull.edu.es)

Large amounts of waste water are produced in households or buildings. Most of this greywater comes from showers, baths, washing machines or dishwashers. Arguably, the most frequently found contaminants of greywater are detergents. They are related to household cleaning products, body health-care or cloth washers. Normally, greywaters are drained to the drainpipes although the contamination level may vary enormously in different houses or buildings or even in different water uses inside a building.

Several investigations have reported that greywater with a relatively low detergent contamination level (< 1 mg/ml) can be safely used for plant irrigation. Therefore, if the quality of the greywater produced in a house could be determined, the reuse of this greywater for gardening could be envisaged.

In this communication we report on our results on an interferometric optical sensor that can be used to control the detergent concentration of greywater from a washing machine. The optical sensor is based on a Fabry-Perot microresonator. The optical resonances of the transmission modes depend on the refractive index of the aqueous medium, which is influenced by the content of detergent.



**Figure 1.** Image of a typical FP microresonator used to monitor the detergent content of greywater from a washing machine.

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## Integration of multispectral camera on low-cost UAVs for precision agriculture

Hernández, E.,<sup>1</sup> Rodríguez, A.,<sup>1\*</sup> Pérez-García, A.,<sup>1\*\*</sup> López, J.F.<sup>1</sup>

<sup>1</sup>Institute for Applied Microelectronics, University of Las Palmas de Gran Canaria, Spain

Corresponding author: \* poster: armolina@iuma.ulpgc.es, \*\* oral presentation: apgarcia@iuma.ulpgc.es

In the last decades, the integration of multi and hyperspectral cameras with unmanned aerial vehicles (UAVs) has resulted in a tool that allows acquiring relevant information for many different fields of study with an unprecedented flexibility. For agriculture, the use of these devices is an advantage in terms of saving time and resources, as well as being beneficial for sustainability and environmental preservation. Spectral technology is crucial in the new revolution called precision agriculture, providing information related to the internal structure of the leaves which is highly related to its reflection of light at different wavelengths. The use of UAVs equipped with the appropriate sensors enables periodic monitoring of crops during cultivation, preventing diseases, pests and other plant needs.

In this work, framed in the Interreg-MAC project called APOGEO, the development of a low-cost UAV that can incorporate different sensors, such as inertial measurement unit (IMU) or multispectral cameras, is presented. A low-cost multispectral camera is also being developed that can be incorporated into the UAV for precision agriculture studies. In addition, an embedded board with advanced processing capabilities has been mounted on the UAV to control its trajectory, manage data acquisition, and enable on-board processing such as the evaluation of different spectral vegetation indices. The system has been experimentally validated using a commercial multispectral camera to calculate vegetation indices in vineyard areas. Specifically, the Normalized Difference Vegetation Index (NDVI) results facilitate the differentiation of canopy cover from other types of surfaces and indicate the general state of health of the vegetation.

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## Nano-enabled strategies to enhance plant tolerance to climate stress

Guo, Z.,<sup>1</sup> Zhang, P.,<sup>1\*</sup> Lynch, I<sup>1</sup>

<sup>1</sup>School of Geography, Earth and Environmental Sciences, University of Birmingham, UK

Corresponding author: p.zhang.1@bham.ac.uk

Plants in their physical environment face various types of abiotic stresses such as high salinity, drought, saturation, high temperature, cold, heavy metals, and biotic stress such as pest and pathogens. The abiotic stresses characterize the main cause of crop fiasco globally, contributing to more than 50% of the average loss for significant crops. Developing tolerant cultivars using genetic manipulation is the most important strategy currently to cope with these stresses; however, the overall progress is slow and complete success is not yet achieved due to the difficulty in identifying key genetic determinants of stress tolerance. A complementary approach is the use of nanotechnology to promote plant growth by mitigating stress-induced signalling pathways, and thus increase crop yields, although its full potential has not yet been discovered and the underlying mechanisms by which NMs enhance seed germination, provide resistance to pathogens and more are not yet fully understood which hampers its progress and widespread adoption. Recently, we used nano-CeO<sub>2</sub> and nano-MoS<sub>2</sub> to protect plants against various stress conditions, taking advantage of their capacity to scavenge reactive oxygen species (ROS), which are the major cause of cell death in plants under stress conditions. Our results showed that both nano-CeO<sub>2</sub> and nano-MoS<sub>2</sub> showed protective effects and such effects can be modulated or enhanced by modifying the physicochemical properties of the nanomaterials. We believe that nano-enabled solutions could provide simple and robust strategies to improve plant resistance to environmental stress because they are based on mechanisms that relies on the materials, which is distinct from genetic-based mechanisms that are highly affected by plant species and other environmental conditions.

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### Supported Ionic Liquids materials to remove insecticides

Francisco, R.,<sup>1</sup> Almeida, C.,<sup>1\*</sup> Sousa, A. C. A.,<sup>2</sup> Neves, M. C.,<sup>1</sup> Freire, M. G.<sup>1</sup>

<sup>1</sup> CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup> Comprehensive Health Research Centre (CHRC) and Department of Biology, School of Science and Technology, University of Évora, 7006-554 Évora, Portugal

\* Corresponding author: Catarina Almeida, ac.almeida@ua.pt

Neonicotinoids insecticides are generally used to control pests in agriculture and for veterinary applications.<sup>1,2</sup> Due to their extensive use, these compounds can be found in various environmental compartments, such as water, soils, and biota, which can be a problem of concern due to their toxicity against non-target organisms.<sup>2</sup> Given this, it is of utmost priority to develop innovative and effective strategies able to remove neonicotinoids, while preventing environmental contamination by their exposure. In this work, supported ionic liquids (SILs) were studied as alternative adsorbent materials to remove insecticides, namely imidacloprid, acetamiprid, thiacloprid, and thiamethoxam, from aqueous matrices. SILs were initially synthesized and characterized and then employed in adsorption studies. The best identified SIL for the adsorption of all neonicotinoids was silica modified with propyltriethylammonium chloride, [Si][N<sub>3888</sub>]Cl. The saturation of the materials was attained in 5 min or less, demonstrating their fast adsorption rate for the target compounds. The best fitting of the experimental kinetic data was achieved with the Pseudo Second-Order model, suggesting that the adsorption process is controlled at the solid-liquid interface, whereas for the experimental isotherm data Freundlich isotherm model was considered the best, exposing the occurrence of multiple layers of insecticides onto the surface of the material. The removal efficiency under continuous mode through solid-phase extraction using [Si][N<sub>3888</sub>]Cl was also evaluated, with maximum adsorption capacities decreasing according to the following order: imidacloprid > thiacloprid > thiamethoxam > acetamiprid. Overall, it was probed the outstanding adsorption performance of SILs for a wide range of insecticides, confirming their potential to be used as adsorbent materials to remove these compounds from aqueous matrices. This will contribute to avoid their introduction in the environment and therefore reduce all the associated environmental and health burdens.

#### Acknowledgements:

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## Agrochemical and bacteriological study of lettuce fertilized with cheese whey based digestate

Álvarez-Méndez, S. J.,<sup>1,2\*</sup> Ramos-Suárez, J. L.,<sup>2</sup> Ritter, A.,<sup>3</sup> Mata González, J.<sup>2</sup>

<sup>1</sup> Inst. Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, Spain

<sup>2</sup> Departamento de Ingeniería Agraria y del Medio Natural, Universidad de La Laguna, Spain

<sup>3</sup> Área de Ingeniería Agroforestal, Universidad de La Laguna, Spain.

\*Corresponding author: salvmen@ull.edu.es

Cheese whey is a by-product from the cheese manufacturing. Within the framework of the circular economy paradigm, anaerobic digestion raises as a technology able to transform organic residues into an energy rich biogas and digestate, which is a nutritive liquid that may be used for crops fertigation. To evaluate the fertilizer potential of a digestate obtained from the anaerobic digestion of an industrial cow cheese whey, 3 cultivations of romaine lettuce (*Lactuca sativa* L var. *longifolia*) were consecutively studied during summer and autumn of 2021 and spring of 2022 in a greenhouse located at San Cristóbal de La Laguna (Canary Islands, Spain). Each experiment was carried out with 80 4-week-old lettuce plantlets grown in 2 liters polypropylene pots, watered regularly and subjected to 5 different treatments once a week (16 plantlets per treatment in a randomized complete block design): control water (pH = 7.1 ± 0.2), cow cheese whey (pH = 3.9 ± 0.1), a nutrient-rich liquid digestate obtained from the anaerobic digestion of the cow cheese whey (pH = 8.3 ± 0.1), a commercial organic horticultural fertilizer (pH = 5.8 ± 0.1) and the same digestate treated by adding an acidity regulator (pH = 6.1 ± 0.2). After 7 weeks, fresh weight, foliar area, head circumference and greenness of the lettuces fertilized with untreated digestate were statistically equivalent to those from lettuces treated with the commercial fertilizer. Similarly, the N, P, K, Fe, Cu, Zn and B content on leaves did not differ significantly between them. Moreover, all the measured physical parameters values of the lettuces fertilized with untreated digestate were significantly higher than those found in lettuces from the rest of the treatments, revealing that the attempt to avoid the high alkalinity of the digestate by adding a pH regulator had negative effects on the lettuces. As no *Salmonella* spp. neither *Escherichia coli* were detected during bacteriological analyses, digestate-fertilized lettuces were suitable for human consumption. These results highlight the power of anaerobic digestion to transform an organic waste into an added value product (digestate).



Figure 1. From left to right lettuces irrigated with: control water, cow cheese whey, cow cheese whey based digestate, commercial fertilizer and pH-corrected cheese whey digestate.

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## Molluscicidal effect of Azorean *Cryptomeria japonica* essential oil against *Radix peregra* (Lymnaeidae)

Arruda, F.<sup>1,2\*</sup> Lima, A.,<sup>1,3</sup> Pavão, A.,<sup>2</sup> Baptista, J.,<sup>1,3</sup> Rosa, J. S.<sup>2,4</sup> Lima, E.<sup>1,3</sup>

<sup>1</sup> Institute of Agricultural and Environmental Research and Technology, University of Azores, Portugal

<sup>2</sup> Department of Biology, University of Azores, Portugal

<sup>3</sup> Department of Physics, Chemistry and Engineering, University of Azores, Portugal

<sup>4</sup> Biotechnology Centre of Azores, University of Azores, Portugal

\* Corresponding author: filipe.mp.arruda@uac.pt

Fascioliasis is a zoonotic disease caused by the liver trematodes *Fasciola hepatica* and *F. gigantica*. Despite neglected, this food-borne trematodiasis has a worldwide distribution, and cause important morbidity and mortality in both livestock and humans. The intermediate hosts of these trematodes are, normally, the freshwater pulmonate snail of Lymnaeidae family and, in Europa (including Azores Archipelago), *Radix peregra* (Müller, 1774) is a susceptible intermediate host of *F. hepatica*. Due to the spread of fascioliasis disease, drug resistance and the lack of an effective vaccine against *Fasciola*, selective plant-based molluscicides remains one of the most effective methods to control snail populations and, thus, the parasite.<sup>1</sup> *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae), the most commercially important forest tree species in Azores, has shown several biological activities, namely against snail crop pest.<sup>2</sup> Integrated in a wider research work of assessing the bioactive potential of Azorean *C. japonica* essential oils (EOs), this study aimed to obtain leaf EOs by different distillation techniques for further chemical characterization and evaluation, for the first time, of the molluscicidal potential of *C. japonica* against *R. peregra*.

*C. japonica* leaf EOs were obtained by hydrodistillation (HD) and water-steam distillation (WSD) techniques. The OE-HD and OE-WSD chemical composition, determined by GC and GC-MS, revealed a  $\alpha$ -pinene plus sabinene *C. japonica* leaf OE chemotype. The molluscicidal activity of these OEs was performed against several development stages of *R. peregra* by the immersion method. A first screened through a single-dose bioassay (30 ppm for eggs and juveniles, and 100 ppm for adult snails), revealed that both EOs were highly active towards eggs and juveniles (100% mortality after 24 h), and adult snails (100% and 97% mortality after 48 h for OE-WSD and OE-HD, respectively). Subsequently, dose- and time-lethality bioassays were performed only against adults to determine lethal parameters (LC<sub>50;90</sub> and LT<sub>50;90</sub>). The LC<sub>50</sub> values were 33 ppm and 62 ppm for OE-WSD and OE-HD, respectively, after 48 h. The LT<sub>50</sub> of OE-WSD required only 21 h for both 16 h and continuous exposure times, while that of OE-HD was slightly superior (26 h and 22 h for 16 h and continuous exposure times, respectively). Overall, Azorean *C. japonica* leaf EOs, namely OE-WSD, seemed to be possible candidates as natural molluscicides to control snails populations responsible for transmitting fasciolosis.

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## Contribution to water saving in crops through the application of macroalgae extracts

Cueto, M.,<sup>1\*</sup> Borges, A. A.,<sup>1</sup> Jiménez-Arias, D.,<sup>2</sup> Luis, J. C.,<sup>3</sup> Morales-Sierra, S.,<sup>3</sup> Rancel, N.<sup>3</sup>

<sup>1</sup> Instituto de Productos Naturales y Agrobiología (IPNA-CSIC). Avenida Astrofísico F. Sánchez, 3, 38206 La Laguna, Tenerife, España

<sup>2</sup> ISOPlexis, Centro de Agricultura Sustentável e Tecnologia Alimentar, Campus Universitário da Penteada, 9020-105 Funchal, Madeira, Portugal

<sup>3</sup> Grupo de biología Vegetal Aplicada (GBVA), Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Farmacia Universidad de La Laguna. Avenida Astrofísico F. Sánchez s/n, 38206 La Laguna, Tenerife, España

\* Corresponding author: mcueto@ipna.csic.es

The increase in temperature and the change in the rainfall pattern caused by climate change have a strong impact on agriculture since it affects crop yields and causes the proliferation of diseases that affect crops.<sup>1</sup>

To avoid the loss of agricultural productive capacity, it is necessary to adapt agriculture to the new climatic conditions. Currently, the use of algae extracts in agriculture to improve germination, yield or crop resistance to biotic and abiotic stresses is widely extended and there are various algal preparations on the market that are used in agriculture as biostimulants and growth promoters.<sup>2,3</sup>

AHIDAGRO (MAC 2 1 1 b/ 279) is an EU-funded project that aims to develop new sustainable agricultural technology that permits saving irrigation water without reducing crop productivity and quality. Within this project, obtaining metabolites with osmoprotective properties from macro- and microalgae from Tenerife and Madeira is one of its activities.

This presentation will be focused on the preliminary results obtained from the study of the extracts of 13 species of seaweeds collected from the coast of Tenerife (Canary Islands). The effect of the aqueous extracts of these species on the growth of tomato plants growing under water stress conditions will be compared with the effect produced by some commercial algal preparations.

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## Resveratrol, a double-edged sword: long life elixir or father of toxic products?

Francioso, A.<sup>1,2\*</sup>, Jiménez Diaz, I. A.,<sup>2</sup> Lopez Bazzocchi, I.,<sup>2</sup> Mosca, L.<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze Biochimiche “A. Rossi Fanelli”, Sapienza Università di Roma, piazzale Aldo Moro 5, 00185 Roma, Italy

<sup>2</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain

\*Corresponding autor: antonio.francioso@uniroma1.it

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a bioactive polyphenol occurring in a variety of agricultural and food products like grapes and wine, and is one of the most important natural polyphenols. However, despite its multiple well documented beneficial effects on human health, Resveratrol use as a drug is strongly limited by its poor solubility, scarce bioavailability, and for its tendency to be unstable mainly due to autooxidation and photochemical induced degradation, features that limits its use in pharmaceutical products<sup>1</sup>.

We explored Resveratrol photochemical reactions, isolating and identifying for the first time a novel Resveratrol photo-induced electrocyclization product, namely 2,4,6-trihydroxyphenanthrene (THP) by means of HPLC-DAD, GC-MS, FT-IR, MS/MS and NMR techniques<sup>2</sup>. The photocyclization process is a formal  $6\pi$  electrocyclization and takes place when the triene system of *cis*-Resveratrol isomer undergoes a  $(4n+2)$ -electron conrotatory pericyclic ring closure. Our results demonstrate that Resveratrol photochemical isomerization and electrocyclization give rise to THP as a byproduct, even when Resveratrol is present in complex matrices such as agricultural or pharmaceutical products (i.e., wine, grape seed oil, medical devices). This new finding represents an important reference for further in vivo investigation of THP as a contaminant in view of its possible side effects on human health. Therefore, we have developed a new GC-MS analytical method for the analysis of THP in complex matrices, which can represent a good tool to analyze the possible presence of THP in biological, pharmaceutical, and agricultural samples<sup>3</sup>.

Being THP a potentially harmful hydroxylated polycyclic aromatic hydrocarbon, we aimed to verify its potential toxic risk for human health. Our data demonstrate cytotoxic and genotoxic effects even in the sub-micromolar range and in particular we demonstrated that the DNA damage is primarily exerted by a pro-oxidant mechanism<sup>4</sup>. Further experiments will be performed to test the possible mutagenic and/or cancerogenic effects of this photoreaction toxic by-product.

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# Flash Communications



## **Effect of variable rate seeding on maize water use efficiency in different geoclimatic locations in Spain**

Ojembarrena, A.,<sup>1\*</sup> Ruiz-González, E.,<sup>1</sup> Pérez-Ruiz, M.,<sup>1</sup> Egea, G.<sup>1</sup>

<sup>1</sup> *Smart Biosystem Laboratory, University of Seville, Spain*

\*Corresponding autor: andojemag@us.es

Currently, most farms manage inputs (seeds, fertilizers, water, etc.) in a homogeneous way, without considering the spatial variability of soil chemical and physical properties. This leads to heterogeneous crop development and non-optimized use of inputs, as input doses are often applied above or below the actual needs of each area. Precision agriculture techniques are focused on measuring and managing soil and crop spatial variability to increase the inputs' efficiency and reduce the environmental impact of agriculture. The objective of this work was to compare the effect of variable versus uniform seeding rate on water use efficiency in five commercial maize fields located in different geographical areas of Spain. The soil spatial variability of the five maize fields was characterized through bulk electric conductivity (ECa) measurements performed with an electromagnetic induction sensor (Geonics EM38). The ECa measurements were used to zone a fraction of the maize fields into three differentiated management areas, which were classified as of low, average, and high potential fertility. The rest of the maize fields were used as control treatment. Based on this criterion, a variable seeding rate was applied in the three management zones identified, while irrigation rate was kept uniform in the three selected zones. Crop water needs (ETc) were determined using the crop coefficient method, whose values were estimated throughout the crop growing cycle from the average NDVI values obtained from Sentinel 2 satellite images for each uniform management zone. Precipitation, irrigation water supply and yield were used to estimate the Relative Irrigation Supply (RIS) and Irrigation Water Use Efficiency (IWUE) indices. The results obtained showed a wide range of RIS and IWUE values observed in the five experimental fields. RIS and IWUE values between *ca.* 0.8 to 1.2 and between *ca.* 1.6 and 4.3 kg/m<sup>3</sup> were observed, respectively. Two of the five experimental fields showed RIS values < 1, indicating that they were deficit irrigated. In these fields, yield and IWUE values were higher in the area where variable rate seeding was applied as compared to the control area where a uniform seeding rate was applied. In contrast, in the over-irrigated fields with RIS > 1, yield and IWUE values were either similar or slightly higher in the control area with uniform seeding rate. Although these are preliminary results from a first-year study and should be treated with caution, these findings suggest that variable rate seeding attending to soil fertility potential may improve irrigation water productivity of deficit irrigated maize fields.

## Multilayer data and artificial intelligence for the delimitation of homogeneous management zones in corn cultivation

Gallardo-Romero, D.J.,<sup>1\*</sup> Martínez-Guanter, J.,<sup>2</sup> Apolo-Apolo, O. E.,<sup>1</sup> Pérez-Ruiz, M.<sup>1</sup>

<sup>1</sup>Smart Biosystem Laboratory, University of Seville, Spain

<sup>2</sup>Digital Agronomy dpt. Corteva Agriscience, Spain

\*Corresponding autor: diegogallardo.gr@gmail.com

Currently, the use of management zones for variable rate application is timidly beginning to appear in the European commercial agricultural sector. Based on the expertise of our research group, this low adoption is attributed to two key aspects: the zones are defined manually by agronomists and farmers, and a not very refined workflow of transferring the prescription file to the machine to execute the field task. Also, the need for high quality user interfaces can represent a challenge that is limiting their widespread adoption.

Thus, straightforward and robust delineation of homogeneous management zones for variable rate application it becoming of high interest in extensive crops such as corn. This study shows the preliminary results of the development and methodology for the delineation of homogeneous agricultural management zones based on the combination of artificial intelligence and multi-source data. These automatically obtained homogeneous zones maps have been compared with previous years corn yield maps.

The automatic zone delineation carried out in this study was done by combining automatic analysis tools such as Google Collab and remote sensing images. Series of Copernicus Sentinel 2 images, combining multiple spectral layers and analysing the seasonal variation of vegetation indexes were employed for crop monitoring and delineation.

Implementation of an artificial intelligence algorithm based on unsupervised learning has been done. By comparing the suitability of different ML approaches, a technique based on K-means algorithm has been optimized which has provided a classification accuracy of more than 80%.

As preliminary results of this evaluated method, a precise zoning map is obtained in which a clear differentiation of zones and a Pearson correlation of 0.530889 can be observed.



Figure 1. Zoning based on 3 clusters.

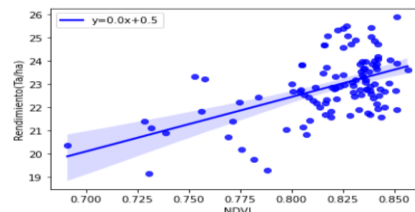


Figure 2. Correlation NDVI-yield.

After these first results, seeing its good computational performance, the next steps will focus on obtaining predicted productivity maps using different vegetation indices and dates to optimize selection and check if the correlation values increase, and the implementation of sensor data to check if they would improve the classifications made on the satellite images.



SPA2022

## Sustainable and Precision Agriculture Symposium 2022

### 5G Ecosystem: The Next Step for Precision Agriculture

Sánchez-Fernández, L.,<sup>1\*</sup> Pérez-Ruiz, M.<sup>1</sup>

<sup>1</sup>Smart Biosystems Laboratory, University of Seville, Spain

\*Corresponding autor: lsanchez1@us.es,

The agricultural sector faces an unprecedented challenge. Agricultural yield must increase by 70% to cope with the demands of a growing population and economy over the next 30 years. Increasing agricultural yield is not an easy task, especially in a context of climate change. Moreover, there is an increasing concern about the environmental impact of plant protection products (PPPs). This concern has resulted in initiatives, such as the Farm to Fork initiative, that aims to reduce by a 50% the use of PPPs in Europe over the next decade. Given that it is not sustainable to increase the agricultural area at the expense of natural ecosystems, this increase in yield must come from a sustainable intensification. Some technologies with potential to contribute to solve this challenge have already been developed. However, the transfer of these new technologies is slow and problematic due to the application costs, complexity of data processing, computational power, and the lack of a user-friendly interface.

Some of these limitations could be solved by the introduction of high-speed and low-latency communication technology 5G. The implementation of this technology will not only contribute to develop and increase the adoption rate of new techniques but will also introduce the Internet of Things (IoT) and near real-time (NRT) processing and decision making in the field. 5G technology has potential to boost the development of autonomous platforms that will contribute to the sustainability of agriculture while reducing transmission power consumption significantly.

While 5G technology has been successful in the industrial sector and pledges to introduce some of the latest technologies into the farm fields, some of its peculiarities might hinder its adoption by the agricultural sector. Even though 5G can be significantly faster than 4G, their average data rates are similar. Its range is limited compared to 4G, and to give coverage to a certain area, ground stations are needed every 250 m. This infrastructure cost might be unaffordable for the average farmer. Thus, it might be unreasonable to think that the application of 5G technology in the field will happen soon when most rural areas are out of 4G coverage. Nevertheless, its potential might be pivotal to the development of smart agriculture.

5G technology will not solve any problems of the agricultural sector by itself, auxiliary technologies such as blockchain technology or algorithms for pest and disease detection must be developed simultaneously. Presently, farmers have alternatives to 5G technology that have not been exploited yet. For example, satellite internet offers worldwide coverage at a lower average latency than current 4G networks. The cost of these platforms might be expensive for the common farmer but will get cheaper in the mid-term and might help to speed up the adoption rate of certain technologies. We must try to get the most out of the technology currently available in the field. It is fundamental to introduce farmers to the benefits of having an interconnected farm before expecting them to invest in technologies they do not trust yet.

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### UAV-based multispectral imagery to detect diseases in *Phoenix canariensis* palm-groves from the Canary Islands (Spain)

Casas, E.J.,<sup>1\*</sup> Arbelo, M.,<sup>1</sup> Reyes-Carlos, J.A.,<sup>2</sup> Hernández-Dorta, A.,<sup>2</sup> González, A.D.<sup>2</sup>

<sup>1</sup> Departamento de Física, Universidad de La Laguna, Spain

<sup>2</sup> Consejería de Agricultura, Ganadería y Pesca, Gobierno de Canarias, Spain

\*Corresponding autor: [ecasasma@ull.edu.es](mailto:ecasasma@ull.edu.es)

The alteration of environmental conditions, as a consequence of climate change, and the abandonment of supply agriculture in recent decades is causing many *Phoenix canariensis* palm groves in the Canary Islands (Spain) to be affected by pests and diseases. To control and eradicate these pathologies, it is necessary to implement methods of determination and monitoring that allow early and efficient detection. In this sense, remote sensing techniques based on multispectral images from UAV in conjunction with adequate field spectroradiometry could prove to be a suitable method for this purpose. In this work, framed within the Project Conservation and Management of Macaronesian island palm trees through sustainable use (GUARAPOMAC) funded by the International Cooperation Programme MAC 2014-2020, Interreg (FEDER), we studied a natural palm grove with specimens affected by *Serenomyces phoenicis* (Rolland) E. Müll. & S. Ahmada cf. This fungus invades the vascular tissues, mainly of mature and older leaves, producing localised drying. The main objective consisted of capturing multispectral images with an Altum-Micasense camera of healthy and affected specimens, the simultaneous measurement with a FieldSpec-3 ASD spectroradiometer of their spectral signatures and the subsequent application of eight spectral indices susceptible to detecting this pathology, to determine which could be the most suitable for its identification. Based on the characteristics of the five camera bands and a spectral separability analysis, the following indices were selected: BNDVI (Blue Normalized Difference Vegetation Index), GNDVI (Green Normalized Difference Vegetation Index), NDRE (Normalized Difference Red Edge Index), NDVI, SIPI 2 (Structure Insensitive Pigment Index 2), OSAVI (Optimized Soil-Adjusted Vegetation Index), NDYI (Normalized Difference Yellowness Index) and SIPI (Structure Insensitive Pigment Index). In general, almost all indices can distinguish healthy leaves from affected leaves to different degrees, but with very unequal numerical values due to the different combinations of bands used. For all the indices, except for both SIPI indices, the leaves of the healthy specimens have the highest values, with NDVI reaching a difference of 0.4 or more for the rest of the leaves. The SIPI indices, on the other hand, show the opposite behaviour. In these cases, it is the completely dry leaves that obtain the highest absolute values, with 1.82 for SIPI2 and 1.28 for SIPI. The SIPI index is proposed as a potential candidate for detecting the pathology under consideration. SIPI not only presents a complete separability between healthy and dry leaves but also places the rest of the leaves with an intermediate degree of affection, in values differentiated from the previous ones. Furthermore, this proposal is supported by the fact that the camera bands used by this index are the most stable and best radiometrically calibrated: B1 (blue), B3 (red) and B5 (near-infrared).

## Evaluation of the antifungal activity of basic substances on growth and germination of *Botrytis cinerea*

Fuentes, A.,<sup>1\*</sup> González-Acosta, S.<sup>1\*</sup> Giaki, K.,<sup>1</sup> Morales-de la Nuez, A.,<sup>1</sup> Guillermo, R.,<sup>3</sup> Pérez de la Lastra, J. M.<sup>1</sup>

<sup>1</sup>Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de la Laguna, Spain.

<sup>2</sup>Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza- Apdo. 456 38200 San Cristóbal de La Laguna, Spain.

<sup>3</sup>Departamento de Química Orgánica, Facultad de Ciencias, Universidad de La Laguna (ULL). Tenerife Spain.

\*Corresponding autor: ari13fm@gmail.com

*Botrytis cinerea* is a phytopathogenic fungus that causes losses in more than 500 crop species around the world. Different types of control are used to reduce its presence, but the most acclaimed by farmers is the chemical one. However, in recent years, the European Union has withdrawn many of these chemicals from the market leaving many farmers with more limited defense methods. In addition, this fungus can generate resistance to these products, decreasing their effectiveness over time. Therefore, alternative elements for its containment are being searched.

Basic substances are a series of products, approved by the European Commission (EC), whose main function is not their use as a phytosanitary, but can have a biocidal activity. Soy lecithin, sodium chloride (NaCl), sodium bicarbonate (NaHCO<sub>3</sub>), vinegar, whey and calcium hydroxide or slaked lime (Ca(OH)<sub>2</sub>), which are approved under Article 23 of Regulation (EC) No. 1107/2009, were used in this study. The objective was to test the antifungal activity of this substances against *B. cinerea*. A study of germination and hyphal growth was carried out where sodium bicarbonate totally reduced growth except at the lowest concentration used, while vinegar achieved inhibition at all concentrations. In the mycelial growth study carried out on plates, the two previously mentioned substances showed again the most favourable results. However, in a final postharvest test on tomatoes, it was lactic whey and soy lecithin that achieved the lowest infection.

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## Study of Alternative Splicing In the Response of *Arabidopsis Thaliana* to Heat Stress

Baca-González, V.,<sup>1\*</sup>, Conesa, C.M.,<sup>1</sup> González-García, M. P.,<sup>1</sup> Garrido-Martín, D.,<sup>2</sup> Caro, E.,<sup>1</sup> del Pozo, J. C.<sup>1</sup>

<sup>1</sup>Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Madrid, Spain

<sup>2</sup>Centro de Regulación Genómica, Barcelona, Spain

\*Corresponding autor: victoria.baca@inia.csic.es

Future plant production is threatened by the unpredicted consequences of global warming. It is then essential to study the mechanisms behind plant adaptation to these adverse conditions, including extreme heat waves. One of the mechanisms that plays an important role in the regulation of the response to heat stress is the alternative splicing (AS) of mRNAs. Nonetheless, published research about the effects of heat stress on plants *in vitro* were mainly made creating a homogeneous high temperature for the entire plate, although in natural fields the temperature of soil is more stable and cooler than that of the air. To overcome this experimental limitation, the group designed a device to cultivate the root system in a temperature gradient similar to that found in the soil.

Here, RNA-seq data from roots and shoots of plants grown under different temperature conditions were bioinformatically analysed to study differential gene expression (DGE) and differential alternative splicing (DAS). *Arabidopsis thaliana* plants grown *in vitro* in standard homogenous temperature (22°C), in high homogenous temperature (32°C) and high temperature of 32°C in the shoot with a thermal gradient in the root area, to imitate the conditions of the soil in a global warming scenario. In all conditions, plants were grown with their roots in darkness.

Our results show that shoots growing at homogeneous 32°C and the ones growing at a high temperature in the shoot with thermal gradient in the root area display a similar molecular response, although the existence of genes that were deregulated in one compared to the other indicated root-shoot communication. In contrast, roots displayed a strong response in both treatments, at the levels of DGE and DAS. Functional enrichment analysis shows that the homogeneous 32 °C condition affects plant processes that are not deregulated when the gradient is applied. In addition, we have seen that a large proportion of the genes undergoing DGE and DAS are not the same, revealing that both mechanisms are involved in the plant heat response at least in part independently. Our results suggest that the use of a gradient of temperature in the area of root growth during heat stress is relevant to identify the processes that plants use to adapt to heat stress.

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## Effects of Proline-Arginine-Rich cathelicidins (LV-RR32 and TT-FR28) against conidia of *Botrytis cinerea*

Otazo-Pérez, A.,<sup>1,2\*</sup> González-Acosta, S.,<sup>1,2</sup> Baca-González, V.,<sup>1</sup> García-Machado, J. F.,<sup>1</sup> Asensio-Calavia, P.,<sup>1,2</sup> Giaki, K.,<sup>1</sup> Klingelhöfer, S.,<sup>1</sup> López, M. R.,<sup>1</sup> Morales-de la Nuez, A.,<sup>1</sup> Pérez de la Lastra, J. M.<sup>1</sup>

<sup>1</sup>Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de la Laguna, Spain.

<sup>2</sup>Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza-Apdo. 456 38200 San Cristóbal de La Laguna, Spain.

*Botrytis cinerea* is a polyphagous fungus capable of infecting more than 200 plant species, affecting both the cultivation and post-harvest phases. It is considered the second most important phytopathogen<sup>1</sup>, producing a great economic impact. In addition, it has a big capacity to generate resistant strains to commercial fungicides, which increases the need to search new products capable of dealing with this phytopathogen. One possible option are cathelicidins, peptides with antimicrobial capacity that are present in the innate immune system of all vertebrates. Specifically, cathelicidins rich in proline and arginine have been shown to have highly antifungal activity.

In this work, the main objective was to study the inhibitory effect on the germination and development of botrytis of two proline-arginine-rich cetacean cathelicidins (LV-RR32 and TT-FR28). A strain of *B. cinerea* was incubated *in vitro* for 10 hours in contact with the peptides, at concentrations of 25, 50 and 100  $\mu$ M. The percentage of germinated conidia and the length of the hyphae were quantified after 4, 6, 8 and 10 hours of incubation.

*In vitro* assays showed that both peptides were able to completely inhibit conidia germination at a concentration of 100  $\mu$ M. However, the LV-RR32 peptide proved to be the most effective, since the percentage of inhibition was very high even at 25  $\mu$ M. In addition, both peptides markedly affected the growth of hyphae, significantly reducing their length. Hyphal development was completely inhibited at 100  $\mu$ M. Although it is necessary to carry out *in vivo* tests, these results highlight the potential of cathelicidins as a source of new antifungal compounds, especially those with a structure rich in proline and arginine.

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## Fungicidal activity of overlapping peptide derived from cathelicidin peptide Lv\_RR32 against *Botrytis cinerea*

Klingelhöfer, S.,<sup>1,2\*</sup> Giaki, K.,<sup>2</sup> González-Acosta, S.,<sup>2,3</sup> Asensio-Calavia, P.,<sup>2,3</sup> Otazo-Pérez, A.,<sup>2,3</sup> López, M. R.,<sup>2</sup> Morales-de la Nuez, A.,<sup>2</sup> Pérez de la Lastra, J. M.<sup>2</sup>

<sup>1</sup>Institute of Pharmacy and Molecular Biotechnology. University Heidelberg. Neuenheimer Feld 364, 69120 Heidelberg, Germany.

<sup>2</sup>Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de la Laguna, Spain.

<sup>3</sup>Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza-Apdo. 456 38200 San Cristóbal de La Laguna, Spain.

\*Corresponding author: s.klingelhoef@stud.uni-heidelberg.de

Cathelicidin active peptides are small, cationic active molecules with amphipathic properties found in the innate immune system of vertebrates. Antimicrobial peptides have a fast mode of action and a low level of induced resistance in pathogens, making them a potential alternative to conventional antibiotics and chemical fungicides. Lv\_RR32, a proline-rich cathelicidin peptide from the Chinese freshwater dolphin (*Lipotes vexillifer*), has been shown to have fungicidal activity.

A total of 5 overlapping peptides, of 10 amino acids each, were designed from the 32 amino acid long Lv\_RR32 peptide, which reduced the synthesis cost. These overlapping peptides were tested against *Botrytis cinerea* to evaluate their antifungal activity. For this purpose, 50 µM peptides were incubated with *B. cinerea* conidia for 8 hours. The effect of each overlapping peptide was analyzed based on the conidia germination and length of the germ tube after 4 and 8 hours.

The overlapping peptide Lv\_RR32(1-10) was shown to significantly reduce both germination and germ tube length. After 4 hours, this peptide did not allow any germination and therefore no tube length could be determined. After a total of 8 hours, germination was decreased by 26 % and the germ tube length was reduced by 59 % compared to the control. Treatment with the remaining four overlapping peptides resulted in no significant difference in germination and showed slightly increased tube length.

The experiments conducted so far have shown promising results in reducing conidial germination and germ tube length of *B. cinerea* by Lv\_RR32(1-10). Further experiments need to be conducted to confirm the antifungal activity of this synthetic overlapping peptide.

### Acknowledgements:

This work was funded by projects APOGEO (INTERREGMAC Call-European Funds for Regional Development-FEDER) “Agencia Canaria de Investigación, Innovación y Sociedad de la Información (ACIISI) del Gobierno de Canarias”, project ProID2020010134 “Bioprospección y biotecnología en el descubrimiento de péptidos antimicrobianos contra patógenos resistentes” and CajaCanarias project 2019SP43 “Nanotecnología para el tratamiento antifúngico poscosecha de la pobredumbre gris (*Botrytis cinerea*). MRL is currently funded by the Cabildo de Tenerife, under the TFinnova Programme, supported by MEDI and FDCAN funds (project number 19-0231). AM-dIN is a recipient of a postdoctoral Marie Curie fellowship under grant agreement 101030604 (IGYMER).

## The quest for the discovery of improved pesticides: Assessing the toxicity of a lipopeptide extract of the olive tree endophytic *Bacillus* PTA13 to the model organism *Lemna minor* L. applying metabolomics

Papadopoulou, E.-A.,<sup>1</sup> Giaki, K.,<sup>1\*</sup> Aliferis, K. A.<sup>1,2</sup>

<sup>1</sup> Laboratory of Pesticide Science, Agricultural University of Athens, Iera Odos 75, Athens, 118 55, Greece

<sup>2</sup> Department of Plant Science, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada

\*Corresponding autor:katerinagiakh@gmail.com

The agrochemical sector is facing great challenges, including, among others, the strict legislation on pesticides, and the public concern on their potential effects on human, the environment and non-target organisms. In this context, there is an urgent need to discover new, alternative, and improved sources of bioactivity that could potentially be developed as commercial pesticides.

The discovery of pesticides with improved toxicological profiles represents a main axis of our research. Recently, we isolated an olive tree endophytic bacterium belonging to the genus *Bacillus* that exhibits superior bioactivity against plant pathogenic fungi. Such bioactivity is largely attributed to its lipopeptide (LP)-producing capacity. A LP extract, composed of the main surfactin, bacillomycin and fengycin, and the minor bacilotetrin and gageotetrin groups, was obtained from liquid cultures of the bacterium.

Since the assessment of the toxicity of bioactive agents to non-target organisms is a primary step in pesticide R&D, here, we have undergone the task to mine the phytotoxicity of the obtained LP extract to the aquatic model organism *Lemna minor* L. In total, six concentrations were tested based on the effects on the fresh weight, the chlorophyll and carotenoid contents and the number of fronds. The results of this assessment were used in order to determine the concentrations that should be applied for the metabolomics analyses.

Chlorophyll  $\alpha$  was proven to be the most sensitive indicator of the LP-caused toxicity. Nonetheless, results revealed that the LP extract is moderate toxic to *Lemna*, exhibiting EC<sub>25</sub> value of 156 ppm and EC<sub>50</sub> of 419 ppm. Such results were in line with the observed phenotypes.

GC/EI/MS metabolomics analysis revealed metabolites-biomarkers of the LP phytotoxicity, such as the carbohydrates  $\alpha$ , $\alpha$ -trehalose, D-glucose and D-fructose, the carboxylic acids malate, glycerate, and the amino acids GABA, L-alanine, L-serine and L-asparagine. The discovered biomarkers play multiple roles in plant responses to stresses, among others, oxidative stress, and function as signals.

The developed pipeline represents a proof-of-concept of the applicability and potential of metabolomics in the risk assessment of bioactive compounds towards their development as plant protection products.

## A new elicitor to increase secondary metabolites in *Artemisia annua* plants

García-García, A. L.,<sup>1,2,\*</sup> García-Machado, F. J.,<sup>1,2</sup> Boto, A.,<sup>2</sup> Jiménez-Arias, D.<sup>3</sup>

<sup>1</sup> Grupo Síntesis de Fármacos y Compuestos Bioactivos, Departamento de Química de Productos Naturales y Sintéticos Bioactivos, Instituto de Productos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, San Cristóbal de La Laguna, España.

<sup>2</sup> Universidad de La Laguna, San Cristóbal de La Laguna, España

<sup>3</sup> Centro de Agricultura Sostenible y Tecnología Alimentaria, Universidad de Madeira, Portugal.

\*Corresponding author: [algg@ipna.csic.es](mailto:algg@ipna.csic.es)

Plant secondary metabolites are an important class of natural products with important applications in nutrition, agronomy, pharmacy or cosmetic. An interesting metabolite is artemisinin, which is a sesquiterpene endoperoxide lactone. Artemisinin is better known for this use as treatment against malaria owing his effect against Plasmodium parasite. Besides, it has activity against several types of cancers, as well as anti-viral and anti-inflammatory properties. The most efficient source of artemisinin is the *Artemisia annua* plant which produced it in small quantities. Also, the chemical synthesis is complex and with low yields. Thus, the most common strategy to increase a secondary metabolite is the use of elicitors. This refers to biotic elicitors such as substances from fungi, bacterial, viral or other plants, physical elicitors (such as water stress) or synthetic chemicals. In this work, the effect of a vitamin derivative is studied in artemisinin production of *A. annua* plants. Plants were treated with different concentrations of elicitor in a growth chamber (0, 0.2, 0.8, 1, 2, 3 and 4 mM). After 48 hours of treatment, artemisinin was quantified. The highest artemisinin content was obtained with 1 mM. After dose optimization, time harvesting was optimised. Leaves samples were taken at 24, 48 and 72 hours. The highest increase was observed at 48 hours. This elicitor produces an increase in artemisinin production of 38.41%.

### Acknowledgements:

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## Subaerophytic Canarian microalgae: a promising source for new biopesticides

Rancel Rodríguez, N. M.,<sup>1\*</sup>, Reyes, C.P.<sup>2</sup>, Giménez Mariño, C.,<sup>1</sup> Moujir Moujir, L.,<sup>2</sup> and Sansón Acedo, M.<sup>1</sup>.

<sup>1</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Farmacia, Universidad de La Laguna, Avenida Astrofísico Sánchez 2, 38206 La Laguna, Tenerife, Spain

<sup>2</sup> Departamento de Bioquímica, Microbiología, Biología Celular y Genética. Instituto Universitario de Bio-Organica Antonio González, Universidad de La Laguna, Avenida Astrofísico Sánchez 2, 38206 La Laguna, Tenerife, Spain

\*Corresponding autor: nrodri@ull.edu.es

The ongoing use of synthetic fertilizers and pesticides (agrochemicals) has driven excessive anthropogenic nutrient pollution and resulted in a large number of environmental impairments. Further, long-term exposure to agrochemicals can cause significant human health problems.<sup>1</sup> Cyanobacteria-based biostimulants and biopesticides represent a promising alternative to reduce these negative effects and achieve greater sustainable value in modern agricultura.<sup>2</sup> However, the cyanobacterial biomass contains numerous amino acids and phytohormones that promote plant growth and increase the productivity of crops by stimulating root and shoot growth.<sup>3</sup> On top of that, these prokaryote organisms have been found to inhibit the growth of several pathogens owing to their antimicrobial properties. Thus, they can be considered a potentially sustainable alternative to synthetic fertilizers and pesticides in the agricultural and horticultural sectors.<sup>4</sup> In this study, we focused on the application of ethanolic extracts of Nostoclean cyanobacteria, isolated from the Canarian Laurel forest, as bactericides and fungicides against the plant pathogenic bacteria such as *Erwinia carotovora* and *Pseudomonas corrugata* and also pathogenic fungi such as *Alternaria alternata*, *Botrytis cinerea*, and *Fusarium oxysporum*.

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## Effects of two alpha-helix cathelicidins against conidia of *Botrytis cinerea*

Asensio-Calavia, P.,<sup>1,2\*</sup> González-Acosta, S.<sup>1,2</sup>, Baca-González, V.<sup>1</sup>, Garcia-Machado, J.F.<sup>1</sup>, Otazo-Pérez, A.<sup>1,2</sup>, López, M.R.<sup>1</sup>, Morales-delaNuez, A.<sup>1</sup>, Pérez de la Lastra, J.M.<sup>1</sup>

<sup>1</sup>Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de la Laguna, Spain.

<sup>2</sup>Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza-Apdo. 456 38200 San Cristóbal de La Laguna, Spain.

\*Corresponding autor: patriciaac@ipna.csic.es

*Botrytis cinerea* is a fungal pathogen of plants that causes the grey mould disease. This pathogen triggers the loss of a huge amount of crops at year and the actual treatment to reduce the infection is mainly based in synthetic fungicides that could cause resistances and environmental problems. Cathelicidins, a group of antimicrobial peptides (AMPs) from the innate immune system of vertebrates, are a good alternative because of their good activity against pathogens and low toxicity. In general, they are cationic peptides and their secondary structure is usually  $\alpha$ -helix. The objective of this study was to obtain cathelicidins from *Alligator mississippiensis* (AM-RV28) and *Talpa occidentalis* (TO-KL37) and prove them against the fungus *Botrytis cinerea* in order to discover new natural active compounds.

In the study, we evaluated *B.cinerea* conidia against several concentrations of the peptides in a 96-well plate. After incubation in appropriate conditions during 4, 6, 8 and 10 hours, we calculated the percentage of germinated conidia and hyphal length using an optical microscope. Finally, we proceed with the statistical data analysis at SPSS software.

The results showed that the two  $\alpha$ -helical peptides tested reduced significantly the percentage of germination. The fungi in presence of AM-RV28 germinated around 15% and TO-KL37 around 30% at 50  $\mu$ M and 10 hours of incubation. Regarding the hyphal length measures, both showed a hypha around 20-25  $\mu$ m at 50  $\mu$ M which statistically differs from the 60  $\mu$ m control hypha at 10 hours of incubation.

Both  $\alpha$ -helical peptides had good results against *Botrytis cinerea* plant pathogen, reducing germination as much as the hyphal growth but these results were done *in vitro*. We need to investigate more about their *in vivo* activity in order to elucidate their real potential to resolve *Botrytis* problem in agriculture.

### Acknowledgements:

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## Chemical screening and antifungal activity of *Origanum vulgari* and *Thymus vulgari* essential Oils against *Botrytis cinerea* and *Erysiphe* plant pathogens

Ferreira, R.M.,<sup>1\*</sup> Santos, V.S.<sup>2</sup> and Castilho, P.C.<sup>2</sup>

<sup>1</sup> Centro de Química da Madeira, Universidade da Madeira, Portugal

<sup>2</sup> Centro de Química da Madeira, Universidade da Madeira, Portugal

\*Corresponding autor: rui.ferreira@staff.uma.pt

In this study, essential oils (EO) from two species with widespread application in the Mediterranean cuisine – *Origanum vulgari* and *Thymus vulgari*, were extracted by hydrodistillation with a Clevenger-type apparatus. The essential oils obtained were subjected to GC-FID quantification. FTIR-ATR spectroscopy and high-resolution <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy were also used for characterization of bioactive compounds.

The fungicidal activity was evaluated on 4 common plant pathogen fungus (*Sphaerotheca pannosa*, *Erysiphe polygoni*, *Erysiphe cruciferarum* and *Botrytis cinerea*) applying the disk diffusion method or moist chamber for holoparasites such as *Erysiphaceae* family. Different formulations were tested for evaluation of *in vitro* efficacy and commercial fungicides such as methyparaben and ARAW were used as control

The essential oils obtained from *Thymus* and *Origanum* were dominated by monoterpenoid phenol derivatives of *p*-cymene: thymol and carvacrol, corroborated by IR and NMR characterization. As for *Origanum vulgari*, the major components identified were carvacrol (73,04%),  $\gamma$ -terpinene (5,97%) and thymol (5,65%). *Thymus vulgari* EO is characterized for its high content in thymol (89,50%),  $\gamma$ -terpinene (3,27%) and *p*-cymene (1,81%).

Results indicate that mycelial grow for *E. polygoni*, *E. cruciferarum* and *S. pannosa* was hindered with a minimum inhibitory concentration (MIC) of 20  $\mu$ g/ml for *Origanum vulgari* and 10  $\mu$ g/ml for *Thymus vulgari*. For *B. cinerea*, the strongest antifungal activity was reported for *Thymus vulgari* EO, with inhibition observed for 5  $\mu$ g/ml of EO in ethanol and 10  $\mu$ g/ml in Tween 20. The MIC of *Origanum vulgari* EO for this pathogen was established at 50  $\mu$ g/ml. Therefore, present results indicated that thymol and carvacrol rich chemotype plants could be considered as alternative to conventional fungicides.

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## *Argyranthemum frutescens*, an endemic canarian plant as a promising biopesticide

Rodríguez-Sabina, S.,<sup>1\*</sup> Hernández, E.A.,<sup>2</sup> Giménez, C.M.,<sup>1</sup> Espinel, G.G.,<sup>1</sup> Cabrera, R.C.,<sup>1</sup> Jiménez, I.A.,<sup>2</sup> Bazzocchi, I.L.<sup>2</sup>

<sup>1</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias Sección de Biología, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>2</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

\*Corresponding autor: samuelrguezsabina@gmail.com

Global population growth means that food production needs to increase 70% in 2050. Annually, up to 40 percent of global crop production is lost to pests, and among them, the polyphagous lepidopteran *Chrysodeixis chalcites* is one of the four most important pests of European greenhouse crops. Furthermore, *Botrytis cinerea*, *Alternaria alternata* and *Fusarium oxysporum* are phytopathogenic fungi responsible for several diseases in economically important crops such as tomato, potato or banana. The long-term use of chemical pesticides has caused environmental, ecological, and human health problems. In this regards, botanical biopesticides offer an eco-friendlier solution for pest control and environmental management.<sup>1</sup>

In the search for plant-derived pesticides, *Argyranthemum frutescens* ssp. *frutescens*, an endemic species to the Canary Islands, was studied as a potential source. Thus, the leaves extract of the plant was tested against *C. chalcites*. Insect anti-feeding activity was evaluated *in vitro* by the leaf-disk bioassay, in a choice and non-choice feeding assay. The surface of leaf-disk was impregnated with 0,2 mg/cm<sup>2</sup> of the tested extract, and fifth-instar larvae were placed on each plate.<sup>2</sup> The results expressed as anti-feeding rate (FR) showed that the leaves extract exhibited strong anti-feeding activity on *C. chalcites* (choice assay FR 78.69%, and non-choice assay FR 85.80%).

Moreover, the roots extract was evaluated against the phytopathogenic fungi, *B. cinerea*, *A. alternata* and *F. oxysporum*, using an *in vitro* dilution agar assay.<sup>3</sup> Results revealed inhibition of all pathogen fungi growth at 1 mg/ml (% inhibition ranging from 100 to 55%). Therefore, *A. frutescens* appeared as a very promising species for the development of biopesticides, and further assays will performed for scaled-up to field conditions.

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SPA2022

## Sustainable and Precision Agriculture Symposium 2022

### Effects of four cathelicidin against *Botrytis cinerea* in tomato leaf infection

González-Acosta, S.,<sup>1,2\*</sup> Baca-González, V.,<sup>1</sup> García-Machado, J. F.,<sup>1</sup> Asensio-Calavia, P.,<sup>1,2</sup> Otazo-Pérez, A.,<sup>1,2</sup> López, M. R.,<sup>1</sup> Morales-delaNuez, A.,<sup>1</sup> Pérez de la Lastra, J.M.<sup>1</sup>

<sup>1</sup>Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de la Laguna, Spain.

<sup>2</sup>Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza-Apdo. 456 38200 San Cristóbal de La Laguna, Spain.

\*Corresponding author: sergi\_glez@hotmail.com

*Botrytis cinerea* is a fungal phytopathogen with the second largest worldwide impact on the agricultural industry. One of the most common methods for its control is the use of chemicals. However, its high capacity to generate resistance, as well as the increasing prohibition by the European Community on the use of these compounds, forces to research alternative antifungals for its control. An interesting alternative is the use of cathelicidins, which are a family of antimicrobial peptides (AMPs) present in most vertebrates as part of their innate immune system. It is remarkable their low rate of resistance induction, mainly due to their multiple mode of action, such as disrupting the cell membrane. Cathelicidins range in size from 12 to 80 amino acids residues presenting diverse structures. We can find  $\alpha$ -helices,  $\beta$ -hairpin structures (with one or two disulphide bonds), and extended structures (proline or tryptophan rich).

In this work we evaluated the ability of four peptides to reduce *B. cinerea* infection in tomato leaves: two proline-rich peptides (LV-RR32 and TT-FR28) and two  $\alpha$ -helix (AM-RV28 and TO-KL37). For this purpose, we inoculated four-week-old tomatoes third leaf with a 5  $\mu$ L droplet of *B. cinerea* ( $2 \times 10^5$  conidia/mL) mixed with each peptide a different concentration (200, 100, 50 and 25  $\mu$ M) and one group with distilled water as control. After three days of incubation at 24°C in dark conditions, the average lesion diameter was determined. From the proline-rich peptides examined, the results showed that LV-RR32 was the most effective, fully inhibiting the infection at 100  $\mu$ M and greatly reducing it at the lowest concentration. TT-FR28, on the other hand, displayed less activity. The larger size and higher proline and arginine content could be responsible of the higher activity of LV-RR32. In the case of  $\alpha$ -helices peptides, AM-RV28 inhibited completely the infection at 100  $\mu$ M and showed good activity at lower concentrations. Meanwhile, TO-KL37 only reduced the infection at the higher concentration tested (200  $\mu$ M). In this case, AM-RV28 presented the higher hydrophobicity in its  $\alpha$ -helix, which could be related with its higher activity against this fungus.

In this preliminary work, we showed that cathelicidins are effective against *B. cinerea* infection in tomatoes leaves. Particularly, LV-RR32 showed promising results so further research on this peptide would be carried out in the future. Also, the diversity of cathelicidins tested provides an interesting alternative to chemical compounds for the control of this fungus.

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## Nitrogen-containing Heterocycles as potential antimicrobials for Agriculture.

González, Z.,<sup>1\*</sup> Martínez, E.,<sup>1</sup> Giménez, C.,<sup>2</sup> González, C.<sup>1</sup>

<sup>1</sup> Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Tenerife, Spain

<sup>2</sup> UDI Fitopatología, Facultad de Ciencias, Universidad de La Laguna (ULL). Tenerife Spain

\*Corresponding autor: zuleima@ipna.csic.es

Heterocyclic compounds, one of the most important classes of organic compounds, are frequently present in molecules of biological interest. Among them, nitrogen-containing heterocycles are of great importance for life sciences, as they are abundant in nature, existing as subunits in several natural products.<sup>1</sup> In relation to agrochemicals, it is known that approximately 70% of the agrochemicals currently in use contain a nitrogen ring in their structure.<sup>2</sup>

2,4-imidazolidinediones, also known as hydantoin, are a class of well-known molecules that have demonstrated broad therapeutic interest, including anti-epileptic, anticonvulsant, antiandrogenic, antidiabetic or antifungal activities. Hydantoin as well as their homologues, thio-hydantoin, represent an important group of heterocycles with a wide variety of biological and pharmacological activities.<sup>3</sup>

Synthetically, hydantoin and thiohydantoin are obtained from the reaction of an  $\alpha$ -amino acid with an isocyanate or isothiocyanate, in a reaction known as Edman degradation.<sup>4</sup>

In this poster, we will present the results obtained in the synthesis and antifungal evaluation of several hydantoin, thiohydantoin and ureas derived from amino acids and other nitrogenous heterocycles against different phytopathogenic fungi (*Botrytis cinerea*, *Alternaria alternata* and *Fusarium oxysporum*).

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## Combinatorial preparation of peptide libraries to identify antifungal agents

Hernández D.,<sup>1\*</sup> Rullo M.,<sup>1,2</sup> Porras M.,<sup>1,2</sup> Giménez C.,<sup>2</sup> Abdala S.,<sup>2</sup> Boto A.<sup>1</sup>

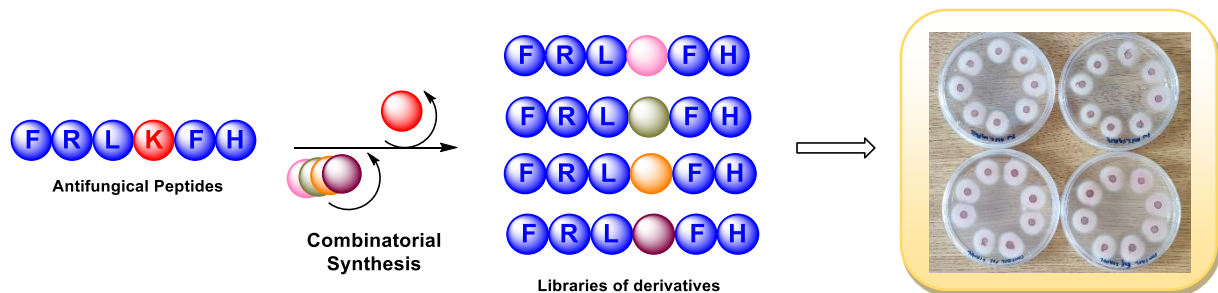
<sup>1</sup> Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico F. Sánchez, 3; 38206-La Laguna, Tenerife, Spain.

<sup>2</sup> Universidad de La Laguna (ULL), La Laguna, Tenerife, Spain

\*Corresponding author: dacil@ipna.csic.es

The development of short antimicrobial peptides (5-50 amino acids) for use in agriculture is eliciting much interest. These peptides present important advantages, such as a broad spectrum of activity, little induction of resistances, and synergic action with conventional antimicrobials (1–3). In this communication, we report a combinatorial strategy to produce libraries of ultra-short peptides with potential antifungal effect.

In the discovery process, the generation of peptide libraries can be accelerated using methodologies for the site-selective modification of peptides, where a few “parent” peptides are converted into a diversity of derivatives (4). Thus, the modification of “customizable units” allows the generation of peptides “à la carte”, with a variety of lateral chains. These libraries were evaluated against three important phytopathogens, *Botrytis cinerea*, *Alternaria alternata* and *Fusarium oxysporum*, using the radial growth test in PDA. Some promising compounds were identified for study as crop protection agents.



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# Poster Communications



## Biochemical and proximate composition of Madeira Island custard apple (*Annona cherimola* Mill.) and its by-products

Gouveia C.S.S.,<sup>1,2,3\*</sup> Freitas G.,<sup>1</sup> Pinheiro de Carvalho, M.Â.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> Faculty of Life Sciences, University of Madeira, Portugal

<sup>3</sup> CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, Portugal

\*Corresponding author: [csgouveia@staff.uma.pt](mailto:csgouveia@staff.uma.pt)

The continuous search for nutritional sources for a healthy diet has aroused worldwide interest in the consumption of tropical fruits, such as custard apple (*Annona cherimola* Mill.). This fruit pulp has flavors from sweet to bittersweet, is low in calories with high content in water, and has a noticeable number of bioactive compounds. Originally from South America, the custard apple was introduced in Europe in the mid-18th century and later in Madeira Island (Portugal) at the end of the 19th century. Currently, the importance of this crop in Madeira Island has led to registration as “Anona da Madeira” in the National Catalog of Varieties of Fruit Species, and a protected origin denomination by the UE since 2000, which allows its differentiation and recognizes the excellence of this regional product. The custard apple is mainly produced locally for self-consumption in fresh or in cakes, ice creams, mousses, and liqueurs. The fruit peel was also used in natural medicine and applied in the form of porridge to the skin to reduce inflammation. Currently, it is the second most exported tropical fruit from Madeira Island, with average productivity of 12.4 t/ha. However, in these past few years, there is a slight decrease in production, mainly due to less precipitation in the winter/spring period, higher temperatures, and relative moisture below 70%. This leads to an increasing need to carry out a more detailed assessment of the regional custard apple edible part (pulp) and by-products, that can ensure the post-harvest quality for commercialization in the region. The peel of the fruit is thin, and it brings quality limitations in terms of exportation. Here, our main objective is to increase its economic valorization and bio-sustainability, by creating new market niches by differentiating the regional custard apple into flours as a new agri-food product, to facilitate exportation and consumption without compromising the quality of this fruit. We performed the harvest of eight Madeiran custard apple varieties for 2 distinct cycles and evaluated them for productivity and quality traits. The pulp and by-product peel were transformed in flours and were analyzed for physico-chemical, proximate, and antioxidant traits. Comparing both cycles for pulp and by-product peel, we registered trait changes with significant correlations. The 2nd cycle showed a decreased pH with increased acidity, and also a decreased glucose, protein, and ashes, with total phenolics content (TPC) increasing. Overall, the pulp had significantly higher glucose content, and kept its nutritional properties, keeping its quality for be applied in the pastry industry. And finally, considering the presence of a higher source of TPC, fiber, and ash content in the by-product peel, it could also be considered a good additive to be used in food products.

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## Biocoating application to extend the postharvest phase of custard apple (*Annona*) from Madeira

Ragonezi, C.,<sup>1,2\*</sup> Barbosa, C.<sup>1</sup>, Ramos, T.,<sup>1</sup> Pinheiro de Carvalho, M.Â.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira (UMa);

<sup>2</sup> Research Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro

<sup>3</sup> Faculty of Life Sciences, University of Madeira (UMa).

\*Corresponding author: Carla Ragonezi, carla.ragonezi@staff.uma.pt

Custard apple fruits are sources of carbohydrates, including fibers and pectin, protein, minerals, antioxidants, and other bifunctional compounds with health benefits. Despite those benefits, the custard apple is climacteric fruit with a quick ripening period, extremely perishable, have a short fresh consumption time window. Mainly due to these features, it is needed to develop strategies for the post-harvest stage optimization. One of these strategies is to delay the fruit ripening using biocoat, thus allowing to enlarge fresh consumption timeline. Custard apple is the 2<sup>nd</sup> most exported fruit from Madeira (Portugal). However, due to the limitation of fruit perishability is mainly commercialized in proxy markets, when a delay in ripening could allow exploring other markets. This work presents the preliminary results of biocoating application in 5 custard apple varieties to prolong the fruits' shelf-life, delay the ripening, and improve the post-harvest conservation, without influencing the quality of the fruits. Briefly, 13 fruits of each variety were collected, rinsed, and identified. Biocoating was applied to 3 fruits per treatment: (I) Control (immersion in water for 10 minutes), (II) agar (immersed, removed, and left to dry - repeated 4X), and (III) chitosan solution + citric acid (submerged for 10 minutes). After coating, fruits were placed in a growth chamber under controlled conditions (8°C and 90% RH) for 10 days, and then removed and kept at room temperature. At 2-day intervals, fruits were weighed and observed for browning. Data analysis was performed via the SPSS (Statistical Package for the Social Sciences) program. Data analysis related to browning showed that there were significant differences between the varieties. Among the treatments, there was only a significant difference between the agar treatment and the other two treatments (control and chitosan + citric acid) in the varieties *francesa* and *faial*. Data analysis related to weight showed that there were no significant differences between the varieties. However, in the *francesa* variety, there was a significant difference between the control treatment and chitosan + citric acid. *Francesa* is one of the custard apple varieties that was not yet cataloged and that can be better explored for production purposes. Other biocoatings need to test to achieve a more significant impact on delaying ripening, thus impacting the domestic market, but mainly on the fruit's exportation. Further, an evaluation of the fruit quality and nutritional features after the biocoating application, the expression patterns of several genes related to maturation, and an analysis of the activity of key enzymes for ripening are foreseen. Data obtained contributes to a wider project regarding custard apple valorization (fruits/by-products) and is embedded in the regional plan of action and RIS3-RAM strategy for bio-sustainability and circular economy. Our work provides important knowledge to support the exceptionality of these varieties, aiming to ensure the quality of the agri-food products commercialized.

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## Study of nutritional and biofunctional components of avocado (*Persea americana* Mill.) fruits from Madeira Island

Gonçalves, D.,<sup>1\*</sup> Gouveia, C.S.S.,<sup>1,2,3</sup> Freitas, G.,<sup>1</sup> Pinheiro de Carvalho, M.Â.A.,<sup>1,2,3</sup>

<sup>1</sup>ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira, Portugal.

<sup>2</sup>Faculty of Life Sciences, University of Madeira, Portugal.

<sup>3</sup>CITAB - Research Center for the Research and Technology of Agro-Environmental and Biological Sciences, Portugal.

\*Corresponding author: joao.d.goncalves@staff.uma.pt

Avocado (*Persea americana* Mill.) is a fruit native to South America, and currently represents the most important and popular tropical fruit in the world, due to its nutritional and chemical characteristics. Geographically, it is distributed worldwide, meanwhile in Europe, it is mainly produced in Spain, Greece, Italy, and Portugal. Portugal production of avocado is essentially made in Algarve and Madeira Island, owing to its Mediterranean temperate climate with short winters and long summers.

Globally, the most produced variety is the commercial “Hass” due to its facility in cultivation, which creates a problem, because these commercial varieties have been replacing the regional varieties, inciting the need to study the regional varieties and their value through a fully quality profile.

The aim of this project is the study the quality of few local avocado varieties from Madeira Island, in different cycles (harvest made in different years), from morphology and phenology to nutritional and biochemical traits, and to compare them with commercial varieties, using all parts of the fruit (pulp, peel, and seeds).

Our preliminary results in the physical-chemical analysis showed that pH and titratable acidity values did not varied much in both cycles, indicating good maturation for consume. The centesimal analysis showed that the crude fiber exists only in avocado peel, meanwhile in pulp and seed is just vestigial, with no difference in both cycles. Both protein and ash contents suffered small variations in both cycles, but not significantly. Glycose showed an increase in the 2nd cycle, mostly in the pulp, however it was not registered a significant difference in peel and seeds. The starch in seeds revealed a decrease from the 1st cycle to the 2nd cycle meanwhile it was not detected in the pulp and peel. Coloration values showed some little differences between 1st and 2nd cycles, which are expected because that color changes with environmental conditions and maturation. The samples were collected in different years with different ambiental factors, however, pulp colors stayed in green/yellow axis, meanwhile peel and seed colors stayed in red/yellow axis.

In short, there is some differences between 1st and 2nd cycles, probably because environmental conditions and different stages of maturation. However, more analysis will be performed to create a full profile about Madeira regional avocado varieties, mainly a fatty acid profile with Gas Chromatography.

### Acknowledgements:

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## Use of rhizospheric bacteria as growth promoters in lettuce crops and study of their effect on root ultrastructure.

Montesdeoca-Flores, D.,<sup>1,4\*</sup> Alfayate-Casañas, M.C.,<sup>3</sup> Hernández-Bolaños, E.,<sup>2,4</sup> Zuleima Estupiñan-Afonso, Z.<sup>3,4</sup> Abreu-Acosta, N.<sup>4</sup>

<sup>1</sup> Department of Botany, Ecology and Plant Physiology, Area of Plant Physiology, ULL, Spain.

<sup>2</sup> Department of Biochemistry, Microbiology, Cell Biology and Genetics, Microbiology Area, ULL, Spain.

<sup>3</sup> Department of Biochemistry, Microbiology, Cell Biology and Genetics, Cell Biology Area, ULL, Spain.

<sup>4</sup> Nertalab S.L.

\*Corresponding autor: dmontesd@ull.edu.es

Soil is an important component of all terrestrial ecosystems, as well as the main source of production in agriculture. Agricultural practices affect soil structure and function and the balance of ecosystems (Young and Crawford, 2004). Among these practices is fertilization, which consists of adding nutrients to the soil in a form that can be assimilated by plants, which, despite having low costs and producing high yields in the short term, in the long term can have serious social and environmental consequences on the ecosystem (Rocha et al., 2012)

An alternative to this practice is biofertilization, which seeks the use of plant and livestock remains that provide the soil with organic matter and nutrients, in addition to the use of microorganisms capable of metabolizing these by-products, thus facilitating their absorption by the root system of plants (Aguado-Santacruz, 2012).

The objectives of this work were to test the effect of the inoculation of rhizospheric bacteria obtained from a developed and healthy crop of lettuce (*Lactuca sativa* L. Var. Romana), exclusively or in combination with a SEFEL type liquid fertilizer (Acosta-Hernández 2013), on the development, yield, and nutritional content of a new crop of the same species. In addition, it was considered to study, at the light and electron microscopy level, how the applied treatments affect the roots, both tissue and cellularly, and what changes are produced when compared to the control roots.

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## Assessment of the capacity of the CAS-HDTMA reagent for the determination of siderophores in microbiological cultures

Montesdeoca-Flores, D.,<sup>1,4\*</sup> Hernández-Bolaños, E.,<sup>2,4</sup> Díaz-González, S.,<sup>3</sup> Hernández-Amador, E.,<sup>1</sup> Abreu-Acosta, N.,<sup>4</sup> Luis-Jorge, J. C.<sup>1</sup>

<sup>1</sup> Department of Botany, Ecology and Plant Physiology, Area of Plant Physiology, ULL, Spain.

<sup>2</sup> Department of Biochemistry, Microbiology, Cell Biology and Genetics, Microbiology Area, ULL, Spain.

<sup>3</sup> Department of Chemistry, Inorganic Chemistry Area, ULL, Spain.

<sup>4</sup> Nertalab S.L.

\*Corresponding autor: dmontesd@ull.edu.es

Siderophores are mainly non-ribosomal peptides synthesized by microorganisms and plants with a high affinity for iron and, to a lesser extent, other metals. As well, other iron chelating compounds are also included in this group. These chelating molecules facilitate the absorption and assimilation of these metallic elements that act as micronutrients, which can appear in oxidized forms not directly assimilated by organisms or at very low concentrations.

The Chrome Azurol S (CAS) together with Hexadecyltrimethylammonium Bromide (HDTMA) form the CAS-HDTMA reagent. This can bind to iron in the form of FeCl<sub>3</sub>, which gives rise to a blue-colored solution. When a chelating agent acts on CAS-HDTMA it sequesters the iron bound to the reagent, causing a change in the color of the solution that can be quantified by a decrease in absorbance at 630 nm.<sup>2</sup>

This is the most widely used reagent for the detection of siderophores in biological samples.<sup>3</sup> However, it is very sensitive to slight pH variations, and there is no defined and optimal value of this parameter in the reaction. The objective of this work was to verify the influence of pH on the reagent and subsequently the reaction in the presence of a chelating agent, ethylenediaminetetraacetic acid (EDTA).

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## A metabolomics approach to assess the combined toxicity of pesticides using *Lemna minor* L. as the model organism

Giaki, K.,<sup>1\*</sup> Papadopoulou, E.-A.,<sup>1</sup> Aliferis, K. A.<sup>1,2</sup>

<sup>1</sup> Laboratory of Pesticide Science, Agricultural University of Athens, Iera Odos 75, Athens, 118 55, Greece

<sup>2</sup> Department of Plant Science, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada

\*Corresponding autor: katerinagiakh@gmail.com

Plant protection products (PPPs) represent the backbone of the agricultural sector. Nonetheless, such dependance on the use of PPPs has come at a price; presence of residues in the environment and the food, and toxicity to non-target organisms, are major challenges for the agri-food industry.

Currently, there is an increasing interest in the toxicity of mixtures of bioactive compounds to target and non-target organisms. Such interest is based on the fact that organisms are often exposed to concentrations of various bioactive compounds simultaneously, with the knowledge relevant to the cumulative effect being largely fragmented, if non-existent.

In the above context, we have aimed to dissect the effect of selected individual active ingredients (a.i.) of PPPs exhibiting various mode(s)-of-action, as well as their mixtures using the aquatic plant *Lemna minor* L. as the model applying GC/EI/MS metabolomics. The a.i. metolachlor (herbicide), pyrimethanil (fungicide), and pirimiphos-methyl (insecticide) were studied.

The bioassays for the assessment of the toxicity of the a.i. revealed that metolachlor was the most phytotoxic, followed by pyrimethanil, and pirimiphos-methyl. Plants were then exposed to the individual a.i. at their respective EC<sub>50</sub> and binary mixtures of various ratios, in order to study their effects on the plants' metabolism applying metabolomics.

Multivariate analyses revealed a substantial effect of the treatments on the metabolomics of *Lemna* as confirmed by the corresponding obtained distinct metabolite profiles. Various metabolites that play key roles in plant responses to stresses were discovered as biomarkers of the observed toxicities. Overall, metolachlor and pyrimethanil had the highest effect on *Lemna*, while pirimiphos-methyl had the lowest. In the case of binary mixtures, those of pyrimethanil with pirimiphos-methyl caused the highest fluctuation of the plants' metabolomes.

Although the assessment of the combined toxicity of a.i. is a very challenging task, our study has further supported the potential of metabolomics in gaining insights into the underlying mechanisms, which could be of paramount importance in our effort to understand the toxicity of mixtures.

## A comparative study of the chemical and biological profile of wild and cultivated *Salvia canariensis*

Hernández-Álvarez, E.,<sup>1\*</sup> Rodríguez-Sabina, S.,<sup>2</sup> Gómez, D.E.,<sup>1</sup> Reyes, C.P.,<sup>3</sup> Giménez, C.M.,<sup>2</sup> Jiménez, I.A.,<sup>1</sup> Cabrera, R.P.,<sup>2</sup> Bazzocchi, I.L.<sup>1</sup>

<sup>1</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>2</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias Sección de Biología, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>3</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Bioquímica, Microbiología, Biología Celular y Genética, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain

\*Corresponding autor: alu0100947311@ull.edu.es

Fungal infections by ascomycetes are among the pests that threaten crops worldwide, highlighting the phytopathogens *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria alternata*, responsible for serious plant diseases in economically important crops. In recent years, various synthetic fungicides used to treat these infections have been withdrawn and/or banned in the European Union.<sup>1</sup> Furthermore, many target pathogens have developed resistance to some of the most important classes of fungicides. Therefore, there is a growing concern to develop alternatives to synthetic pesticides. In this regard, biopesticides represent promising candidates,<sup>2</sup> less persistence in the environment, and less toxic towards humans.

The Canary archipelago has a surprising ecological diversity due to its special geographical and climatic conditions, with a flora rich in endemism. These endemic plants could be potential sources of biofungicides, and a viable alternative to synthetic pesticides.

The present work reports a comparative study of the chemical and fungicide profile of wild (collected in Galdar, Gran Canaria) and cultivated (Centro Ambiental, La Tahonilla, Cabildo de Tenerife) *Salvia canariensis* L., an endemic species from the Canary Islands. Thus, *S. canariensis* leaves, wild and cultivated, were extracted by maceration and Soxhlet techniques, using different solvents. The ethanolic extracts, the most active ones, were further fractionated by liquid-liquid partition to obtain the water, hexanes and ethyl acetate fractions. All extracts and organic fractions, enriched in bioactive components, showed remarkable inhibitory activity against *B. cinerea*, *F. oxysporum* and *A. alternata*. This study reinforces the potential of *S. canariensis* as a promising source of botanical biopesticide against phytopathogenic fungi, and revealed its culture is an eco-friendly and sustainable alternative to its potential application in integrated crop protection plans.

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## Determination of the antifungal activity of actinomycetes strains against *Botrytis cinerea* and *Fusarium oxysporum*.

Hernández-Bolaños, E.,<sup>1, 3\*</sup> Montesdeoca-Flores, D.,<sup>2,3</sup> Abreu-Acosta, N.,<sup>3</sup> León-Barrios, M.,<sup>1</sup> Luis-Jorge, J.C.<sup>2</sup>

<sup>1</sup> Department of Biochemistry, Microbiology, Cell Biology and Genetics, Microbiology Area, ULL, Spain.

<sup>2</sup> Department of Botany, Ecology and Plant Physiology, Area of Plant Physiology, ULL, Spain.

<sup>3</sup> Nertalab S.L.

\*Corresponding autor: [eduardo.hernandez@ull.edu.es](mailto:eduardo.hernandez@ull.edu.es)

Actinomycetes are a wide group of Gram-positive prokaryotes, the soil being their most characteristic habitat, where they play important ecological functions. Their immense biotechnological potential as producers of bioactive molecules of great commercial and industrial interest is exemplified by most antibiotics for clinical use being derived from this group of bacteria (Hernandez-Bolaños et al., 2020).

On the other hand, phytopathogenic fungi, such as *Botrytis cinerea* and *Fusarium oxysporum*, are the most important cause of losses in agriculture. These two fungi are among the 10 most scientifically and economically important phytopathogenic fungi, causing serious damage both pre- and post-harvest, as they are necrotrophic fungi causing root, fruit and flower rot, vascular wilt and ultimately plant death (Fernandez-San Millan et al., 2021).

Currently, to fight phytopathogenic fungi, the most widely used are synthetic chemical fungicides to deal with post-harvest crop diseases. However, the excessive use of these synthetic fungicides has caused a great impact on human health, due to the presence of fungicidal residues in food and in the environment. In this context, natural products such as those produced by actinomycetes are an encouraging alternative to the use of synthetic chemical fungicides currently used to control phytopathogenic fungi (Parra-Amin et al., 2019).

The objective of this work was to verify in vitro the antifungal activity of 4 strains of actinomycetes against these phytopathogenic fungi, as well as an extract obtained from the liquid culture of the same strains, considering the possibility of future application in tests on plants and fruits.

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## Application of bioinformatics in the phytosanitaries discovery: Mining and selection of antimicrobial peptides.

Lobo, F.,<sup>1\*</sup> Pérez-De La Lastra, J.M.,<sup>2</sup> Boto, A.<sup>2</sup>

<sup>1</sup> Programa Agustín de Betancourt, Universidad de La Laguna, 38206-La Laguna, Tenerife, Spain

<sup>2</sup> Instituto de Productos Naturales y Agrobiología del CSIC (IPNA-CSIC), Avda. Astrofísico Fco. Sánchez, 3-38206-La Laguna, Tenerife,

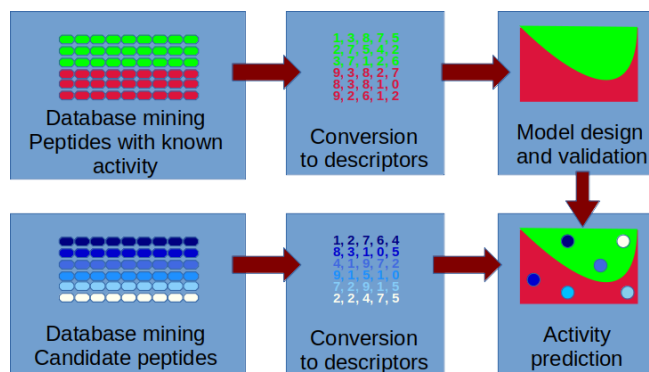
\*Corresponding author: flobopal@ull.edu.es

Bioinformatics is emerging as an useful tool in the development of new bioactive compounds with utility in the agrosanitary area.<sup>1</sup> We are particularly interested in the discovery of host-defense peptides (HDP) as new generation phytosanitaries. These compounds have been used by animals and plants during millions of years to defend themselves against pathogens, and unlike current phytosanitaries, have a very low induction of resistances.<sup>2</sup> Therefore, we are developing a platform that will facilitate the application of bioinformatic tools to the discovery of these antimicrobial peptides.<sup>1</sup>

In this communication, we show an example of the selection of host-defense peptides using a bioinformatic QSAR tool. To that end, we developed a machine learning model that predicts if a candidate peptide is likely to be bioactive as antimicrobial agent and useful in a potential application as phytosanitary.

As shown in the figure, this method involves six steps:

1. Mining of databases to select peptides with are known to be either bioactive or not.
2. Conversion of peptide sequences to numerical values using descriptors or sequence vectorization methods.
3. Design and validation of a machine learning model.
4. Mining of candidate peptides whose bioactivity will be predicted.
5. Conversion of candidate sequences to numerical values.
6. Prediction of the candidate bioactivity applying the validated method.



This method allows a quick and cost effective selection of peptides which reduces the amount of compounds to synthesize and assay in subsequent stages of phytosanitaries development.

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## MACBIOPEST: *Salvia canariensis*, a sustainable tool to replace synthetic pesticides in food production against phytopathogenic fungi

Jiménez, I.A.,<sup>1\*</sup> Gómez, D.E.,<sup>1</sup> Rodríguez-Sabina, S.,<sup>2</sup> Hernández-Álvarez, E.,<sup>1</sup> Llaría-López, M.A.,<sup>3</sup> Cabrera, R.P.,<sup>2</sup> Bazzocchi, I.L.<sup>1</sup>

<sup>1</sup> Instituto Universitario de Bio-Orgánica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>2</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias Sección de Biología, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>3</sup> Área de Medio Natural y Seguridad, C/ Las Macetas s/n, Pabellón Insular Santiago Martín, 38108 La Laguna, Tenerife, Spain.

\*Corresponding autor: ignadiaz@ull.edu.es

The indiscriminate use of chemical pesticides has led to many well-known problems, including resistance of pest species, products residues, toxicity to non-target organism, biodiversity loss, and acute or chronic poisoning to farmer, consumers, and the environment [1]. In this context, the use of biopesticides based on botanical preparation could be an eco-friendly alternative to traditional pesticides [2]. The purpose of MACBIOPEST project is development botanical preparations for crop protection using the Macaronesia biodiversity as platform. The project has allowed the screening of several plants and select some candidates to carry out a bioassay-guided isolation of crude extracts to target the active compounds.

The present communication describes the bioassay-guide fractionation of the ethanolic extract from leaves of cultivated *Salvia canariensis* (Labiatae) against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*, three of the most damaging fungi for the agricultural of the Macaronesian region. In this sense, the active EtOH extract was fractionated by liquid-liquid partition to yield hexane, ethyl acetate and waters fractions. The most active hexane fraction was further subjected to multiple chromatographic step on silica gel and Sephadex LH-20, including vacuum liquid chromatography, medium pressure liquid chromatography as well as preparative TLC and high-performance TLC, to yield the active compounds.

These results suggested that *S. canariensis* could be potential candidate for developing botanical preparations, providing new insight in the search for plant-derived biopesticides of crop pests management.

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## Brazilian flora, an alternative to pest management and sustainable agriculture

Aguilar, M. A.,<sup>1\*</sup> Jiménez, I. A.,<sup>2</sup> Bazzocchi, I. L.<sup>2</sup>

<sup>1</sup> Departamento de Química, Universidade Federal de Minas Gerais, Avenida Presidente Antonio Carlos 6627, 31270-901 Belo Horizonte, Minas Gerais, Brazil.

<sup>2</sup> Instituto Universitario de Bio-Orgánica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 3806 La Laguna, Tenerife, Spain.

\*Corresponding autor: marianag.a9@gmail.com

Brazil has a long history of the use of biological control (BC) of pests. The first attempt to use was reported in the 1930s, and the first successful case dates to 1967. For a long period, agrochemical products were the most widespread control measure among Brazilian growers. However, the growing popularity of sustainable food due to the increasing consumer concerns towards negative health impact of chemical pesticides is propelling the biopesticides demand. In fact, the culture of the growers is beginning to change, with more and more of them opting to use biological products, and definitely, Institutional support for environmentally friendly agricultural practices is a very important tool to promote their adoption and increase their efficiency among Brazilian rural producers.<sup>1</sup>

In line with this, the rising focus by the Ministry of Agriculture is promoting agricultural sustainability and maintaining environmental safety, and as a consequence the Brazilian biopesticides market showed strong growth during 2015-2020. Brazil ranks fourth in the biopesticide development ranking, second only behind USA, Spain, and Italy. By the year 2020, 29 biopesticides were registered in Brazil for the control of agricultural import pests, representing a 500% increase in the use of biopesticide formulations. Despite their increased, they are used only in a 5% of all crops.<sup>2</sup>

Brazil has the largest flora on the planet, housing a total of 49,990 species of which 2,113 are threatened with extinction. Nonetheless, a large portion of them has few or no studies on their potential as plant-derived biopesticides.<sup>3</sup> Some Piperaceae species, one of the largest families found in the Amazon Forest, showed potential as a promising source of biopesticides. Thus, the leaves extract of *Piper aduncum* presents a potent insecticidal activity against *Euschistus heroes*, a pest of soybean plantations. In addition, species of the Annonaceae family, including *Annona muricata* and *Annona squamosa*, are widely studied for their insecticide properties. Given the extraordinary richness of the Brazilian flora, and chemical diversity offered from nature, developing plant-derived biopesticides as an alternative to pest management and sustainable agriculture has remained challenging.

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## Fungicidal activity of compounds derived from the amino acid L-Valine against phytopathogenic fungus

González Hernández, S.,<sup>1,2\*</sup> González Martín, C.,<sup>1</sup> Luis Jorge, J. C.,<sup>2</sup> Montesdeoca Flores, D.<sup>2</sup>

<sup>1</sup>Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Tenerife, Spain

<sup>2</sup>Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Farmacia, Universidad de La Laguna (ULL), Tenerife, Spain

\*Corresponding autor: saragonzalezhernandez546@gmail.com

*Botrytis cinerea* is a phytopathogenic fungus that affects a wide range of plant species, being able to infect any of its parts (cotyledons, stems, leaves, fruits, etc.), during growth or in the post-harvest stage, causing enormous economic losses. It usually colonizes healthy, deteriorated or dead tissues and even acts as a secondary pathogen in plants that have already been infected by other organisms. It causes the disease commonly known as "gray rot" as it produces the browning of the tissue it infects and, subsequently, its death.

This species in particular, as well as the rest of those belonging to the genus *Botrytis*, attack mainly through three mechanisms, the release of enzymes that degrade the plant wall, the excretion of toxins causing the death of plant cells and the mechanical action of the hyphae on the epidermis of the plant species. Moreover, in most cases, a combination of these mechanisms of action occurs, making treatment and control more complex.

The study of this phytopathogenic fungus, as well as others with importance in agriculture and economy such as *Alternaria alternata* and *Fusarium oxysporum*, has been an area of interest for a long time. A wide variety of fungicides have been developed, but in practice, however, they are not very effective and, above all, have a great negative impact on the environment. Therefore, the use of biocontrol agents has been found to be more appropriate, for example, fungal species such as *Gliocladium roseum* and *Trichoderma harzianum* have an antagonistic effect on *Botrytis* or *Bacillus subtilis* against *A. alternata*.

This poster will show the preliminary results of the evaluation of different derivatives of the amino acid L-Valine, chemically modified, with the idea of finding new compounds that inhibit the growth of the fungi *B. cinerea*, *A. alternata* and *F. oxysporum*.

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## Biochar and vermicompost leachate combination as a strategy for crop improvement

Arco-Lázaro, E.,<sup>1\*</sup> and Raya, V.<sup>1</sup>

<sup>1</sup> Instituto Canario de Investigaciones Agrarias (ICIA). Finca La Estación. Lugar Finca San Antonio, 3. Santa Lucía de Tirajana (Gran Canaria), Spain.

\*Corresponding autor: earco@icia.es

The use of palm pruning biochar as a soil amendment, involves both the recovery of an organic resource and the soil improvement. Biochar is a carbonous material generated through a pyrolysis process, characterised by its high stable carbon content, pore volume and specific surface area, which give it a high water retention capacity and a favourable microenvironment susceptible to microbial colonization. However, the application of biochar does not provide available nutrients to the soil, so mixing it with nutrient-rich materials prior to its application could be a strategy to improve crop productivity. In this regard, earthworm leachate contains large amounts of plant nutrients and is normally diluted when applied, to avoid plant damage due to its high electric conductivity. The aim of this study was to evaluate the effect of soil application of biochar (10 tn·ha<sup>-1</sup>) amended with different dilutions of vermicompost leachate on a radish crop. The following treatments were tested: Control + (C+): no addition of biochar; Control: biochar with distilled water; BL1: biochar with 75% diluted leachate; BL2: biochar with 50% diluted leachate; BL3: biochar with 25% diluted leachate; and BL4: biochar with undiluted leachate. Highest radish fresh weight were obtained in treatments BL2 and BL3, in relation to the rest of the treatments, showing a negative effect of biochar amended with undiluted leachate, possibly associated with its high content of elements such as sodium. Therefore, biochar nutrient enrichment with earthworm leachate is a simple practice that can contribute to increase soil fertility and crop productivity. Depending on its composition, it will be convenient to work with different leachate concentrations to avoid crop problems derived by its high electric conductivity.

### Acknowledgements:

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## Preparation of libraries of diketopiperazine derivatives in the search of new biostimulants

Saavedra, C. J.,<sup>1,2</sup> and Boto, A.<sup>2</sup>

<sup>1</sup>Programa Agustín de Betancourt, Departamento de Química Orgánica de la Universidad de La Laguna, 38206-La Laguna, Tenerife, Spain.

<sup>2</sup>Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico Fco. Sánchez, 3-38206, La Laguna, Tenerife

Although most of the work on the bioactivity of 2,5-diketopiperazines<sup>1</sup> has been carried out in biomedicine,<sup>1</sup> applications of interest in agriculture have recently been described, mainly as antifungals and herbicides (as shown in Figure 1),<sup>1,2</sup> but also as antifouling agents.<sup>3</sup>

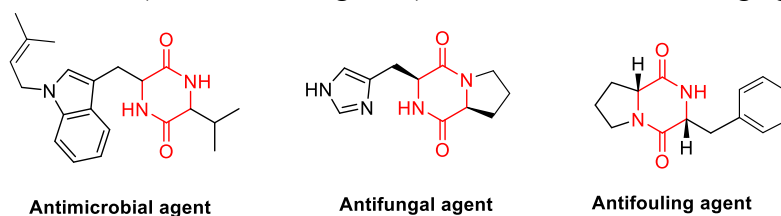


Figure 1

However, the use of diketopiperazines to induce tolerance against abiotic stresses or resistance against pathogens has been scarcely studied.<sup>4</sup> We report herein the preparation of a library of 2,5-diketopiperazines with a variety of  $\alpha$ -chains, including aliphatic, aromatic, polar and cationic substituents (some examples shown in Figure 2). The rationale for the design of the library and the efficient procedure to prepare these compounds will be commented. A detailed discussion of their biological evaluation is presented in the short talk “*Libraries of host-defense peptides in the search of new phytosanitarios with low induction of resistances*”

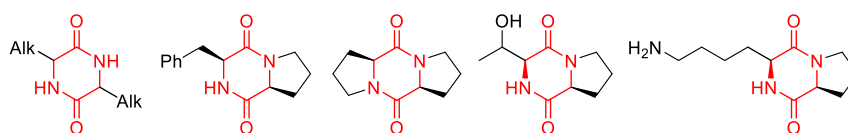


Figure 2

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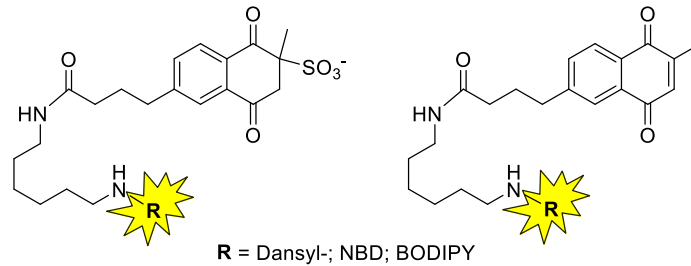
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## Preparation of new Fluorogenic Vitamin K Analog Probes to Untangle Their Biological Function in Plants

Pérez-Martín, I.,<sup>1</sup> Jiménez-Arias, D.,<sup>1</sup> Herrera, A.J.<sup>1\*</sup>

<sup>1</sup>Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico Fco. Sánchez, 3-38206, La Laguna, Tenerife

\*Corresponding author: ajherrera@ipna.csic.es



Menadione sodium bisulfite (MSB) has been used in plants as an elicitor against parasitic stress (1-4) as well as a priming agent for improving salt tolerance (5-8), both by root and foliar treatments. MSB is a water-soluble form of menadione (MND), also known as vitamin K<sub>3</sub>, which belongs to the Vitamin K class of compounds.

Herein, we present the design and synthetic approach toward fluorogenic vitamin K analog probes to mimic their original activity and untangle their biological function, both from a plant and cellular perspective. The establishment of a possible biological relationship between MSB and MND is pursued.

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## Isolation, characterization, and identification of plant growth promoting bacterial isolates from agriculture soils of Tenerife

Hernández-Amador, E.,<sup>1,3\*</sup> Mascareño-Pérez, R.,<sup>1</sup> Baquero-Machado, M.,<sup>1</sup> González-Hernández, S.,<sup>1,2</sup> Abreu-Acosta, N.,<sup>1,3</sup> Luis-Jorge, J.C.<sup>1</sup>

<sup>1</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, Spain

<sup>2</sup> Instituto de Productos Naturales y Agrobiología (IPNA), Spain

<sup>3</sup> Nertalab S.L, Spain

\*Corresponding autor: ehernana@ull.edu.es

The use of fertilizers and pesticides has meant an improvement of quality and quantity in the agriculture output. Nevertheless, its long-term application has produced environmental contamination in soils, waters, and sediments, threatening all kinds of life on earth. Therefore, phytopathogenic fungi, such as *Botrytis cinerea* and *Fusarium oxysporum*, are one of the main causes in agriculture losses. These fungi produce numerous diseases pre- and post-harvest, such as root, fruit and flower rot, vascular wilt and finally plant death (Fernandez-San Millan et al., 2021).

As a result of these problems, the use of plant-growth promoting bacteria (PGPB) has become the main alternative to reduce the synthetic chemicals (Goswanu et al., 2020). The Bacillus species have become the most common PGPB. These bacteria have probed their traits in phosphate and potassium solubilization, nitrogen fixation, phytohormone production, siderophore production and antifungal activity (Shafi et al., 2017).

The current study was carried out to assess the potential of native microorganisms from agricultural soils to plant growth promotion and development of a future biofertilizer. Biochemical characterization and antagonistic activity were performed with the aim of determining which bacterial strains present PGPB and biocontrol attributes. Once the characterization results were obtained, a couple of the bacteria were genetic analyzed to identify them.

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## Fluorides and nitrates as sustainable indicators of marine pollution in the Canary Islands

García-Báez, J.,<sup>1,2\*</sup> Niebla-Canelo, D.,<sup>1,2</sup> Alejandro-Vega, S.,<sup>1,2</sup> Gutiérrez-Fernández, A.J.,<sup>1,2</sup> Rubio-Armendáriz, C.,<sup>1,2</sup> Hardisson, A.,<sup>1,2</sup> Paz-Montelongo, S.<sup>1,2</sup>

<sup>1</sup>Área de Toxicología, Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, España.

<sup>2</sup>Grupo interuniversitario de Toxicología Alimentaria y Ambiental, Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, España

\*Corresponding autor: alu0101226552@ull.edu.es

Since the 20th century, coinciding with the industrial revolution, there has been an increase in the deposition and leaching of pollutants into the marine environment, with serious consequences for the marine environment. This pollution produces a phenomenon called eutrophication, which is an enrichment of nutrients, mainly nitrogen and phosphorus. It can occur naturally, but it is gradual and slow over time, unlike anthropogenic eutrophication, causing an accelerated increase in the production of organic matter and decreasing the concentration of oxygen in the water, leading to anoxic conditions. In addition, it favours the development of dinoflagellates, capable of forming toxic compounds that, through biomagnification in the trophic chain, can produce adverse effects on human health through the seafood consumption (Luque, 2004). The main nutrients that limit this phenomenon are nitrogen and phosphorus, although there are also others that could be relevant, such as fluoride. Therefore, the aim of this study is 1) to determine the content of nitrate ( $\text{NO}_3^-$ ) and fluoride (F) in intertidal water samples and 2) to verify if there is a relationship between fluoride and nitrate as a possible indicator of eutrophication.

A total of 124 samples were taken on the island of Tenerife (Canary Islands). Nitrate analysis was carried out by UV-Vis spectrophotometry. The determination of fluorides was carried out by fluoride ion selective potentiometry.

The nitrate content between the sampling areas showed significant differences ( $p < 0.05$ ). However, the fluoride content only shows significant differences ( $p < 0.05$ ) between the metropolitan and northern areas. In addition, it is worth noting that the northern area recorded the highest nitrate concentrations ( $2.28 \pm 0.16$  mg/L), which may be due to the intense agricultural activity in this area. As for fluoride, the highest concentrations have been reported in the metropolitan area ( $2.31 \pm 0.60$  mg/L). A correlation study (Pearson's study) has been carried out showing a negative correlation ( $r = 0.160$ ) between fluoride and nitrates. This shows that the higher the fluoride concentration, the lower the nitrate levels. This may be due to the strong activity of fluoride as an enzyme inhibitor and inhibitor of bacterial metabolism (Zhang et al., 2019). This relationship will be further studied to demonstrate the usefulness of fluoride ion as a possible indicator of eutrophication.

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## Avocado Water Footprint in the Canary Islands

Cruz-Pérez, N.,<sup>1\*</sup> Álvarez-Acosta, C.,<sup>2</sup> Gamallo, I.,<sup>1</sup> and Santamarta, J.C.<sup>1</sup>

<sup>1</sup> Universidad de La Laguna, Departamento de Ingeniería Agraria y del Medio Natural, Spain.

<sup>2</sup> Instituto Canario de Investigaciones Agrarias (ICIA). Departamento de Producción Vegetal en Zonas Tropicales y Subtropicales, Spain.

\*Corresponding autor: nacruzper@ull.edu.es

Most of the water resources produced in the Islands are used for agriculture, in some cases more than 80% of the water. Groundwater is the basic water resource for agricultural activity in the Canary Islands, although the industrial production of seawater (desalination) is increasing, especially on islands such as Fuerteventura and Lanzarote. This is why it is particularly important to know the water footprint of the main crops in the Canary Islands, in order to be able to take strategic measures to save agricultural water in the archipelago.

The avocado is positioning itself as a highly demanded food worldwide. In the Canary Islands it has doubled the cultivated area reaching around 2.000 ha in 2020. In order to calculate the water footprint, the methodology proposed by the Water Footprint Network was applied, taking data from the nearest weather stations proposed by the Spanish Network of Agroclimatic Information System for Irrigation (SiAR), to obtain the green water footprint.

For the blue water footprint, although it has yielded values above the world's average footprint for avocado of 237 m<sup>3</sup>/t (ranging from 0 to 2.295 m<sup>3</sup>/t), alternate bearing in avocado and different evapotranspiration areas need to be considered, in order to compare the efficient use of water.

Also, the irrigation systems of farmers on the islands have been analyzed, and conclusions have been drawn regarding the type of irrigation and the actual irrigation value.

## Three seasons agronomic study of lettuce and chemical analyses of soils after prolonged cheese whey based digestate fertilization

Álvarez-Méndez, S. J.,<sup>1,2\*</sup> Ramos-Suárez, J. L.,<sup>2</sup> Ritter, A.<sup>3</sup> and Mata González, J.<sup>2</sup>

<sup>1</sup>Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, Spain

<sup>2</sup>Departamento de Ingeniería Agraria y del Medio Natural, Universidad de La Laguna, Spain

<sup>3</sup>Área de Ingeniería Agroforestal, Universidad de La Laguna, Spain

\*Corresponding autor: salvmen@ull.edu.es

In terms of circular economy and sustainable agriculture, fertilizers obtained from organic wastes exhibit an added value. A digestate obtained from the anaerobic digestion of an industrial cheese whey was successfully employed as fertilizer in romaine lettuce (*Lactuca sativa* L var. *longifolia*) grown in pots during 3 consecutive seasons in San Cristóbal de La Laguna (Canary Islands, Spain). In summer 2021, 16 digestate-treated lettuces showed a 4-fold increase in harvested weight when compared to 16 control lettuces. From this experiment a set of 16 pots with watered soil (WS) and 16 with soil fertilized with digestate (DS) were available for the next growing season. In autumn 2021, 32 new lettuces were transplanted into both pot types and treated with control water or with digestate (8 lettuces per soil and treatment). On the one hand, comparing the lettuces which were only irrigated, a 1.4-fold fresh weight increase was found in lettuces cultivated on DS vs. WS. On the other hand, the fresh weight of both lettuces' groups cultivated on WS and DS and treated with digestate doubled the fresh weight of irrigated WS-grown lettuces. After this second seasonal study, 4 different soils were obtained: soils watered during summer and autumn (WWS), soils watered in summer and treated with digestate in autumn (DWS), soils treated with digestate in summer and watered in autumn (WDS), and soils treated with digestate in both seasons (DDS). In spring 2022, additional 32 plantlets were transplanted into these soils in sets of 8 plants and treated with control water to yield a third lettuce harvest and four final soils (WWWS, WDWS, WWDS and WDDS, respectively). Harvests from soils treated with digestate in autumn (i.e., DWS and DDS) were identical each other and significantly higher than harvests from WWS and WDS (1.3-fold). Furthermore, soil chemical analyses revealed higher values of P, Ca, Mg and K in WDWS and WDDS vs. WWWS. Thus, the benefits of the digestate fertigation for lettuce cultivation remains even beyond its first application.



Figure 2. Second season lettuces irrigated with control water and cultivated in soils which came from water irrigation (left) or digestate fertilization (right) in the previous season.

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## Study of proximate composition and phytonutrient properties of cactus pear (*Opuntia ficus-indica* (L.) Mill.) cladodes and fruits

Gouveia C.S.S.,<sup>1,2,3\*</sup> Gomes G.M.F.,<sup>4</sup> Rodrigues M.,<sup>4</sup> Nunes R.,<sup>4</sup> Ragonezi C.,<sup>1,3</sup> Pinheiro de Carvalho M.Â.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> Faculty of Life Sciences, University of Madeira, Portugal

<sup>3</sup> CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, Portugal

<sup>4</sup> Regional Secretary of Agriculture and Rural Development, Portugal

\*Corresponding author: csgouveia@staff.uma.pt

The cactus pear, *Opuntia ficus-indica* (L.) Mill., endemic to America, is used for food consumption and livestock feed. The interest in this crop is currently increasing, due to its good adaptation to arid lands, heat tolerance, and low water requirements, as it is expected to worsen the effects of drought weather events, leading to lower levels of water availability, accelerating degradation and dryness of the soil and, consequently, the desertification of arable land. In the Madeira Archipelago (Portugal), the cactus pear is a “marginalized” crop, considered invasive, and fully embedded into the landscape, growing mainly in semi-arid areas that are considered inadequate for agriculture practice. This crop contributes to environmental sustainability by sequestering atmospheric carbon and has some economic interest in fruit production which is appreciated by local consumers. However, its consumption is still underexplored and undervalued. Here, our main objective is to valorize the local cactus pear crop by creating new and more attractive food products destined for use in the local agri-food industry, by adding value and food differentiation. Flours from cladodes and fruit from 3 ecotypes from Madeira Island were evaluated for biomass production, yield, and quality. Among ecotypes, the cladodes showed significant differences in the proximate composition (ash, protein, carbohydrates), phytonutrient properties (total phenolics), and photosynthetic pigments fraction (total carotenoids, chlorophyll *a*). The biomass showed a negative significant correlation between protein, total carotenoids, and chlorophyll *a*. The fruit maturation showed a significant decrease in ashes, starch, and total phenolics, with the peel showing higher values than the pulp. Meanwhile, glucose and total carotenoids increased significantly with maturation, with the pulp showing higher glucose content and the peel upper total carotenoid content. This preliminary study showed that the cactus pear cladodes and pulp fruit flours demonstrated a vital source of compounds with good nutritional components and antioxidant activity, showing that this crop can be consumed in the form of flour, or it can also be added to other food products. The fruit peel can also be used as a by-product source of carotenoids that act as free-radical scavengers and fibers conferring nutraceutical properties. To conclude, the flour transformation of the cactus pear cladodes and fruits can be a good quality food source. This new food application could generate crop added value, being more attractive to commercialization, leveraging the profitability of the producers, and ensuring environmental sustainability by expanding the plantation area of this drought-tolerant crop in rough areas not applied for agriculture.

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## Screening of functional genes in a collection of fungi isolated from agrosystems under monoculture and polyculture

Oliveira C.<sup>1,3\*</sup>, Pinheiro de Carvalho M.Â.A.<sup>1,2</sup>, Alves A.<sup>3</sup>

<sup>1</sup> ISOPlexis - Center for Sustainable Agriculture and Food Technology, University of Madeira (UMa),

<sup>2</sup> Research Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB).

<sup>3</sup> Biology Department, University of Aveiro

\*Corresponding autor: maria.oliveira@staff.uma.pt

Soil fungi play a central role in agroecosystems. They may contribute to a variety of ecosystem functions such as organic matter decomposition, nutrient delivery for plant growth and carbon storage. However, fungal communities' structure is influenced by several factors, including soil physicochemical factors, which in turn are affected by geological and climatic factors and land use. Thus, understanding the relationship between functional diversity of soil fungi and agroecosystems dynamic is a key issue.

This work presents the preliminary results for search of the target genes related to soil fungal functions. A fungi collection isolated from 18 mono and polyculture agrosystems, including vineyards, banana plantation and horticulture plantation were used.

A total of 114 isolated fungi were obtained and stored at 4°C (active collection) and at -80 °C for long-term storage. DNA was extracted from all isolates and ITS region was amplified through PCR and sequenced for species identification. The DNA samples were also used for amplification of functional target genes. A total of 4 target genes related to organic matter degradation, antagonism and nitrogen cycling were already screened.

Data analysis shows differences in functional fungal communities between mono and polyculture agrosystems. Communities from horticulture plantations seem to be more diverse and more likely to harbour genes involved in organic matter degradation. This means that fungal communities from polyculture may participate more actively in this ecosystem function than in monoculture.

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## The chemical composition of ethanolic extracts with potential biopesticide effects

Seca A.M.L.,<sup>1,2\*</sup> Viveiros M.M.,<sup>1</sup> Tavares W.R.,<sup>1</sup> Barreto M.C.<sup>1</sup>

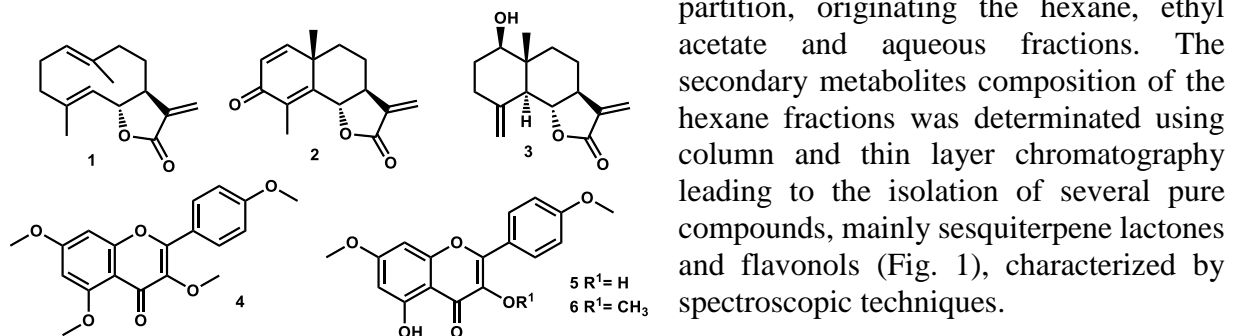
<sup>1</sup> cE3c- Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Portugal

<sup>2</sup> LAQV-REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal

\*Corresponding autor: ana.ml.seca@uac.pt

The emergence of insect pests in crops represents a serious problem, since they decrease the yield of production, leading to considerable losses of time and money invested. Research already carried out show that nature is a privileged source of plants with significant biopesticide effects, as shown by their extracts.<sup>1</sup> It is the case of *Laurus azorica* (Seub.) Franco (endemic) and *Hedychium gardnerianum* Sheppard ex Ker Gawl. (invasive), whose biopesticide potential against the Mediterranean fruit fly (*Ceratitis capitata*) was recently assessed (see short lecture, M<sup>a</sup> Carmo Barreto). However, little is known about the chemical composition of these two species.

The maceration of the dried aerial parts of the *Laurus azorica* and *Hedychium gardnerianum* with ethanol 96%, provided the ethanolic extracts. Each one was fractionated by liquid-liquid partition, originating the hexane, ethyl acetate and aqueous fractions. The secondary metabolites composition of the hexane fractions was determined using column and thin layer chromatography leading to the isolation of several pure compounds, mainly sesquiterpene lactones and flavonols (Fig. 1), characterized by spectroscopic techniques.



**Fig. 1:** Chemical structure of some compounds isolated from *Laurus azorica* and *Hedychium gardnerianum* ethanolic extracts.

Compounds 4, 5 and 6 may be related to the near absence of pests in the species *H. gardnerianum*, since the pesticidal action of flavonoids such as kaempferol and quercetin is reported in the literature.<sup>2</sup> More details about this work will be presented and discussed.

### Acknowledgements:

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## *Salvia canariensis* constituents as potential biopesticides in the control of phytopathogenic fungi

Gómez D.E.,<sup>1\*</sup> Rodríguez-Sabina S.,<sup>2</sup> López-Cabeza R.,<sup>3</sup> Reyes C. P.,<sup>4</sup> Giménez C. M.,<sup>2</sup> Jiménez I. A.,<sup>1</sup> Cabrera R P.,<sup>2</sup> Bazzocchi I. L.<sup>1</sup>

<sup>1</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Química Orgánica, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>2</sup> Facultad de Ciencias Sección de Biología, Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

<sup>3</sup> Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina Mercedes 10, 41012, Sevilla, Spain.

<sup>4</sup> Instituto Universitario de Bio-Organica Antonio González, Departamento de Bioquímica, Microbiología, Biología Celular y Genética, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain.

\*Corresponding autor: mgomezex@ull.edu.es

*Salvia canariensis* L. is an endemic plant of the Canary Islands widely used in the popular medicine of the Archipelago. Previous phytochemical studies have been reported the isolation of diterpenoids as the main components in this species, but also triterpenoids and essential oils from different parts of the plant [1].

In the search for eco-friendly solutions to control pests, the interest in plants and their chemobiodiversity as source of biopesticides has increased over the time. Plants are able to biosynthesize a number of metabolites with bactericidal, fungicidal, insecticidal, nematocidal or repellent activity to defend themselves from predators and diseases. Therefore, these plant-derived pesticides may be a green alternative as compared to synthetic ones since they are less toxic, biodegradable, environment friendly and safe to humans' health [2].

The present work aims to investigate the potential of *S. canariensis* as a source of biopesticides, offering an alternative to hazard pesticides, and which can be used in an integrated pest management. A bio-guided fractionation of ethanolic extract of *S. canariensis* leaves was carried out based on fungicidal activity against *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria alternata*, phytopathogens affecting crops worldwide, and causing important economic losses due to reduce yield and quality of food products [3]. Biological evaluation revealed that organic fractions were active against the three selected fungi, and were further investigated. Five diterpenoids and one triterpenoid were identified as promising biopesticide components in this plant.

**Acknowledgments:** This research was supported by Cooperation Program INTERREG V-A MAC 2014-2020, cofunded by FEDER (MACBIOPEST Project, MAC2/1.1a/289).

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## Assessing the effect of the application of aqueous extracts from four marine macroalgae in the development of maize and tomato plantlets under water stress.

Ganança, J.F.T.,<sup>1\*</sup> Ferraz, S.,<sup>1</sup> de Nóbrega, H.G.M.,<sup>1</sup> Antunes, G.N.,<sup>1</sup> Nunes, N.,<sup>1,3</sup> Pinheiro de Carvalho, M.Â.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis Centre Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> Faculty of Life Sciences, University of Madeira, Portugal

<sup>3</sup> CITAB, Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, Portugal

\*Corresponding autor: jofitei@staff.uma.pt

The application of bio stimulants, including algae extracts, is one of the most promising strategies to induce protective responses against drought in plants. The effect of various concentrations of aqueous extracts from *Sargassum vulgare*, *Halopteris scoparia*, *Grateloupia lanceola* and *Nemalion elminthoides*, harvested from Madeiran Sea waters, in the growth of maize and tomato plantlets, was tested.

Seeds were germinated on germination substrate, in 5 racks with 24 individual alveoli (1 seed per alveolus), from 7 to 10 days. These plantlets were then grown under water stress for 7 days, under controlled conditions. Experiment was run in duplicate, and plantlets were irrigated daily with Hoagland solution. One rack was maintained fully irrigated (control), while 4 were irrigated with 25% of the volume used in the control. Three of these racks were treated with 1%, 5% and 10% of the aqueous algae extract, 24 hours prior to stress imposition, and one of the racks was maintained as treatment control. Extracts were produced using high pressure liquid extractor. Ten plantlets were harvested at stress imposition, and ten plantlets per treatment were harvested after 7 days. Root and shoot length and dry weight were recorded. Relative growth rate (RGR), Relative Water Content (RWC) and Stress Tolerance Indices (STI) were calculated.

The stress significantly reduced plantlets RGR and biomass accumulation during the experiments, in both studied crops. All the extracts showed some, although seldom significant, protective effect in the crops. In maize, *S. vulgare* extract had consistently the better effect in stimulating plantlets growth and biomass accumulation under stress, followed by *N. elminthoides*, both at 10% concentration. *G. lanceola* showed the smallest effect. In tomato, *S. vulgare* also showed to be overall the best protective extract, having almost the same effect independently of the used concentration, and being effective in most of the studied parameters. Interestingly, however, 1% *N. elminthoides* extract showed the best protective effect in the parameter shoot biomass, while 10% *N. elminthoides*, for example, was prejudicial to plant biomass accumulation. On the other hand, *G. lanceola* and *H. scoparia* showed the best results in shoot length. Therefore, tomato plantlets showed a more complex pattern of responses to the extract application than maize. We hypothesize that extracts provide some protective effect, but role and mechanism of action shows species and plant organ specificity.

## Detection of Acetic Acid Using an Optical Fiber Sensor

Freitas A.I.,<sup>1, 2\*</sup> Bierlich J.,<sup>3</sup> Kobelke J.,<sup>3</sup> Marques J.C.,<sup>1, 2</sup> Ferreira M.S.<sup>2, 4</sup>

<sup>1</sup> Faculty of Exact Sciences and Engineering & ISOPlexis – Center for Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> i3N, University of Aveiro, Portugal

<sup>3</sup> Leibniz Institute of Photonic Technology IPHT, Germany

<sup>4</sup> Department of Physics, University of Aveiro, Portugal

\*Corresponding autor: ana.isabel.freitas@staff.uma.pt

Food security and safety are an issue of growing concern to our society. Acetic acid is a compound that is often used as a food preservative and additive, but it is also produced during food processing and constitutes an important marker of food safety and quality. Traditionally, enzymatic reactions, chromatography, and spectroscopy are used in the analysis of acetic acid. However, while accurate, sensitive, and selective, these methods are also costly and time-consuming. Optical fiber sensors have emerged as a promising and versatile alternative for the detection of food contaminants, with several advantages over traditional methods, providing fast and sensitive responses, while remaining relatively small and inexpensive.

In this work, we propose a balloon-shaped optical fiber sensor, consisting of a section of capillary tube spliced between two sections of single-mode fiber, for the simultaneous measurement of refractive index and temperature. The sensor was characterized in regard to its response to the refractive index, using thirteen aqueous solutions of increasing concentrations of acetic acid in the range of 0 to 60% (v/v), and to its response to temperature in the range between 22.7 and 48.0 °C. A sensitivity of 5 pm/°C was attained for temperature. Measurements were performed at 1594.8 nm, providing a maximum sensitivity of 181.2 nm/RIU for the refractive index variations, with a resolution as low as  $1.93 \times 10^{-2}$  % (v/v) of acetic acid.

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## Food security assessment of pesticides residues in the scope of the PERVEMAC II Project. Wines commercialized in the Canary Islands 2017-2020

Santana-Mayor, Á,<sup>1\*</sup> Rodríguez-Ramos, R.,<sup>1</sup> Socas-Rodríguez, B.,<sup>1</sup> Rodríguez-Delgado, M.A.<sup>1</sup>

<sup>1</sup> *Departamento de Química, Unidad Departamental de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL). Avda. Astrofísico Francisco Sánchez, s/n 38206 San Cristóbal de La Laguna (Tenerife), Spain*

\*Corresponding autor: asantanm@ull.edu.es

Food safety is important because of the need to ensure the protection of human health by consuming high-quality foodstuffs and establish global connections. In this sense, ensuring the correct handling and storage of foodstuff, as well as minimizing or eliminating food-borne illnesses are considered crucial issues<sup>1</sup>.

The control of contaminants, such as pesticide residues is one of the main challenges in this area, due to their potential health risk.<sup>1</sup> In addition, different regulatory authorities, including the European Commission (EC) and the United States Food and Drug Administration (FDA) have established monitoring plans and different directives that regulate this issue, in order to protect consumer safety and ensure products quality. A clear example is the abusive use of pesticides as a measure against pests that attack different crops. For these reasons, it is fundamental to the development of analytical methodologies that allow monitoring these compounds, and guarantee safety in food consumption.

For this reason, in this work, an analytical method has been applied for the determination of pesticide residues in wines from different origins commercialized in the Canary Islands, using both gas and liquid chromatography coupled to mass spectrometry in combination with the QuEChERS method<sup>2</sup> as extraction procedure. The method was validated following the SANTE European guidelines.<sup>3</sup> In this study, 243 wine samples acquired in the Canary Islands between 2017 and 2020 were analyzed. Finally, a non-accumulative risk assessment was carried out following the margin of safety approach,<sup>4</sup> finding that in general terms the consumption of these cereals does not entail a risk.

**Acknowledgements:** This work has been supported by the European Project Pervemac II (Mac/1.1a/049). Á.S.M. and R.R.R. would like to thank their FPI fellowships co-financed by Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Conocimiento y Empleo y Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%). The authors would like to acknowledge the use of the Research Support General Service (SEGAI) of the University of La Laguna.

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## Lead levels in cereals and cereal-based products consumed in Macaronesia

Niebla-Canelo, D.,<sup>1\*</sup> Alejandro-Vega, S.,<sup>1</sup> Gutiérrez-Fernández, A. J.,<sup>1,2</sup> Paz-Montelongo, S.,<sup>1,2</sup> Revert-Girones, C.,<sup>1,2</sup> Gonzáles-Weller, D.,<sup>1,2</sup> Hardisson, A.,<sup>1,2</sup> Rubio-Armendáriz, C.<sup>1,2</sup>

<sup>1</sup>Grupo interuniversitario de Toxicología Alimentaria y Ambiental, Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, España; <sup>2</sup>Proyecto MAC-Pervemac2 (MAC/1.1a/049)

\*Corresponding autor: alu0100798203@ull.edu.es

Lead, in its inorganic species, is classified as a potentially carcinogenic substance in humans (IARC Group 2A). Likewise, it is known for its hematological, gastrointestinal, cardiovascular (BMDL: 1.50 µg/kg bw/day), renal (BMDL: 0.63 µg/kg bw/day) and nervous (BMDL for neurodevelopmental effects: 0.50 µg/kg bw/day) toxicity. Among the different Pb exposure sources for humans, diet stands out. Cereals and cereal products represent, according to EFSA, the group with the highest exposure to Pb due to their high consumption rather than potential contamination. To minimize the risks from dietary exposure to Pb, Commission Regulation (EU) 2021/1317 sets a maximum permitted Pb level of 0.20 mg/kg fresh weight for cereals and 0.020 mg/kg fresh weight for cereal-based products.

To determine the Pb content in cereals and cereal-based products consumed in the Macaronesia's archipelagos (Canary Islands, Cape Verde, Azores and Madeira) participating in the MAC-PERVEMAC2 project (MAC/1.1a/049) entitled *Research and Development Cooperation Project in the field of Agriculture and Food Security*.

Materials and method: 544 samples were collected from Cape Verde (228); Canary Islands (216); Madeira (60); Azores (40). According to the type of matrix, a total of 335 samples corresponded to cereal grains (162 maize, 124 rice, 31 wheat), 126 flour samples and 83 gofio samples. Samples were digested in acid medium by microwave digester and Pb content was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Conclusions: Lead is a toxic food contaminant that poses risks to the consumers' health. Based on the levels of Pb observed in cereals and cereal-based commercialized in the Macaronesia region, there is still a need for a continuous follow-up of the dietary exposure to this toxic metal from these and other food groups. Likewise, further progress should be made for a coordinated management and communication of this pollutant in the region.

**Acknowledgements:** This research was funded by PERVEMAC II: Programa de Cooperación INTERREG V-A España-Portugal MAC (Madeira-Azores-Canarias) 2014–2020, grant No. MAC/1.1a/049, project “Sustainable Agriculture and Food Security in Macaronesia: Investigation of the benefits and risks of the intake of plant products for the health of consumers and development of minimization strategies.”

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## Anti-penicillium activity of essential oils from fresh or dried leaves of *Cymbopogon citratus* (DC) Stapf.: A comparative study

Lima, A.,<sup>1,2</sup> Arruda, F.,<sup>1,3,\*</sup> Baptista, J.,<sup>1,2</sup> Medeiros, J.,<sup>2</sup> Fernandes-Ferreira, M.,<sup>4</sup> Lima, E.<sup>1,2</sup>

<sup>1</sup> Institute of Agricultural and Environmental Research and Technology, University of Azores, Portugal

<sup>2</sup> Department of Physics, Chemistry and Engineering, University of Azores, Portugal

<sup>3</sup> Department of Biology, University of Azores, Portugal

<sup>4</sup> Department of Biology, Faculty of Science, Centre for the Research and Technology of Agro-Environmental and Biological Sciences-GreenUP, University of Porto, Portugal

\*Corresponding autor: filipe.mp.arruda@uac.pt

Postharvest fungal diseases are among the significant factors that limit the storage period and shelf life of fruits, and even result in serious economic losses worldwide. In particular, *Penicillium* spp. are the main fruit pathogens, which can compromise human health due to the production of mycotoxins.

In order to manage postharvest diseases and reduce the use of synthetic fungicides, extensive research has been carried out to find environmentally safe fungicides. Plant-derived products, such as essential oils (EOs), are promising alternatives since they are less hazardous to mammals, easily biodegradable, and less susceptible to the development of resistance, as compared to their synthetic pesticides equivalents.

However, as well known, the yield, chemical composition and bioactivity value of EOs depend on many factors, including EO chemotypes, environmental parameters and post-processing conditions of plant material (e.g., drying).

The present comparative study aimed to determine the antifungal activity of Azorean *Cymbopogon citratus* leaf EOs against *Penicillium* spp., using different leaves status (i.e., fresh and dried).

Fresh or dried leaves of *C. citratus* were subjected to hydrodistillation using a Clevenger-type apparatus. The EOs chemical profiles were performed by gas chromatography (GC) with flame ionization and mass selective detectors, and their antimicrobial activities were evaluated by the agar disk diffusion method.

The EO yield of the samples was 0.41%, v/w (FW) and 0.80%, v/w, (DW) for fresh and dried plant material, respectively. GC analysis showed that the major compounds of *C. citratus* EOs were citral (24–28% of neral and 37–42% of geranial) and myrcene (4.9–9.2%). Regarding the *in vitro* antifungal activities, both EOs were highly effective in inhibiting the growth of *Penicillium* spp., with diameter inhibition zones higher than 23 mm at 24 h. There were no significant differences on the antifungal activity between fresh and dried samples ( $P > 0.05$ ).

In summary, our data suggest that *C. citratus* EOs (from fresh or dried leaves) could be considered as natural antimicrobial agents, namely against species of *Penicillium*, for application in the storage of food products to prolong their shelf life. In addition, once there were no differences between the effectiveness of *C. citratus* EOs from fresh or dried samples, it seems better to dry fresh leaves for further extraction as the first step in industrial scale extractions.

## Pesticide residues monitoring and risk assessment in Rice from Cape Verde: 2018-2020

Rodríguez-Ramos, R.,<sup>1\*</sup> Santana-Mayor, Á,<sup>1</sup> Socas-Rodríguez, B,<sup>1</sup> Rodríguez-Delgado, M.A<sup>1</sup>

<sup>1</sup> *Departamento de Química, Unidad Departamental de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL). Avda. Astrofísico Francisco Sánchez, s/n 38206 San Cristóbal de La Laguna (Tenerife), Spain*

\*Corresponding autor: rrodrira@ull.edu.es

Nowadays, society is increasingly aware of the importance of consuming high-quality food products and concerned about certain groups of compounds that could be harmful to public health.<sup>1</sup> The Macaronesia Region, made up of Azores, Madeira, Canary Islands and Cape Verde Archipelago, as an outermost region, is an area especially susceptible to food contamination due to external agents coming from products on the international market. The Archipelago of Cape Verde imports 80% of the cereal needed to cover the needs of its citizens.<sup>2</sup> In 2020, Cape Verde imported \$15.9M in Rice mainly from Thailand, Brazil, and Vietnam.<sup>3</sup> Thus, this area is especially susceptible to food contamination due to external agents coming from products on the international market. Among these contaminants, pesticides have been the ones that have caused the greatest concern due to their intensive use in agricultural crops to prevent, mitigate or eliminate pests during harvesting and storage of cereals. For these reasons, national and international organizations have proposed the control and monitoring of these high-risk substances in order to protect consumers' health and quality of agricultural products.<sup>1</sup>

In this work, an analytical methodology has been developed for the determination of 165 pesticide residues in rice samples, using both, ultra-high performance liquid chromatography (UHPLC) and gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS) systems, combined with the QuEChERS method as extraction and clean-up procedures. The method was validated following the SANTE European guidelines.<sup>4</sup> In this study, a total of 98 rice samples consumed in Cape Verde of harvesting periods between 2018 and 2020 were analyzed. Finally, a non-accumulative risk assessment was carried out following the margin of safety approach,<sup>5</sup> finding that in general terms the consumption of these cereals does not entail a risk.

**Acknowledgements:** This work has been supported by the European Project Pervemac II (Mac/1.1a/049). R.R.R. and Á.S.M. would like to thank their FPI fellowships co-financed by Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Conocimiento y Empleo y Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%). The authors would like to acknowledge the use of the Research Support General Service (SEGAI) of the University of La Laguna.

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## Dietary Cd risk characterization from cereal-based products in the Macaronesia archipelagos

Alejandro-Vega, S.,<sup>1</sup> Paz-Montelongo, S.,<sup>1,2</sup> Gutiérrez-Fernández, A. J.,<sup>1,2</sup> Niebla-Canelo, D.,<sup>1</sup> Revert-Gironés, C.,<sup>1,2</sup> Gonzales-Weller, D.,<sup>1</sup> Hardisson, A.,<sup>1,2</sup> Rubio-Armendáriz, C.<sup>1,2</sup>

<sup>1</sup> Grupo interuniversitario de Toxicología Alimentaria y Ambiental, Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, España

<sup>2</sup> Proyecto MAC-Pervemac2 (MAC/1.1a/049)

\*Corresponding autor: Samuel Alejandro-Vega, alu0100992397@ull.edu.es

Cereals and cereal-based products are fundamental for the global food industry, both in human diets and animal feeds. Therefore, there is a great interest in the analysis of their safety and projects as MAC-Pervemac2 (MAC/1.1a/049) are of great relevance for the monitoring, management, and communication of the associated potential risks. Cd concentrations in cereals have been studied to assess the dietary exposure of the Macaronesia's archipelagos population and to characterize the health risks. Cd is well known for its nephrotoxicity; however, it has been also classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer. The EC Regulation 1881/2006 sets a maximum concentration of 0.10 mg/kg of Cd in cereals, excluding rice, wheat, bran, and germ, as the maximum level set for those products is 0.20 mg/kg. Meanwhile the European Food Safety Authority (EFSA) has set the tolerable weekly intake (TWI) for Cd in 2.5 µg/kg bw·week.

**Objectives:** To determine Cd in different cereals and cereal-based products commercialized in the Macaronesia's archipelagos (Canary Island, Madeira, Azores, and Cape Verde); to assess the Cd dietary exposure (EDIs) derived from cereal-based products and rice intake for different population groups and to characterize the potential health risks

**Materials and methods:** 544 samples were digested in a microwave oven and analyzed by Inductively coupled plasma optical emission spectrometry (ICP-OES). The samples were collected in Cape Verde (N=228), the Canary Islands (N=216), Madeira (N=60) and Azores (N=40). The matrix distribution was 335 cereal (162 corn, 124 rice, 31 wheat) and 209 cereal-based products (126 flour and 83 gofio samples). The estimated daily intakes (EDI) were assessed considering a daily intake of 100 g of each food product and then evaluated considering the Cd TWI.

**Conclusions:** The appreciable number of samples with Cd concentrations below the LOQ and under the parametric levels set by the European Regulation reveal that these agricultural products are safe in terms of Cd levels and no health risk are expected in our consumers.

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## Algae from the Canary Islands: A possible sustainable foodstuff

Paz-Montelongo, S.,<sup>1\*</sup> García-Meléndez, M.C.,<sup>1</sup> Pérez-Godiño, M.,<sup>1</sup> Niebla-Canelo, D.,<sup>1</sup> Alejandro-Vega, S.,<sup>1</sup> Martín-León, V.,<sup>2</sup> Gutiérrez-Fernández, A.J.,<sup>1</sup> Rubio-Armendáriz, C.,<sup>1</sup> Hardisson A.<sup>1</sup>

<sup>1</sup>Grupo interuniversitario de Toxicología Alimentaria y Ambiental, Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, España

<sup>2</sup>Public Health Laboratory of Las Palmas (Laboratorio de Salud Pública), Canary Health Service (Servicio Canario de Salud), 35004 Las Palmas de Gran Canaria, Spain

\*Corresponding autor: spazmont@ull.edu.es

Seaweed is a nutritious and functional food that is increasingly consumed by the Western population. However, the edible seaweed currently on the market comes from industrialized areas with high anthropic pressure, such as China and Japan. Among the contaminants that stand out in seaweed are toxic metals (Cd, Pb, Hg) and nitrates which, in high concentrations, can cause damage to human health (Chen et al., 2018; Paz et al., 2018).

The Canary Islands, due to its geographical location, could be an optimal region for the exploitation of edible algae that are safe for consumers. In addition, it is worth highlighting the great variability of algal species that grow on the Canary coasts and the sustainability of being a zero-kilometer food.

The content of toxic metals (Cd, Pb, Hg) and nitrate has been determined in 44 samples of seaweed from the coast of Tenerife (south, north and metropolitan area). The algae collected belong to the brown algae, red algae and green algae, choosing those that may be of interest from a food point of view.

The study of the content of toxic metals by zones indicates a higher concentration of these metals in the algae collected in the southern zone. In the study by species, the species *Gelidium spp* is the one with the highest concentration of Cd (0.165 mg/kg) and Pb (1.564 mg/kg). On the other hand, the species *Rhododymenia pseudopalmata* contains the highest concentration of Hg (0.06 mg/kg).

The study of nitrate content was carried out both in the water and in the algae in order to study possible correlations. This study reports that the highest concentration in water was recorded in the metropolitan area (2.24 mg/L) and the species with the highest amount of nitrate is *Taonia atomaria* (5530 mg/kg).

As for the evaluation of dietary intake of toxic metals and nitrates, a consumption scenario of 4 g/day of dehydrated seaweed was assumed. Contribution percentages to the reference values for acceptable daily intakes were obtained which are generally low (< 10%). Therefore, the consumption of seaweed from the waters off the coast of Tenerife would not pose a health risk.

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## Biochar as a sorbent of ethofumesate and sulfamethoxazole in agricultural soil: changes of its sorption capacity over time

López-Cabeza, R.,<sup>1\*</sup> Gámiz, B.,<sup>1</sup> Galán-Pérez, J.A.,<sup>1</sup> Cox, L.<sup>1</sup>, Celis, R.<sup>1</sup>

<sup>1</sup> Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina Mercedes 10, 41012 Sevilla, Spain.

\*Corresponding autor: rlopezc@irnas.csic.es

An important issue regarding the presence of pesticides and other organic pollutants in agricultural soils relates to their mobility, which may imply surface and groundwater contamination risks. Soil application of sorbents that increase the retention capacity can be an excellent strategy to reduce the dispersion of organic contaminants in the environment. Biochar is a sustainable carbon-rich product prepared by pyrolysis of biomass that has been proposed as a soil amendment due to its great sorption capacity for organic compounds. Furthermore, biochar has been proven to enhance CO<sub>2</sub> sequestration, increase water retention, and supply nutrients. However, once in the soil, biochar undergoes biogeochemical changes with residence time (aging) that may alter its physicochemical properties and modify its sorption capacity. Therefore, research on biochar aging in the field and its simulation in laboratory experiments is of great interest.

In this work, the sorption of the pesticide ethofumesate (ETFM) and the antibiotic of veterinary use sulfamethoxazole (SFMX) in an agricultural soil with low retention capacity amended with a commercial wood-derived biochar was assessed. In addition, the possible change of biochar sorption capacity over time was studied, simulating aging in the field by incubating the unamended soil and the soil amended with biochar (5%) at 40 °C and a soil water content of 30% for 48 days. At selected times, the incubated soils were sampled and the sorption of the two compounds was determined by the batch technique. To evaluate sorption over time, 24 h-distribution coefficients,  $K_d$  (l/kg), were determined at each sampling time from the expression  $K_d = C_s/C_{aq}$ , where  $C_s$  (mg/kg) is the amount sorbed and  $C_{aq}$  (mg/l) is the concentration remaining in the aqueous phase.

The results showed that the addition of biochar to the soil markedly increased the sorption of both ETFM ( $K_d = 168 \pm 40$  l/kg) and SFMX ( $K_d = 3.89 \pm 0.58$  l/kg) in comparison with the unamended soil ( $K_d = 1.90 \pm 0.08$  l/kg for ETFM and  $0.06 \pm 0.01$  l/kg for SFMX). However, the sorption capacity of the biochar applied to the soil decreased over time. In one week, the  $K_d$  values of the amended soil decreased to  $27.6 \pm 7.7$  l/kg for ETFM and to  $2.14 \pm 0.33$  l/kg for SFMX. This decrease continued over time, reaching values of  $K_d = 10.5 \pm 0.4$  l/kg for ETFM and  $0.38 \pm 0.01$  l/kg for SFMX after 48 days. From the results obtained in this work, it can be concluded that biochar increased the retention of ETFM and SFMX in the soil, but this retention capacity is rapidly reduced over time, although sorption in the biochar-amended soils still remained higher than in unamended soil all over the time frame of the experiment.

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## Volatile characterization of Verdelho during grape maturation

Martins A.,<sup>1,2\*</sup> Pereira V.,<sup>1,2,3</sup> Pereira A.,<sup>1,2,3,4</sup>, Marques J.C.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis Centre of Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> Faculty of Exact Sciences and Engineering, University of Madeira, Portugal

<sup>3</sup> Institute of Nanostructures Nanomodelling and Nanofabrication (I3N), University of Aveiro, Portugal

<sup>4</sup> CIEPQPF, University of Coimbra, Portugal

\*Corresponding autor:::anisia.martins@staff.uma.pt

Precision viticulture merges science, new technologies and practical knowledge to maximize the oenological potential of vineyards. It is a new approach to vineyard management which aims to improve the efficiency and quality of production, while also reducing its environmental impact [1]. Nowadays, sustainable viticulture remains an underdeveloped concept, so more studies in this field are essential to allow winegrowers to improve their sustainability performance [2].

This research aims to add further data about the ripening process of Verdelho grapes, one of the most important white varieties grown in Madeira, which is often used to produce medium-dry Madeira Wines (1.5 to 2.5 ° Baumé) [3]. In the present work, we characterize the maturation phase of Verdelho grapes, in terms of volatile composition by comparing different management systems, rootstocks and plantation years. Fifty-nine volatile compounds were identified and, in all samples at both maturation points, the major compounds identified were hexanal and (E)-2-hexanal, with abundances of about 46% and 27%, respectively. Our results allow us to infer that younger vines and vines conducted in espalier produced grapes with higher concentrations of carbonyl compounds at harvest time, evidencing that they reached maturation earlier. Grapes from vines conducted in *latada* presented a lower content of volatile compounds, with a lower increase in carbonyl compounds (20% increase compared to 40% in espalier) and a higher concentration of higher alcohols at harvest time, which may indicate a delay in maturation.

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SPA2022

## Sustainable and Precision Agriculture Symposium 2022

### Amino alcohols- derived thioureas. Synthesis and evaluation as potential antifungal against *Botrytis cinerea*.

Herrera, P.,<sup>1</sup> González, Z.,<sup>1</sup> Giménez, C.,<sup>2</sup> Guillermo, R.,<sup>3</sup> González, C.<sup>1\*</sup>

<sup>1</sup> Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Tenerife, Spain

<sup>2</sup> UDI Fitopatología, Facultad de Ciencias, Universidad de La Laguna (ULL). Tenerife Spain

<sup>3</sup> Departamento de Química Orgánica, Facultad de Ciencias, Universidad de La Laguna (ULL). Tenerife Spain.

\*Corresponding autor: ccgm@ipna.csic.es

Aminoalcohols, urea and thioureas are considered suitable synthons for the preparation of compounds with wide and varied biological activities. Compounds synthesized by combining amino alcohols and thioureas exhibit medicinal properties and have been used in the preparation of several natural compounds. Studies on the properties and structure of amino alcohols and their derivatives have been conducted since the middle of the last century, and interest in these compounds has increased over time. Amino alcohols and their various derivatives play a very important role in the functioning of a living organism.<sup>1</sup>

N-substituted thioureas also have broad physiological activity such as antibacterial, antiviral, antitumour and antihypertensive. Some of them are used in plant growth as well as in agriculture as fungicides and herbicides.<sup>2</sup>

Since amino alcohols and their corresponding thioureas derivatives show important bioactivity, their synthesis and bioactivity are among the topics of interest and since nitrogen-containing heterocyclic compounds have broad pharmaceutical properties, it is very important to develop new methods of synthesis and to obtain new derivatives. In the framework of the project, we are developing different thioureas derivatives have been synthesized, which are new functional heterocyclic compounds based on amino alcohols. These compounds have been tested against different phytopathogens (*Botrytis cinerea*, *Alternaria alternata* and *Fusarium oxysporum*). In this poster, the synthesis and preliminary results obtained from these tests will be presented.

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## **Correlation between CWSI<sub>si</sub> and others vegetation indexes in Madeira vineyards**

Macedo, F.L.,<sup>1,2\*</sup> Pinheiro de Carvalho, M.A.A.,<sup>2</sup> Nóbrega, H.G.M.<sup>2</sup>

<sup>1</sup> UMA, Portugal

<sup>2</sup>Universidade da Madeira (UMa), Portugal

\*Corresponding autor: fabriciolmacedo@hotmail.com

The vine culture is one of the most emblematic and important in Madeira, therefore, it is extremely necessary to analyze and consequently propose strategies to overcome the obstacles arising from climate change. In simulations carried out by the IPCC, considerable increases in temperature and decreases in precipitation volumes are often presented for the whole world. The conscious use of water resources becomes extremely important, since crops, to be economically viable, need to continue producing even under the effect of climate change, however, water will become increasingly scarce. The use of new technologies such as Unmanned Aerial Vehicles (UAV), has been gaining increasing prominence in agriculture, becoming a real alternative in the process of sustainable optimization of natural resources. Associated with UAV's, the use of multispectral and thermal cameras makes it possible to check the water stress of crops almost in real-time. However thermal cameras are reasonably expensive. The present study aimed to determine the water stress on the vine culture in two study sites (Quinta das Vinhas and Tiquinho) on two dates (July and September 2021) using the index known as Crop Water Stress Index Simplified (CWSI<sub>si</sub>) which uses temperature data obtained from the thermal band and correlate the results with 3 vegetation indexes Normalized Difference Vegetation Index (NDVI), Normalized Difference Red Edge (NDRE), and Green Normalized Difference Vegetation Index (GNDVI). A test of their correlation with the CWSI<sub>si</sub> was also performed. The results for the CWSI<sub>si</sub> show that the vineyard, in both cases of study, presents a great heterogeneity concerning water requirements, these results can provide great benefits for the producer, because it allows him to properly provide water to the crop, more accurately, efficiently, and economically, seeking to maintain crop production and still using water resources more efficiently. Regarding the correlation test, for Quinta das Vinhas the NDVI showed the best negative correlations with the CWSI<sub>si</sub>, with values of -0.55 and -0.79 (July and September, respectively); for Tiquinho the indexes were NDRE (July) with -0.78 and GNDVI (September) -0.57.

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## Efficient irrigation water management for salinity control under Mediterranean greenhouse conditions: tomato fruit quality

Romero-Sanz, A.G.,<sup>1</sup> Jaime-Fernández, E.,<sup>1</sup> Ramos-Martín, J.M.,<sup>1</sup> González-Gil, G.,<sup>1</sup> Ferez-Gómez, A.,<sup>1</sup> López-Serrano, L.,<sup>1</sup> Romero-Aranda M.R.<sup>1\*</sup>

<sup>1</sup>Plant Breeding and Biotechnology Dept. IHSM-La Mayora, UMA- CSIC, Málaga, Spain

\*Corresponding author: mr.romero@csic.es

A mixture of salts is present in all types of substrates used for greenhouse production of horticultural crops. This mixture includes salts originally dissolved in the water used to prepare the irrigation nutrient solution and from an intensive fertilization using chemical fertilizers. If the concentration of these salts becomes excessive, crop yield is reduced because of the decrease of osmotic potential of the soil water. To prevent harmful accumulation of salts, the soil profile must be leached periodically with an amount of water in excess to that used by evapotranspiration. Thus, the concept of efficient water use should be expanded to include the adequate amount of water to meet the leaching requirement. In this study, we used soil sensors to record the volumetric water content and the electrical conductivity in a tomato crop grown in a mixture of peat, coconut fiber and vermiculite. The objective was to evaluate whether these parameters could help to manage the irrigation schedule to avoid excessive water loss as drainage, and consequently induce a water economy while reducing the negative environmental impacts of high volume of lixiviates from greenhouses. The implementation of this technology is discussed in relation to tomato fruit quality.

Tomato crop is relatively resistant to salt concentration in the growing substrate (Romero-Aranda *et al.*, 2001) and to the low relative humidity inside the Mediterranean greenhouses, mainly during the midday hours (Romero-Aranda *et al.*, 2002). However, research has shown that salinity and drought stresses causes changes in the quality of the tomato fruit, which indirectly reduce the marketable yield (Romero-Aranda *et al.*, 2020). So a second objective of this study was to determine the effects of salinity and drought stresses on some indicators of the nutritional value of tomato fruits, such as lycopene, phenols, total flavonoids, which are well known for their antioxidant capacity. Refractometric sugar content and total titrable acidity were also determined. In addition, color of epidermis was recorded in order to evaluate whether colorimetric analyses could be used to determine the best moment to harvest tomato fruits in order to guaranty the highest quality and nutritional value.

Results showed that increased salinity in the irrigation solution increased the recorded values of volumetric water content in the substrate. The time evolution of the EC recorded by the sensors followed a similar pattern than that showed by data from laboratory analysis of water extracts from the substrate, although the EC values from the analysis of extracts were one order of magnitude higher than those recorded by the sensors. Saline irrigation led to an increase of some of the fruit quality parameters and some of them were closely related with the colorimetric analysis, which could be relevant from an economic point of view.

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## Monitoring of the action of Organic Compost in Amendment of Degraded Soil in Madeira Island

Valente S.,<sup>1,\*</sup> Freitas G.,<sup>1</sup> Gouveia C.,<sup>1,2,3</sup> Macedo F.,<sup>1,2</sup> Nóbrega H.,<sup>1</sup> Pinto L.,<sup>1</sup> Rosa J.,<sup>1</sup> Carvalho M.A.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis, Centre Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> CITAB, Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes, Portugal

<sup>3</sup> Faculty of Life Sciences, University of Madeira, Portugal

\*Corresponding author: sofia.valente@staff.uma.pt

Soil degradation, as result of human activities and environmental changes, arouse as a threat for sustainable development. Degraded soils have low water retention, as result of high drainage, and organic matter content, negatively influencing soil's functions and nutrient uptake and energy supply to crops development. The organic compost incorporation to achieve the soil amendment is a strategic measure, providing the increase of organic matter providing the enhancement of soil edaphic, physicochemical and biological properties, and consequently improving soil's nutrients recycling and fertility. Soil physicochemical and biological parameters are sensitive quality indicators of soil, potential allowing to access compost effect on soil improvement.

This work shows the results of a 6-month monitorization of amended with organic compost added in two different quantities (5 and 10 kg.m<sup>-2</sup>) in fire-ravaged area of Natural Park, Madeira Island. Physicochemical evaluation of the compost was previously performed. Physicochemical and microbiological properties of soil samples collected periodically, after the organic compost incorporation were also determined. Experimental areas were periodically monitor using a drone platform to obtain a Normalized Difference Vegetation Index (NDVI).

In the compost production was used as raw material local agriculture residues, such as banana straw, garden waste and vine pruning remains, and as source of organic matter cow manure. Compost shows to be an alkaline product, pH 7.53, 34.83% of organic matter and a C:N ratio of 16,.67. Significant Pearson correlations between the quantity of organic compost applied and the following soil physicochemical parameters pH, Electrical Conductivity, N %, and Water Holding Capacity. Significant Pearson correlations were also obtained for soil microbiological activity, such as Basal Respiration, Metabolic Quotient and Bacteria counting. Soil's monitoring since the start showed significant correlations with the Organic Matter and Nitrogen content, as well as the microbiological activity parameters. So far, NDVI slightly increased in the plots with organic compost comparing to control plot.

Long term results are needed to access the effect of organic compost on soil features, so the monitorization is planned to extend for one year period.

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## The use of Sewage Sludge in the Soil Amendment: preliminary study in Madeira Island

Valente S.,<sup>1\*</sup> Freitas G.,<sup>1</sup> Gouveia C.,<sup>1,2,3</sup> Macedo F.,<sup>1,2</sup> Nóbrega H.,<sup>1</sup> Pinto L.,<sup>1</sup> Rosa J.,<sup>1</sup> Carvalho M.A.A.<sup>1,2,3</sup>

<sup>1</sup> ISOPlexis, Centre Sustainable Agriculture and Food Technology, University of Madeira, Portugal

<sup>2</sup> CITAB, Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes, Portugal

<sup>3</sup> Faculty of Life Sciences, University of Madeira, Portugal

\*Corresponding author: sofia.valente@staff.uma.pt

Degraded soils areas are prone to desertification and generally can be affected by climatic conditions such as low precipitation. These soils show low levels of organic matter and water retention capacity. Consequently, these soils have high drainage due to absence of organic matter and evaporation ratios due to the absence of vegetation. To avoid these negative effects the use of agroforestry and agriculture residues, waste of urban and industrial origin, such as sewage sludge, is promoted to produce amendments and fertilizers as a strategic measure for soil regeneration. These amendments represent a source of organic matter to increase its content in the soil. The sewage sludge is a worrisome waste product that is produced in large volumes, notwithstanding, with high potential to be recycled as a soil corrective.

This work shows a 4-month soil plot monitoring with sewage sludge application in a previously fire-ravaged area in the Natural Park, Madeira Island, nearby Funchal. The experimental design consists in a soil plot submitted to a 0.8 kg m<sup>-2</sup> of sewage sludge and a control plot, unamended. Soil samples were collected periodically for physicochemical and microbiological analysis. Drone captured images were taken for Normalized Difference Vegetation Index (NDVI) evaluation, during the trial time.

Statistical analysis (Pearson, SPSS) shows that during the monitored period, the amendment with sewage sludge presented significant correlations with soil's Organic Matter (OM) and Nitrogen content, influencing soil's microbiological activity, according with Basal Respiration, Microbial Biomass and Metabolic Quotient. However, the added OM shows a continuous decrease since its incorporation, which could result from rapid mobilization, through nutrient uptake by plants and vegetation grown in the experimental area or as result of leaching.

The NDVI in the experimental area is considerably higher in the amended plot, than in control plot. After the soil amendment plot has covered by native grasses coverage. The amendment with sewage sludge significantly increased the plant biomass in the plot and influenced the physicochemical and microbiological parameters under study.

Long term results of soil monitoring with the application of sewage sludge amendment are crucial to access its effects and safety use for eroded areas restoration and ecological purposes.

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## Preparation of $\alpha$ -keto- $\beta$ -amino esters and peptide derivatives and antimicrobial evaluation against *Pseudomonas syringae*

Hernández D.,<sup>1\*</sup> García-Machado J.,<sup>1,2</sup> Jiménez D.,<sup>3</sup> Boto A.<sup>1</sup>

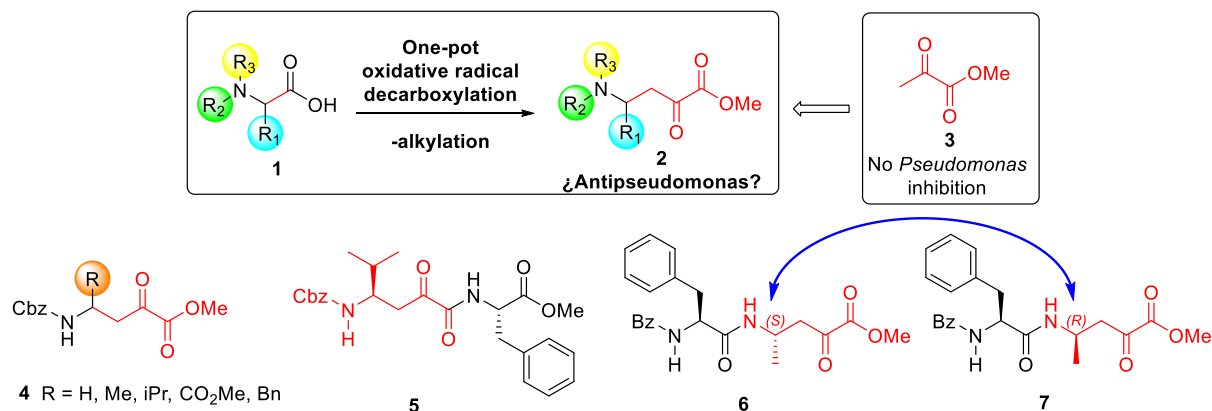
<sup>1</sup> Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico F. Sánchez, 3; 38206-La Laguna, Tenerife, Spain.

<sup>2</sup> Universidad de La Laguna (ULL), La Laguna, Tenerife, Spain

<sup>3</sup> Currently Research contract in BIOVERT SL.

\*Corresponding author: dacil@ipna.csic.es

The preparation of peptides containing  $\alpha$ -keto  $\beta$ -amino acids has attracted much attention, since many are antitumoral and antimicrobial agents.<sup>1</sup> In contrast, reports on bioactive compounds containing the related  $\alpha$ -keto  $\gamma$ -amino acids (or their derivatives) are scarce.<sup>2</sup> Herein, we report an efficient method for the transformation of  $\alpha$ -amino acids into  $\alpha$ -keto  $\gamma$ -amino esters, using a new sequential oxidative radical decarboxylation–alkylation process (conversion **1**→**2**). The process was also applied to the selective modification of the C-terminal residue in peptides.<sup>2</sup> The libraries of these compounds (some examples shown, compounds **4**–**7**) were tested against the phytopathogen *Pseudomonas syringae*, which causes extensive losses in a variety of crops. Unlike  $\alpha$ -keto  $\beta$ -amino acids, the direct inhibition of  $\alpha$ -keto  $\gamma$ -amino acid derivatives was low. However, this study has allowed the detection of a series of compounds capable of reducing the percentage of survival of these bacteria in relation to growth control, data that will be useful for the development of a second generation of peptides with improved activity against *Pseudomonas Syringae*.



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## Synthesis and evaluation of a AHL library against *Pseudomonas syringae*.

Porras M.,<sup>1,2\*</sup> Hernández D.,<sup>1</sup> Boto A.<sup>1</sup>

<sup>1</sup> Instituto de Productos Naturales y Agrobiología (IPNA.-CSIC), La Laguna (Tenerife), España.

<sup>2</sup> Programa de Doctorado en Ciencias Médicas y Farmacéuticas, Desarrollo y Calidad de Vida. Universidad de La Laguna, La Laguna (Tenerife), España.

\*Corresponding author: mporras@ipna.csic.es

Currently, one of the main health and food security problems is the emergency of bacterial resistance to known antimicrobials and phytosanitaries agents. Therefore, the WHO and the FAO have stressed the importance of developing new agents to face this challenge<sup>1a,b</sup> Among the different strategies, a promising one is the development of quorum sensing (QS) modulators. These molecules interfere with the bacterial communication needed for their coordinated actions, such as host invasion, virulence, or the formation of biofilms. Among the QS signals, also called autoinducers, *N*-acyl homoserine lactone (AHL) is found in Gram negative bacteria.<sup>2</sup> This communication describes a synthetic methodology to readily prepare AHL analogues. The process involves a radical scission of hydroxyproline “customizable” units and subsequent reduction of the intermediate to afford AHL derivatives.<sup>3</sup> The evaluation of this AHL library against *Pseudomonas syringae* is reported too.

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## Evaluation of 4-oxo-L-homoalanine derivatives against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*

Porras M.,<sup>1,2\*</sup> Hernández D.,<sup>1</sup> Gimenez, C,<sup>3</sup> Boto A.<sup>1</sup>

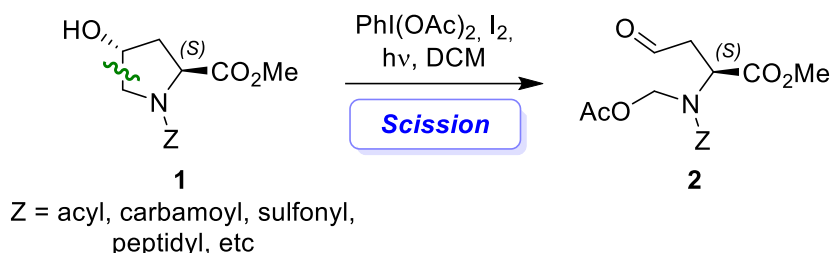
<sup>1</sup> Instituto de Productos Naturales y Agrobiología (IPNA.-CSIC), La Laguna (Tenerife), España.

<sup>2</sup> Programa de Doctorado en Ciencias Médicas y Farmacéuticas, Desarrollo y Calidad de Vida, Escuela de Doctorado y Estudios de Posgrado. Universidad de La Laguna, La Laguna (Tenerife), España.

<sup>3</sup> Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, La Laguna (Tenerife), España.

\*Corresponding author: mporras@ipna.csic.es

In this communication, we report a synthetic methodology in order to create a library of 4-oxo-L-homoalanine derivatives. The synthetic process involves a radical scission of hydroxiproline units (1), in which its amine function has previously been protected. Furthermore, we detail the results of the evaluations of this chemical library against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*, using the radial growth test in potato dextrose agar (2). This study allowed the identification of promising candidates with activity against these fungi.



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## Influence of green bananas storage temperature on the green banana flour physicochemical parameters

Martín Lorenzo, M.,<sup>1</sup> Piedra-Buena, A.,<sup>2</sup> Lobo, M.G.,<sup>3\*</sup> Rodríguez-Rodríguez, E.M.<sup>1</sup>

<sup>1</sup> Área de Nutrición y Bromatología. Departamento de Ingeniería Química y Tecnología Farmacéutica. Universidad de La Laguna, Tenerife, Spain

<sup>2</sup> Unidad Protección Vegetal, Instituto Canario de Investigaciones Agrarias, Valle de Guerra, Tenerife, Spain

<sup>3</sup> Departamento de Producción Vegetal en Zonas Tropicales y Subtropicales, Instituto Canario de Investigaciones Agrarias, Valle de Guerra, Tenerife, Spain

\*Corresponding author: [globo@icia.es](mailto:globo@icia.es)

15% of the harvested bananas are daily discarded in Canary Islands at the packing houses because they do not reach the requirements of marketing standards which are mainly based on external characteristics (shape, size, absence of skin defects, color, presence of pests and diseases, etc.), or are retired in order to avoid falls in the market price of bananas. However, the discarded green bananas, maintain an interesting nutritional and functional value. They are an excellent source of resistant starch, potassium and other minerals, phenolic compounds and other antioxidants, and phytosterols [1,2]. Therefore, the production of green banana flour (GBF) has attracted significant attention in the food industry because it is gluten free. The aim of this research was to study the storage conditions (temperature and time) of green bananas for the GBF elaboration to recover this waste.

Green banana (*Musa acuminata* Colla AAA cv. Dwarf Cavendish) samples were kept in cold storage rooms at two temperatures (6 and 12 °C). Ten bananas from each storage room were removed at the following times: 0, 2, 4, 5 and 7 weeks, to elaborate the banana flours. The parameters analyzed in these GBFs were: moisture, starch and antioxidant capacity (DPPH and ABTS methods).

GBFs produced from green bananas stored at 12 °C showed greater changes in their physicochemical parameters than the one produced with bananas stored at 6 °C. In this sense, it was even not possible to elaborate the flour from bananas stored for more than 3 weeks at 12 °C because at this temperature the fruit continue ripening developing the typical peel yellow color and diminishing the starch content. Thus, when the banana storage increased, a decrease ( $p < 0.05$ ) in the starch content occurred, since it was gradually transformed into sugars. On the contrary, an increase ( $p < 0.05$ ) in its antioxidant capacity (DPPH and ABTS) was observed as the bananas ripened. Moreover, the flours made with bananas stored at 12 °C had banana aroma and were much darker and slightly sweeter than those obtained from bananas stored at 6 °C. Thus, when the storage temperature was 6 °C, the shelf life of bananas to make GBF was 7 weeks. It is important to denote that although the banana skin turns completely dark during the storage at 6 °C, the pulp remained with not perceptible color change.

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## Participant List

Surname	First Name	Afiliation	Country	e-mail
Alejandro Vega	Samuel	Universidad de La Laguna	Spain	alu0100992397@ull.edu.es
Almeida	Catarina	Universidade de Aveiro	Portugal	ac.almeida@ua.pt
Alonso Lorenzo	Jana	Instituto de Productos Naturales y Agrobiología (IPNA-CSIC)	Spain	jana.alonso@csic.es
Alonso Vega	Mercedes	Dirección General de Agricultura. Gobierno de Canarias	Spain	maloveg@gobiernodecanarias.org
Álvarez Méndez	Sergio Joaquín	Universidad de La Laguna	Spain	salvmen@ull.edu.es
Arco Lázaro	Elena	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	earco@icia.es
Arruda	Filipe	Universidade dos Açores	Portugal	filipearruda1995@hotmail.com
Asensio Calavia	Patricia	Instituto de Productos Naturales y Agrobiología (IPNA-CSIC)	Spain	pacma1394@gmail.com
Baca González	María Victoria	CBGP (UPM - INIA/CSIC)	Spain	victoria.baca@inia.csic.es
Baquero Machado	María	Universidad de La Laguna	Spain	alu0101223803@ull.edu.es
Barreto	Maria do Carmo	Universidade dos Açores	Portugal	maria.cr.barreto@uac.pt
Blais	Mylene	Agriculture and Agri-Food Canada	Canada	mylene.blais@agr.gc.ca
Bonet	Enric	BIOVERT-MANVERT	Spain	enric.bonet@manvert.com
Borges	Andrés A.	Instituto de Productos Naturales y Agrobiología (IPNA-CSIC)	Spain	aborges@ipna.csic.es
Boto Castro	Alicia	IPNA-CSIC	Spain	alicia@ipna.csic.es
Cabrera Pérez	Raimundo	Universidad de La Laguna	Spain	rcabrera@ull.edu.es
Casas Mas	Enrique José	Universidad de La Laguna	Spain	ecasasma@ull.edu.es
Casas Morales	Hugo	Universidad Autónoma del Estado de Morelos	México	hugo.casasmor@uaem.edu.mx

Surname	First Name	Afiliation	Country	e-mail
Correa Delgado	Raquel	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	raquelcorreadelgado@gmail.com
Correia Levy	Ana	INIDA-Instituto Nacional de Investigaçã o e Desenvolvimento Agrário de Cabo Verde	Cabo Verde	awacorreia2000@yahoo.fr
Cruz Pérez	Noelia	Universidad de La Laguna	Spain	ncruzper@ull.edu.es
Cueto	Mercedes	IPNA-CSIC	Spain	mcueto@ipna.csic.es
Curbelo Cruz	Carmen	Universidad Autónoma de Madrid (UAM)	Spain	curbelopa@gmail.com
Da Silva Mendonça	Duarte Manuel	Universidade dos Açores	Portugal	duarte.ms.mendonca@uac.pt
De Diego Sánchez	Nuria	University of Palacky, Olomuc	Czech Republic	nuria.de@upol.cz
Delgado Bello	José Alberto	Cabildo de Tenerife	Spain	jalberto@tenerife.es
Díaz Díaz	Ricardo	Instituto Tecnológico de Canarias	Spain	rdiaz@itccanarias.org
Dos Santos Delgado	António	Direção Geral de Agricultura, Silvicultura e Pecuária de Cabo Verde	Cabo Verde	Antonio.S.Delgado@maa.gov.cv
Duarte Silva	Gilbert	INIDA-Instituto Nacional de Investigaçã o e Desenvolvimento Agrário de Cabo Verde	Cabo Verde	inida@inida.gov.cv
Expósito Rodríguez	Marino	Sainsbury Laboratory-Cambridge University	UK	marino.exposito@slcu.cam.ac.uk
Fernandes	Andreia	Direção Regional de Agricultura Desenvolvimento Rural Madeira	Portugal	andreia.fn.fernandes@madeira.gov.pt
Fernández Luque	José Enrique	IRNAS-CSIC	Spain	jefer@irnase.csic.es
Ferraz Alves	Sónia	Centro ISOPlexis - Universidade da Madeira	Portugal	sferraz81@staff.uma.pt



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Surname	First Name	Afiliation	Country	e-mail
Ferreira	Rui	Universidade da Madeira	Portugal	rui.ferreira@staff.uma.pt
Francioso	Antonio	Universidad de La Laguna	Spain	antonio.francioso@uniroma1.it
Freitas	Ana	Universidade da Madeira	Portugal	ana.isabel.freitas@staff.uma.pt
Fuentes	Ariadna	Universidad de La Laguna	Spain	alu0101231075@ull.edu.es
Gallardo-Romero	Diego José	Universidad de Sevilla	Spain	diegogallardo.gr@gmail.com
García-Báez	Jasmina	Universidad de La Laguna	Spain	alu0101226552@ull.edu.es
García García	Ana Libertad	IPNA-CSIC	Spain	ana2293@gmail.com
García Machado	F. Javier	IPNA-CSIC	Spain	fjaviergarma@gmail.com
Giaki	Aikaterini	Agricultural University of Athens	Greece	katerinagiakh@gmail.com
Gonçalves	David	Universidade da Madeira	Portugal	joao.d.goncalves@staff.uma.pt
Gómez Expósito	María Daniela	Universidad de La Laguna	Spain	mgomezex@ull.edu.es
González Acosta	Sergio	IPNA-CSIC	Spain	sergi_glez@hotmail.com
González Avilés	Maily Selena	IPNA-CSIC	Spain	maily.gonzalez@yachaytech.edu.ec
González Hernández	Sara	IPNA-CSIC	Spain	saragonzalezhernandez546@gmail.com
González Martín	Concepción C.	IPNA-CSIC	Spain	ccgm@ipna.csic.es
Gonzalez Montelongo	Cristina	Universidad de La Laguna	Spain	cgonzalm@ull.edu.es
González Rodríguez	Zuleima	IPNA-CSIC	Spain	zuleima@ipna.csic.es
Grajal Martín	María José	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	mjgrajal@icia.es
Guerra de Aguilar	Mariana	Universidad de La Laguna	Spain	marianag.a9@gmail.com
Guerra García	José Asterio	GMR Canarias SAU-Área de Agricultura	Spain	joseagg@gmrcanarias.com
Gutiérrez-Fernández	Angel J.	Universidad de La Laguna	Spain	ajguti@ull.edu.es
Hardisson de la Torre	Arturo	Universidad de La Laguna	Spain	atorre@ull.edu.es
Haroun Tabraue	José Antonio	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	jharoun@icia.es
Hernández Álvarez	Eduardo	Universidad de La Laguna	Spain	alu0100947311@ull.edu.es

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Surname	First Name	Afiliation	Country	e-mail
Hernández Amador	Eduardo	Universidad de La Laguna	Spain	eduardohernador@gmail.com
Hernández Bolaños	Eduardo Antonio	Universidad de La Laguna	Spain	ehernabo@ull.edu.es
Hernández Dorta	Aranzazu	Gobierno de Canarias	Spain	aherdor@gobiernodecanarias.org
Hernández González	María Mercedes	IPNA-CSIC	Spain	mercedes@ipna.csic.es
Hernández Mesa	Dácil	IPNA-CSIC	Spain	dacil@ipna.csic.es
Hernández Suárez	Emma Cristina	Universidad de Las Palmas de Gran Canaria	Spain	ehernandez@iuma.ulpgc.es
Herrera González	Antonio J.	IPNA-CSIC	Spain	ajherrera@ipna.csic.es
Herrera Herrera	Antonio V.	Universidad de La Laguna	Spain	avherrer@ull.es
Horta Lopes	David	Universidade dos Açores	Portugal	david.jh.lopes@uac.pt
Jaizme Vega	M <sup>a</sup> Carmen	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	mcjaizme@icia.es
Jiménez Arias	David	Universidade da Madeira	Portugal	david.j.a1983@gmail.com
Jiménez Díaz	Ignacio	Universidad de La Laguna	Spain	ignadiaz@ull.edu.es
Lahoz Zamarro	Fernando	Universidad de La Laguna	Spain	flahoz@ull.es
Llaría López	María Angeles	Cabildo de Tenerife	Spain	mllaria@tenerife.es
Lobo Palacios	Fernando	Universidad de La Laguna	Spain	flobopal@ull.edu.es
Lobo Rodrigo	M <sup>a</sup> Gloria	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	globo@icia.es
Loizou	Maria Stella	IPNA-CSIC	Spain	mariastella.loizou@gmail.com
Lopes de Macedo	Fabricio	Universidade da Madeira	Portugal	fabriciomacedo@hotmail.com
López Bazzocchi	Isabel	Universidad de La Laguna	Spain	ilopez@ull.edu.es
López Cabeza	Rocío	Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC)	Spain	rlopezc@irnas.csic.es
López Feliciano	José Francisco	Universidad de las Palmas de Gran Canaria	Spain	lopez@iuma.ulpgc.es
Machado Ferreira Castilho	Paula Cristina	Universidade da Madeira	Portugal	pcastilho@staff.uma.pt

Surname	First Name	Afiliation	Country	e-mail
Marques da Silva	Jorge ML	Universidade de Lisboa	Portugal	jmlsilva@fc.ul.pt
Martíns	Anísia	Universidade da Madeira	Portugal	anisia.martins@staff.uma.pt
Montesdeoca Flores	David Tomás	Universidad de La Laguna	Spain	dmontesd@ull.edu.es
Mora Mendoza	Albert	Escuela de Capacitación Agraria de Tacoronte-DGA-Gobierno Canarias	Spain	amormenj@gobiernodecanarias.org
Morales de la Nuez	Antonio José	IPNA-CSIC	Spain	morales.delanuez@ipna.csic.es
Niebla Canelo	Daniel	Universidad de La Laguna	Spain	alu0100798203@ull.edu.es
Ojembarrena Magister	Andrea	Universidad de Sevilla	Spain	andojemag@us.es
Oliveira	Maria Cristina	Universidade da Madeira	Portugal	maria.oliveira@staff.uma.pt
Otazo	Andrea	IPNA-CSIC	Spain	andreaotazopz@gmail.com
Paz Montelongo	Soraya	Universidad de La Laguna	Spain	spazmont@ull.edu.es
Pedraza-Torres	Angie Marcela	Instituto de Ciencias Ambientales, Universidad de Castilla-la Mancha	Spain	Angie.Pedraza@uclm.es
Peña-Rey Lorenzo	Isabel	Agencia Española de Seguridad Alimentaria y Nutrición (AESAN)	Spain	direccion@aesan.gob.es
Pérez de la Lastra	José Manuel	IPNA-CSIC	Spain	jm.perezdelalastra@csic.es
Pérez García	Ámbar	Universidad de Las Palmas de Gran Canaria	Spain	apgarcia@iuma.ulpgc.es
Pérez-Godiño García	Marta	Universidad de La Laguna	Spain	alu0100910735@ull.edu.es
Pérez-Martín	Inés	IPNA-CSIC	Spain	ines@ipna.csic.es
Pérez Pérez	Luisa del Pilar	Dirección General de Agricultura. Gobierno de Canarias	Spain	lpperper@gobiernodecanarias.org
Pérez Reyes	Carolina	Universidad de La Laguna	Spain	cpreyes@ull.es
Pérez Ruiz	Manuel	Universidad de Sevilla	Spain	manuelperez@us.es
Pinacho Crisóstomo	Fernando Rodrigo	Universidad de La Laguna	Spain	fpinacho@ull.edu.es; fernandopc@ecobertura.es
Pinheiro de Carvalho	Miguel Angelo	Universidade da Madeira	Portugal	miguel.carvalho@staff.uma.pt

Surname	First Name	Afiliation	Country	e-mail
Porras Romero	Marina	IPNA-CSIC	Spain	mporras@ipna.csic.es
Ragonezi Lopes	Carla	Centro ISOplexis - Universidade da Madeira	Portugal	carla.ragonezi@staff.uma.pt
Rancel Rodríguez	Nereida	Universidad de La Laguna	Spain	nrrodri@ull.edu.es
Raya Ramallo	Vanesa	Instituto Canario de Investigaciones Agrarias (ICIA)	Spain	vraya@icia.es
Rodríguez Egea	Pedro Luís	Instituto de Biología Molecular y Celular de Plantas CSIC-UPV	Spain	prodriguez@ibmcp.upv.es
Rodriguez Molina	Adrián	Universidad de Las Palmas de Gran Canaria	Spain	armolina@iuma.ulpgc.es
Rodríguez Sabina	Samuel	Universidad de La Laguna	Spain	srodri@ull.edu.es
Romero Aranda	María Remedios	IHSM-La Mayora (CSIC)	Spain	rromero@eelm.csic.es
Rubio Armendáriz	Carmen	Universidad de La Laguna	Spain	crubio@ull.edu.es
Ruiz González	Estefanía del Rocío	Universidad de Sevilla	Spain	fanirg@hotmail.com
Saavedra	Carlos J.	Universidad de La Laguna	Spain	csaavedr@ull.edu.es
Salazar Martínez	Raúl Omar	Universidad Autónoma del Estado de Morelos	México	omar.salazar1997@outlook.com
Sanchez Fernández	Luis	Universidad de Sevilla	Spain	lsanchez1@us.es
Schätz	Alexander	Syngenta SA	Switzerland	alexander.schaetz@syngenta.com
Seca	Ana M.L.	Universidade dos Açores	Portugal	ana.ml.seca@uac.pt
Semedo Monteiro	João Francisco	INIDA	Cabo Verde	joaofmonteiro4@gmail.com
Sequeira	Erik Augusto da Cruz	Universidade de Cabo Verde	Cabo Verde	erik.sequeira@docente.unicv.edu.cv
Silva Gouveia	Carla Susana	Centro ISOplexis - Universidade da Madeira	Portugal	csgouveia@staff.uma.pt
Spíchal	Lukáš	University of Palacky, Olomuc	Czech Republic	lukas.spichal@upol.cz
Teixeira Ganança	José Filipe	ISOplexis - Universidade da Madeira	Portugal	jofitei@staff.uma.pt
Torregrosa	Laurent	L'Institut Agro Montpellier	France	laurent.torregrosa@supagro.fr



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## Sustainable and Precision Agriculture Symposium 2022

Surname	First Name	Afiliation	Country	e-mail
Travieso	José	Fundación Universitaria de Las Palmas	Spain	jrobaina@fulp.es
Usenco Usenco	Natalia	IPNA-CSIC	Spain	nataliausenco@gmail.com
Valente	Sofía	Universidade da Madeira	Portugal	sofia.valente@staff.uma.pt
Vieira	Carolina	Universidade da Madeira	Portugal	ana.vieira@staff.uma.pt
Zhang	Peng	University of Birmingham	UK	p.zhang.1@bham.ac.uk

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