

VOL VIII

AGRÁRIAS

PESQUISA E INOVAÇÃO NAS CIÊNCIAS QUE
ALIMENTAM O MUNDO

EDUARDO EUGÊNIO
SPERS
(Organizador)

 EDITORA
ARTEMIS

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2022



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APRESENTAÇÃO

As Ciências Agrárias são um campo de estudo multidisciplinar por excelência, e um dos mais profícuos em termos de pesquisas e aprimoramento técnico. A demanda mundial por alimentos e a crescente degradação ambiental impulsionam a busca constante por soluções sustentáveis de produção e por medidas visando à preservação e recuperação dos recursos naturais.

A obra **Agrárias: Pesquisa e Inovação nas Ciências que Alimentam o Mundo** compila pesquisas atuais e extremamente relevantes, apresentadas em linguagem científica de fácil entendimento. Na coletânea, o leitor encontrará textos que tratam dos sistemas produtivos em seus diversos aspectos, além de estudos que exploram diferentes perspectivas ou abordagens sobre a planta, o meio ambiente, o animal, o homem e a sociedade no ambiente rural.

É uma obra que fornece dados, informações e resultados de pesquisas tanto para pesquisadores e atuantes nas diversas áreas das Ciências Agrárias, como para o leitor que tenha a curiosidade de entender e expandir seus conhecimentos.

Este Volume VIII traz 26 artigos de estudiosos de diversos países, divididos em quatro eixos temáticos: *Cultura e Sociedade no Contexto Rural; Produção Sustentável; Produção Vegetal e Solos e Aquacultura, Produção Animal e Veterinária.*

Desejo a todos uma proveitosa leitura!

Eduardo Eugênio Spers

SUMÁRIO

CULTURA E SOCIEDADE NO CONTEXTO RURAL

CAPÍTULO 1..... 1

DESAFIOS DE UMA PAISAGEM CULTURAL MEDITERRÂNICA: O MONTADO, O TIRADOR DE CORTIÇA E A TRANSMISSÃO DO SABER-FAZER TRADICIONAL

Sónia Bombico

Carlos Manuel Faísca

 https://doi.org/10.37572/EdArt_2608226821

CAPÍTULO 2.....28

DISEÑO DE UN SISTEMA DE BUENAS PRACTICAS AGRICOLAS COMO ESTRATEGIA DE IMPLEMENTACION EN LA ASOCIACION APRIMUJER UBICADA EN EL MUNICIPIO DE SAN VICENTE DE CHUCURI

Leidy Andrea Carreño Castaño

Mónica María Pacheco Valderrama

Héctor Julio Paz Díaz

Miguel Arturo Lozada Valero

Rafael Calderón Silva

Jhoan Arley Ochoa Martínez

Angélica María Montoya Hernández

Irina Alean Carreño

Shirley Mancera

Daniel Augusto Buitrago Ibañez

Ana Milena Salazar

Sandra Milena Montesino Rincón

 https://doi.org/10.37572/EdArt_2608226822

CAPÍTULO 3..... 38

ESPECIES FORESTALES DE IMPORTANCIA CULTURAL DE BADIRAGUATO SINALOA

Yulisa Rodríguez López

Heréndira Flores Almeida

Gilberto Sandoval Varela

Bladimir Salomón Montijo

Aidé Avendaño Gómez

 https://doi.org/10.37572/EdArt_2608226823

CAPÍTULO 4..... 50

CONTRIBUCIÓN POTENCIAL DE LAS SEMILLAS DE *Carica papaya* Linn Y SU ACEITE EN LA SALUD

Amelia Andrea Espitia Arrieta
Jennifer Judith Lafont Mendoza
Ana Karina Paternina Zapa

 https://doi.org/10.37572/EdArt_2608226824

CAPÍTULO 5.....62

PROTOTIPOS DE INNOVACIÓN SOCIAL EN PESCA ARTESANAL, REGIÓN DE LOS RÍOS – CHILE

Griselda Ilabel Pérez
Meyling Tang Ortiz
Claudio Barrientos Aguila

 https://doi.org/10.37572/EdArt_2608226825

PRODUÇÃO SUSTENTÁVEL

CAPÍTULO 6.....70

CONCEPTO DE BIORREFINERÍA: DESARROLLO SOSTENIBLE Y PROPUESTA DE PROCESO LIMPIO EN LA EXTRACCIÓN DE COMPUESTOS FENÓLICOS DE RESIDUOS INDUSTRIALES DE PISTACHO (*Pistacia vera* var. *Kerman*)

Daniela Zalazar-García
Rosa Rodriguez
María Paula Fabani
Germán Mazza
Marcelo Echegaray
Romina Zabaleta
Eliana Sanchez
Erick Torres

 https://doi.org/10.37572/EdArt_2608226826

CAPÍTULO 7..... 83

REDUCCIÓN DE LA CANTIDAD DE VINAZA POR AUMENTO DE LA CONCENTRACIÓN FINAL DE ETANOL POR FERMENTACIÓN DE *Saccharomyces cerevisiae*

María Laura Muruaga
María Gabriela Muruaga
Cristian Andrés Sleiman
Nora Inés Perotti

 https://doi.org/10.37572/EdArt_2608226827

CAPÍTULO 8.....97

EVALUACIÓN DE LA *CHLORELLA SP* Y LA *DUNALIELLA TERTIOLECTA* COMO FUENTE POTENCIAL DE ÁCIDOS GRASOS PARA LA PRODUCCIÓN DE BIODIESEL

Dally Esperanza Gáfaró Álvarez
Mónica María Pacheco Valderrama
Daniel Augusto Buitrago Ibañez
Yuleisi Tatiana Caballero Hernandez
Leidy Andrea Carreño Castaño
Ana Milena Salazar Beleño
Miguel Arturo Lozada Valero
Leidy Carolina Ortiz Araque
Olga Cecilia Alarcón Vesga
Sandra Milena Montesino Rincón
Cristian Giovanni Palencia Blanco
Nora Milena Ortiz Garcia

 https://doi.org/10.37572/EdArt_2608226828

CAPÍTULO 9..... 110

A TEMPORARY IMMERSION SYSTEM (TIS) BIOREACTOR USED FOR THE IN VITRO PROPAGATION OF *PRUNUS* AND *PYRUS* ROOTSTOCKS

Carlos Rolando Mendoza
Ramon Dolcet-Sanjuan

 https://doi.org/10.37572/EdArt_2608226829

CAPÍTULO 10.....125

CARACTERIZAÇÃO DE CORANTES PARA ELABORAÇÃO DE CEREJAS CANDEADA: ERITROSINA VERSUS VERMELHO GARDENIA

Juan Ignacio González Pacheco
Mariela Beatriz Maldonado
Ariel Fernando Márquez Agüero
Emanuel Félix Condori Laura
Paula Anabella Giorlando Videla

 https://doi.org/10.37572/EdArt_26082268210

PRODUÇÃO VEGETAL E SOLOS

CAPÍTULO 11..... 141

THE QUALITY OF APPLE FRUIT PRODUCTS WHEN USING THE GROWTH BIOREGULATOR ALBIT IN THE SYSTEM OF PROTECTION

Svetlana Levchenko
Elena Stranishevskaya

Elena Matveikina
Vladimir Boiko
Nadezhda Shadura
Vitalii Volodin
D. Belash
Ya. Volkov
Marina Volkova

 https://doi.org/10.37572/EdArt_26082268211

CAPÍTULO 12 151

THE EFFECT OF VEGETATIVE TREATMENT OF GRAPES WITH A PREPARATION
BASED ON AMINO ACIDS ON THE PHENOLIC COMPLEX OF BERRIES

Svetlana Levchenko
Elena Ostroukhova
Sofia Cherviak
Vladimir Boyko
Dmitriy Belash
Irina Peskova
Nataliya Lutkova
Mariya Viugina
Olga Zaitseva
Aleksandr Romanov

 https://doi.org/10.37572/EdArt_26082268212

CAPÍTULO 13 162

ANÁLISIS FÍSICOQUÍMICO DE ACEITES SEMILLAS CON APROVECHAMIENTO
POTENCIAL ZONAS TROPICALES

Amelia Andrea Espitia Arrieta
Jennifer Judith Lafont Mendoza

 https://doi.org/10.37572/EdArt_26082268213

CAPÍTULO 14 175

PLAGAS DESENCADENANTES DE EPIFITIAS DEL CULTIVO DE PLATANO &
ESTRATEGIAS DE CONTROL

Francisco Angel Simón Ricardo
Renso Oswaldo Lozano Gámez
Cristhian Andrés Méndez Cedeño
Luis Pérez Vicente

 https://doi.org/10.37572/EdArt_26082268214

CAPÍTULO 15 191

EFFECTOS ABIÓTICOS DE LA SALINIDAD EN CULTIVOS DE ARÁNDANO BAJO RIEGO POR GOTEO, EN LA PROVINCIA DE BUENOS AIRES

Alejandro Pannunzio

Pamela Texeira

Luciana Tozzini

 https://doi.org/10.37572/EdArt_26082268215

CAPÍTULO 16 200

EVALUACIÓN DEL RENDIMIENTO DEL GRANO CON LOS TRES HÍBRIDOS ASOCIADOS CON TRES NIVELES DE LA FERTILIZACIÓN NITROGENADA EN EL CULTIVO DE MAÍZ ENTRE LA ASPERSIÓN Y GOTEO POR FERTIRIEGO DURANTE LA ESTACIÓN SECA EN UN SUELO VERTISOL

Kentaro Tomita

Jaime Proaño

 https://doi.org/10.37572/EdArt_26082268216

CAPÍTULO 17 209

APLICAÇÃO DE TÉCNICAS DE MACHINE LEARNING PARA CLASSIFICAÇÃO DA APTIDÃO DOS SOLOS PARA O REGADIO

Pedro Torres

António Canatário Duarte

João Gerales

Sílvia Marques

 https://doi.org/10.37572/EdArt_26082268217

AQUACULTURA, PRODUÇÃO ANIMAL E VETERINÁRIA

CAPÍTULO 18 225

INFLUENCIA DE LAS VARIABLES MORFOLÓGICAS Y POBLACIONALES DE *Eichornia crassipes* Y *Pistia stratiotes* SOBRE LA COMUNIDAD DE MACROINVERTEBRADOS ACUÁTICOS EN UNA MADRE VIEJA DEL VALLE DEL CAUCA

Daniel Feriz Garcia

Jency Nathaly Palacio Bayer

Laura Melissa Muños Burbano

 https://doi.org/10.37572/EdArt_26082268218

CAPÍTULO 19239

AVALIAÇÃO DO CRESCIMENTO DE ACHIGÃS PRODUZIDOS EM AQUACULTURA

António Moitinho Rodrigues

António Vasco de Mello

Miguel de Mello

Filipa Inês Pitacas

 https://doi.org/10.37572/EdArt_26082268219

CAPÍTULO 20250

EFICÁCIA DO TRATAMENTO COMBINADO DE AMITRAZ E FLUMETRINA NO CONTROLO DA VARROOSE

Maria Alice Carvalho Hipólito

Catarina Manuela Almeida Coelho

Sância Maria Afonso Pires

Jorge Belarmino Ferreira de Oliveira

 https://doi.org/10.37572/EdArt_26082268220

CAPÍTULO 21263

CAPTACIÓN Y DISTRIBUCIÓN DE AGUA PARA RIEGO DE PASTURAS EN CHIPAUQUIL (DPTO. VALCHETA). ARGENTINA

Juan José Gallego

Ciro Adrián Saber

Germán Cariac

Pablo Giovinne

Julio Argentino Llampá

Horacio Alberto Pallao

Diego Milipil

Hernán Zelmer

Roberto Angel Molina

Ines Mora Jara

María Victoria Cortés

 https://doi.org/10.37572/EdArt_26082268221

CAPÍTULO 22270

POTENCIALES MECANISMOS POR LOS CUALES SE MANIFIESTAN LAS ENFERMEDADES INFECCIOSAS EMERGENTES DEL CERDO

Carlos J. Perfumo

Mariana Machuca

Alejandra Quiroga

 https://doi.org/10.37572/EdArt_26082268222

CAPÍTULO 23285

CONFORTO TÉRMICO PARA FRANGOS DE CORTE EM CENÁRIOS DE MUDANÇA CLIMÁTICA NO RS

Zanandra Boff de Oliveira
Emanuel Luis Christmann
Eduardo Leonel Bottega
Tiago Rodrigo Francetto

 https://doi.org/10.37572/EdArt_26082268223

CAPÍTULO 24298

GANADERÍA EQUINA EXTENSIVA, FIESTAS Y PRODUCTOS TRADICIONALES: COOPERATIVA MONTE CABALAR Y RAPA DAS BESTAS DE SABUCEDO (A ESTRADA, PONTEVEDRA)

Francisco Xavier Barreiro
Adolfo Cano Guervós

 https://doi.org/10.37572/EdArt_26082268224

CAPÍTULO 25316

VINCRISTINA SUBCUTÁNEA COMO VIA ALTERNATIVA PARA EL TRATAMIENTO DE TUMOR VENÉREO TRANSMISIBLE EN PERROS

Gloria Beatriz Cabrera Suarez
David Octavio Rugel González

 https://doi.org/10.37572/EdArt_26082268225

CAPÍTULO 26326

A MASTITE E SEU EFEITO NO DESEMPENHO ZOOTÉCNICO E QUALIDADE DO LEITE

Greyce Kelly Schmitt Reitz
Mariana Monteiro Boeng Pelegrini
Pietra Viertel Molinari
Fabiana Moreira
Ivan Bianchi
Juliano Santos Gueretz
Vanessa Peripolli
Elizabeth Schwegler

 https://doi.org/10.37572/EdArt_26082268226

SOBRE O ORGANIZADOR.....332

ÍNDICE REMISSIVO333

CAPÍTULO 9

A TEMPORARY IMMERSION SYSTEM (TIS) BIOREACTOR USED FOR THE IN VITRO PROPAGATION OF *PRUNUS* AND *PYRUS* ROOTSTOCKS

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ABSTRACT: A new type of bioreactor, which has been patented (Dolcet-Sanjuan and Mendoza, 2018; Patent Pending ES201831164), is described herein and the results on the propagation of fruit tree rootstocks are detailed. The “GreenTray” (GT) bioreactor based on the temporary immersion system (TIS) principle, is a mid-sized unit reactor, from 1 to 4L capacity, with the distinction in the fact that, firstly, transforms glass, transparent and commercial flasks into a bioreactor; secondly, the flask containing the shoots is placed in horizontal position, and

more importantly, its concept allows for the extraction and cut of all the shoots at once, making more efficient the explants culture and shoot extraction out of the bioreactor. As described here, the GT bioreactor consists of two flasks, one for the shoot explants and the other for the liquid culture media, joined through a perforated adaptor piece that permits the flow of the liquid media from one vessel to the other, driven by pressured air and gravity. The structural simplicity and the modular and independent nature of the bioreactor, helps its operation and reduces the amount of hand labor required for shoot transfers, thereby reducing the cost of the whole micropropagation process, when compared with other commercial TIS bioreactors or with the cultures in vessels with semisolid media. The system has been applied to propagate RootPack-20® (RP-20), a commercial *Prunus spp.* rootstock, and “Py170”, an experimental *Pyrus spp.* hybrid rootstock. Compared with the conventional culture in semisolid medium, GT bioreactor improves by a 10% the multiplication rates of both rootstocks, and more significantly it improves shoot length by a 33% and 26%, and shoot fresh weight by a 21.9% and 30%, for RP-20 and “Py170” respectively. Shoot cultures produced with the GT bioreactor had better leaf development and less hyperhydricity, therefore improved shoot quality, than with the conventional cultures in semisolid media. Culture time required for

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plantlet development was reduced, hence costs to produce high quality commercial plants was 0.11€/plant with the GT bioreactor, significantly less than with the semisolid agar containing media.

KEYWORDS: “GreenTray”. GT bioreactor. IRTA-reactor. Temporary immersion system. TIS. Liquid culture. In vitro plant micropropagation.

1 INTRODUCTION

Micropropagation has great commercial impact and potential due to the speed of propagation, the high quality of plants that are obtained and the possibility of producing plants free of pathogens (Steward et al., 1970; Ammirato, 1985). Traditional micropropagation methods on semisolid media are limited by the high labor cost. In vitro culture systems based on liquid culture medium are considered to be more effective than culture systems in semisolid medium due to better accessibility of medium components by the plant tissue (Martínez, 2019), ease handling, and possibility of better scaling up and automation. However, as direct liquid immersion of some plant material causes vitrification or hyperhydricity, and sometimes inhibits growth and development of the plant material, TIS bioreactors have emerged as a workable alternative, capturing the benefits of liquid medium and semisolid support. In comparison with both solid and liquid culture systems, TIS bioreactors have been shown to offer technological and quantitative benefits such as higher multiplication rates and reduction of production cost (Etienne, H. and Berthouly, 2002). or explant quality improvements (Tisserat and Vandercook, 1985; Aitken-Christie and Davies, 1988; Etienne and Berthouly, 2002).

The development of propagation systems to produce affordable certified, true-to-type and disease-free fruit tree rootstocks or self-rooted varieties, is a worldwide objective in particular in the Mediterranean areas, such as in Catalonia, leader in the production of stone and pit fruits (MAPAMA, 2016). In order to meet these market demands, it is proposed to develop a technology that maximizes productivity by optimizing the use of resources, guaranteeing the quality of production and innovating in the design.

Unlike traditional micropropagation, bioreactors use liquid media based on the principles of temporary immersion (TIS). This principle is simply immersing the plant material into liquid growth media for short periods of time, sufficient for the plant to uptake nutrients. TIS technology profits from the advantages of liquid cultures, while growing the plants under high gas- exchange environments. Bioreactors have been considered as a promising alternative to the semi-solid culture system. However, there are no profitable bioreactors available for commercial production of fruit tree rootstocks.

There are various TIS bioreactor conformations, which have been described by Georgiev and Schumann (2014). From those, Rita® and Setis® are the most widely used in commercial and research propagation, although they need improvements to facilitate plant manipulation and reduce hyperhydricity in some plant materials. The GT bioreactor described herein, in comparison with the commercial ones, is cheaper, easy to assemble, occupying materials that can be found anywhere in the World. Its adaptability, practicality and low cost make it a good option to make escalations for mass production of plants and to carry out research works.

Herein it's described and evaluated the performance of the GT bioreactor, developed and patented by IRTA, previously called "IRTA-reactor" (Mendoza and Dolcet-Sanjuan, 2017) in comparison with the conventional in vitro culture in vessels. with semisolid medium, through the micropropagation process of the commercial *Prunus spp.* rootstock "RootPack-20®" (RP20), and a new *Pyrus spp.* hybrid pear rootstock "Py170", which is a clonal selection of an IRTA breeding program (Clavería and Asin, 2012).

2 MATERIALS AND METHODS

2.1 PARTS, ASSEMBLY, AND LIQUID FLOW IN THE GTBIOREACTOR

Two commercial glass flasks are adapted to be used as two different parts of the bioreactor. A first one, placed in its upright position, containing the liquid medium. The other, in horizontal position, having the explants inside through the whole growth process. The reactor is assembled using the following parts (Figure 1): one 2 liter and one 850 mL glass flask and their corresponding metallic lids; four hose plastic fitting; two 0.2 µm PTFE Midisart® 2000 air filters; one 6 mm external diameter glass elbow; 6 mm external diameter silicone hose to connect all parts; one 6 mm external diameter glass tubing; a stainless steel 2-mm-opening mesh and a stainless steel 15-mm-opening grid. These parts are assembled as indicated in Figure 2 to get one bioreactor unit. The bioreactor, without the medium, was autoclaved at 120°C during 30 minutes, and sterile liquid culture media poured in the vertical 850 mL glass flask after opening it under a flow hood.

Figure 1. Parts of the bioreactor: (1) metallic cap with 2 hose plastic fittings for the 2-liter-capacity glass flask (2); (3) Metallic cap with 2 hose plastic fittings for the 850-mL-capacity glass flask (4); (5) 0.2 μm PTFE Midisart® 2000 filter; (6) Glass elbow; (7) Silicone hose; (8) glass tubing; (9) stainless steel 2-mm-opening mesh; and (10) 15-mm-opening stainless steel grid.

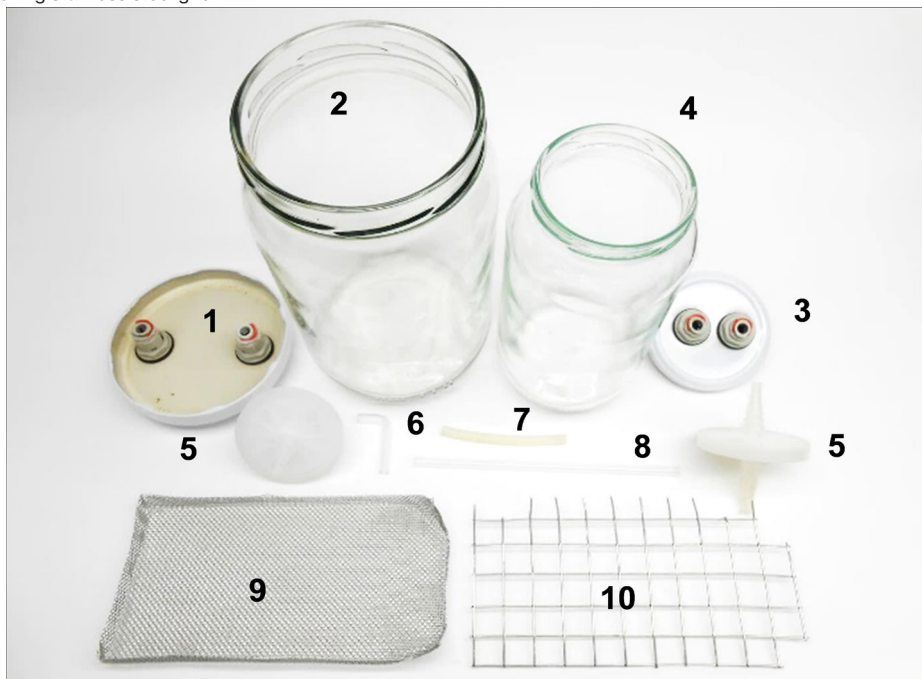
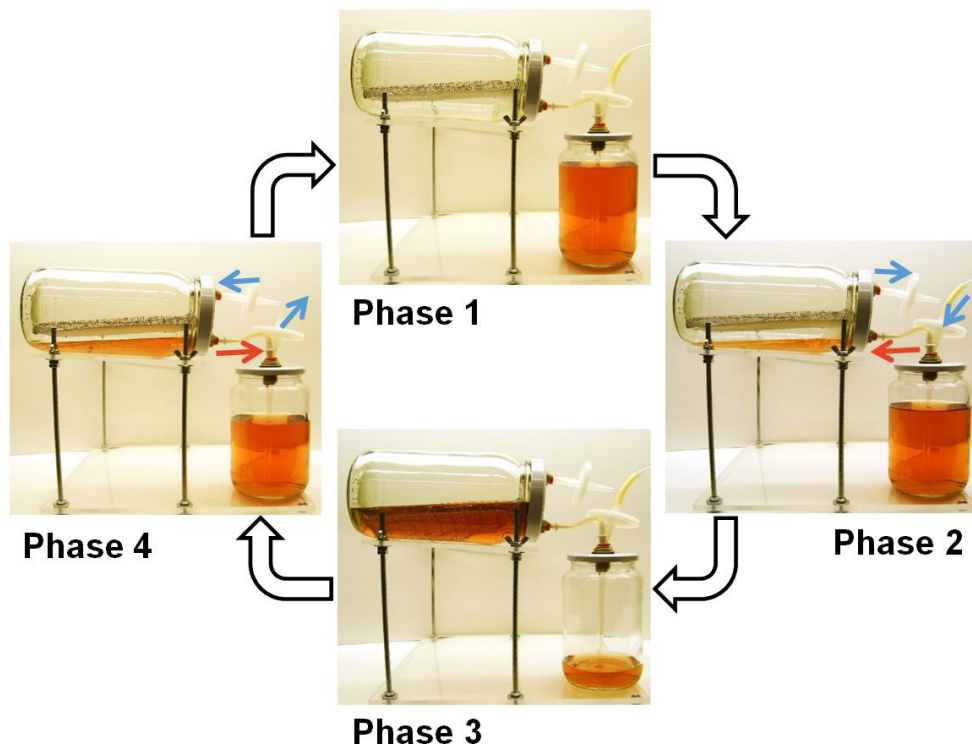


Figure 2. Assembly of the bioreactor.



The liquid medium passes from the first flask to the second flask using air under pressure. The incoming air is supplied by a 4 bar line and passes through a 0.2 μm Millex-FG50PTFE Midisart® 2000 filter (Merck KGaA, Darmstadt, Germany), to avoid contamination (Figure 3). The explants were placed in a stainless steel grid which is inserted into the horizontal flask. The immersion frequencies were controlled by a timer that kept the explants in contact with the culture medium for 1 minute, every 3 hours.

Figure 3. Liquid media (←) and air (↔) flow in the bioreactor. (Phase 1) media rests in the vertically oriented 850-mL-capacity glass flask, until pressured air pushes the media to the horizontally oriented 2-Liter-capacity glass flask (Phase 2), while air gets out of the latter. Pressured air stops flowing (Phase 3) when stainless steel mesh and grid, holding the explants, are completely immersed in the liquid medium. Media returns by gravity to the original flask moving the air out, while atmospheric air flows into the horizontally oriented flask.



2.2 PLANT MATERIALS

Rootstocks of commercial interest in fruit production were used in this study. A *Prunus* rootstock marketed by the industry, named Rootpac®20 (RP-20) (Agromillora Group), and a *Pyrus* rootstock, named “Py170”, under agronomic evaluation by IRTA., RP-20 is a natural hybrid between the “Myrobalan” plum (*Prunus cerasifera* Ehr.) and an almond (*Prunus dulcis*) (Pinochet et al., 2011), can be used as a rootstock for Japanese plums,

peaches, nectarines, almond and some apricot cultivars (Pinochet, 2010). The Py170 is a hybrid between “OH11” (Simard and Michelesi, 2002) and *Pyrus amygdaliformis*, which is in the last phase of selection in an IRTA breeding program, oriented to have tolerance to iron chlorosis and reduced vigor. Shoot tip cultures of both clones were established in vitro as follows.

2.3 ESTABLISHMENT OF SHOOT IN VITRO CULTURES

New actively growing shoots, collected from greenhouse potted RP-20 plants or from field grown Py170 trees, were used as source of explants. Explants were 1 to 2-cm-long nodal segments with one to three axillary buds each. Explants were washed for 1 h in stirring distilled water, and surface-sterilized in 70% ethanol for 1 minute, followed by 0.5 % (w/v) NaOCl with 0.1 % (v/v) Tween-20 for 10 minutes. Each explant was rinsed individually three times with sterile distilled water, trimmed, and cultured in a tube containing 15 mL of MS with 5 μ M BA. Shoots that appeared uncontaminated were screened further for bacterial contamination. Explants that showed bacterial growth on this medium were discarded (Iglesias et al., 2004).

2.4 CULTURE MEDIA

Micropropagation in flasks with semisolid agar-containing media were used as the standard culture conditions described earlier (Iglesias et al., 2004). MS (Murashige and Skoog, 1962) supplemented with 3% sucrose and 5mM 6-Benzylaminopurine (BAP), pH to 5.7, agar (7 g/L), autoclaved at 121 ° C for 20 minutes, The same MS+5BAP media, but without agar, was used in the GT bioreactor culture system. . In both culture systems, explants were kept during 3 weeks in MS+5BAP medium, and then moved to MS, without BAP medium, to promote shoot elongation for an additional 3-week-long period.

2.5 GREEN TRAY VERSUS CONVENTIONAL IN VITRO CULTURE CONDITIONS

Nodal segments, 1 to 2-cm-long, derived from shoot tip cultures in the multiplication phase, were used as explants to initiated new cultures in the GT bioreactor or in glass flasks. 70 explants per bioreactor and 10 explants per flask, with three replicates per clone, were used. All cultures were kept at 26 \pm 1°C under a photoperiod of 16h of cool-white fluorescent light (70 μ mol·m⁻²·s⁻¹), and 8h darkness.

The TIS bioreactor was set at an immersion frequency of 1 minute every 6 hours. After 3 weeks of culture, the culture medium in the bioreactors was replaced with fresh

medium of the same composition but without BAP. In the case of flasks, 50ml of the same medium was dispensed to each flask.

2.6 SHOOT MULTIPLICATION EVALUATION

In the case of flasks, with semisolid media, shoot clumps were moved out with the help of forceps, and in the case of the GT bioreactor, were all moved at once by pooling out the tray holding the whole plant material. Individual shoots were separated from the clumps with the help of scapel and forceps. The number of new shoots from each initial explant, their length, fresh weight, and multiplication rate was determined for each container. Additionally, the incidence of vitrification or hyperhydricity was scored for each shoot clump.

2.7 ACCLIMATION TO SOIL IN THE GREENHOUSE

Ninety shoots per clone, 4 to 10-cm-long, were removed from the GT bioreactors, rinsed with tap water and planted in trays with 28 wells, of 90-mL-capacity each, containing a mixture peat moss and vermiculite (2:1, v:v). The peat moss Pro Start (Castillo Arnedo S.L.) contained perlite (10%). Plantlets were acclimated to low atmospheric humidity was done gradually from 100 to 60%, in a 3-week-long period, in which soil temperature was kept above 20°C, and air temperature of 26-30°C. The photoperiod was supplemented to 14h light, with LED light (AlternativaLED, Valencia, Spain) to 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Another ninety shoots per clone were treated with 1000 ppm indole-3-butyric acid (IBA) and planted in the same conditions.

The percent survival of acclimated plantlets was determined.

2.8 COST EVALUATION AND OCCUPIED IN VITRO GROWTH CHAMBER AREA

The needed in vitro growth chamber area (CA_b) and the cost to produce new shoots (CS) were separately analyzed for the micropropagation in flasks or in the bioreactor.

The occupied chamber area per shoot produced (CA_b) was calculated by dividing the area, in cm^2 , needed to hold each container (Ab) by the number of new explants produced (NE).

$$CA_b = \frac{Ab}{NE}$$

The cost to produce each plant, in € per plant (*CP*) was obtained after adding the cost of each container (*CC*), the costs of all the culture media used (*CM*), and the workforce cost needed to complete the culture process (*CWF*), and dividing the sum by the number of new explants produced (*NE*).

$$CP = \frac{CC + CM + CWF}{NE}$$

2.8 STATISTICAL ANALYSIS

The experiment was set up as a completely randomized design, with two culture systems, two plant materials, and three containers per treatment. Data was statistically analyzed with JMP Statistics software (version 13.1.0, SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$) was performed.

3 RESULTS

3.1 ASSEMBLY AND OPERATION OF THE GREEN TRAY BIOREACTOR

The principal advantage of the GT bioreactor operation is the facility to move the plant material out of the recipient, or placing it back into it after is being cut, since it is held in a tray easily moved with a pair of forceps. This is the main claim of the patented bioreactor (Dolcet-Sanjuan and Mendoza, 2018; Patent Pending ES201831164). This operation is always done with the recipient in horizontal position, avoiding placing the hands above or close to the flask entrance, and this way reducing the possibilities to contaminate the cultures.

Other advantages of the GT bioreactor are (1) the high illumination of the plant material, since the lid is not shading it, (2) the elimination of all the medium and condensed water in the recipients walls closer to the leaves, since the flask surface is curved and the liquid moves to the bottom where it returns by gravity to the original, and (3) atmospheric air renewal in the recipient holding the plant material each time there is a movement of the liquid media. Such GT bioreactor characteristics enhance leaf development, shoot elongation, and avoids vitrification or hyperhydricity of the plant tissues observed with the plant materials used herein, when cultured in flasks with semisolid media o in other TIS bioreactors.

3.2 SHOOT PROLIFERATION IN THE GREEN TRAY BIOREACTOR VERSUS CONVENTIONAL CULTURES IN FLASKS

As shown in Figure 4 and 5, leaf number and stem thickness of shoots produced from both plant materials were comparable after culture in the two culture systems. Although differences in the multiplication rates were observed, after the 6-week-long culture process, no statistically significant differences were found, for Py170 or RP-20, between cultures in flasks with semisolid agar-containing medium or in the temporal immersion system with the GT bioreactor (Table 1). However, significant statistical differences were observed on the shoot length and the fresh weight per shoot between both culture systems. Both rootstocks, Py170 and RP-20, were 35% and 49% longer respectively, when grown in the GT bioreactor than in flasks with semisolid media. This was in concordance with a 43% and a 28% increase in the shoot fresh weight per new shoot, for Py-170 and RP-20 respectively.

Figure 4. Shoot growth after 42 weeks of culture in conventional semisolid agar-containing media (A and C), and culture in the bioreactor (B and D), of RP-20 (A and B) and Py170 (C and D) clones.

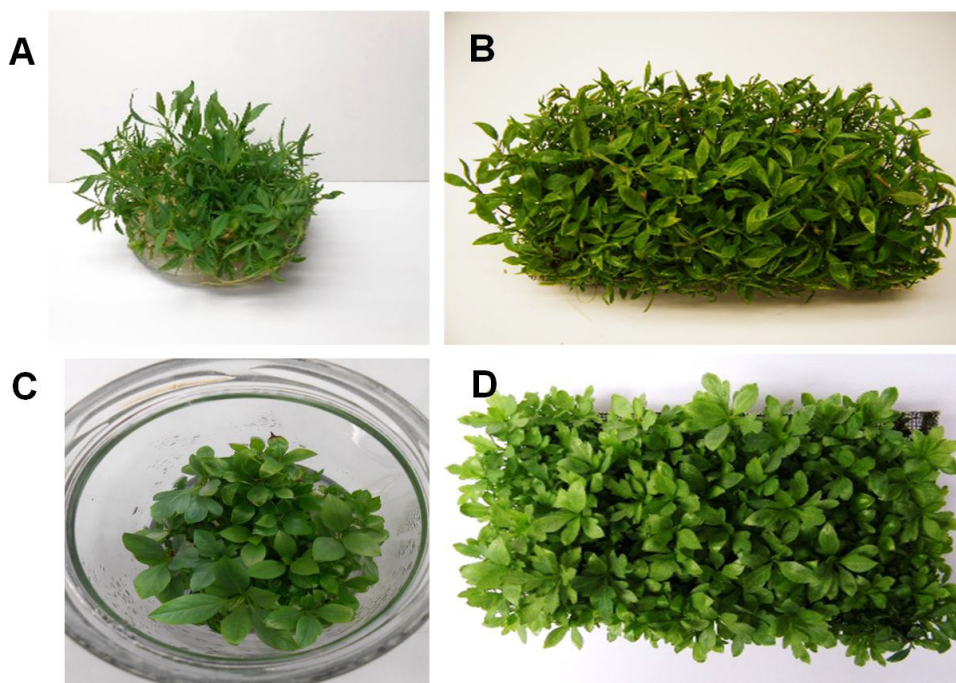


Figure 5. Evolution of the RP-20 and Py170 clones in the bioreactor. Initial explants (0), after 3 weeks of immersion in 5mM BAP (21), after 3 weeks of immersion in 0mM BAP (42), and after direct rooting of shoots in acclimation tunnels.

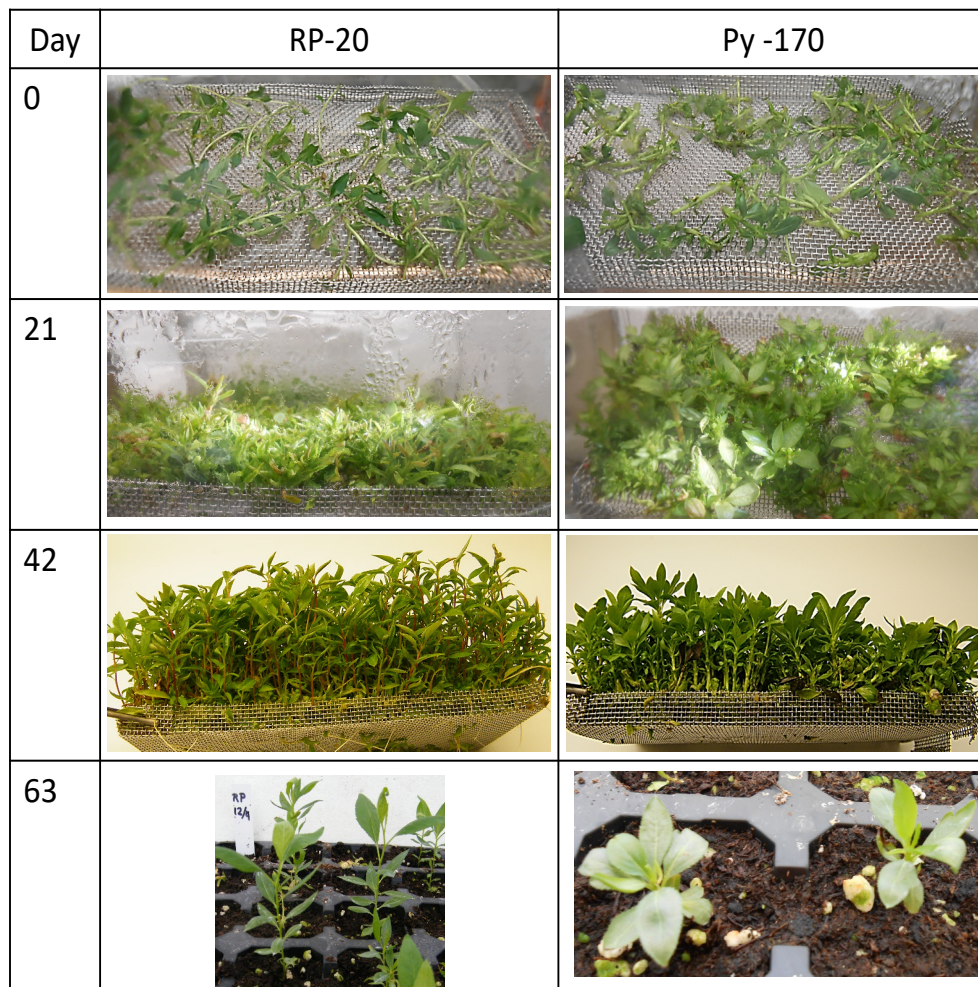


Table 1. Shoot multiplication rates and growth, of RP-20 and Py170 clones, after 6 weeks of culture in semisolid agar-containing medium or in the temporal immersion system with GT IRTA bioreactor.

Clon	Culture System	Multiplication Rate (Shoots / Explant)	Shoot Length (cm)	Fresh weight (grs / explant)
Py170	Semisolid medium	5.03 ± 0.44 a*	16.90 ± 0.82 a	0.42 ± 0.03 a
Py170	GT bioreactor	5.59 ± 0.45 a	22.84 ± 0.68 b	0.60 ± 0.05 b
RP-20	Semisolid medium	7.29 ± 0.79 a	26.60 ± 0.80 a	0.57 ± 0.06 a
RP-20	GT bioreactor	8.10 ± 0.48 a	39.70 ± 0.69 b	0.73 ± 0.04 a

Values represent mean ± SE (Standard Error) out of three repetitions. *Means with different letter, within each measured variable, are significantly different according to Tukey HSD (P=0,05)

3.3 EVALUATION OF GT BIOREACTOR IN THE ACCLIMATION PHASE

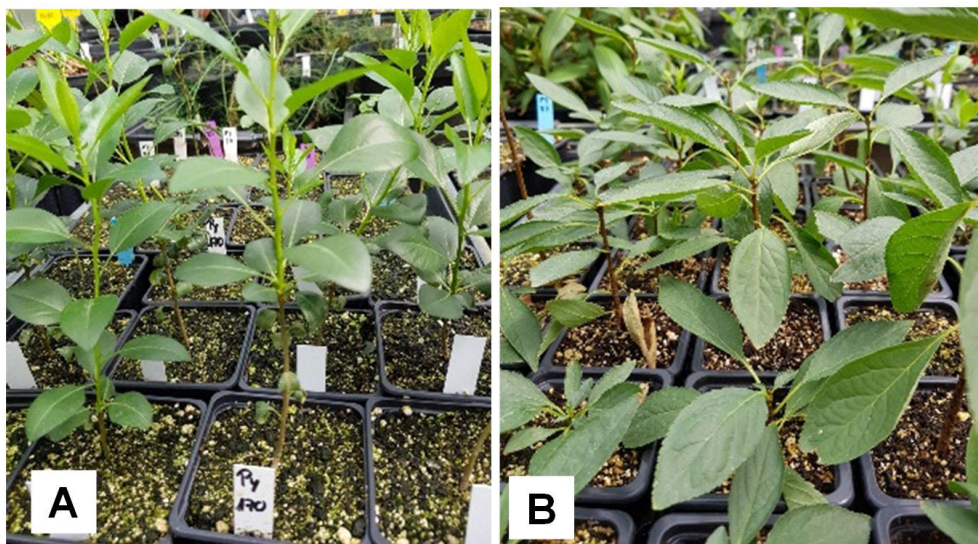
Shoot acclimation and rooting to the greenhouse conditions (Figure 5) and further plant growth and endurance (Figure 6) were highly successful. And 81% or 78% of shoots, Py170 and Rp-20 respectively, derived from the GT bioreactor directly rooted in the acclimation tunnels and survived in greenhouse conditions, without IBA treatment. Indeed the percentage of rooting dropped to 60% and 59%, for Py170 and Rp-20 respectively, when the shoot basis was dipped in IBA before planting, though the root development was better when the explants were treated with IBA (Table 2).

Table 2. Rooting of RP-20 and Py170 shoots derived from the GT IRTA bioreactor, treated or not with IBA, after 3 weeks of acclimation and 4 additional weeks of culture in the greenhouse.

Clon	IBA (ppm)	Rooting (%)
Py170	0	81.1 ± 4.0
Py170	1000	60.0 ± 8.8
RP-20	0	78.3 ± 7.3
RP-20	1000	58.6 ± 8.3

Values represent mean ± SE (Standard Error) out of three repetitions.

Figure 6. Actively growing 3-month-old Py170 (A) and RP-20 (B) rooted plants derived from shoots produced in the bioreactor.



3.4 COST EVALUATION AND OCCUPIED CHAMBER AREA

Following the protocol described here the cost to produce an acclimated plant of RP-20 with the GT bioreactor was significantly lower than with the conventional method in flasks with semisolid medium (Table 3). While the cost of the container and materials to assemble a GT bioreactor, is sixty times more expensive than that of the flask with semisolid medium, the hand labor is less than one half since the time to produce and acclimated plant is reduced to 9 weeks. In consequence, the cost to produce one plant with the GT bioreactor is 0,11€, seven times less than with the semisolid medium. In addition, the in vitro growth chamber surface needed to get one shoot of RP-20 with the GT bioreactor is 0,32 cm², two and a half times less than with the conventional culture in agar containing medium.

Table 3. Comparative costs between the micropropagation in semisolid agar-containing media and the temporal immersion with GT bioreactor, of the RP-20 clone.

Plant growth requirements for each container	Semisolid medium	GT bioreactor
Number of initial explants	15	70
Total time needed for plant production (weeks)	14	9
NE: Number of shoots produced	100	700
Ab: Growth chamber area needed for each container (cm ²)	81	230
CAb: Growth chamber area needed for each shoot (cm ² /shoot)	0.81	0.32
Costs for each container		
CC: Container cost (€)	0.45	47.23
CM: Culture media cost (€)	0.28	1.38
CWF: Workforce cost (€)	75.00	30.00
Total Costs (€)	75.73	78.61
CP: Total Cost / Plant (€)	0.76	0.11

4 DISCUSSION

The results show that this newly developed Green Tray or GT bioreactor is suitable for mass production of fruit tree species, with survival rate and plant quality similar to those from semisolid medium. Similar results have been obtained in different species such as *Mentha x piperita* L. (Vaidya, 2018), red currant (J. Hautsaloa, 2018), sugarcane (Carrillo-Bermejo, 2018) using liquid media in different temporary immersion systems. The multiplication rate was either similar to or better than that from semisolid medium. Depending on the rootstock, between 400 and 700 shoots could be obtained per bioreactor, whereas in agar containing medium, over 100 shoots per flask were obtained. The average

shoots length was higher in the GT than in the semisolid medium. In addition, while shoots produced had the same quality in both systems, the time needed was shortened two weeks when cultures were done in the GT. In this reactor, shoot hyperhydricity was controlled and eliminated, when immersion time and frequency were corrected for each rootstock. Overall, the analysis of the culture systems results show that the GT is stable, reliable and with greater productivity than the culture in semisolid media.

The results of this study demonstrated the utility of the GT for the micropropagation of the two clones studied, obtaining more plants in a shorter time, occupying less chamber area per explant and reducing costs. The high plantlet survival rate obtained with this system assured the success of the micropropagation process because the *in vitro* rooting phase could be eliminated.

It is reported in the literature that the positive effects of TIS are due to a better aeration and renewal of chemical components at each immersion, otherwise limited in solid condition by the agar matrix. In this way the risk of anoxia and hyperhydricity, frequently observed in constant immersion as well as in solid culture, is significantly reduced (Etienne et al., 1997). The results obtained in the present work are consistent with those obtained in other micropropagation studies using temporal immersion systems in other species (Ramírez et al 2016; Escolana et al 1999, Bernal et al 2008, Niemenak et al 2008; Roels et al 2005) however most of these studies did not make the comparison between different temporal immersion system, or with the culture in semisolid media as here presented.

Microshoots under *in vitro* culture conditions can have a negative net photosynthetic rate and depend on sucrose uptake from the medium for their growth. Few results are available on the mechanisms of sugar uptake in shoot tissue culture systems. It is accepted that sugar uptake is an energy dependent process. Most publications dealing with sucrose uptake by cell cultures assume that sucrose molecules supplied via the culture medium are hydrolysed by cell wall. The use of LED lamps to improve the development of explants in the different stages of micropropagation in temporary immersion systems should be studied in greater depth, always seeking to improve the systems, some works show it that way J. Hausaloa (2018). In our experience (data not shown) the higher light intensity available with the LED lights increases the fixation of CO₂ available in the inner atmosphere of the OGT bioreactor. One of the main drawbacks of the establishment of a micropropagation protocol in a temporary immersion system are the morphological and fisiological abnormalities that occur in the shoots and explants (Ruffoni and Savona 2013). In temporary immersion systems, hyperhydricity problems usually occur, but in the GT bioreactor there were no symptoms of vitrification.

The main advantages of this reactor are, first, the plant material can be easily manipulated, and second, the shoots produced have well developed leaves, which can be easily acclimated in the greenhouse. The production capacity of explants in the GT bioreactor can be 4 to 7 times greater than in semi-solid medium, with the same plant quality, and at lower costs when compared to the conventional micropropagation in semi-solid medium. Consequently, the bioreactor GT is stable and reliable and with higher productivity, and has possibilities to be employed in large scale plant production.

5 ACKNOWLEDGEMENTS

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SOBRE O ORGANIZADOR

EDUARDO EUGENIO SPERS realizou pós-doutorado na Wageningen University (WUR), Holanda, e especialização no IGIA, França. Possui doutorado em Administração pela Universidade de São Paulo (USP). Foi Professor do Programa de Mestrado e Doutorado em Administração e do Mestrado Profissional em Comportamento do Consumidor da ESPM. Líder do tema Teoria, Epistemologia e Métodos de Pesquisa em Marketing na Associação Nacional de Pós-Graduação e Pesquisa em Administração (ANPAD). Participou de diversos projetos de consultoria e pesquisa coordenados pelo PENSE e Markestrat. É Professor Titular no Departamento de Economia, Administração e Sociologia, docente do Mestrado em Administração e Coordenador do Grupo de Extensão MarkEsalq no campus da USP/Esalq. Proferiu palestras em diversos eventos acadêmicos e profissionais, com diversos artigos publicados em periódicos nacionais e internacionais, livros e capítulos de livros sobre agronegócios, com foco no marketing e no comportamento do produtor rural e do consumidor de alimentos.

ÍNDICE REMISSIVO

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Aceite 1, 28, 38, 50, 52, 53, 56, 57, 58, 59, 62, 70, 83, 97, 98, 99, 101, 102, 103, 106, 107, 108, 110, 125, 130, 141, 151, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 191, 200, 209, 225, 239, 250, 263, 270, 285, 298, 309, 316, 326

Aceites 33, 56, 57, 100, 107, 109, 162, 163, 165, 166, 168, 169, 170, 171, 172

Agua 33, 42, 47, 71, 72, 73, 74, 77, 78, 80, 81, 86, 87, 99, 101, 102, 103, 104, 105, 106, 107, 126, 130, 131, 133, 136, 163, 164, 167, 168, 169, 180, 187, 191, 192, 193, 194, 195, 196, 197, 198, 203, 204, 208, 211, 215, 216, 217, 225, 226, 227, 228, 230, 231, 236, 239, 241, 242, 244, 245, 246, 247, 263, 264, 265, 266, 267, 268, 269, 294, 295

Alimento composto 239, 244, 245

Amitraz 250, 251, 252, 254, 255, 256, 257, 258, 259, 261, 262

Análisis exergético 71, 75

Análisis fisicoquímicos 162, 163, 169

Apis mellifera 251, 252, 253, 260, 261

Aprendizagem Supervisionada 210, 212, 214

Aptidão solos regadio 210

Arándanos 191, 193, 195, 198

Aspersión 200, 202, 203, 204, 205, 208

Aumento de temperatura 286

Autoevaluación 29, 31, 32, 36

B

Beneficio neto 200, 201

Berry skin 152, 155, 157

Biocombustibles 84, 85, 86, 96, 98, 99, 101, 102, 107, 108, 162, 163, 172

Biocultural 39, 49

Bioetanol 83, 84, 95, 109

Biological effectiveness 142, 146, 147, 148, 150

Biomarcadores 327, 328, 329

Biomasa vegetal 98, 99, 100, 102

C

Cabalo de Pura Raza Galega 298, 299, 303, 310, 312, 313, 314

Carica papaya Linn 50, 51, 52, 53, 54, 55, 56, 57, 58, 60

Cepa 84, 89, 90, 91, 94, 95, 98, 99, 100, 103, 105, 106, 107, 139, 279
Cepas hiperproductoras 84
Cerdo 270, 271, 272, 274, 275, 276, 277, 278, 279, 308
Cerezas 125, 126, 128, 129, 130, 131, 135, 136, 139
Co-diseño 63
Colorantes naturais 125, 126, 129, 130, 137, 138, 139
Complex of amino acids 152, 154
Comprimento 239, 243, 244, 245, 246, 247, 254
Conditional parameters 142, 145, 148
Curros 298, 299, 300, 310, 311, 314, 315

E

Eficácia 143, 180, 217, 250, 251, 254, 256, 257, 258, 259, 260, 261, 307, 324
Enfermedades Infecciosas Emergentes 270, 271
Epifitias 175, 176, 177, 185
Eritrosina 125, 126, 128, 130, 131, 132, 133, 135, 136
Especies nativas 39, 40, 47
Estabilidad 57, 126, 127, 130, 131, 136, 162, 169, 170, 172, 271
Estresse Térmico 286, 294
Extracción de compuestos fenólicos 70, 71, 80

F

Fator K 239, 242, 243, 244, 245, 246, 247
Fermentación 84, 85, 86, 87, 89, 90, 91, 94
Fertilización nitrogenada 200, 202, 203, 206, 207
Flumetrina 251, 254, 255, 256, 257, 258, 259
Fruits 59, 60, 111, 142, 144, 145, 146, 148, 149

G

Ganadería equina 298
Glândula mamária 326, 327, 328, 329, 330
Goteo por fertiriego 200, 202, 203, 204, 205, 206, 208
GreenTray 110, 111
GT bioreactor 110, 111, 112, 115, 116, 117, 118, 120, 121, 122, 123

H

Humedal 225, 226, 227, 228, 231, 237, 238

I

Immune 142, 143, 144

Influenza 3, 80, 102, 225, 226, 228, 234, 235, 236, 246, 296

Innovación social 62, 63, 66, 67, 68, 69

In vitro plant micropropagation 111

IRTA-reactor 111, 112

L

Lactação 326, 327, 329, 330

Lípidos 50, 54, 57, 58, 99, 104, 105, 107, 244, 246

Liquid culture 110, 111, 112, 124

M

Machine Learning 209, 210, 211, 212, 214, 223, 224

Macrófitas acuáticas 225, 226, 229, 230, 235, 236

Macroinvertebrados acuáticos 225, 226, 227, 228, 229, 238

Madre vieja 225, 226, 227, 228

Mal de Panamá 175, 176, 178

Mayos 39, 48

Mecanismos para su presentación 270

Mediterráneo 1, 3, 6

Métodos de extracción 72, 98, 106, 162

Microalgas 98, 99, 100, 101, 102, 103, 107, 108, 109

Micropterus salmoides 239, 240, 247, 248, 249

Moko bacteriano 175, 176

Morfología 190, 226

N

Nematodos 175, 176, 177, 178, 179, 180, 182, 183, 184, 186, 187, 188, 189, 190

O

Optimización de extracción 71

P

Paisagem cultural 1, 2, 3, 22, 25
Parrilla costal 316, 318, 323, 324
Pasturas 263, 264, 265, 269
Património cultural imaterial 1, 13, 22
Perro 52, 316, 317, 318, 324
Pesca artesanal 62, 63, 64, 69
Peso 57, 73, 88, 92, 143, 166, 167, 168, 193, 215, 225, 229, 230, 239, 241, 242, 243, 244, 245, 246, 247, 252, 287, 318, 327, 329
Phenolic compounds 59, 71, 72, 81, 82, 152, 153, 156, 159
Phenolic maturity 152, 153, 154, 158, 160
PH y temperatura 126, 131, 136
Picudo negro 175, 176, 177, 180
Potencialidades 4, 24, 50, 52, 53, 58, 162, 300
Prácticas 28, 29, 30, 31, 32, 35, 36, 37, 40, 187, 188, 310
Produção Animal 286, 326
Productividad 191, 193, 316
Productivity 111, 122, 123, 142, 143, 144, 149, 150, 192
Prototipos 21, 62, 63, 68, 69

Q

Questionários 1
Quimioterapia 316, 317, 324

R

Rapa das Bestas 298, 299, 310, 311, 314
Razas autóctonas 298
Represa 264, 266, 267, 268, 269
Residuos industriales de pistacho 70, 71, 80
Resolución 29, 31, 35, 37
Resultados 1, 12, 16, 18, 19, 21, 22, 29, 32, 34, 39, 43, 47, 57, 58, 69, 71, 73, 74, 76, 79, 81, 88, 90, 95, 100, 106, 126, 131, 132, 133, 136, 168, 169, 170, 172, 182, 183, 184, 185, 187, 188, 194, 200, 201, 205, 207, 208, 209, 211, 213, 218, 222, 223, 230, 233, 239, 243, 245, 247, 251, 256, 257, 258, 267, 270, 279, 280, 289, 291, 304, 307, 316, 319, 324
Riego 33, 180, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 204, 263, 264, 265, 266

Rojo gardenia 126

S

Salinidad 102, 103, 104, 191, 192, 193, 194, 195, 196, 197, 198, 199

Salud 28, 29, 35, 50, 51, 52, 53, 54, 58, 72, 97, 125, 128, 129, 164, 271, 272, 273, 278, 279, 316, 324

Scikit-Learn 210

Seeds 51, 59, 60, 82, 152, 158, 159, 160, 173, 174

Semillas 47, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 85, 162, 163, 164, 165, 168, 169, 170, 172, 173, 174, 179, 208

Simulación numérica 71

Sistemas agroforestales 38, 39, 40, 41, 43, 47, 48

Sobreiro 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21, 24, 26

T

Temporary immersion system 110, 111, 121, 122, 123, 124

Tiradores de cortiça 1, 2, 10, 11, 14, 16, 22, 23, 24

TIS 110, 111, 112, 115, 117, 122, 124

Tumor 316, 317, 319, 320, 321, 323, 324, 325

T.V.T 316, 317

V

Valcheta 263, 264, 265

Validación de la innovación social 62, 63, 66, 67

Varroa destructor 250, 251, 252, 255, 259, 260, 261, 262

Vertiente 264, 265, 266, 267

Vertisol 200, 201, 202, 205

Vía subcutánea 316, 318, 323, 324

Vinaza 83, 84, 94, 95, 96