6th International Conference on Olive Tree and Olive Products

OLIVE BIOTEQ’18

Seville / October 15th-19th

Olive Management, Biotechnology and Authenticity of Olive Products

Book of Abstracts
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The Organizing Committee thanks
the Sponsors of OLIVEBIOTEQ’18

The Conveners

Welcome

Dear participants,

We welcome you to OLIVEBIOTEQ’18, the 6th International Conference on the Olive Tree and Olive Products. This edition has the subtitle of Olive Management, Biotechnology and Authenticity of Olive Products, and thanks to all of you, we have succeeded on gathering state of the art information on those topics, as reflected in the contents of this Book of Abstracts.

We will enjoy learning from 268 contributions by 218 registered participants from 16 countries. In addition, a number of related activities have been organized, as detailed in the program inside, including a visit to the World Germplasm Bank of Centro IFAPA Alameda del Obispo (Córdoba), and to both traditional and technologically advanced almazaras (oil extraction mills) in the countryside of Baena, one of Spain’s top olive producing areas.

The conference content will be completed with the presence and contributions of a variety of companies and associations related to olive management and the olive industry. The social events of our Programme are highlighted by the opportunity to visit key Seville landmarks, to enjoy a cata of selected olive oils, and to gather together at the Gala dinner in a restaurant of Triana, a neighbourhood with a rich cultural heritage.

We all wish you a fruitful and enjoyable time during your stay in Seville for OLIVEBIOTEQ’18.

The Conveners
Committees

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- José Manuel Martínez-Rivas - Instituto de la Grasa (CSIC), Sevilla, Spain
- Wenceslao Moreda - Instituto de la Grasa (CSIC), Sevilla, Spain

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- Sebastiano Roussos - Institut de Recherche pour le Développement, Marseille, France
- Luca Sebastiani - Scuola Superiore Sant’Anna, Pisa, Italy
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<tr>
<td>8:30</td>
<td>REGISTRATION</td>
<td>S5: Abiotic Stresses</td>
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<td>Chairs: B. Dichio / J.M. Torres-Ruiz</td>
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<td>Key speaker: L. Sebastiani</td>
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<td>10:00</td>
<td>OPENING CEREMONY</td>
<td>Posters Awards S1-S8</td>
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<td>11:00</td>
<td>COFFEE</td>
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<td>S1: Genetic Resources, Breeding and Propagation</td>
<td>S6: Irrigation</td>
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<td></td>
<td>Key speaker: B. Khadari</td>
<td>Key speaker: A. Díaz-Espejo</td>
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<td>13:00</td>
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<td>14:30</td>
<td>S2: Molecular Biology, Genomics and Biotechnology</td>
<td>S7: Fertilization</td>
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<td>Key speaker: L. Baldoni</td>
<td>Key speaker: R. Fdez-Escobar</td>
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<td>COFFEE + POSTER SESSION S1-S8</td>
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<td>16:30</td>
<td>S3: Reproductive Biology and Fruit Development</td>
<td>S8: Tillage and Mechanization of Pruning and Harvesting</td>
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<td>Key speaker: H. Rapoport</td>
<td>Key speaker: G. Ben-Ari</td>
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<td>S4: Plant Protection</td>
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<td>Chairs: J. Mercado-Blanco / F.J. López-Escudero</td>
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<td>Key speaker: D. Boscia</td>
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<td>FIELD TRIP</td>
<td>S9: Olive Oil Quality</td>
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<td>Chairs: M. Servilli / J.M. Martínez-Rivas</td>
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<td>Key speaker: D. García-González</td>
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<td>Dissemination Session</td>
<td>Posters Awards S9-S14</td>
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<td>COFFEE + POSTER SESSION S9-S14</td>
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<td>11:30</td>
<td>S10: Olive Oil Authenticity</td>
<td>S14: Nutrition and Health</td>
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<td>Key speaker: M. Fitó</td>
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<td>COFFEE + POSTER SESSION S9-S14</td>
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<td>14:30</td>
<td>S11: Olive Oil Technology and By-products</td>
<td>S12: Economics and Marketing</td>
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<td>Key speaker: J. Vilar</td>
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<td>GALA DINNER</td>
<td>VISIT HISTORICAL AREAS</td>
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14:30 hrs.  S2  Molecular Biology, Genomics and Biotechnology
Chairs: P. Kalaitzis  F. Pliego
Key speaker: L. Baldoni

14:30 a 15:00 hrs.  T02-K  HOW GENOMICS AND BIOTECHNOLOGY ARE CONTRIBUTING TO UNLOCK THE POTENTIAL OF OLIVE?
SORAYA MOUSAVI, ROBERTO MARIOTTI, NICOLO' CULTRERA
CONSIGLIO NAZIONALE DELLE RICERCHE INSTITUTE OF BIOSCIENCES AND BIORESOURCES

15:00 a 15:15 hrs.  T02-O1  A ROBUST PHYLOGENY OF CULTIVATED AND WILD OLIVE TREES SUGGESTS THAT DOMESTICATION TOOK PLACE IN TWO DIFFERENTIATED EVENTS
JORGE A. RAMÍREZ-TEJERO, JAIME JIMÉNEZ-RUIZ, MARÍA DE LA O LEYVA-PÉREZ, ANGELINA BELAJ, RAÚL DE LA ROSA, AURELIANO BOMBARELLY, FRANCISCO LUQUE
UNIVERSIDAD DE JAÉN CENTRO DE ESTUDIOS AVANZADOS EN OLIVAR Y ACEITE DE OLIVA

15:15 a 15:30 hrs.  T02-O2  OLIVES IN ITALY: SNP MARKER DIVERSITY AND GEOGRAPHICAL ORIGIN OF THE REPRESENTATIVE ITALIAN GERMPLASM
FRANCESCA TARANTO, NUNZIO D'AGOSTINO, GIACOMO MANGINI, VALENTINA DI RENZO, VALENTINA FANELLI, SUSANNA GADALETA, MONICA MARILENA MIAZZI, WILMA SABETTA, SARA SION, SAMANTA ZELASCO, ENZO PERRI, SALVATORE CAMPOSEO, CINZIA MONTEMURRO
COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA) CER

15:30 a 16:00 hrs.  T02-O3  COMPARATIVE TRANSCRIPTIONAL PROFILING FROM OLIVE SEED AND MESOCARP TISSUES RELATED TO FATTY ACID AND OIL BIOSYNTHESIS
M LUISA HERNÁNDEZ, ANTONIO MUÑOZ-MÉRIDA, M DOLORES SICARDO, OSWALDO TRELLES, VICTORIANO VALPUESTA, JOSÉ M MARTÍNEZ-RIVAS
1 INSTITUTO DE LA GRASA - CSIC BIOCHEMISTRY AND MOLECULAR BIOLOGY OF PLANT PRODUCTS, 2 UNIVERSIDAD DE MÁLAGA INTEGRATED BIOINFORMATICS

16:00 hrs.  COFFEE + POSTER SESSION S1 - S8

Program

Monday 15th

8:30 hrs.  8:30 hrs.  REGISTRATION
10:00 hrs.  10:00 hrs.  OPENING CEREMONY
11:00 hrs.  11:00 hrs.  COFFEE
11:30 hrs.  S1  Genetic Resources, Breeding and Propagation
Chairs: T. Caruso  L. Rallo
Key speaker: B. Khadari
11:30 a 12:00 hrs.  T01-K  FROM DOMESTICATION AND DIVERSIFICATION TO ADAPTATION OF MEDITERRANEAN OLIVE TREE TO CLIMATE CHANGE
BOUCHAIB KHADARI
AMÉLIORATION GENÉTIQUE ET ADAPTATION DES PLANTES (INRA, UMR 1334)

12:00 hrs.  12:00 hrs.
12:00 a 12:15 hrs.  T01-O1  SILVOLIVE, A COLLECTION OF WILD OLIVE GENOTYPES AS A SOURCE OF RESISTANCE GENES AND ROOTSTOCKS FOR OLIVE GROVE CULTIVATION
JOSÉ MANUEL COLMENERO-FLORES, PABLO DÍAZ-RUEDA, NIEVES CAPOTE-MAÍNEZ, ANA AGUADO, LAURA ROMERO-CUADRADO, LORENZO LEÓN, CARLOS CARRASCOSA
INSTITUTO DE RECURSOS NATURALES Y AGROBIOLÓGIA DE SEVILLA, IRNAS (CSIC) PLANT BIOTECHNOLOGY

12:15 hrs.  12:15 hrs.
12:15 a 12:30 hrs.  T01-O2  NEW OLIVE CULTIVARS FOR HEDGEROW ORCHARDS
CONCEPCIÓN MUÑOZ-DÍEZ, DIEGO CABELLO, JUAN MORAL, PABLO MORELLO, DIEGO BARRANCO, LUIS RALLO,
UNIVERSIDAD CORDOBA AGRONOMÍA

12:30 hrs.  12:30 hrs.
12:30 a 12:45 hrs.  T01-O3  GENOTYPE, ENVIRONMENT AND THEIR INTERACTION IN OLIVE
RAUL DE LA ROSA, JOSE F NAVAS-LOPEZ, ALCIA SERRANO, GUACIMARA M MEDINA-ALONSO, ROCIO ARIAS-CAVALLERI, MARIA DE LA CRUZ BLANCO, CRISTINA SANTOS, JUAN CANO, DANIEL PEREZ, EDUARDO TRENTOCASTO, DOMINGO RIOS-MESA, LEONARDO VELASCO, CARLOS SÁNCHEZ, ANA O PÉREZ, JOSE BEJARANO, Dolores Rodríguez, Hava F. Raapor, Angelina Belaj, Ignacio Lorig, Lorenzo León
IFAPA, JUNTA DE ANDALUCÍA BREEDING AND BIOTECHNOLOGY

12:45 hrs.  12:45 hrs.
12:45 a 13:00 hrs.  T01-O4  IBA-INDUCED IN VITRO ADVENTITIOUS ROOTING IN OLIVE: A MOLECULAR RESPONSE TO CUT INJURY AND AUXIN APPLICATION
ISABEL VELADA, HÉLIA CARDOSO, DARUISZ GRZEBIELUS, DIANA LOUISA, CLÁUDIO M. SOARES, ELISETE S. MACEDO, BIRGIT ARNHOLDT-SCHMITT, AUGUSTO PEIXE
INSTITUTO DE CIÊNCIAS AGRÁRIAS E AMBIENTAIIS MEDITERRÂNICAS

13:00 hrs.  13:00 hrs.  LUNCH
16:30 hrs.  S3 Reproductive Biology and Fruit Development

16:30 a 17:00 hrs.  T03-K

**OLIVE FRUIT CELLULAR PROCESSES: IMPLICATIONS FOR GROWTH, QUALITY AND MANAGEMENT**

HAVA RAPOPORT

*Instituto de Agricultura Sostenible-CSIC Protección de Cultivos*

17:00 a 17:15 hrs.  T03-O1

**COMPARATIVE LARGE-SCALE ANALYSIS OF LIPID METABOLISM DURING THE EARLY STAGES OF OLIVE POLLEN GERMINATION**

ANTONIO JESÚS CASTRO, MARÍA JOSÉ JIMÉNEZ-QUESADA, JUAN DE DIOS ALCÉ, JOSÉ MANUEL MARTÍNEZ-RIVAS, MARÍA LUISA HERNÁNDEZ

*Estación Experimental del Zaidín (CSIC) Biochemistry, Cell and Molecular Biology of Plants*

17:15 a 17:30 hrs.  T03-O2

**CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF ENZYMES INVOLVED IN GLUTATHIONE METABOLISM IN OLIVE REPRODUCTIVE BIOLOGY**

JOSE CARLOS JIMÉNEZ-LOPEZ, ELENA LIMA-CABELLO, ESTEFANIA GARCÍA-QUIRÓS, ROSARIO Mª CARMONA, ADORACIÓN ZAFRA, ANTONIO JESÚS CASTRO, JUAN DE DIOS ALCÉ

*Estación Experimental del Zaidín (CSIC) Plant Reproductive Biology Laboratory. Department of Biochemistry, Cell and Molecular Biology of Plants*

17:30 a 17:45 hrs.  T03-O3

**STUDYING INTERACTIONS BETWEEN WARMER WINTERS AND GIBBERELLIN IN THE CONTROL OF OLIVE FLOWERING**

TAHEL WECHSLER, ORTAL BAKHSHIAN, GORIA BEN-ARI, ALON SAMACH

*Hebrew University of Jerusalem Plant Sciences in Agriculture*

17:45 a 18:00 hrs.  T03-O4

**THE NETWORK CONNECTION BETWEEN FROST ADAPTATION AND FLOWERING DETERMINATION: THE CASE OF ARBEQUINA AND ROYETA DE ASIQUE**

FABIANO GATTABRIA, BEATRIZ BILSA PÉREZ, MARCO CIRILLO, ANGEL FERNÁNDEZ I MARTÍ, MARÍA JOSÉ RUBIO-CABETAS, MAURO ZECCHINI, CALODERGO ACONA, ROSARIO MULEO

*Tuscia University DAFNE, *Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) Unidad de Horticulcutura, *University of Milano-Bicocca*

18:00 hrs.  S8 Reproductive Biology and Fruit Development

**COFFEE + POSTER SESSION S1-S8**

18:30 hrs.  S4 Plant Protection

18:30 a 19:00 hrs.  T04-K

**THE EPIDEMIC OF XYLELLA FASTIDIOSA IN APULIA: CURRENT SITUATION AND FUTURE PERSPECTIVE FOR THE CONTROL**

DONATO BOSCIA, MARIA SAPONARI

*Consiglio Nazionale delle Ricerche Istituto Per la Protezione Sostenibile delle Pianta*

19:00 a 19:15 hrs.  T04-O1

**MICROBIAL COMMUNITIES INHABITING THE ROOT SYSTEM OF HEALTHY OLIVE TREES**

ANTONIO JOSÉ FERNÁNDEZ-GONZÁLEZ, PABLO JOSÉ VILLADAS, CARMEN GÓMEZ-LA MA CABANÁS, ANTONIO VAUVERDE-CORREDO, ANGJELINA BELAJ, JESÚS MERCADO-Blanco, MANUEL FERNÁNDEZ-LÓPEZ

1*Estación Experimental del Zaidín (EEZ) - Spanish National Research Council (CSIC) Soil Microbiology and Symbiotic Systems, 2*Estación Experimental del Zaidín (EEZ) - Soil Microbiology and Symbiotic Systems, 3*Institute for Sustainable Agriculture (IAS) - Spanish National Research Council (CSIC) Crop Protection*

19:15 a 19:30 hrs.  T04-O2

**A LONG-TERM STUDY ON AERIAL OLIVE DISEASES: EMPIRICAL APPROACH TO DEVELOP USER-FRIENDLY MODELS**

JOAQUÍN ROMERO RODRÍGUEZ, CARLOS AGUSTÍ BRISACH, LUIS FERNÁNDEZ-TILLO, JUAN MORAL MORAL, ELISA GONZÁLEZ DOMÍNGUEZ, VITTORIO ROSSI, ANTONIO TRAPERO CASAS

*Universidad de Córdoba Agronomía*

19:30 a 19:45 hrs.  T04-O3

**CULTIVABLE FUNGI PRESENT IN TUNISIAN OLIVE ORCHARDS: DIVERSITY AND BIOPROSPECTING OF ENTOMOPATHOGENIC ACTIVITY**

INES KSENTINI, HOUDA GHARSALLAH, SOUROUR NAAYMA, KARAMA HADJ TAIEB, CHRISTINA SCHUSTER, ANDREAS LECLERQUE

*Institut de l’Olivier Laboratory of Improvement and Protection of Genetic Resources of the Olive Tree*

19:45 a 20:00 hrs.  T04-O4

**POPULATION DYNAMIC OF OLIVE PIT SCALE, POLLINIA POLLINI COSTA (HEMIPTERA: ASTEROLECANIDAE) ON TWO OLIVE CULTIVARS IN NORTH REGION OF JORDAN**

HAIL SHANNA, NAWAF FREHAT, MAZEN AL-KILANI, JOHN CAPINERA, SALAM AYOUB

*Jordan University of Science and Technology Plant Production Department*
**Tuesday 16th**

### 08:30 hrs.  
**S5 Abiotic Stresses**

**Chairs:** B. Dichio  
J.M. Torres-Ruiz  
**Key speaker:** L. Sebastiani

#### 08:30 a 09:00 hrs.  
**T05-K**  
**ABIOTIC STRESS IN OLIVE: DISSECTING THE PHYSIOLOGICAL AND MOLECULAR MECHANISMS**

**Luca Sebastiani**  
*Scuola Superiore Sant'Anna Institute of Life Sciences*

#### 09:00 a 09:15 hrs.  
**T05-O1**  
**SAP FLOW RESPONSES TO ELEVATED TEMPERATURE AND FRUIT LOAD IN YOUNG OLIVE TREES**

**María Cecilia Rousseaux,**  
**Andrea Miserere,**  
**Guadalupe Manchó,**  
**Pablo Maseda,**  
**María Magdalena Broquela,**  
**Peter Stoughton Searles**

*Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de la Rioja (CRILAR) Ecfisiología de Olivo*

#### 09:15 a 09:30 hrs.  
**T05-O2**  
**INVESTIGATION OF THE INVOLVEMENT OF OLIVE PROLYL 4 HYDROXYLASES AND ARABINOGLACTAN PROTEINS UNDER DUAL STRESS OF SALINITY AND HEAT**

**Panagiotis Kalaitzis,**  
**Aristotelis Azaridis,**  
**Konstantinos Blazakis,**  
**Faten Dandanchi,**  
**Mohamed Kouhen,**  
**George Kostelenos**

*Ciemam-Mediterranean Agronomic Institute of Chania Horticulural Genetics & Biotechnology*

#### 09:30 a 09:45 hrs.  
**T05-O3**  
**TRANSGENIC OLIVE (OLEA EUROPAEA L.) SHOOTS OVER-EXPRESSING OSMOTIN GENE ARE LESS SENSITIVE TO IN VITRO-INDUCED SALT STRESS**

**Muhammad Ajmal Bashir,**  
**Cristian Silvestri,**  
**Stefania Astolfi,**  
**Eleonora Cipri,**  
**Valerio Cristofori,**  
**Eddo Rugini**

*Tuscia University DAFNE-DEPARTMENT OF AGRICULTURE AND FOREST SCIENCES*

#### 09:45 a 10:00 hrs.  
**T05-O4**  
**COORDINATION BETWEEN WATER SUPPLY AND DEMAND AT LEAF AND PLANT LEVEL HELPS TO EXPLAIN DIFFERENTIAL GROWTH PATTERNS IN OLIVE GENOTYPES IN RESPONSE TO MILD WATER STRESS**

**Virginia Hernandez-Santana,**  
**Pablo Díaz-Rueda,**  
**Antonio Díaz-Espejo,**  
**María Dolores Raya-Sereno,**  
**Saray Gutiérrez-Gordillo,**  
**Antonio Montero,**  
**Alfonso Pérez-Martín,**  
**José Manuel Colmenero-Flores,**  
** CELIA MODESTA RODRÍGUEZ-DOMINGUEZ**

*IRNAS*

### 11:00 hrs.  
**COFFEE + POSTER SESSION S1-S8**

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### 11:30 hrs.  
**S6 Irrigation**

**Chairs:** A. Vivaldi  
L. Testi  
**Key speaker:** A. Díaz-Espejo

#### 11:30 a 12:00 hrs.  
**T06-K**  
**THE FUTURE OF PRECISION AGRICULTURE AND THE RATIONAL APPLICATION OF DEFICIT IRRIGATION**

**Antonio Díaz-Espejo,**  
**Virginia Hernandez-Santana,**  
**José Enrique Fernández**

*IRNAS Protection of the Soil, Plant, Water System*

#### 12:00 a 12:15 hrs.  
**T06-O1**  
**EFFECT OF SALTY RECLAIMED WATER AND DEFICIT IRRIGATION ON SOME AGRONOMICAL AND PHYSIOLOGICAL PARAMETERS OF OLIVE TREES.**

**Gaetano Alessandro Vivaldi,**  
**Salvatore Campossio,**  
**Emilio Nicolas,**  
**Antonello Lo Paduano,**  
**Cristina Romero Trigueros,**  
**Giuseppe Loprile,**  
**Francisco Pérez-Dróper Salcedo**

*University of Bari Aldo Moro Agricultural and Environmental Science*

#### 12:15 a 12:30 hrs.  
**T06-O2**  
**IDENTIFICATION OF WATER STRESS LEVEL IN OLIVE TREES DURING PIT HARDENING USING TRUNK GROWTH RATE INDICATOR.**

**Mireia Corell,**  
**María José Martín-Palomó,**  
**Ignacio Girón,**  
**Luis Andreu,**  
**Emiliano Trigo,**  
**Arturo Torrecillas,**  
**Ana Centeno,**  
**David Pérez-López,**  
**Alfonso Moriana**

*Universidad de Sevilla Dpto Ciencias Agroforestales*

#### 12:30 a 12:45 hrs.  
**T06-O3**  
**THE INTERACTION BETWEEN FRUIT LOAD AND IRRIGATION REQUIREMENT IN OLIVE TREES**

**Arnón Dag,**  
**Anmon Bustan,**  
**Amos Naor,**  
**Alon Ben-Gal**

*Agricultural Research Organization Fruit Tree Sciences*

#### 12:45 a 13:00 hrs.  
**T06-O4**  
**MONITORING CROWN PARAMETERS OF OLIVE TREES SUBJECTED TO DIFFERENT IRRIGATION REGIMES BY AN UAV AND RGB-NIR CAMERAS**

**Giovanni Caruso,**  
**Pablo J. Zarco-Tejada,**  
**Victoria Gonzalez-Dugo,**  
**Marco Moriondo,**  
**Letizia Tozzini,**  
**Giacomo Palai,**  
**Giovanni Rallo,**  
**Alberto Horne-Ro,**  
**Jacoop Primicerio,**  
** Riccardo Gucci**

*University of Pisa DIPARTIMENTO DI SCIENZE AGRARIE ALIMENTARI E AGRO-AMBIENTALI, Consejo Superior de Investigaciones Científicas (CSIC) INSTITUTO DE AGRICULTURA SOSTENIBLE (IAS)*

### 13:00 hrs.  
**LUNCH**
14:30 hrs.  S7  Fertilization

**14:30 a 15:00 hrs.**  T07-K  OLIVE NUTRITION AND TOLERANCE TO BIOTIC AND ABIOTIC STRESSES
  
  **Ricardo Fernández-Escobar**  
  **Universidad de Córdoba Agronomía**

**15:00 a 15:15 hrs.**  T07-O1  OLIVE TREE RESPONSE TO PHOSPHORUS APPLICATION ASSESSED FROM FIELD AND POT EXPERIMENTS

**Margarida Arrobas**  
**Instituto Politécnico de Bragança CIMO - Mountain Research Center**

**15:15 a 15:30 hrs.**  T07-O2  SEASONAL CHANGES IN MINERALS AND CARBOHYDRATES STATUS OF OLIVE LEAVES AFTER FOLIAR FERTILIZER APPLICATION

**Imen Zouari**  
**Olive Institute of Tunisia Laboratory of Durability in Semi-Arid and Arid Regions: Ameelioration of Productivity of Olive Tree and Quality of Products**

**15:30 a 15:45 hrs.**  T07-O3  OPTIMIZING FERTILIZATION MANAGEMENT IN OLIVE ORCHARDS TO IMPROVE FRUIT AND OIL YIELDS UNDER IRRIGATED FARMING CONDITIONS

**Bouzid Benabdellaziz**  
**Instituto Politécnico de Bragança CIMO - Mountain Research Center**

**15:45 a 16:00 hrs.**  T07-O4  FROM THE DIAGNOSIS TO THE RESEARCH: THE ROLE OF BEST NUTRIENT MANAGEMENT OF OLIVE ORCHARDS IN NORTH AFRICA REGION

**Hakim Boulaiz**  
**Instituto Politécnico de Bragança CIMO - Mountain Research Center**

16:00 hrs.  COFFEE + POSTER SESSION S1-S8

16:30 hrs.  S8  Tillage and Mechanization of Pruning and Harvesting

**16:30 a 17:00 hrs.**  T08-K  ETHERPHON INDUCED OXIDATIVE STRESS IN THE OLIVE LEAF ABSCISISON ZONE ENABLES DEVELOPMENT OF A SELECTIVE ABSCISISON COMPOUND

**Shiri Goldental-Cohen**  
**P. Searles**, **S. Castro-García**  
**Volcani Center Institute of Plant Sciences**

**17:00 a 17:15 hrs.**  T08-O1  UNRAVELLING FRUITLET DROP IN OLEA EUROPaea (CV. FRANTOII)

**Athanassia-Maria Dourou**  
**Iris Biton**, **Yair Mani**, **Benjamin Avidan**  
**Giора Ben Ari**  
**Argol Center Institute of Plant Sciences**

**17:15 a 17:30 hrs.**  T08-O2  CULTIVARS AND PLANTING SYSTEMS FOR THE NEW SI-CILIAN OLIVE OIL INDUSTRY

**Tiziano Caruso**  
**Laura Macaluso**, **Giulia Marino**, **Francesco Paolo Marra**, **Placidio Volol**  
**University of Palermo Sciences Agrarie, Alimentari, Forestali**

**17:30 a 17:45 hrs.**  T08-O3  EVALUATION OF OLIVE PRUNING EFFECT ON THE PERFORMANCE OF THE ROW-SIDE CONTINUOUS CANOPY SHAKING HARVESTER IN A HIGH DENSITY

**António F. Bento Dias**  
**José O. Peça**, **Anacleto Pinheiro**, **José M. Falcão**  
**Universidade de Évora/ICIAM Engenharia Rural**, **ICIAM, Universidade de Évora Engenharia Rural**

**17:45 a 18:00 hrs.**  T08-O4  VIBRATION OLIVE TREE RESPONSE TO MECHANICAL HARVESTING IN NARROW HEDGEROW ORCHARD

**Sergio Castro-Garcia**  
**RaFAel R. SOLA-Guirado**, **fernando aragón-Rodriguez**, **jesus a Gil-Ribes**  
**Universidad de Córdoba Department of Rural Engineering**

18.30 - 20.30  Guided Visit to Historical Areas

Average Duration: 2 hours

Meeting with the guide at the main entrance of the venue, Hotel Silken Al-Andalus, to start a panoramic tour by bus.

This will include landmarks such as some of the Pavilions of the Latin-American’s exhibition held in Seville at 1929, the America Square ans the Spanish Square, where we will make a stopover. From there we will head the Murillo Gardens, to start a walking tour through the Sta Cruz and Jewish neighborhoods, with explanatory details on k ey spots: Doña Elvira square, Sta Cruz square, the narrow Kios street, or the Venerables Square. These will include references to the myths of the operas related to Seville, like Carmen and Don Giovanni.

The visit will end at the Triumph Square, from where we can see nice views of both the Cathedral and Royal Alcazar.

(All buildings and monuments will be seen from outside, without entrance. Bus Transfer back to the hotel will not be provided.)
### Thursday 18th

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<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>08:30 hrs.</td>
<td>S9</td>
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<td><strong>Olive Oil Quality</strong></td>
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<td>Chairs: M. Servili</td>
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<td>J.M. Martínez-Rivas</td>
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<td>Key speaker: D. García-González</td>
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<td>08:30 a 09:00 hrs.</td>
<td>T09-K</td>
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<td><strong>VOLATILE MARKERS FOR VIRGIN OLIVE OIL QUALITY CONTROL: CONTRIBUTION TO THE DEVELOPMENT OF AROMA REFERENCE MATERIAL</strong></td>
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<td></td>
<td>DIEGO L. GARCÍA-GONZÁLEZ, RAMÓN APARICIO-RUIZ, CLEMENTE ORTIZ, NOELIA TENA, ANA LOBO-PRIETO, SARA BARBIERI, ENRICO VALLI, ALESSANDRA BENDINI, TULLIA GALLINA TOSCHI</td>
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<tr>
<td>09:00 a 09:15 hrs.</td>
<td>T09-O1</td>
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<td><strong>VERIFICATION OF VIRGIN OLIVE OIL SENSORY QUALITY BY VOLATILE FRACTION FINGERPRINTING</strong></td>
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<td>BEATRIZ QUINTANILLA-CASAS, JULEN BUSTAMANTE, FRANCESC GUARDIOLA, DIEGO L. GARCÍA-GONZÁLEZ, SARA BARBIERI, ALESSANDRA BENDINI, TULLIA GALLINA TO- SCHI, ALBA TRES, STEFANIA VICHI</td>
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<td>09:15 a 09:30 hrs.</td>
<td>T09-O2</td>
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<td><strong>SELECTIVE AND STRAIGHTFORWARD “HOST-GUEST” MAGNETIC RESPONSIVE TOOL FOR THE TRACE ANALYSIS OF DIMETHOATE IN OLIVE OIL SAMPLES</strong></td>
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<td>RAQUEL GARCÍA, ELISABETE P. CARREREO, ANTHONY J. BURKE, ANA MARIA COSTA FREITAS, MARCO GOMES DA SILVA, MARIA JOÃO CABRITA</td>
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<tr>
<td>09:30 a 09:45 hrs.</td>
<td>T09-O3</td>
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<td><strong>CHEMICAL CHARACTERIZATION OF ‘ARBEQUINA’ EXTRA-VIRGIN OLIVE OILS PRODUCED IN DIFFERENT GROWING AREAS OF SPAIN</strong></td>
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<td>JOAN TOUS, VICTORINO VEGA</td>
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<td>09:45 a 10:00 hrs.</td>
<td>T09-O4</td>
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<td><strong>MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF GENES AND ENZYMES INVOLVED IN VIRGIN OLIVE OIL QUALITY</strong></td>
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<td>M LUISA HERNÁNDEZ, M DOLORES SICARDO, PATRICIA M ARJONA, MARIA N PADILLA, LOURDES GARCIA-VICO, DAVID VELAZQUEZ-PALMERO, ANA G PEREZ, CARLOS SANZ, JOSE M MARTÍNEZ-RIVAS</td>
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#### Dissemination Session

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<td>10:00 hrs.</td>
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<tr>
<td>11:00 hrs.</td>
<td><strong>COFFEE + POSTER SESSION S9-S14</strong></td>
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### S10 Olive Oil Authenticity

**Chairs:** S. Vichi, W. Moreda  
**Key speaker:** T.G. Toschi

<table>
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| 11:30 a 12:00 hrs. | **T10-K** NEW INSTRUMENTAL AND SENSORY STRATEGIES TO AUTHENTICATE OLIVE OILS: THE 2018 SCENARIO  
TULLIA GALLINA TOSCHI, ALESSANDRA BENDINI, ENRICO VALLI, FLORENCIA LACOSTE, FLORENT JOFFRE, JOSE MANUEL MARTINEZ RIVAS, MARIA DEL CARMEN PEREZ CAMINO, WENCESLAO MOREDA  
ALMA MATER STUDIORUM - UNIVERSITY OF BOLOGNA DEPARTMENT OF AGRICULTURAL AND FOOD SCIENCES |
| 12:00 a 12:15 hrs. | **T10-O1** APPROXIMATIONS TO THE DETECTION OF LEGAL BLENDS OF OLIVE OILS WITH SEED OILS  
INSTITUTO DE LA GRASA-CSIC QUALITY AND CHARACTERIZATION OF LIPIDS |
| 12:15 a 12:30 hrs. | **T10-O2** DETECTION OF SOFT DEODORIZED OIL IN VIRGIN OLIVE OIL THROUGH DIACYLGLYCEROL DETERMINATION. RELATIONSHIP WITH FREE ACIDITY  
RAQUEL B. GÓMEZ-COCA, MARÍA DEL CARMEN PÉREZ-CAMINO, JOSE MANUEL MARTÍNEZ RIVAS, ALESSANDRA BENDINI, TULLIA GALLINA TOSCHI, WENCESLAO MOREDA  
INSTITUTO DE LA GRASA CHARACTERIZATION AND QUALITY OF LIPIDS |
| 12:30 a 12:45 hrs. | **T10-O3** TRACEABILITY OF TUNISIAN OLIVE OILS USING GEOCHEMICAL FINGERPRINTS AND CHEMOMETRICS: RELATIONSHIP WITH THE SOIL OF ORIGIN  
FADWA DAMAK EP HMANI, MAKI ASANO, KOJI BABA, AOMI SUDA, DAISUKE ARAKOA, AHMED WALI, HIROKO ISODA, MITSUTOshi NAKAJIMA, MOHAMMED KSIBI, KENJI TAMURA  
TSUKUBA UNIVERSITY SCHOOL OF INTEGRATIVE AND GLOBAL MAJORS- ENVIRONMENTAL MANAGEMENT |
| 12:45 a 13:00 hrs. | **T10-O4** Medomics - Mediterranean Extra Virgin Olive Oil Omics: Profiling and Fingerprinting  
MARIA CABRITA, MARCO GOMES DA SILVA, YVELINE LA DREAU, MOHAMED BOUAZZA, SERKAN SELLI, HASIM KELEBEK  
ICAM, UNIVERSIDADE DE ÉVORA, LAGV-REQUIUMTE, DEPARTAMENTO DE QUÍMICA, FACULDADE DE CIÊNCIAS E TECNOLOGIA, AIX MARSEILLE UNIVERSITÉ, UNIVERSITÉ AVIGNON, UNIVERSITY OF SFAK, HIGH INSTITUTE OF BIOTECHNOLOGY OF SFAK DEPARTMENT OF FOOD TECHNOLOGY, CUUKOVA UNIVERSITY, FACULTY OF AGRICULTURE DEPARTMENT OF FOOD ENGINEERING, ADANA SCIENCE AND TECHNOLOGY UNIVERSITY DEPARTMENT OF FOOD TECHNOLOGY |
| 13:00 hrs.  | LUNCH |

### S11 Olive Oil Technology and By-products

**Chairs:** S. Roussos, J. Fernández-Bolaños  
**Key speaker:** K. Chartzoulakis

<table>
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| 14:30 a 15:00 hrs. | **T11-K** TREATMENT METHODS AND TECHNOLOGIES FOR OLIVE MILL BY-PRODUCT MANAGEMENT IN MEDITERRANEAN COUNTRIES  
KOSTANTINOS CHARTZOULAKIS, NIKOLAOS KALogerakis, GEORGE PSARRAS, FRANSESCHI SANTORI  
| 15:00 a 15:15 hrs. | **T11-O1** IMPACT OF NEW TECHNOLOGIES ON EXTRACTABILITY AND QUALITY OF EXTRA VIRGIN OLIVE OIL: PULSED ELECTRIC FIELD AND ULTRASOUND  
GIANLUCA VENEZIANI, SONIA ESPOSTO, AGNESE TATICCHI, STEFANIA URBANI, ROBERTO SELVAGGINI, BEATRICE SORDINI, ANTONIETTA LOREFICE, MAURIZIO SERVILI  
UNIVERSITY OF PERUGIA AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES |
| 15:15 a 15:30 hrs. | **T11-O2** PARAMETERS THAT INCREASE THE CONTENT OF ETHANOL AND METHANOL DURING POST-HARVEST AND PROCESSING OF ‘ARBEQUINA  
BOUDEBOUZ ABDELAZIZ, AGUSTI ROMERO, RICARD BOQUE, MONTSERRAT MESTRES  
INSTITUTO DE RECERCA I TECNOLOGÍA AGROALIMENTARIA (IRTA-MAS DE BOVER) OLVICULTURA I FRUTA SECA |
| 15:30 a 15:45 hrs. | **T11-O3** PILOT-SCALE FEASIBILITY OF VERMICOMPOSTING PROCESS TO REDUCE TOXICITY OF OLIVE BY-PRODUCTS USING EISENIA ANDREI EWTHAWORMS  
LAKHTAR HICHAM, KHARBOUCH BARHOUM, ROUSSOS SEVASTIANOS, AMAT SANDRINE, DUPUY NATHALIE, EL MOUSADIK ABDELHAMID  
IBN ZOHIR UNIVERSITY BIOLOGY |
| 16:00 hrs.  | COFFEE  + POSTER SESSION S9-S14 |
Friday 19th

08:30 hrs.  S13  Table Olives
Chairs: P. Rallo  M. Brenes
Key speaker: M. Brenes

08:30 a 09:00 hrs.  T13-K  FERMENTATIVE ALTERATIONS AND TEXTURAL DAMAGES IN OLIVE FRUIT DURING THE PROCESSING TREATMENTS
BARBARA LANZA
COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA) RESEARCH CENTRE FOR ENGINEERING AND AGRO-FOOD PROCESSING (CREA-IT)

09:00 a 09:15 hrs.  T13-O1  TOWARDS A BETTER UNDERSTANDING OF BRUISING DAMAGE IN THE OLIVE FRUIT
PILAR RALLO, ANA MORALES-SILLERO, LAURA CASANOVA, MARÍA PAZ SUÁREZ, MARÍA ROCÍO JIMÉNEZ
UNIVERSIDAD DE SEVILLA CIENCIAS AGROFORESTALES

09:15 a 09:30 hrs.  T13-O2  EFFECTS OF SONICATION AND OZONATION ON THE NATURAL BLACK OLIVE FERMENTATION OF GEMLIK CULTIVAR OLIVES
ECE YILDIZ, AYSEGUL KUMRAL
UNIVERSITY OF ULUDAG FOOD ENGINEERING

09:45 a 10:00 hrs.  T13-O4  ARE PHENOLS ABLE TO REDUCE ACRYLAMIDE CONTENT IN CALIFORNIA STYLE BLACK OLIVES?
JONATHAN DELGADO-ADÁMEZ, FRANCISCO PEREZ-NEVADO, MANUEL CABRE-RA-BAÑEGIL, MANUEL MARTINEZ, CONCEPCION DE MIGUEL, DANIEL MARTIN-VERT-EDOR
"CICTEX MICROBIOLOGÍA, "UNIVERSIDAD DE EXTREMADURA DEPARTMENT OF ANIMAL PRODUCTION AND FOOD SCIENCE"
OLIVE OIL, MEDITERRANEAN DIET AND CARDIOVASCULAR DISEASE

MONTSERRAT FITÓ1 AND RAMÓN ESTRUCH2

1 HOSPITAL DEL MAR MEDICAL RESEARCH INSTITUTE, BARCELONA 2 CIBER OB (OBESITY AND NUTRITION), INSTITUTO DE SALUD CARLOS III, MADRID

EFFECT OF DIETARY FATTY ACIDS ON BONE MARROW NEUTROPHILS LIPID ACCUMULATION

ALMUDENA ORTEGA-GÓMEZ, LOURDES M. JARELA, SERGIO LÓPEZ, SERGIO MONSER-RAT DE LA PAZ, ROSARIO SÁNCHEZ, FRANCISCO J. GARCÍA MURCIA, BEATRIZ BERMÚDEZ, ROCIO ABIA

INSTITUTO DE LA GRASA (CSIC) ALIMENTACIÓN Y SALUD

ASSESSMENT OF THE ANTI-INFLAMMATORY POTENTIAL OF PURIFIED POLYPHENOLS FROM EVOO IN INDUCED PANCREATIC CELLS CULTURED IN VITRO

ELENA LIMA-CABELLO, JOSE CARLOS JIMENEZ-LOPEZ, PAULA MARTINEZ-MAZON, DAMIÁN MAESTRI, JUAN DE DIOS ALCHÉ

ESTACIÓN EXPERIMENTAL DEL ZAFÍN, CSIC PLANT REPRODUCTIVE LABORATORY, DEPARTMENT OF BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY OF PLANTS

ACYLATED DERIVATIVES OF OLEOEUPEIN REDUCE LPS-INDUCED INFLAMMATORY RESPONSE IN MURINE PERITONEAL MACROPHAGES

MARIA LUISA CASTEJÓN, MARINA APARICIO-SOTO, MARINA SÁNCHEZ-HIDALGO, ALEJANDRO GONZÁLEZ-BENJUMEA, JOSÉ G. FERNÁNDEZ-BOLAÑOS, CATALINA ALARCÓN DE LA LASTRA

UNIVERSITY OF SEVILLE FARMACY

EFFECT OF MODIFIED OLIVE PECTIN ON PROLIFERATION OF BLADDER CANCER CELLS

ALEJANDRA BERMÚDEZ-ORIA, GUILLERMO RODRÍGUEZ-GUTIÉRREZ, AFRICA FERNÁNDEZ-PRIOR, ELISA RODRÍGUEZ-JUAN, JUAN FERNÁNDEZ-BOLAÑOS

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INSTITUTO DE LA GRASA - CSIC FOOD PHYTOCHEMISTRY
Abstracts of Oral Communications

NEW OLIVE CULTIVARS FOR HEDGEROW ORCHARDS

CONCEPCIÓN MUÑOZ-DÍEZ, DIEGO CABELLO, JUAN MORAL, PABLO MORELLO, DIEGO BARRANCO, LUIS RALLO
UNIVERSIDAD CORDOBA AGRONOMÍA

Super high-density (SHD) olive orchards (>1500 trees ha−1) are rapidly expanding in Spain since the 1990s. Only few cultivars are adapted to this system, being ‘Arbequina’, ‘Arbosana’ and ‘Koroneiki’ the most popular ones. Thus, it is necessary to increase the number of cultivars adapted to high density conditions to strengthen the viability of these systems worldwide. Over 11 years we evaluated the performance of three new potential olive cultivars, UC1-2-35, UC1-6-9 and UC1-7-8, coming from the olive breeding program of the University of Cordoba and IFAPA, and compared them with ‘Arbequina’, ‘Arbosana’ and the recently release cultivar ‘Sikitita’. The average annual oil production was 1.9, 1.6 and 1.7 t ha−1 for the three new selections and 1.9, 2.1 and 2.1, for ‘Arbequina’, ‘Arbosana’ and ‘Sikitita’, respectively. The accumulated fruit and oil production of all the cultivars increased linearly over time. ‘Arbequina’, ‘Sikitita’ and UC1-6-9 showed the highest fresh fruit oil content, being above 20 %. The less vigorous cultivars, according to their hedgerow dimensions and annual pruning waste, were ‘Arbosana’, ‘Sikitita’ and UC1-2-35. These cultivars were about 20 % smaller than the most vigorous genotype, UC1-6-9. We observed clear differences in the ripening time of olive fruits and oil accumulation. Only in the case of flowering and oil accumulation, fatty acid composition and phenol content (flowering phenology, flower quality, pattern of oil accumulation, fatty acid composition and phenol content and composition), significant genotype and environment interactions have been observed. For example, olive cultivars grown in Tenerife under much milder winter temperatures than in the Iberian Peninsula showed substantially earlier flowering and oil accumulation. Only in the case of flowering phenology was no significant genotype effect found. Furthermore, a strong genotype x environment effect was highly consistent in all characters considered. Regarding resistance to disease, such as Verticillium wilt, the variability of results from both natural and artificial inoculations also tends to indicate a considerable environmental effect and the need for careful testing of disease evolution. All this information strongly suggests the necessity of comparative trials of olive cultivars for both adequate choice of cultivar and final selection in breeding programs.

GENOTYPE, ENVIRONMENT AND THEIR INTERACTION IN OLIVE

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The wide olive genetic patrimony has revealed high variability for most of the agronomic and oil quality traits of interest in olive growing. Few studies, however, have addressed the interaction of this variability with the environment, a subject of particular interest considering the natural high instability of the Mediterranean climate and the challenge of the predicted climate change. The current work presents results on the interaction between genotype and environment from multi-environment trials of olive cultivars and breeding selections, planted in different edaphoclimatic conditions of Andalusia, Southern Peninsular Spain and Canary Islands. For most of the agronomic and oil quality characters evaluated (flowering phenology, flower quality, pattern of oil accumulation, fatty acid composition and phenol content and composition), significant genotype and environment interactions have been observed. For example, olive cultivars grown in Tenerife under much milder winter temperatures than in the Iberian Peninsula showed substantially earlier flowering and oil accumulation. Only in the case of flowering phenology was no significant genotype effect found. Furthermore, a strong genotype x environment effect was highly consistent in all characters considered. Regarding resistance to disease, such as Verticillium wilt, the variability of results from both natural and artificial inoculations also tends to indicate a considerable environmental effect and the need for careful testing of disease evolution. All this information strongly suggests the necessity of comparative trials of olive cultivars for both adequate choice of cultivar and final selection in breeding programs.

IBA-INDUCED IN VITRO ADVENTITIOUS ROOTING IN OLIVE: A MOLECULAR RESPONSE TO CUT INJURY AND AUXIN APPLICATION

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Olive (Olea europaea L.) comprises several varieties with reduced capacity to form adventitious roots (AR) by semi-hardwood cuttings. This will prevent their propagation and consequently their availability in the nurseries, ending up by neglecting some of the most agronomically interesting cultivars. One example is the cv. ‘Galega vulgar’, showing a rooting rate of 5-10% by this propagation method. Nevertheless, our laboratory increased this rooting rate, to approximately 90%, by establishing an auxin-induced in vitro adventitious rooting protocol, using the indole-3-butyric acid (IBA) to induce AR formation in microcuttings. Aiming to understand the molecular mechanisms underlying the adventitious rooting process, we decided to follow, at a first place, a candidate-gene-based approach. Genes involved in plant stress responses and in auxin transport were here characterized and investigated in terms of their expression during the process. Our findings showed an increased expression of the alternative oxidase gene (mitochondrial marker of plant stress response) in induction, pathogen resistance and abiotic stress tolerance have been identified, and the effects of environmental factors, such as drought or salt stress, on shaping the epigenetic pathways in olive, are currently under study for some cultivars. Genetic mapping and genome-wide association studies are carried out on segregating cross progenies and wide set of varieties in order to detect loci and QTLs controlling phenotypic traits of agronomical interest. New biotechnological approaches of cisgenesis and genome editing are being applied in olive both, for the study of gene function and for the development of new genotypes. The combination of these works and the availability of a huge amount of data will allow, in the near future, to overcome the great research gap that has affected the most important oil fruit crop of the Mediterranean area and beyond.
A ROBUST PHYLOGENY OF CULTIVATED AND WILD OLIVE TREES SUGGESTS THAT DOMESTICATION TOOK PLACE IN TWO DIFFERENTIATED EVENTS

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UNIVERSIDAD DE JAÉN CENTRO DE ESTUDIOS AVANZADOS EN OLIVO Y ACEITE DE OLIVA

OLIVE (Olea europaea L.) is one of the most important crops along the Mediterranean Basin, given its social and economic relevance. Cultivated among centuries, olive tree is a priceless source for a large number of products, such as olives, wood, biomass as energy source and one of the most well-known edible in the world, the olive oil. There is a myriad of cultivated olive’s varieties spread across the Mediterranean Basin, ranging from Spain (Royal, Manzanilla, or Picual, among others) to Middle East countries (for instance, Abbadri-Abou-Gabbara, Uulu or Kalamoni). Relevant traits in olive industry like yield, fruit size and oil quality are remarkably different among cultivars. However, the genetic mechanisms behind this evolutive differentiation remain unknown. We have analyzed 100,000 genetic variants among 52 cultivars from the Mediterranean Basin (42 cultivated and 10 wild varieties). The phylogeny obtained clearly divides the cohort into three subpopulations: (i) wild cultivars, (ii) cluster 1 (composed by Southern Spanish cultivars) and (iii) cluster 2 (comprising Eastern Spanish, Greek and Italian cultivars). This results point to an evolutionary tendency, probably posed by Eastern Spanish, Greek and Italian cultivars) and (iii) cluster 2 (composed by Southern Spanish cultivars) and (iii) cluster 2 (composed by Southern Spanish cultivars). The development of new cultivars with increased oil content in the olive fruit mesocarp or with improved fatty acid composition in their oils are two of the more important aims of olive breeding programs. In the present study, sequences related to lipid metabolism from the olive transcriptome generated in the project OLEAGEN have been further manually annotated. In addition, in silico expression analysis of non-normalized 454 cDNA collections corresponding to seed and Picual mesocarp tissue at two different developmental stages has been carried out. The larger number of ESTs provided reliable estimates of genes expressed at low levels. Furthermore, comparison of expression of members from multi-genе families allowed the identification of specific isoforms with conserved functions in oil biosynthesis. Analyses of selected lipid metabolic pathways in both olive tissues revealed patterns similar to that of other oil-rich species. However, some predominant orthologs were identified in the mesocarp tissue. In particular, the accumulation of triacylglycerols rich in oleic acid was associated with higher transcript levels of acetyl-CoA carboxylase, acyl carrier protein, stearoyl-ACP desaturase and endoplasmic reticulum-associated acyl-CoA synthetases. Genes involved in chloroplastic glycerolipid biosynthesis were also identified, although showing low expression levels. In addition, our results indicate that both acyl-CoA-depen dent and -independent mechanisms might play a role in terminal steps of triacylglycerol biosynthesis. Expression levels of transcription factors involved in oil biosynthesis were analyzed as well. Furthermore, the contribution of cytosolic and plastidial glycolytic pathways involved in the carbon supply for oil biosynthesis will be discussed.
OLIVE FRUIT CELLULAR PROCESSES: IMPLICATIONS FOR GROWTH, QUALITY AND MANAGEMENT

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Fruit developmental depends on the cellular processes in the fruit tissues - cell division, expansion, and differentiation. Studies to better define and quantify these processes provide a more comprehensive view of their role, timing, interaction, and the factors which influence them, sometimes contrasting with traditional perceptions. While the highest rate and amount of mesocarp cell division is confirmed to occur immediately following bloom, increase in cell number continues throughout much of fruit development, although at a reduced rate. Cell number forms the basis of cultivar differences in fruit size, water status tends to impact cell size rather than number, and crop load, closely linked to substrate interactions, appears to affect cell number to a greater degree than cell size. Olive mesocarp oil metabolism may be less linked to cell division and expansion than previously hypothesized. Pit hardening entails the initiation and completion of individual cell scission, events which respond to different factors and vary among cells, and are coordinated with endocarp expansive growth. Endocarp scission is affected by both water and assimilate status, and appears to drive the end of endocarp expansion rather than vice versa. The developmental patterns of the different fruit tissues, and their associated substrate demands, provide new information for interpreting and thus optimizing olive fruit growth by crop management practices.

COMPARATIVE LARGE-SCALE ANALYSIS OF LIPID METABOLISM DURING THE EARLY STAGES OF OLIVE POLLEN GERMINATION

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ESTACIÓN EXPERIMENTAL DEL ZAIDÍN (CSIC) BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY OF PLANTS

Lipids are essential for pollen function as the corresponding knockout mutants impair its development or result in an embryo-lethal phenotype. The pollen lipidsome shows a unique composition and the set of genes required for lipid metabolism displays a different expression pattern compared with other tissues. Since most studies were focused on mature pollen, our current knowledge regarding lipid dynamics in growing pollen tubes is still scarce. Here, we carried out a comparative lipidomic and transcriptomic profiling of olive pollen at the early stages of germination. Overall, lipid metabolism accounted for ~2% of total transcripts. Thus, olive pollen grains expressed up to 297 genes involved in the synthesis of all lipid classes, excluding suberin and cutin. Neutral acyl-lipids were the most abundant lipid class. During germination, the triacylglycerol pool came down to about a third, suggesting that lipid bodies were mobilized. The observed increase of lipase and lipoxygenase activities is consistent with this scenario. Olive pollen tubes appear to have no glyoxylate cycle, and lipoxygenase activities is consistent with this scenario.

Glutathione is a multifunctional metabolite considered essential for plant growth and development. On top of its production through the primary metabolism of C, N and S, it plays functions in defense and detoxification against both biotic and abiotic stresses, and is an important factor for redox signaling. Such functions are little known in detail in plant reproduction, in spite of the fact of alterations in its metabolism resulting in highly significant reproductive phenotypes.

We have identified the main components of the gene network controlling generation and levels of glutathione in the olive mature pollen, the gynoecium and the seed by functional analysis of enzymes involved in glutathione metabolism in olive reproductive biology. We have characterized the differential expression of the genes related to glutathione-synthesis, glutathione-reduction, glutathione peroxidase and glutathione S-transferase, and their reliable expression profiles have been determined in different reproductive tissues (gynoecium, anthers, pollen, seeds) and at different developmental stages (flower developmental stages previously defined, dehiscent pollen, pollen through in vitro germination, mature seeds, hydrated seeds along in vitro germination). Finally, models for the participation of key elements in the control of glutathione metabolism, and their interactions with the metabolism of ABA and NO under different reproductive scenarios are being constructed, as well as models of the putative interaction of glutathione-S-transferase with the humane immune system for the generation of allergies.

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STUDYING INTERACTIONS BETWEEN WARMER WINTERS AND GIBBERELLIN IN THE CONTROL OF OLIVE FLOWERING

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In olive, cold temperatures enhance flowering induction while crop load reduces it, in a process known as “alternate bearing”. In a previous study, our lab found a positive correlation between expression levels of two genes encoding proteins similar to FLOWERING LOCUS T (FT) in late winter and flowering intensity. The expression levels in leaves of both genes increases during the winter in trees which had low fruit load (towards ON trees). This increase during winter is reduced in trees which had heavy fruit load (towards OFF trees). It seems that in olive, flower induction occurs during the winter and is mediated by the increase in FT proteins formed in response to cold temperatures. The memory of the fruit load (harvested in November) inhibits the induction by reducing the expression of these genes. Previous research showed that pre-winter treatment with exogenous gibberellin GA3 inhibits olive flowering. In the last couple of years we tested whether GA3 or Uniconazole (GA biosynthesis inhibitor) application can modulate gene expression and the flowering response of olive trees exposed to different winter conditions. Here we will report the results of this study.
processes. how plant connects frost perception and adaptive response measures supported molecular results. The biological well as membrane target genes (FADs) resulted independently down-regulated. CBF and downstream regulated genes, as perception were quickly up-regulated, while genes related to temperature -4°C, indicate that in R.d.A genes linked to temperature connects frost-tolerance and flowering determination. The flowering under frost is crucial for understanding frost adaption and that exist for plants to safeguard their functional integrity when cultivation moves towards Northern latitude and higher by the anthropic pressures. Due to climate changes olive cultivation moves towards Northern latitude and higher altitudes and understanding the molecular mechanisms that exist for plants to safeguard their functional integrity under frost is crucial for understanding frost adaption and olive improvement. In olive, inflorescence development occurs later in winter and/or earlier spring. The flowering regulatory network related to frost adaptation remains still unclear in olive. The olive Spanish cultivars, Royeta de Asque (R.d.A) and Arbequina (Arb.), were used to explore the role of molecular elements in the network which connects frost-tolerance and flowering determination. Cultivars show different adaptive behaviour to frost after naturally occurring of late frost vernalization. Molecular and physiological analyses, determined in plants exposed to -4°C, indicate that in R.d.A genes linked to temperature perception were quickly up-regulated, while genes related to the repression of flowering induction were quickly down-regulated. CBF and downstream regulated genes, as well as membrane target genes (FADs) resulted independently regulated. Ion electrolyte leakage and gas exchange measures supported molecular results. The biological model proposed offers a framework for understanding how plant connects frost perception and adaptive responses to flowering destiny and the relationships between the mechanisms involved in the regulation of both biological processes.

THE EPIDEMIC OF XYELLA FASTIDIOSA IN APULIA: CURRENT SITUATION AND FUTURE PERSPECTIVE FOR THE CONTROL

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CONSIGLIO NAZIONALE DELLE RICERCHE ISTITUTO PER LA PROTEZIONE SOSTENIBILE DELLE PIANTE

In 2013, the quarantine bacterium Xyella fastidiosa was detected in olives in Apulia (southern Italy), whose infections were consistently associated to a severe hiberto disease named olive quick decline syndrome (QODS), a plant health emergency of unprecedented proportions for the EU. The bacterium, belonging to the subspecies pauca was found to be efficiently spread by the meadow spittlebug Philaenus spumarius, and able to infect more than 30 plant species. The initial foci rapidly expanded over the past 4 years, with infections affecting now almost one third of the territory of the region. Phytosanitary measures to contain the spread and mitigate the impact of the bacterial infections, included restrictions for new plantations, for the movement of propagating materials, removal of infected trees and vector control. The EU Commission mobilized dedicated resources to build EU research actions to fulfill research gaps for this emerging pathogen threatening the entire EU territory. Between 2015 and 2016, two multiactor research projects in the framework of the H2020 programs have been funded: the project “Pest Organisms Threatening Europe” (POTHE) and the project “Xyella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy” (XF-ACTORS) the latter targeting exclusively X. fastidiosa. From the intense research activity developed in the past three years some major results have been already achieved, providing data on the genetic and biological properties of the population of the bacterium, the range of hosts, the identification and biology of the vector, the identification of olive cultivars with promising traits of resistance.

MICROBIAL COMMUNITIES INHABITING THE ROOT SYSTEM OF HEALTHY OLIVE TREES

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‘ESTACIÓN EXPERIMENTAL DEL ZADIN (EEZ) - SPANISH NATIONAL RESEARCH COUNCIL (CSIC) SOIL MICROBIOLOGY AND SYMBIOTIC SYSTEMS, EXPERIMENTAL FIELD CULTIVATION’ (EEZ) - SPANISH NATIONAL RESEARCH COUNCIL (CSIC) SOIL MICROBIOLOGY AND SYMBIOTIC SYSTEMS, ‘INSTITUTE FOR SUSTAINABLE AGRICULTURE (IAS)’ - SPANISH NATIONAL RESEARCH COUNCIL (CSIC) CROP PROTECTION

The importance that cultivated olive (Olea europaea L. subsp. europaea var. europaea) has in the Mediterranean Basin is outstanding. In some countries such as Spain, olive crop has undisputable social, economic and agro-ecological relevance. Nevertheless, its future as a strategic commodity within the Mediterranean Agriculture is challenged by a range of threats. Verticillium wilt of olive (VWO), caused by the soil-borne fungus Verticillium dahliae Kleb., constitutes one of the most serious biotic constraints in many areas where this tree is cultivated. Moreover, VWO is very difficult to control, and there is no single effective measure when applied individually. Knowing that plants live in close association with an enormous diversity of microorganisms, and their composite genotype is considered as “the plant’s second genome”, it’s not surprising that members of these plant-associated microbial communities may play an important antagonistic role against phytopathogens. Within this framework, we aimed to implement a strategy to reduce V. dahliae infection based on the whole characterization of the microbe associated with olive roots (rhizosphere and root endosphere) of a range of olive cultivars growing in a single location (Olive World Germplasm Bank, Córdoba, Spain). These olive varieties, originating from 9 different olive-growing countries and accounting for the 95% of the genetic variability of this species, also have different susceptibility/tolerance levels to VWO. Our goals are to identify the cultivar-based microbial profile present in olive roots, and to characterize specific microorganisms which could be associated with tolerance to this disease.

A LONG-TERM STUDY ON AERIAL OLIVE DISEASES: EMPIRICAL APPROACH TO DEVELOP USER-FRIENDLY MODELS

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Scab (OS), anthracnose (OA) and cercospora leaf spot (OC) are the most widespread olive diseases. However, factors involved on their development in natural conditions are not well known. For this reason, 92, 76 and 120 epidemics of OS, OA and OC, respectively, were evaluated in different locations (14, 13 and 3), olive cultivars (11, 9 and 22) and years (16, 13 and 8). The follow-up period was comprised between 1994 and 2015, carrying out one OS or two OA (and OC) evaluations of disease severity per year. Pearson (r) or Spearman (ρ) correlation coefficients were calculated between OS, OA and OC severities and monthly weather variables, disease severity in the previous years (n-1 and n-2) and cultivar resistance category. The most explanatory variables were selected to fit lineal regression models for OS and OA. OS, OA and OC were observed in 90.2, 57.9 and 75.8% of the cases, respectively. OS, OA and OC severities were correlated with several monthly weather variables (P < 0.05). The direction of some correlations indicated that abiotic stressful condition could favor disease development. OS and OA severities were correlated with the n-1 year ones, whereas the OC severity was correlated with the n-2 one. OS, OA and OC severities were correlated with cultivar resistance categories. Models of OS (R2 = 0.725) and OA (R2 = 0.825) were significant. This study provides key information about the long-term factors affecting OS, OA and OC and complements the previously developed models, grouped in a decision support system.
**CULTIVABLE FUNGI PRESENT IN TUNISIAN OLIVE ORCHARDS: DIVERSITY AND BIOPROSPECTING OF ENTOMOPATHOGENIC ACTIVITY**

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INSTITUT DE L’OLIVIER LABORATORY OF IMPROVEMENT AND PROTECTION OF GENETIC RESOURCES OF THE OLIVE TREE

This study investigated the diversity of fungi randomly isolated from olive cultivars in Sfax region (Tunisia) and screened for their entomopathogenic activity.

Molecular biology techniques were used to identify 35 fungi from samples collected. Identification included comparison of their nucleotide sequence analysis of the ITS–5.8S ribosomal DNA region. These consisted of 7 different genera belonging to the Ascomycota. Aspergillus, Cladosporium and Penicillium were the most frequently isolated genera and Cladosporium cladosporioides followed by Aspergillus ustus were the most abundant species. The selected fungal isolates were tested for their antifungal activities against different fungal genus. All isolates exhibited activity against at least one of the tested fungi except Talaromyces verruculosus.

Outcomes exhibited that Grossa de Spain cultivar was significantly more liable to attack by *P. pollinii* than Nabalbi Baladi as indicated by building higher population density all through the year. There was a strong evidence that *P. pollinii* hibernated as adult. This pest completed three generations yearly. The incidence of first generation took place in February and March followed by second and third one from May to June and from August to November, respectively. The first and second generations were significantly more abundant in population density in comparison with the last one. Based on this finding, it is advised to apply insecticides by sever infestation in the mid of March and May, since the vast majority of insect population during this time consists of crawlers with no wax layer covering their body.

In addition, local olive farmers are encouraged to cultivate the native Nabalbi Baladi cultivar in place of introduced one. Grossa de Spain in areas suffering from heavy attack.

**POPULATION DYNAMIC OF OLIVE PIT SCALE, POLLINIA POLLINI COSTA (HEMIPTERA: ASTEROLECANIDAE) ON TWO OLIVE CULTIVARS IN NORTH REGION OF JORDAN.**

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Olive tree (*Olea europaea* L.) is considered the most important tree in Jordan. There are several arthropod pests attacking the olive trees. Among them, *Pollinia pollini* becomes increasingly a destructive threat in some olive groves in the country. A field study was conducted in an olive grove located in north Jordan during the growing season 2015 in aim to identify the performance of population dynamics of this pest on two olive cultivars, namely Grossa de Spain and Nabalbi Baladi, grown under rain-fed conditions.

Outcomes exhibited that *Grossa de Spain* cultivar was significantly more liable to attack by *P. pollinii* than Nabalbi Baladi as indicated by building higher population density all through the year. There was a strong evidence that *P. pollinii* hibernated as adult. This pest completed three generations yearly. The incidence of first generation took place in February and March followed by second and third one from May to June and from August to November, respectively. The first and second generations were significantly more abundant in population density in comparison with the last one. Based on this finding, it is advised to apply insecticides by sever infestation in the mid of March and May, since the vast majority of insect population during this time consists of crawlers with no wax layer covering their body.

In addition, local olive farmers are encouraged to cultivate the native Nabalbi Baladi cultivar in place of introduced one. Grossa de Spain in areas suffering from heavy attack.

**SAP FLOW RESPONSES TO ELEVATED TEMPERATURE AND FRUIT LOAD IN YOUNG OLIVE TREES**

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CENTRO REGIONAL DE INVESTIGACIÓN ECOLÓGICA DE LA RIOJA (CRIEL) ECOFISIOLOGÍA DE OLIVO

Global warming is resulting in temperature increases in South America where temperatures are already considered to be high for many crop species, and greater increases are expected by the end of the century. In perennial crops, there is little understanding as to how water use and other aspects of crop functioning will be affected. Thus, our objectives were to: i) evaluate the response of olive tree sap flow and xylem anatomy to elevated temperature; and ii) determine whether fruit load may affect temperature responses. Two temperature levels were applied in the summer and fall using open top chambers: a control near air temperature and a treatment 4°C above the control. Whole tree sap flow was measured under these treatment conditions using an energy balance methodology in well-watered, three-year-old olive trees (*cv. Arbequina*). The number, diameter, and area of xylem elements was also assessed in shoots formed under the two treatments. Sap flow increased under the elevated temperature treatment, and it was also greater at a given temperature in trees growing under elevated temperature compared to the control trees. This apparent acclimation to elevated temperature may be a function of the greater xylem area observed at elevated temperature. Similar trees were evaluated at ambient temperature with a range of fruit loads. With increasing fruit load, sap flow and stomatal conductance increased linearly up to a threshold above which they remained constant. Thus, other crop factors such as fruit load will likely affect the physiological responses to elevated temperature.

**ABIOTIC STRESS IN OLIVE: DISSECTING THE PHYSIOLOGICAL AND MOLECULAR MECHANISMS**

LUCA SEBASTIANI

SCUOLA SUPERIORE SANT’ANNA INSTITUTE OF LIFE SCIENCES

Olive (*Olea europaea* L.) is an iconic Mediterranean tree that in the last decades has been cultivated in many other warm-temperate regions of the world (North and South America, Australia, New Zealand, and South Africa). Under Mediterranean climate this species is well adapted but when climatic and edaphic conditions become extreme (drought, high, or low temperatures, salinity, low oxygen, nutrient deficiencies) olive plant undergo abiotic stresses. The negative effects caused by environmental constraints are not easily recognized in orchards, especially in those poorly managed by farmers. Since olive cultivation worldwide is gradually moving toward high-density and high-input growing systems with improved olive profitability, a deeper understanding on the effect of abiotic stresses on olive physiology is necessary. In this key lecture, a focus on the molecular and physiological knowledge achieved by the scientific community on olive responses under abiotic stress will be given. Data will be discussed to highlight some of the future research directions and their significance for future genetic improvement of olive cultivars.
INVESTIGATION OF THE INVOLVEMENT OF OLIVE PROLYL 4 HYDROXYLASES AND ARABINOGALACTAN PROTEINS UNDER DUAL STRESS OF SALINITY AND HEAT

PANAGIOTIS KALAITZIS, ARISTOTELIS AZARIADIS, KONSTANTINOS BLAZAKIS, FATEN DANDANCHI, MOHAMED KOUIHEN, GEORGE KOSTELENOUS

CHIEAM-MEDITERRANEAN AGRONOMIC INSTITUTE OF CHANIA HORTICULTURAL GENETICS & BIOTECHNOLOGY

Constant decline in water resources for agriculture and limited rainfall often leads to the use of either low quality or saline water for irrigation indicating that salinity stress might be a major problem for olive culture in the years to come. Most of the times, this stress is accompanied by high temperatures during the summer period. The main objectives of this work is to identify key molecular components of the adaptive mechanism to this dual abiotic stress of salinity and heat. In this study, Greek cultivars were exposed to NaCl stress for a 90-day period under heat stress conditions during the summer. The olive trees were placed in barrels in order to simulate in the degree that was possible, olive growing conditions in the field. Phenomics approaches were applied to determine growth alterations in response to salinity treatment by using in house image analysis algorithms. Previous reports indicated that cell wall glycoproteins such as Arabinogalactans (AGPs) might be involved in the response of plants to salinity stress; therefore transcrip- tions encoding AGPs were identified in the olive genome as well as transcripts encoding prolyl 4 hydroxylases which are involved in the hydroxylation of hydroxyproline rich glyco- proteins (HRGPs) such as AGPs. The expression patterns of the olive P4Hs and AGPs were determined in roots and leaves during the time course of the salinity treatment. In this study, the salt tolerance of olive cultivars Canino and Sirole was compared with two transgenic lines of Canino cultivar, expressing osmotin gene from tobacco, obtained by agro- bacterium-mediated transformation. Shoot cultures were in vitro exposed to salinity stress by adding sodium chloride (NaCl) at different concentrations: 0, 50, 100, and 200 mM, corresponding to electric conductivity of 7.64, 13.75, 19.53 and 40.70 dS m-1 respectively. After four weeks in cultivu- le, most of the shoots of wt plants, in salt-enriched media, showed shrunken growth and ultimate leaf drop contrary to transgenic shoots of both lines, which did not show any injur- ies and exhibited a normal growth. The possibility that S assimilatory pathway could be involved in alleviating the adverse effects of salt stress has been here evaluated. For this purpose, photosynthetic capacity parameters in different olive genotypes under a whole year under well-watered and water-stressed con- ditions. We observed a different response to water stress in the RGR of the genotypes which was best explained by the net CO2 assimilation rate (NAR). Further, net pho- tosynthesis, closely related to NAR, was mainly determi- ned by hydraulic traits, both at leaf and whole plant levels, mediated through their effect on stomatal conductance. We observed a decrease in the ratios of leaf area:sapwood area and leaf area:root area in water-stressed plants which was more remarkable in the olive genotype Olea europea subsp. Guanchica (GUA8) whose RGR was less affected by water deficit. In addition, at leaf level, water-stressed plants of GUÁ8 presented a better photosynthetic capaci- ty through a higher mesophyll conductance to CO2 and foliar N. We conclude that hydraulic allometry adjustments of whole plant and leaf physiological response were well coordinated, helping to buffer the water stress experi- enced by GUÁ8. That explained its higher RGR compared to the rest under water stress conditions.

TRANSGENIC OLIVE (OLEA EUROPEA L.) SHOOTS OVEREXPRESSION OF OSMOTIN GENE ARE LESS SENSITIVE TO IN VITRO-INDUCED SALT STRESS

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Most of the worldwide agricultural lands are unfavourable for plants, due to scarcity of water availability in the soils or due to soil salinization, caused by prolonged use of water containing high concentrations of salts. Olive is considered moderately resistant to soil salinity as compared to other trees, but anyway it is advisable to avoid the use of irrigation water exceeding 3.4 dS m-1. In this study, the salt tolerance of olive cultivars Canino and Sirole was compared with two transgenic lines of Canino cultivar, expressing osmotin gene from tobacco, obtained by agro- bacterium-mediated transformation. Shoot cultures were in vitro exposed to salt stress by adding sodium chloride (NaCl) at different concentrations: 0, 50, 100, and 200 mM, corresponding to electric conductivity of 7.64, 13.75, 19.53 and 40.70 dS m-1 respectively. After four weeks in culture, most of the shoots of wt plants, in salt-enriched media, showed shrunken growth and ultimate leaf drop contrary to transgenic shoots of both lines, which did not show any injuries and exhibited a normal growth. The possibility that S assimilatory pathway could be involved in alleviating the adverse effects of salt stress has been here evaluated. For this purpose, photosynthetic capacity parameters in different olive genotypes over a whole year under well-watered and water-stressed con- ditions. We observed a different response to water stress in the RGR of the genotypes which was best explained by the net CO2 assimilation rate (NAR). Further, net pho- tosynthesis, closely related to NAR, was mainly determi- ned by hydraulic traits, both at leaf and whole plant levels, mediated through their effect on stomatal conductance. We observed a decrease in the ratios of leaf area:sapwood area and leaf area:root area in water-stressed plants which was more remarkable in the olive genotype Olea europea subsp. Guanchica (GUÁ8) whose RGR was less affected by water deficit. In addition, at leaf level, water-stressed plants of GUÁ8 presented a better photosynthetic capaci- ty through a higher mesophyll conductance to CO2 and foliar N. We conclude that hydraulic allometry adjustments of whole plant and leaf physiological response were well coordinated, helping to buffer the water stress experi- enced by GUÁ8. That explained its higher RGR compared to the rest under water stress conditions.

COORDINATION BETWEEN WATER SUPPLY AND DEMAND AT LEAF AND PLANT LEVEL HELPS TO EXPLAIN DIFFERENTIAL GROWTH PATTERNS IN OLIVE GENOTYPES IN RESPONSE TO MILD WATER STRESS

VIRGINIA HERNANDEZ-SANTANA, PABLO DIAZ-RUEDA, ANTONIO DIAZ-ESPEJO, MARIA DOLORES RAYA-SERENO, SARAY GUTIERREZ-GORDILLO, ANTONIO MONTERO, AL- FONSO PEREZ-MARTIN, JOSÉ MANUEL COLMENERO-FLO- RES, CELIA MODESTA RODRIGUEZ-DOMINGUEZ

IRNAS

Hydraulic traits could help to anticipate the impacts of climate change and improve crop productivity. However, the mechanisms explaining their role on plant photosyn- thesis and thus, plant growth and yield, are just emerging. We conducted an experiment to recognize differences in growth patterns among different olive genotypes and to determine whether hydraulic traits may help to explain such differences through their effect on photosynthesis. We estimated the relative growth rate (RGR), its compo- nents, gas exchange, hydraulic traits and photosynthetic capacity parameters in different olive genotypes over a whole year under well-watered and water-stressed con- ditions. We observed a different response to water stress in the RGR of the genotypes which was best explained by the net CO2 assimilation rate (NAR). Further, net pho- tosynthesis, closely related to NAR, was mainly determi- ned by hydraulic traits, both at leaf and whole plant levels, mediated through their effect on stomatal conductance. We observed a decrease in the ratios of leaf area:sapwood area and leaf area:root area in water-stressed plants which was more remarkable in the olive genotype Olea europea subsp. Guanchica (GUÁ8) whose RGR was less affected by water deficit. In addition, at leaf level, water-stressed plants of GUÁ8 presented a better photosynthetic capaci- ty through a higher mesophyll conductance to CO2 and foliar N. We conclude that hydraulic allometry adjustments of whole plant and leaf physiological response were well coordinated, helping to buffer the water stress experi- enced by GUÁ8. That explained its higher RGR compared to the rest under water stress conditions.

THE FUTURE OF PRECISION AGRICULTURE AND THE RATIONAL APPLICATION OF DEFICIT IRRIGATION

ANTONIO DIAZ-ESPEJO, VIRGINIA HERNANDEZ-SANTA- NA, JOSE ENRIQUE FERNÁNDEZ

IRNAS PROTECTION OF THE SOIL, PLANT, WATER SYSTEM

We are witnessing to the implementation of sensor tech- nology and ITCs in agriculture. This is producing a huge amount of information from the soil, plant and atmosphe- re which is difficult to interpret to take decisions in irri- gation scheduling. A short-term solution to deal with this Big Data accumulation is coming from the application of Machine Learning approaches which in the long-term are expected to provide no novel insights into the mechanis- ms involved in the response observed and what are the key physiological traits explaining the differences among cultivars or species. On the other hand, during the last three decades we have assisted to intense research efforts in the study of physiological mechanisms involved in the response of fruit trees and crops to water stress. This accu- mulated knowledge should start to yield new approaches to overcome the difficulty in the interpretation of plant-ba- sed sensors for irrigation scheduling. The choice of the key target variable to maximise or optimise yield is crucial and up-to-date there is not consensus in the scientific commu- nity about it. Stomatal conductance emerges as the best candidate due to several reasons that will be dissected in the presentation. It has been demonstrated recently that we can monitor automatically and in-continuous stomatal conductance in commercial orchards and estimate pho- tosynthesis with confidence. It will be explained how sto- matal conductance can be used to decide, with a rational basis, the level of stress we are implementing in our olive orchard during the application of a regulated deficit irri- gation strategy.
Due to water scarcity, many countries of Mediterranean area are using municipal reclaimed water and deficit irrigation as alternative techniques in oliviculture, and its sustainability and long-term use is becoming a challenge. Although the deficit irrigation techniques have been tested many times by using conventional sources, the combined effects of saline reclaimed water and deficit irrigation have not been fully investigated. The physiological and agronomic effects of irrigating olive trees (Olea europaea L. cv. Arbosana) planted on 100 L/16 h with loam soil (Puglia Region, Italy), and with saline reclaimed water (RW) combined with regulated deficit irrigation (RDI) strategy were analyzed. Two irrigation water sources, DESERT water (DW) (is a result of secondary treated wastewater with ECw of 1 dS m-1), RW (secondary treated wastewater coming from Bari WWTP with ECw of 1.2 dS m-1 mixed with the brine produced on the DESERT prototype till reach an ECw of 3 dS m-1) and two irrigation treatments: a control treatment, FT (irrigated at 100% of the crop evapotranspiration; ETo) and a RDI treatment (irrigated at 50% of ETo from 15th June to 20th August) were examined. RW had high concentration on valuable agro-nomic nutrients such as N, K, P, but also on phytotoxic elements (Na and Cl). Na leaf concentration on RW treated plants did not reach toxic levels, and toxicity symptoms were not shown. However, a significant increase of Na level in olive paste was detected. Regarding N content, a significant increase in plants irrigated with RW with respect to plants irrigated with DW was observed. Despite the saline and water stress induced to plants, significant differences in gas exchange (stomatal conductance Sc and net photosynthesis Pn) were not observed throughout growth season, except during the deficit irrigation period, in which DW-RDI and RW-FI treatments showed significant lower Pn than the others treatments. About plant water status, during the deficit irrigation period, stem water potential on RDI treatments (both RW and DW), decreased below the control treatments but without significant differences. These results manifest that, in arid and semi-arid areas, such a combination of RW and RDI, can be a promising future practice on olive irrigation, but long-term studies to establish suitable management practices must be developed.
The role of nutrients in plant growth are usually explained in terms of its functions in plant metabolism. However, there are evidences that tolerance or resistance to plant pathogens, which are genetically controlled, could be affected by the nutritional status of the plants. These relationships are not well studied. However, it is considered that an adequate nutritional status that ensures optimal plant growth is also optimal to plant resistance. In this sense, it has been found that an excess of nitrogen increases susceptibility to olive leaf spot and to verticillium wilt. Also, an excess of N may reduce K uptake by the roots. Potassium plays an important role in the regulation of water status of the olive. Silicon is not an essential element for plant growth, but it is considered a beneficial element. Its role on the control of pests and diseases is the formation of a physical barrier since Si is deposited in the epidermal cells of the leaves. But K deficiency or N excess may reduce this accumulation of Si, which is considered a beneficial element. Its role on the control of pests and diseases is the formation of a physical barrier since Si is deposited in the epidermal cells of the leaves. But K deficiency or N excess may reduce this accumulation of Si, which is deposited in the epidermis of the leaves. In the absence of Si, it is possible to start evaluating olive yield. The second field trial started from just planted young cuttings to evaluate the biomass produced and phosphorus uptake. One of the pot experiments consisted on the use of four phosphorus rates and the other on the use of four different soils and two phosphorus rates. In the first trial, there was no significant response to phosphorus application in oligo yield or biomass parameters of the fruit such as fruit size and pulp/pit ratio. In the other three trials, only in the second pot experiment an increase in biomass production by the application of phosphorus was observed. This experiment included acidic soils, which may have greatly influenced the availability of phosphorus to the plants. In three of the four experiments leaf phosphorus concentration increased in response to phosphorus application. The pot experiments showed that roots accumulate appreciable amounts of phosphorus and that the application of phosphorus increased proportionally more the concentration of phosphorus in roots than in leaves or stems. In one of the experiments the root/shoot ratio increased with the application of phosphorus. These results seem to indicate that roots are important tissues for phosphorus accumulation which can buffer phosphorus in the shoots in periods of lower phosphorus availability in the soils and may contribute to explain the difficulty to find a response of the olive tree to the phosphorus application under field conditions.

Olive trees in Tunisia have been traditionally cultivated under rainfall conditions. Fertilization practices in olive growing are widely used in order to improve yields and status of trees conducted in severe conditions. The objective of this study was to assess the effect of foliar fertilization on mineral composition and primary metabolites (soluble sugars) in leaves of Olea europaea L. The experiment was conducted on ‘Chemilali’ olive cultivar grown under rain-fed conditions located in central Tunisia. Foliar fertilizers were sprayed separately at different seasons and the treatments were as following: CON (Control, without foliar fertilization), F1 (nitrogen based fertilizer), F2 (rich in boron), F12 (combination of F1 and F2). Leaf samples were collected at three phenological stages, such as full bloom, pit-hardening and fruit growth from the different blocks.

Leaf nutrients were analyzed and carbohydrates composition and oil yields under irrigated farming conditions. The aim of this on-farm study is to optimize the amount of NPK nutrients required annually. For this purpose four fertilization methods were assessed in three consecutive years (2014-2016): T1= Control (no fertilizer supply), T2= farmer used, T3= nutrient foliar analysis and T4= trees nutrient removal. Results showed that T3 had higher olive fruit yield compared to T2 (22%, 39.5% and 36.4%) in first, second and third year respectively. T4 had improved yields: 24.1%, 19.4% and 38.9% in first, second and third year respectively. Average 100 olive fruits weight were 346, 383 and 383 g noted under T2, T3 and T4 respectively. For two consecutive years (2015 and 2016), the olive oil content was significantly improved by optimizing NPK supplies. Compared to T1, olive oil content was increased by 20.1% and 24.1% under T3 and T4 respectively for the first year and by 24.5% and 24.8% respectively in second year. High values of olive phenols content were obtained by T3 (468 ppm) and T4 (495 ppm) compared to T1 (335 ppm). T3 and T4 induced increase of oil chlorophyll content and a decrease in oil peroxide content. No significant differences were observed between studied fertilization methods for fatty acids content and composition. Taking in account amounts of fertilizers supplied, T3 induced best equilibrium among olive oil and oil yields under irrigated farming conditions.

T07-O1
OLIVE TREE RESPONSE TO PHOSPHORUS APPLICATION ASSESSED FROM FIELD AND POT EXPERIMENTS
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Phosphorus is a macronutrient regularly applied in olive groves even though no studies exist demonstrating the need for its application. In this work, results of two field trials and two pot experiments are presented where the response of olive tree to phosphorus application was studied from 2013 to 2017. One of the field trials was installed in a three-year-old olive grove where it was already possible to start evaluating olive yield. The second field trial started from just planted young cuttings to evaluate the biomass produced and phosphorus uptake. One of the pot experiments consisted on the use of four phosphorus rates and the other on the use of four different soils and two phosphorus rates. In the first trial, there was no significant response to phosphorus application in olive yield or biomass parameters of the fruit such as fruit size and pulp/pit ratio. In the other three trials, only in the second pot experiment an increase in biomass production by the application of phosphorus was observed. This experiment included acidic soils, which may have greatly influenced the availability of phosphorus to the plants. In three of the four experiments leaf phosphorus concentration increased in response to phosphorus application. The pot experiments showed that roots accumulate appreciable amounts of phosphorus and that the application of phosphorus increased proportionally more the concentration of phosphorus in roots than in leaves or stems. In one of the experiments the root/shoot ratio increased with the application of phosphorus. These results seem to indicate that roots are important tissues for phosphorus accumulation which can buffer phosphorus in the shoots in periods of lower phosphorus availability in the soils and may contribute to explain the difficulty to find a response of the olive tree to the phosphorus application under field conditions.

T07-O2
SEASONAL CHANGES IN MINERALS AND CARBOHYDRATES STATUS OF OLIVE LEAVES AFTER FOLIAR FERTILIZER APPLICATION
IMEN ZOUIARI, MOUNA AÏACHI-MEZGHANI, BELIEGH MECHRI, HASSOUNA GOUTA, HENRIQUE RIBEIRO, MIGUEL MARTINS, FAOUIZI ATTIA, FOUED LABILID, IMED CHERIAIF, IBTISSEM LAARIBI, KOHLOUD ANNABI, AMEL MAGUIDCHE, MOHAMMED HAMMAMI
OLIVE TREE INSTITUTE TUNISIA LABORATORY OF DURABILITY IN SE-MARZO AND ARID REGIONS: AMELIORATION OF PRODUCTIVITY OF OLIVE TREE AND QUALITY OF PRODUCTS
Olive trees in Tunisia have been traditionally cultivated under rainfall conditions. Fertilization practices in olive growing are widely used in order to improve yields and status of trees conducted in severe conditions. The objective of this study was to assess the effect of foliar fertilization on mineral composition and primary metabolites (soluble sugars) in leaves of Olea europaea L. The experiment was conducted on ‘Chemilali’ olive cultivar grown under rain-fed conditions located in central Tunisia. Foliar fertilizers were sprayed separately at different seasons and the treatments were as following: CON (Control, without foliar fertilization), F1 (nitrogen based fertilizer), F2 (rich in boron), F12 (combination of F1 and F2). Leaf samples were collected at three phenological stages, such as full bloom, pit-hardening and fruit growth from the different blocks.

Leaf nutrients were analyzed and carbohydrates composition were observed through the season and the treatments. The most abundant soluble sugar was mannitol followed by glucose. For the full bloom, mannitol and the glucose levels were the highest in olive leaves from trees that were treated with F12 and were equal to 29.48 µg/mg dry weight (d.w) and 17.43 µg/mg d.w, respectively. For the two other sampling dates, mannitol contents decreased following the treatments compared to the control. This study brought out the impact of foliar fertilization on the mineral status and elucidated an association between nutrients and sugar metabolism in rain-fed conditions.

T07-O3
OPTIMIZING FERTILIZATION MANAGEMENT IN OLIVE ORCHARDS TO IMPROVE FRUIT AND OIL YIELDS UNDER IRRIGATED FARMING CONDITIONS
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INRA MOROCCO PLANT BREEDING, PLANT BREEDING AND QUALITY
Olive (Olea europaea L.) is among fruit trees having important economic and social impacts in Morocco. The area covered by this crop is estimated to 1 Million hectares. However, olive yields are still very low because of insufficient fertilization management. Thus, new recommendations based on scientific approach are needed to improve olive and oil yields under irrigated farming conditions. The aim of this on-farm study is to optimize the amount of NPK nutrients required annually. For this purpose four fertilization methods were assessed in three consecutive years (2014-2016): T1= Control (no fertilizer supply), T2= farmer used, T3= nutrient foliar analysis and T4= trees nutrient removal. Results showed that T3 had higher olive fruit yield compared to T2 (22%, 39.5% and 36.4%) in first, second and third year respectively. T4 had improved yields: 24.1%, 19.4% and 38.9% in first, second and third year respectively. Average 100 olive fruits weight were 346, 383 and 383 g noted under T2, T3 and T4 respectively. For two consecutive years (2015 and 2016), the olive oil content was significantly improved by optimizing NPK supplies. Compared to T1, olive oil content was increased by 20.1% and 24.1% under T3 and T4 respectively for the first year and by 24.5% and 24.8% respectively in second year. High values of olive pheno-nols content were obtained by T3 (468 ppm) and T4 (495 ppm) compared to T1 (335 ppm). T3 and T4 induced increase of oil chlorophyll content and a decrease in oil peroxide content. No significant differences were observed between studied fertilization methods for fatty acids content and composition. Taking in account amounts of fertilizers supplied, T3 induced best equilibrium among olive oil and oil yields and fertilization costs.
Olive orchard is one of the most important fruit crops in North Africa. It plays an important economic and social role for smallholder farmers. Although that the olive area has increased markedly in the last ten years in North Africa, olive production still low than the potential of the region. Poor nutrient management is one of the main constraints of olive production in North Africa. Farmers fertilizer practices are in general traditional based mostly on organic fertilizer with few complement of mineral fertilization based mostly on nitrogen without any specific knowledge on the best technical practice. Best fertilization practice should cover the need of the tree and provide the right quantities of nutrients with the right source at the right time and the right placement which represent the component of the 4R nutrient stewardship. Since 2013, on-farm research undertaken by International Plant Nutrition Institute (IPNI) over 4 years in Morocco and Tunisia showed that olive responds well to N, P and K fertilizers. Foliar diagnosis conducted in irrigated and rainfed olive orchards in Tunisia and Morocco showed a high spatial variability of nutrient contents with high nutrient deficiency under rainfed compared to irrigated olive orchards. By comparing best nutrient management practice (BNM) to fertilizer farmer practices (FFP) olive yield gained more than 30% compared to FFP. The present work is a review of the main results of research undertaken by IPNI and national research institutions about the nutrient management of olive orchards in North Africa region.
In 2009, the Side-Row Continuous Canopy Shaking Harvester project was set to develop such technology. The prototype comprises two symmetrical harvesters trailed by a farm tractor. Each harvester has a vibratory rotor with flexible rods, a catching platform with conveyors belts delivering fruits to a temporary storage bag (Peça et al., 2014). The detachment efficiency of canopy shakers are influenced by factors like shaking frequency, ground speed as well as the dimension and shape of olive canopy.

In 2014, authors started a trial to evaluate the influence of pruning in the performance of the Side-Row Continuous Canopy Shaking Harvester. The trial was established in an irrigated olive orchard of Picual cultivar planted in 1996 with the array 7m x 3.5m. The narrow hedgerow olive orchards are increasing due to, among other factors, the possibility of using straddle harvester. The harvester requirements are the main parameters to design both the plantations and the trees. However, the analysis of the tree response under forced vibration should allow the improvement of the tree training and the future development of the machines. In this study, the tree’s response to mechanical harvesting is analyzed in 22 Arbequina® olive trees in a 7 years-old plantation. In each tree, two measurement points were recorded in the central leader, ranged from 50 to 250 cm above ground, with a set of two 3D wireless accelerometers. The trees were excited with a frequency value of 7.8±0.1 Hz during a vibration time of 6.9±1.4 s. The tree vibration was characterized by the combination of a resultant acceleration value (101±31 m/s²) and by impact events (973±216 m/s²). During the harvesting process, the trees were subjected during 4.4% of vibration time to an acceleration level above 1000 m/s². The tree response was 3 times higher in the cross-hedge direction than the forward machine direction. The vertical acceleration represented 17.7% of the resultant acceleration of the tree. The resultant acceleration and impact values increased according to the tree height from the ground and were reduced according to the branch diameter. So, the tree parts located 2 m above the ground had acceleration and impact levels of 1.6 and 1.7 times, respectively, higher than the parts of the tree located below 1 m.

The study of volatile compounds allows selecting those chemical markers that are responsible for aroma, and in particular, for the sensory defects that are determinant for the category classification (e.g., rancid, winey-vinegary, musty-humidity). The analysis of the volatile compounds of a sample set including the three quality categories served to demonstrate the occurrence of particular volatile compounds in certain sensory defects. The most relevant compounds were considered for developing reference materials (RMs) for aroma. Virgin olive oil sensory assessment requires the use of RMs providing the most representative aromas of sensory defects. The RMs that are used today are real virgin/lampante olive oils sensory qualified with these defects. However, those samples may differ in the defect intensity and some of them are characterized with more than one defect. Thus, a new approach has been addressed by providing RMs formulated with volatiles responsible for each defect. The objective is to achieve formulations that are able to reproduce the aroma of particular sensory defects. In this work, two RMs for the defects rancid and winey-vinegary have been developed. When developing the formulations, the synergic and the antagonistic effects in the sensory perceptions of volatile compounds added in the same matrix were taken into account. This work has been carried out inside the OLEUM project “Advanced solutions for assuring the authenticity and quality of olive oil at global scale” funded by the European Commission within the Horizon 2020 Programme (2014-2020, grant agreement no. 635690).

Olive Management, Biotechnology and Authenticity of Olive Products
T09-O2
SELECTIVE AND STRAIGHTFORWARD “HOST-GUEST” MAGNETIC RESPONSIVE TOOL FOR THE TRACE ANALYSIS OF DIMETHOATE IN OLIVE OIL SAMPLES
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Trace analysis of pesticide residues in olive oil is a challenging task. Generally, analytical procedures used to quantify these contaminants encompass a previous and tedious sample preparation methodology, enabling the isolation of the target analyte without the co-extraction of fatty interfering species, followed by its quantification using chromatographic techniques. In this work will be presented a new straightforward approach focused on the development of a magnetic and controllable sample preparation methodology for the selective extraction of dimethoate from olive oil and further quantification of their levels by chromatographic techniques. To achieve this goal, magnetic molecularly imprinted polymers (MMIPs)- based sorbents have been designed, synthesized and used on the enrichment of dimethoate from olive oil samples. In this approach, MMIPs are directly dispersed into an olive oil sample, enabling the selective trapping of the target pesticide, followed by an easily separation of the imprinting material from the matrix using an external magnetic field without additional centrifugation or filtration steps, which is highly advantageous. Further, the target pesticide is eluted from MMIP and quantified by chromatographic techniques.

It will be proved the suitability of MMIPs for selective enrichment of dimethoate from spiked olive oils and highlighted the straightforward feature of this analytical tool with less handling and less time consuming.

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T09-O3
CHEMICAL CHARACTERIZATION OF ‘ARBEQUINA’ EXTRA-VIRGIN OLIVE OILS PRODUCED IN DIFFERENT GROWING AREAS OF SPAIN
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1EMP AGRICOLA TECHNICAL COORDINATOR, 2IFAPA, JUNTA DE ANDALUCÍA CENTRO ALAMEDA DEL OIBSO

‘Arbequina’ is one of the main Spanish olive oil cultivars, which is well known in the international oil market for its excellent taste and flavour and, also, used in different plantation models (intensive and super-high density). This is the most important cultivar in Catalonia (North-eastern of Spain). It is also found extensively in other regions (Andalusia, Aragon, etc.), and other countries (Argentina, Chile, Tunisia, Morocco, Portugal, USA, etc.). Extra-virgin olive oil (EVOO) composition determines its intrinsic quality and could be influenced by several factors, such as cultivar, fruit ripening, cultural practices, processing methods and agroclimatic conditions. However, geographical area is greatly responsible for the specific functional characteristics of olive oil, mainly in this cultivar. The aim of this work was to characterize ‘Arbequina’ EVOOs from different crop seasons and locations in Spain in terms of their fatty acid profile, polyphenols, bitterness (R225) and oxidative stability, to show the classification of oil samples with respect to geographic area. The data in this study are taken from different sources, such as bibliographic reviews and unpublished results of oil samples (several harvest) from different regions of Spain, mainly in Catalonia and Andalusia. The obtained results indicate that some quality parameters of the ‘Arbequina’ oil are variable, being cultivated in different environments, mainly certain fatty acids, polyphenols and oxidative stability.

T09-O4
MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF GENES AND ENZYMES INVOLVED IN VIRGIN OLIVE OIL QUALITY
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Virgin olive oil (VOO) is a natural fruit juice having exceptional organoleptic and nutritional properties. Metabolites profiles of VOO responsible for its nutritional, organoleptic and technology quality are determined by the level of certain components that are transferred directly from fruit to oil, such as fatty acids, and the level of other components that are specifically formed during the oil extraction process from precursors already present in the fruit by the action of different enzymes, which is the case of volatile and phenolic compounds. The final content of these metabolites related to VOO quality is controlled by the levels of expression/activity of the corresponding genes/enzymes involved in their biosynthesis which depends on the oil cultivar. However, the characterization of olive cultivars has been traditionally based on purely agronomic criteria without a systematic study devoted to the identification of markers directly related to oil quality.

To this aim, we have performed the isolation, functional identification and transcriptional analysis of genes involved in unsaturated fatty acids, volatiles and polyphenols biosynthesis. In addition, we have identified the first QTLs controlling fatty acid composition in olive, generated olive transgenic lines overexpressing or silencing the 13-hydroperoxide lyase gene responsible for VOO volatile formation, and carried out the immunological characterization and subcellular localization of the beta-glucosidase enzyme involved in the phenolic composition of VOO. All these data will be discussed in relation to the improvement of VOO quality characteristics.
Commission Regulation (EC) No 1019/2002 of 13 June 2002 on marketing standards for olive oil specifies the conditions according to which olive oil may be mixed with seed oil and then commercialized. Specifically, it is mentioned that the word olive oil may be on the labelling of a blend only when it accounts for more than 50%. This Regulation is directly applicable in all Member States. However, no analytical protocol exists to check that an olive oil-seed oil blend actually contains the percentage of olive oil indicated on the percentage. Therefore, we have decided to undertake the study of “legal blends” from the point of view of the triglyceride (TAG) aliphatic hydrocarbon (AHC), sterol and tocopherol compositions: After analysing a large number of genuine (extra virgin) olive oils (EVOO and OO) from different varieties and origins, we have concluded that POQ, an ECN48 (equivalent carbon number) TAG has a constant concentration of 23%. Admixtures with normal or high oleic types sunflower oil (NTSO and HOSO) gave experimental values of 11% and 15%, respectively, meaning that those minimum percentages would guarantee the olive oil presence at least at 50%. Besides, total sterol values below 2400 ppm will also indicate the presence of at least 50% olive oil. Regarding AHC, C23 and C25 are almost absent in most seed oils therefore, for NTSO and HOSO we have established the minimum percentages for both soil and olive oil represented by: Rb, Mg, Sr, Ba, Sr, Ba, Ba, Fe, Mn and Zn. The soils differ geochemically in such a manner that permits the transfer of that signature to olive oils and their traceability.

The elemental composition of foodstuff proved to be a powerful geographical marker. However, no link could have been created between the elemental composition of olive oil and its potential precursor: the soil geochemical composition. In this study, we aimed at using the elemental composition of olive oils and their paired soils to study their discriminating power within the Tunisian context and to understand the control over the distribution of multielements in olive oils. For the first time, fourteen elements (Ca, Na, Mg, Fe, Mn, V, Cr, Cu, Zn, As, Al, Sr, Ba, and Pb) were determined in Tunisian olive oils using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The same elements were also quantified in the paired soil samples coming from four regions using X-Ray Fluorescence and Laser Ablation ICP-MS. All elements in soils, except Cu, showed a significant difference between the four origins compared to only 10 elements in olive oils. Principal component analysis applied to the composition of olive oils demonstrated that elemental profile is substantially shaped by geochemical processes. Linear discriminant analysis allowed 95.2% and 84.1% correct classification and validation rates respectively of olive oils to their origin. Spearman’s correlation analysis revealed a link only between soil and olive oils concentrations. However, a correspondence was identified between the geochemical discriminating elements for both soil and olive oil represented by: Rb, Mg, Sr, Ba, Fe, Mn and Zn. The soils differ geochemically in such a manner that permits the transfer of that signature to olive oils and their traceability.

Olive oil is one of the oldest vegetable oils and is the major constituent of the Mediterranean diet. Its consumption has also spread remarkably outside the Mediterranean Basin. Nowadays, consumers awareness concerning quality, safety and traceability of food products, as olive oil, is increasing. More information is required aiming to maintain and improve the overall quality of this product. In this regard issues related to olive oil authenticity and traceability are considered crucial.

The Project MedOOmics (Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting) aims to profile and fingerprint monovarietal extra virgin olive oils made of the most typical varieties from different regions of four countries, addressing issues related to varietal and geographical traceability and authentication. The objectives can be summarized as 1) the establishment of chemical markers of geographical and varietal traceability and 2) establish a pattern comprising the “markers of authenticity” to be used in the identification of olive oil adulterations. MedOOmics is based on an integrated approach, comprising a deep knowledge of some of the least studied olive oil varieties in the Mediterranean Basin. MedOOmics activities will span 8 working packages, gathering four countries, Portugal as coordinator, France, Turkey and Tunisia. Results obtained so far, using different methodological and analytical tools will be disclosed.

Acknowledgements:

NTSO or HOSO thus, b/g values below 2.4 will guarantee the absence of the former ones.

The detection of soft deodorized olive oils in virgin oil has become a challenging task ever since it was demonstrated that: 1. The process did not form the typical refining markers, i.e. stigmastadienes and trans-fatty acids, and 2. The determination of the fatty acid alkyl esters rendered useful only in those cases in which the deodorized matrix came from oils with fermentative defects.

In the last times a number of strategies have been developed to detect such kind of illegal blends, being one of them based on the fact that both diacylglycerol (DAG) and free fatty acid contents may be enhanced during mild refining activities.

Actually, the DAG presence in virgin olive oils, normally below 3%, and the 1,2- to 1,3-isomer shift are enhanced during oil extraction, refining and storage. Presently, the determination of the free acidity/total DAG ratio is proposed to detect blends of soft deodorized oils in virgin olive oils, being values above 0.20 indicative of the absence of the former ones.

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T11-O1
IMPACT OF NEW TECHNOLOGIES ON EXTRACTABILITY AND QUALITY OF EXTRA VIRGIN OLIVE OIL: PULSED ELECTRIC FIELD AND ULTRASOUND.

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The main operators of olive oil sector are continuously involved in the development of olive oil mechanical extraction process with the common aim of both increasing quality and oil extraction yield coupled with the potential enhancement of working efficiency of the olive mill. Pulsed electric field and ultrasound are two of the recent technological innovations studied during the last years to improve the olive oil extraction technology. The impact on the diffusion of oil and micro-constituents, determined by the disruption effects on olive cell tissues carried out by the two different techniques, has been evaluated. Pulsed electric field and ultrasound can increase the permeability and the breaking of cell membranes with a consequent positive result on oil extractability and quality, mainly related to the compounds involved in the health and sensory properties of extra virgin olive oil. Pulsed electric field was tested on four Italian cultivar (Carolea, Coratina, Ottobratica and Peranzana), whereas ultrasound technology was applied to Ogliastra Garganica olives harvested at different maturity indices. Results showed that the impact of the new technologies on oil yield and concentration of α-tocopherols, hydrophilic phenols and volatile compounds was significantly K content in the soil. Phenols were decomposed rapidly, while the nutritional status, the physiology and yield of olive trees is not affected by the application of OMW. The disposal of olive mill-by-products produced during oil extraction in Mediterranean countries creates a significant environmental problem due to great volumes of effluent produced (20-30 Mm3/year), heavy pollution load (40-80 g/l BOD, 50-150 g/l COD) and phytotoxic properties (due to phenolic compounds). During the last 30 years many treatment methods have been proposed and tested, but their application at olive mill level is limited due to high investment and/or running cost and technical expertise required. Within the LIFE+ OLEICO+ project have been identified and evaluated existing technologies following certain criteria that are currently being used in Portugal, Spain, Italy and Greece. The results show that several viable technologies for OMW treatment do exist (composting, electro-coagulation, hydrolysis-oxidation, phytoremediation, co-digestion, energy production); however, they require capital investment and maintenance costs which cannot be afforded by a small or medium olive oil mills. On the other hand, application of OMW to olive orchards can be a low-cost alternative method for OMW treatment, in regions with small size olive oil mill enterprises. Controlled annual application of OMW up to 1,500 l/tree in five equal doses during the period of the production increased significantly K content in the soil. Phenols were decomposed rapidly, while the nutritional status, the physiology and yield of olive trees is not affected by the application of OMW. The cost of soil application seems reasonable compared with sophisticated methods. Detailed study for each case to determine the application dose according to soil and climatic conditions of the area is required. The results show that several viable technologies do exist; however, the implementation cost is not negligible.

T11-O2
PARAMETERS THAT INCREASE THE CONTENT OF ETHANOL AND METHANOL DURING POST-HARVEST AND PROCESSING OF ‘ARBEQUINA’ OLIVES

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Alkyl esters content is limited in the quality standards of virgin olive oil, adopted by the European Union and the International Olive Oil Council. Short chain alcohols, mainly ethanol and methanol, are responsible for the formation of alkyl esters in olive oil. It is known that methanol is produced during fruit ripening and ethanol derives from both the fruit metabolism and sugars fermentation by microbiota. The objective of this work is to study these short chain alcohols in ‘Arbequina’ fruits during postharvest and during the virgin oil extraction process under real conditions. Olive samples were taken when delivered and at three different times during the hopper download, just before the milling operation. Concerning the centrifugation process (decanter), three different water to paste ratios were tested. Methanol and ethanol contents were measured in both the pomace and the oil at the decanter exit. Alcohols in fruits and in pomace were analysed by HS-SPME-GC-MS and by HS-GC-MS. Results show that during postharvest the content of ethanol increases significantly during the fruit storing step only for good quality fruits. No significant differences among different layers in the fruit hopper were observed with early harvest fruits. However, when ripe fruits were stored in the hopper the content of ethanol raised significantly in those damaged fruits that remain in the hopper until it is totally empty. Similar results were observed for methanol. Regarding centrifugation process, results show a significant increase of ethanol content in pomace when mass injection is lower, providing the same water injection ratio. This means that ethanol is washed from the oil during centrifugation. Concerning the water injection effect, higher ethanol levels in the pomace were observed when more water was added, specially when good quality olives were processed. In Morocco, large quantities of olive by-products (olive mill wastes and olive cake ) are produced annually. These by-products create serious problems for the environment, particularly groundwater and surface. Several biotechnological processes of olive wastes disposal including, composting, anaerobic digestion, evaporation, have been proposed but highly cost-effective in terms of operation and maintenance. However, the present study proposes a management system in which vermicomposting process could be adapted to the technical and economical requirements of the moroccan olive sector. Indeed, the optimised mixture including olive cake, nitrogen source, bulking material was moistened by OMW with different dilution rates (M1: 0%, M2: 25%, M3: 50%, M4: 75%). Vermicomposting versus combined thermophilic pre-composting and vermicomposting of the defined mixtures were compared as ways of phenolic removal and biocconversion of organic matter into vermicompost with high nutrient content. The vermicomposting parameters related to biomass gain and reproduction rate of earthworms were monitored. Similarly, C/N ratio and phenolic removal were recorded. The mixtures without OMW (M1) or with 50% of OMW (M3) proved more suitable for both vermicomposting and combined thermophilic composting - vermicomposting respectively as well as their vermicomposts were more stabilized and with higher nutrient contents than those made from other mixtures. In addition, the reduction in phenolic compounds observed in mixtures M1 and M3 caused a decrease in phytotoxicity which results in an increase in germination index (GI >80%).
ANAEROBIC CO-DIGESTION OF TWO-PHASE OLIVE MILL SOLID WASTE WITH
Chlamydomonas reinhardtii 6145 and
Chlamydomonas reinhardtii cw15: EVALUATION OF METHANE YIELDS AND PROCESS PERFORMANCES.

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In the two-phase olive oil production process, more than 800 kg of olive mill solid waste (OMSW) are generated for each ton of processed olives. Anaerobic Digestion (AD) is a good alternative for treatment of several wastes due to the excellent stabilization and high energy recovery. OMSW has high content of complex compounds and unbalanced C/N ratio, which avoids obtaining high methane yields in the AD. Co-digestion of OMSW-microalgae allows to balance C/N ratio and may improve the AD results. However, the use of microalgae as substrate in AD presents a drawback, the high resistance of the cell wall to be degraded by the anaerobic microorganisms. The aim of this study was to compare the influence of the cell wall of the microalgae Chlamydomonas reinhardtii 6145 and its mutant without cell wall (mutant cw15) in the AD with OMSW. The results showed that the cell wall was not a drawback, being able to become an advantage by producing intracellular bio-mass to be degraded slowly, providing a constant concentration of nitrogen throughout the degradation process of the substrates. The influence of the substrate composition in the mixtures OMSW-Chlamydomonas reinhardtii studied was assessed through the Transference Function model. Deviations lower than 10% between experimental and predicted methane yield values were obtained for all cases studied. The mixture 50% OMSW-50% C. reinhardtii 6145 allowed obtaining the highest methane yield (529 ± 4 mL CH4/g VS), which was 36.3% and 38.1% higher than that obtained for single OMSW and C. reinhardtii 6145, respectively. The above-mentioned substrate mixture also resulted in one of the highest maximum methane production rates (129 ± 3 mL CH4/g VS-d).

INTERNATIONAL OLIVE GROWING ANALYSIS: STRATEGIC IMPLICATIONS

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JUAN VILAR CONSULTORES ESTRATÉGICOS, S.L. CEO & FOUNDER

At present, international olive growing is located, from a productive point of view, in five continents and more specifically in 58 countries. If we stick to the scope of the consumption of its products, there are already 179 nations where olives are in demand. The expansion that this international sector is experiencing is such that in the last 5 campaigns more than 1 million hectares were planted on the planet, which amounts to a growth of almost 10%. Not only that, olive trees are an everlasting crop of great prominence on the planet, covering 11.5 million hectares of land, which is to say, somewhat more than 1 percent of arable land on the planet. All this is increasingly generating an environment subject to competition and scarcity of fresh water. The situation is aggravated mainly by the different degrees of innovation and efficiency of the different operators and economic categories that work within it, which establishes different realities of economic management.

In the present project, a detailed description of international olive growing is carried out focusing us on important areas such as surface, supply or demand. Subsequently, the different fields of activity or economic categories that perform their activity in the described environment are analyzed, and later determine what the most suitable competitive strategies are for each of the environments and economic categories of activity. The culmination of this work is a series of conclusions and final reflections.

LONG TERM FINANCIAL ASSESSMENT OFregulated DEFICIT IRRIGATION SCHEDULING IN SUPER HIGH-DENSITY OLIVE ORCHARDS

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The financial feasibility of using leaf turgor pressure (TP) and crop coefficient (CC) based irrigation scheduling in an ‘Arbequina’ super high density olive orchard under regulated deficit irrigation (RDI) has been evaluated and compared to fully irrigated trees (FI). The experiment was conducted in a commercial orchard near Seville (Spain). A 6-year dataset, covering the transition from young trees to fully productive trees, has been used. Two RDI treatments, providing 30 and 60% of the crop water needs, were used in the transition period (2010-2012), while in the fully productive period (2013-2015) two RDI treatments were scheduled providing 45% of the crop water needs, of which one used TP related measurements (30-45RDI; 60-45RDI-TP) while the other used the CC approach (30-45RDICC; 30-45RDICCC) to schedule irrigation. The Discounted Cash Flow Analysis for the entire life of the orchard provided economic indicators that demonstrated that both, 30-45RDII and 60-45RDI strategies guaranteed the profitability of SHD olive orchards in the long-term showing positive Net Present Value and Internal Rate of Return above the interest on capital. All the financial indicators suggested higher financial performance of RDITP as compared to RDICCC, but differences between the RDI strategies used in the transition period were not significant. Finally, the FI treatment showed the most profitable indicators suggesting that RDI would be justified for high water prices or reduced water irrigation allotments.

PRODUCTION INEFFECTIVENESS AND COSTS OF BEING SMALL AND FRAGMENTED: IS FARMERS’ COOPERATION A POSSIBLE SOLUTION?

SERGIO COLOMBO, ANTONIO RÚZ CARMON

IFAPA, JUNTA DE ANDALUCÍA AGRICULTURAL ECONOMICS, COOPERATIVA AGRARIA SAN ROQUE INNOVATION AND DEVELOPMENT

Olive growing is typically farmed on a familiar base on a set of small plots. In the literature it is acknowledged that this production form bears high costs and it is barely profitable thanks to the common agricultural policy subsidies and the family labour. These two income contributions are likely to decrease in the near future and alternative production forms are necessary to support smallholder agriculture. Farmers’ cooperation has been suggested as a valid option to increase the scale of production and dilute fixed costs in an attempt to generate higher margins. Nonetheless, there are no studies that quantify the production gains of cooperation. In this article, we estimate the production inefficiencies due to the lack of scale and demonstrate how by means of simple farmers’ cooperation would be possible to reduce significantly the production costs. To do that, we create specific software, (GESTOL V2.2) that allows calculating the production costs under a very diverse set of production assumptions that covers most of the typical production forms in traditional olive grove. Results indicate that significant economies of scales arise when the farming area increases. However, the savings are noteworthy when the size increases from small to medium and become insignificant from there on. The efficiency is gained from the reduction of parcel fragmentation and to the access of advanced technologies. The main conclusion is that the cooperation between a small set of farmers would significantly increase small farm profitability.
Abstracts of Oral Communications

**T12-O3**

**PUBLIC PROCUREMENT OF INNOVATIVE SOLUTIONS FOR OLIVE SECTOR: CPP INNOLIVAR**


UNIVERSIDAD DE CORDOBA, SPAIN DEPARTMENT OF RURAL ENGINEERING

The olive growing presents diverse typologies of plantations based on traditional and historical changes. The traditional, intensive or continuous canopy orchards are promoted based on different levels of research, technology and innovation. However, traditional orchards present a lack of innovations despite its wide cultivation area, social and environmental attributes. From 2014 to 2016, the Spanish Olive Oil Interprofessional (SOOI), co-funding with 80% FEDER founds, promoted a Pre-Commercial Public Procurement 'Mecaolivar' to increase the innovation applied to mechanization and pesticide application on traditional orchards. The successful results produce a second PCP 'Innolivar', from 2018 to 2021, where SOOI and Spanish Table Olive Interprofessional promoted an open accommodation ecosystem of innovation and entrepreneurship based on knowledge and aimed at improving the R+D+i Public Service through interaction with olive sector companies to increase their competitiveness, international positioning, technological capacity and profitability. Innolivar involves 8 research group of the University of Cordoba and it is structured in 12 lines and 5 thematic axes: mechanization, environmentally friendly actions, biotechnology, industry and traceability. About the mechanization, there are activities to improve the harvesting of traditional and intensive orchards with special requirements. The soil erosion, phytosanitary diffuse contamination and burning of pruning remains are focus as environmental challenges. About the industrial transformation, the olive and oil quality, process improvement, traceability and chemical panel test are topics in improve the innovation. Biotechnology results are applied to innovate on biological control of Verticillium and fruit fly together with the development of new olive varieties to high density systems.

**T12-O4**

**OLIVE OIL IS BECOMING A LUXURY**

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Olive oil is a lubricant for cooking which is desired source of local dishes. Major consumers of the olive oil are China, Japan, Russia, Brazil and India and major suppliers are Italy and Spain. There is a greater demand for olive oil from the food and beverage industry who are the major end-users of olive oil. There is a fluctuation of the olive oil prices in the global olive oil market which is influenced by the fluctuating demand and supply. The global olive oil market is about to suffer a shortage and soon it will become a luxury good. Due to the erratic weather in Italy and Spain who are the biggest producers of olive oil, the global olive oil market is rippling and it will get worse. According to the Interna- tional Olive Council in Madrid, the prices of the olive oil are increasing and in Italy, extra virgin olive oil has scored one-third increase which in Spain the increase is 10%. According to Bourne End, England-based Mintec, because of the erratic weather in past years, the prices of the olive oil are becoming more volatile and the global olive oil production is dropped about by 8%. However, the demand for olive oil is continuously increasing. For instance, China is consuming nearly $200 million worth of olive oil. With the less supply and increased demand, the prices are climbing. Worldwide consumers of the olive oil are susceptible to the impact of shortage supply and rising prices of olive oil.

**T13-O1**

**TOWARDS A BETTER UNDERSTANDING OF BRUISING DAMAGE IN THE OLIVE FRUIT**

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Bruising is the most common type of mechanical damage in the olive fruit caused by impacts during harvesting. It severely decreases the final product quality in table olives, but virgin olive oil quality may also be affected. Although commercial regulations of table olives only refer to the appearance of dark spots on the surface of the fruit, bruising also causes internal fruit damage within the mesocarp including tissue discoloration, loss of cell wall thickness and cell breakage. Besides, there is a physiological response to internal damage with an increase in the respiration rate and ethylene production. Important differences in susceptibility to bruising among cultivars are known. In an attempt to better understand olive bruising phenomenon and, particularly, to explore the reasons of this different response among genotypes, our group is currently using a methodology based on the quantification of both external and internal damage (bruise area, volume and bruising index) and internal damage in mesocarp portions and histological preparations (total damaged area, location and extension of tissue ruptures). Other fruit features that may be related to the different bruising susceptibility such as the cuticle thickness or epidermal cell dimensions in the mesocarp, among others, are being explored. This communication summarizes the main results obtained so far by our group.

**T13-K**

**FERMENTATIVE ALTERATIONS AND TEXTURAL DAMAGES IN OLIVE FRUIT DURING THE PROCESSING TREATMENTS**

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Fermentative alterations due to bacteriophages, microorganisms, killer yeasts and other spoilage microorganisms produce, in addition to chemical substances that generate bad smells and bad tastes and inhibition of natural/starter culture, also damages in the fruit texture. The occurrence of negative sensations has a negative impact on gustative and kinaesthetic attributes. The softening of the fruit is due to the action of spoilage microorganism degrading enzymes that act on pectic substances in the middle lamella and on cellulose, hemicellulose and poly saccharides in cell walls, contributing to cell separation. At microstructural level, the action of degrading enzymes causes a noticeable thinning of the normal pecto-celullo- sic thickness of the cell walls, breakage and disruption of the cell structure with negative effects on the consisten- cy of the finished product. In the olive with a tender pulp (as Ascolana tenena cv.), such alterations can be critical and may compromise the marketability of the end product. Moreover, the shape and thickness of the olive epicarp play an important role in table olive processing favoring the colonization of tissues by microorganisms, penetration of alkaline substances during debittering process, osmotic exchange of polyphenols and sugars. The ultrastructure of epicuticular waxes could be of paramount importance during fermentation, promoting the formation of biofilm, skin-adhesion and selection of the microflora.
Abstracts of Oral Communications

T13-O2

EFFECTS OF SONICATION AND OZONATION ON THE NATURAL BLACK OLIVE FERMENTATION OF GEMLIK CULTIVAR OLIVES

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Turkey is an important table olive-producing country, and natural black table olive processing is an old tradition in the country. Gemlik variety is the typical olive cultivar in the northwest part of Turkey and due to its thin peel, small pit, high flesh-to-pit ratio, aromatic taste and smooth, round shape, it is the ideal Turkish olive cultivar for preparing natural black olives in brine. In this research, ozonation and sonication processes were applied to black olives of Gemlik cultivar as debittering pre-treatments prior to fermentation. Two different brines having different NaCl (5% and 3%) and CaCl2 (1% and 3%) concentrations were selected during pre-treatment and fermentation processes. The olive brines of all trials (except control groups) were inoculated with two strains of L. plantarum and left for fermentation. Changes in pH, total acidity, reducing sugar, salt, total phenolic content and biomas were observed. Alternating aeration allowed to increase the microbial growth of the LAB strain which plays the key role in fermentation. This result was confirmed by organoleptic evaluation placing L3V at the top of evaluated samples, surpassing the industrial one where a chemical treatment was applied.

T13-O3

INVESTIGATION ON MIXED-STARTER CULTURES FOR POSSIBLE USE IN TABLE OLIVE FERMENTATION FOR ARTISAN AND INDUSTRIAL APPLICATION

MAJID MOUNIR, HAMMOUDA ALLAL, TALEB OTHMANE, JIHAD HAMMOUCHA, ISMAILI ALAOUI MUSTAPHA

Turkish olives are produced using ancient methods in the region. In the last decades, the table olive industry underwent an important change. The increasing demand for high-quality table olives and the need to extend the shelf-life of finished products, have led to the development of modern post-harvest technologies. In this context, the use of starter cultures is a promising approach for the improvement of the fermentation process. In this study, 30 olive starters were selected during pre-treatment and fermentation processes. Changes in pH, total acidity, reducing sugar, salt, total phenolic content and biomass of both AAB and LAB strains along with yeast one, were observed. Alternating aeration allowed to increase the microbial growth of the LAB strain which plays the key role in fermentation. This result was confirmed by organoleptic evaluation placing L3V at the top of evaluated samples, surpassing the industrial one where a chemical treatment was applied.

T13-O4

ARE PHENOLS ABLE TO REDUCE ACRYLAMIDE CONTENT IN CALIFORNIAN STYLE BLACK OLIVES?

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Acrylamide had already been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) some years ago. It is formed during high-temperature processing of several carbohydrate in certain foods. In the Scientific Opinion on acrylamide in food (2015), Californian-style table olives once appeared as a potential source of acrylamide with acrylamide levels comparable to other well-known acrylamide content food. The aim of the study was to reduce the acrylamide content in ripe olive through the implementation of certain actions and phenolic changes during elaboration process. The acrylamide content in oxidized black olives presented a wide variation in acrylamide concentration both in olives and brine. In fact, the 47% and the 53% of the analysed olives and brines respectively presented more than 250 mg/kg. Those companies that applied a much more aggressive sterilization heat treatment in terms of time and temperature, had highest acrylamide content in olives and brines. Furthermore, some olives varieties presented highest content than others. Different actions conducted to reducing acrylamide precursors, reduced its concentration after sterilization process. Also, the application of different phenolic extracts in canned olives produced a reduction of acrylamide formation after sterilization process in the industry. For that reason, data can be used to reduce acrylamide during elaboration process of ripe olive in the industries.

S14. Nutrition and Health

OLIVE OIL, MEDITERRANEAN DIET AND CARDIOVASCULAR DISEASE

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Olive oil is one of the key foods of the Mediterranean diet and is the main source of vegetable fat, especially monounsaturated fatty acids (MUFA). In addition, virgin extra virgin olive oils (EVOO) also contain interesting bioactive compounds such as polyphenols, phytosterols and vitamin E. Several studies have pointed out the effects of EVOO on health. In the EUROLIVE study we demonstrated that phenolic compounds in EVOO increase plasma HDL-cholesterol concentration in a dose-dependent manner. EVOO consumption reduces blood pressure, improves lipid profile and endothelial function and exhibits anti-thrombotic properties, mainly due to the anti-oxidant and anti-inflammatory properties of polyphenols. However, these protective effects of EVOO on cardiovascular disease (CVD) are even greater when it is included in a healthy dietary pattern. In the PREDIMED (Prevenión con Dieta Mediterránea) trial, that included 7,447 high-risk participants who were follow-up a mean of 4.8 years, we observed that those who consume a traditional Mediterranean diet plenty of EVOO showed a significant reduction of cardiovascular events (myocardial infarction, stroke or cardiovascular death), a lower overall mortality and a diminishing incidence of diabetes mellitus, atrial fibrillation, cognitive decline and some types of neoplasms (i.e. breast cancer).

More recent studies have evaluated additional mechanisms by which EVOO and Mediterranean diet exert these protective effects on CVD. In fact, EVOO has the capacity to modulate gene expression related CVD and also improves anti-inflammatory properties of polyphenols. Olive oil is one of the key foods of the Mediterranean diet and is the main source of vegetable fat, especially monounsaturated fatty acids (MUFA). In addition, virgin extra virgin olive oils (EVOO) also contain interesting bioactive compounds such as polyphenols, phytosterols and vitamin E. Several studies have pointed out the effects of EVOO on health. In the EUROLIVE study we demonstrated that phenolic compounds in EVOO increase plasma HDL-cholesterol concentration in a dose-dependent manner. EVOO consumption reduces blood pressure, improves lipid profile and endothelial function and exhibits anti-thrombotic properties, mainly due to the anti-oxidant and anti-inflammatory properties of polyphenols. However, these protective effects of EVOO on cardiovascular disease (CVD) are even greater when it is included in a healthy dietary pattern. In the PREDIMED (Prevenión con Dieta Mediterránea) trial, that included 7,447 high-risk participants who were follow-up a mean of 4.8 years, we observed that those who consume a traditional Mediterranean diet plenty of EVOO showed a significant reduction of cardiovascular events (myocardial infarction, stroke or cardiovascular death), a lower overall mortality and a diminished incidence of diabetes mellitus, atrial fibrillation, cognitive decline and some types of neoplasms (i.e. breast cancer).

In conclusion, EVOO consumption have a high protective effect on CVD throughout multiple mechanisms, but these effects are even greater when EVOO is included in a healthy dietary pattern such as Mediterranean diet.
T14-O1

EFFECT OF DIETARY FATTY ACIDS ON BONE MARROW NEUTROPHILS LIPID ACCUMULATION

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INSTITUTO DE LA GRASA (CSIC) ALIMENTACIÓN Y SALUD

Scope: Postprandial triglyceride-rich lipoproteins (TRLs) promote atherosclerosis. Recent research points the bone marrow (BM) as a primary site in atherosclerosis. We elucidated how the acute administration of monounsaturated fatty acids (MUFAs) enhances triglyceride (TG) accumulation in murine BM neutrophil lipids and functionality.

Methods and results: Postprandial hypertriglyceridemia increased inflammatory mediators such as IL-1β and TNFα. BM neutrophils and macrophages responded to TRLs with antioxidant down-regulation, keeping NO concentration at the control level. Our results suggest a protective effect against oxidative stress and inflammation associated with numerous diseases.

Conclusion: Postprandial hypertriglyceridemia increased neutrophil oxidative burst and inflammation and may serve as a potential therapeutic target for atherosclerosis.

T14-O2

ASSESSMENT OF THE ANTI-INFLAMMATORY POTENTIAL OF PURIFIED POLYPHENOLS FROM EVOO IN INDUCED PANCREATIC CELLS CULTURED IN VITRO

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ESTACIÓN EXPERIMENTAL DEL ZAIDÍN. CSIC PLANT REPRODUCTIVE LABORATORY. DEPARTMENT OF BIOCHEMISTRY. CELL AND MOLECULAR BIOLOGY OF PLANTS

Fatty acids, vitamins and polyphenols of EVOO are considered key components for the healthy properties of this oil, as a part of the Mediterranean diet. Polyphenols are believed to reduce and/or slow down the excessive formation of reactive oxygen species and inflammation associated with numerous diseases. Our study was aimed to evaluate the ability of six polyphenols present in olive oil such as eleanolic acid, hydroxytyrosol, oleacenic, oleocanthal, oleuropein and tyrosol to counteract the anti-inflammatory effects induced in vitro on a pancreatic cell culture line. Pancreatic cells were treated with different inflammatory stimuli, and then challenged with the purified polyphenols, which were assessed in their protective actions. The treatments with the inflammatory inducers over 24h significantly altered the inflammatory status of PANC-1 cells, leading to an increase of NO release (due to the induction of iNOS), as well as changes in the production of tumor necrosis factor-α, Interleukin-1β, -2, -6, -8, -12p40, -27 and interferon-γ. However, pretreatment with the six different polyphenols assayed, significantly counteracted these inflammatory stimulus effects at different degrees. These six different polyphenols also promoted iNOS down-regulation, keeping NO concentration at the control level. Our results suggest a protective effect at pancreatic level of extra virgin olive oil polyphenols, able to prevent or limit the onset and progression of chronic inflammation disease.

This work was supported by ERDF co-financed grants RTC-2016-4824-2 and BFU-2016-77243 from MINECO, and 201504065 (CSIC).

T14-O3

ACYLATED DERIVATIVES OF OLEUROPEIN REDUCE LPS-INDUCED INFLAMMATORY RESPONSE IN MURINE PERITONEAL MACROPHAGES

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Experimental and epidemiological data have proved the beneficial effect provided by olive derivatives. In this regard, particular attention has been drawn to oleuropein (OL), the main phenolic component of olive leaves and unprocessed olive drupes, which is hydrolyzed during fruit maturation and forms different products, including hydroxytyrosol. OL has been reported as an antioxidant, with anti-thrombic and antiatherogenic, hepato-protective, anti-microbial and anti-cancer activities. Moreover, OL has also been identified as a molecule with a potential utility against neurodegenerative diseases, including Parkinson and Alzheimer. OL has also shown anti-inflammatory effects, and has decreased the severity on dextran sodium sulphate-induced acute colitis. Nevertheless, its bioavailability is low because it is fast metabolized into more polar compounds, mainly hydroxytyrosol.

We have investigated the potential anti-inflammatory activity of three acyl derivatives from OL in an ex vivo model of murine peritoneal macrophages stimulated with LPS compared with OL, and clarified the potential molecular mechanisms involved. The mono and tetra-O-acyl derivatives were obtained by using lipases, and the hexa-O-acyl derivative was obtained by chemical synthesis.

Isolated murine peritoneal macrophages were treated with OL or its derivatives in the presence or absence of LPS. OL derivatives reduced significantly COX-2 and iNOS overexpression, showing better results than the observed in the natural compound.

We could conclude that the new OL derivatives exhibited better anti-inflammatory effects that OL; that seems to be related to the increased lipophility in comparison to OL. Therefore, these new synthetic derivatives may constitute an interesting alternative in the management of inflammatory-related pathologies.

T14-O4

EFFECT OF MODIFIED OLIVE PECTIN ON PROLIFERATION OF BLADDER CANCER CELLS

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INSTITUTO DE LA GRASA - CSIC FOOD PHYTOCHEMISTRY

The alperujo, a byproduct of the continuous extraction process in two phases from the olive oil industry, is being treated to recover the maximum amount of oil and the bioactive compounds.

Low molecular weight pectins have been isolated by direct steam thermal treatment from alperujo. The term “modified pectin” (MP) includes pectins with different composition and common characteristics. They are fragments of low PM that in theory can be absorbed in the small intestine, some of which can bind and block the pro-metastatic protein galectin-3 (Gal3). Gal3 is a multi-faceted protein whose expression is altered in the processes of cancer, inflammation, fibrosis, heart disease and heart attacks.

The anticancer effect of modified pectins obtained from olives, their comparison with PectaSol-C, a commercial modified citrus pectin (MCP) and known Gal3 inhibitor was tested in vitro in four bladder cancer cells lines. The olive pectin showed an important antiproliferative effect in bladder cancer cells, similar to cisplatin in SCaBER cells, and higher than MCP in all cell lines tested.

These modified pectins of olives emerge as a promising anti-cancer compound and very useful in combination with conventional anticancer compounds.
The objective of this study was to investigate the effect of shoot girdling on the rooting potential of ‘Kalamata’ olive leafy cuttings. The experiments were performed on ten mature olive trees grown in the orchard of the Agricultural University of Athens. Girdling was applied at the base of 30-1 year old shoots per tree in late March and late September. Sub-samples of cuttings were taken for analysis of free polyamines and total phenolic compounds. The percentage of rooted cuttings was recorded three months later. Subsamples of cuttings were taken for analysis of free polyamines and total phenolic compounds before planting. Girdling significantly increased rooting of ‘Kalamata’ cuttings (16% in autumn and 11% in spring) compared to control ones (3% and 2%, respectively). Cuttings from girdled shoots exhibited higher free polyamine concentration than cuttings from non-girdled shoots in both seasons, with the exception of spermine in spring. Furthermore, girdling increased concentrations of total phenols, o-diphenols, flavonoids and flavanols in cuttings in autumn and spring. In conclusion, increased rooting ability of girdled ‘Kalamata’ cuttings appears to be associated with an increase in free polyamine and total phenolics.
PRELIMINARY RESULTS ON FLOWERING PHENOLOGY OF THE MEDITERRANEAN DIVERSITY OF CULTIVATED OLIVE IN EX-SITU COLLECTION OWGB MARRAKECH

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A set of 591 olive accessions from the world olive collection of Marrakech was characterized in 2014, 2015 and 2016 for flowering phenology traits according to the BBCH scale (the time of inflorescence buds start to swell, flower opening, full flowering, full petal fall, end of flowering, fruit set). Degree days of flowering stages 51 to 69 were higher in 2015 compared to 2014. The highest flowering period was observed in 2016. A significant positive correlation was detected between all flowering stages except for stage 51, which is negatively correlated with flowering period estimated by the difference between the time of stage 69 and stage 61. The phenological stage 51 seems to be key for identification purposes and for an efficient management of the olive germplasm. Nowadays this Germplasm Collection which belongs to a multi-disciplinary and multi-institutional coordinated project, counts 900 accessions from 26 countries most of them from Mediterranean Basin. The identification of the olive cultivars maintained in this collection is an important ongoing task which has been carried out by both morphological and different molecular markers. In this sense, a set of 1043 EST-SNP markers has been successfully used for identification and variability studies in the mentioned collection. In the present study, we report the selection of a sub-set of 96 EST-SNPs for an efficient, high-throughput and economic identification of olive cultivars. This set was selected based on their discrimination capacity and amplification accuracy. And its use has revealed synonymy and homomycy cases, mislabelling and propagation errors in the collection. At the same time, the subset of EST-SNPs has made possible the identification of some new recently introduced accessions before their introduction in the field. Besides, high levels of diversity in the olive germplasm have been evidenced. The preliminary results obtained showed that the set of selected EST-SNP markers was as efficient as SSRs and DAIRs for identification purposes and for an efficient management of the collection.

USE OF EST-SNP MARKERS AS A MEANS OF CULTIVAR IDENTIFICATION AND GERMPLASM MANAGEMENT OF THE WORLD OLIVE COLLECTION OF IFAPA

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The World Olive Germplasm Collection of Córdoba, Spain which was established more than 45 years ago at the ‘Ala-medada del Obispo’ Centre of IFAPA, represented the first international attempt of conservation and management of the olive germplasm. Nowadays this Germplasm Collection which belongs to a multi-disciplinary and multi-institutional coordinated project, counts 900 accessions from 26 countries most of them from Mediterranean Basin. The identification of the olive cultivars maintained in this collection is an important ongoing task which has been carried out by both morphological and different molecular markers. In this sense, a set of 1043 EST-SNP markers has been successfully used for identification and variability studies in the mentioned collection. In the present study, we report the selection of a sub-set of 96 EST-SNPs for an efficient, high-throughput and economic identification of olive cultivars. This set was selected based on their discrimination capacity and amplification accuracy. And its use has revealed synonymy and homomycy cases, mislabelling and propagation errors in the collection. At the same time, the subset of EST-SNPs has made possible the identification of some new recently introduced accessions before their introduction in the field. Besides, high levels of diversity in the olive germplasm have been evidenced. The preliminary results obtained showed that the set of selected EST-SNP markers was as efficient as SSRs and DAIRs for identification purposes and for an efficient management of the collection.

OLIVE BREEDING TRIALS 3D CHARACTERIZATION USING UAV ACQUIRED PHOTOGRAMMETRIC POINT CLOUDS

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The geometric features of agricultural trees such as canopy area, tree height and crown volume provide useful information about plantation status and crop production. However, these variables are mostly estimated after a time-consuming and hard field work, and applying equations that treat the trees as geometric solids, which usually produce inconsistent results. To overcome these limitations, this work presents an innovative procedure for computing the 3-dimensional geometric features of trees in orchards by applying two consecutive phases: 1) generation of photogrammetric point clouds with Unmanned Aerial Vehicle (UAV) technology, and 2) analysis of the point clouds using object-based image analysis techniques (OBIA). For testing this approach, an UAV with a RGB camera attached was flown over an olive breeding trial for computing the 3-dimensional geometric features of trees in orchards by applying two consecutive phases: 1) generation of photogrammetric point clouds with Unmanned Aerial Vehicle (UAV) technology, and 2) analysis of the point clouds using object-based image analysis techniques (OBIA). For testing this approach, an UAV with a RGB camera attached was flown over an olive breeding trial under a super-intensive planting pattern. The flight plan was programmed for acquiring images with high percentage of overlap, which was necessary for the generation of complete and accurate point clouds. The OBIA algorithm developed in this research achieved successful results, reporting over 90% accuracy on tree limits delineation and minimal deviations compared to in-field estimations of tree heights. The information derived from the OBIA algorithm can be used for generating maps which would be useful to understand the linkages between tree growth and field-related factors according to every olive genotype or variety or also to optimize crop management operations in the context of precision agriculture with relevant agro-environmental implications.
PRELIMINARY AGE ESTIMATION OF SOME ANCIENT OLIVE TREES IN SOUTH LEBANON

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Lebanon has a long history of olive tree culture (Olea europaea L.) dating back to the old antiquity. Ancient olives continue to thrive across the country, but there is yet no scientific study about their age. However, dating of these trees is important to have an idea on the approximate era of plantation and to understand their adaptation to the hosting environment and to the changing climatic conditions through time. In this study, we established an age estimation for some ancient olives growing in four ancient groves in South Lebanon using three different algorithms based on the trunk circumference at soil level, 1.0 m and 1.3 m respectively above soil level. Results showed that the first algorithm, based on trunk circumference at soil level, gave ages between 700 and 1249 years with 7 trees above 1000 years. The second algorithm, based on trunk circumference at 1.0 m, gave lower ages comprised between 366 and 611 years. Finally, the third algorithm based on trunk circumference at 1.3 m, estimated ages between 348 and 553 years. Although preliminary, these results indicate that our olive trees could be as old as the ones reported in other countries in the Mediterranean and even older. Nevertheless, this age estimation should be extended to a large array of olive trees growing across the country for a better understanding of the era of plantation in parallel to further actions of evaluation and preservation of this valuable gene pool.

ENRICHMENT OF THE WORLD OLIVE GERMLASM COLLECTION OF IFAPA-CORDOBA BY MEANS OF NEW PROSPECTING SURVEYS IN ANDALUSIA.

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IFAPA, JUNTA DE ANDALUCIA AGRIFOOD ENGINEERING AND BIOTECHNOLOGY

The study of olive (Olea europaea L.) genetic resources is crucial for conservation and breeding purposes. In Andalusia, southern Spain, as in the rest of Mediterranean olive growing regions, the genetic pool of the olive tree includes cultivated and wild forms. In this sense, thanks to a multi-disciplinary and multi-institutional coordinated work and counting more than 900 accessions from 28 countries, the World Olive Germplasm Collection (WOGC) of IFAPA-Cordoba plays an important role at national and international level. Despite the high diversity maintained at this collection, recent studies have proved the presence of unknown cultivars in restricted areas of Andalusia. Likewise, the survey, evaluation and conservation of wild olive tree populations could be helpful for future olive-breeding programs. The present study describes the preliminary results of a new survey conducted throughout Andalusia aiming to enrich and broaden the WOGC with new and unknown cultivated and wild material. Thus, a total of 110 trees (95 cultivated and 15 wilds) were sampled from diverse sites covering all Andalusian provinces. Each tree was geo-referenced and plant material collected for vegetative propagation as well as for morphological and molecular description. A sub-set of 96 EST-SNPs, recently developed, was used for cultivar identification and diversity studies. The preliminary identification results have shown that some of the prospected trees belong to unknown and uncatalogued cultivars, enriching thus the WOGC IFAPA-Cordoba with new and unexploited diversity. Besides, these surveys will help to increase the representativeness of Andalusian wild olive trees into the wild ex-situ collection at IFAPA-Cordoba.

SELECTION OF NEW OLIVE GENOTYPES RESISTENT TO VERTICILLIUM WILT IN COMPARATIVE FIELD TRIALS

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The olive breeding program developed at IFAPA (Cordoba, Spain) aims to obtain new cultivars resistant to verticillium wilt (VW), a disease caused by the soil-borne fungal pathogen Verticillium dahliae Kleb. As a result of previous works by artificial inoculation of the fungus in growth chamber under controlled environmental conditions, 20 genotypes from open pollination progeny of different cultivars and crosses including cultivars of known resistance level ('Changlot Real'x'Dolce Agogia', 'Frantoio'x'Arbosana' and 'Koroneiki') were selected for further evaluation. Selected genotypes were planted in four infested fields under different environmental conditions to confirm their resistant level under natural conditions. The same genotypes were also planted in a microplot assay with artificial inoculation of the fungus to identify genotypes with different resistance levels. In all experiments, 'Picual' were used as a susceptible reference control, and 'Frantoio' as resistant control. In the different experiments allowed to identify genotypes that behave as resistant in all cases, improving even the resistant cultivar control.

This research was financially supported by IFAPA project AVA201601.2, partially funded by European Regional Development Fund (ERDF). Alicia Serrano is grateful for the funding received from the Researcher Training Program in INIA-Autonomous Communities Research Centers (FPI-INIA). Also thank the farmers for their cooperation.

BIOMETRIC AND BIOCHEMICAL CHARACTERS OF FRUITS OF OLEASTER (OLEA EUROPEA L. VAR. SYLVESTRIS) CLONES

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Twenty years ago, in the framework of the research program aimed to the study of the oleaster (Olea europaea var. sylvestris) genetic resources, some clones have been selected from wild populations and agamically propagated in a repository at Oristano (Central Western Sardinia, Italy). This collection is submitted to experimental observation in order to find valuable characters useful for the breeding of the cultivated olive. In particular, oleaster fruits have been harvested and observed for biometric characteristics and biochemical composition of pulp using fruits of the oleaster cultivar 'Bosana' as comparison. In the years 2012 and 2013, olives of this cultivar showed mean weights of 2.04 and 2.54 g and pulp/pit ratio of 2.38 and 2.77, while values recorded for oleaster clones ranged from 0.30 to 0.65 g and 0.59 to 1.38 respectively. Pulp acidity was comprised between 0.67 to 2.2% for oleaster clones. Oil content of the 'Bosana' pulp ranged from 14 and 18%, while for oleaster from 1.5 to 6.67%. 'Bosana' olives showed maximum polyphenols content of 30685 mg/kg of gallic acid and of anthocyanins of 4300 mg/kg of malvine, while contents of polyphenols of 32131 mg/kg of gallic acid of anthocyanins of 12376 mg/kg of malvine were observed in oleaster clones. The 'Bosana' olives have a quite good content of polyphenols in the oil and in the different experiments allowed to identify genotypes that have a resistant level under natural conditions. The same genotypes were also planted in a microplot assay with artificially inoculated soil by a highly virulent isolate of the defoliant pathotype. In all experiments, 'Picual' were used as a susceptible reference control, and 'Frantoio', as resistant control. In field trials, molecular characterization of the fungus allowed the identification of three different molecular patterns, specific defoliant, specific non-defoliant and unspecific of pathotype by 73%, 20% and 7%, respectively. Although the disease incidence index was highly variable between experimental approaches, from 55.8% in the microplot assay to 23.9%, 14.8%, 6.8% and 2.7% in each field trial, the symptoms developed in the evaluated material and in the different experiments allowed to identify genotypes that behave as resistant in all cases, improving even the resistant cultivar control.
HOW MOLECULAR MARKERS CAN ELUCIDATE THE ORIGIN OF OLIVE CULTIVARS: THE CASE STUDY OF OLIVE FRENCH GERMPLASM

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NATIONAL INSTITUT OF AGROMOMY RESEARCH AMELIORATION GENETIQUE ET ADAPTATION DES PLANTES TROPICALES ET MEDITERRANEENNES

Molecular characterization brings powerful tools to elucidate the origin of varieties, manage and preserve local genetic resources. Here we used twenty microsatellites to characterize the French local olive germplasm in ex-situ collection at the island of Porquerolles, including 113 accessions (FOGB) and to elucidate their origins. Compared to Mediterranean olive varieties from the Worldwide Olive Germplasm Bank of Marrakech, Morocco (WOGB), we observed a high genetic diversity within the local French varieties and a high admixture level, with an almost equal contribution from the three Mediterranean gene pools as evidenced by previous investigations. We obtained similar or close genotypes between French and Italian varieties and a high proportion of parent-offspring relationships among French local varieties and between French and some Mediterranean varieties, especially those from Italy and Spain. Our investigations suggest that French olive germplasm may result from diffusion of material from multiple origins and diversification events with early generations of hybrids between introduced varieties. Subsets of 22, 43, and 75 French varieties were proposed which could be optimal for conservation, diffusion to nurseries and even to conduct further admixture mapping studies as a complement to both association and family-based mapping.

TOWARDS THE AUTHENTICATION OF OLIVE WORLD GERMPLASM BANKS OF MARRAKECH (MOROCCO) AND CORDOBA (SPAIN)

AHMED EL BAKKALI, LAILA ESSALOH, ABDELMAJID MOUKHLI, HAYAT ZAHER, LHASSANE SIKAOU, CHRISTINE TOLLON, ABDEBRAHMAN MEKKAOU, AMAL HADDIDOU, DIEGO BARRANCO, CONCEPCION M. DIEZ, LUIS RALLO, ISABEL TRUJILLO, BOUCHAIB KHADARI

NATIONAL INSTITUT OF AGROMOMY RESEARCH AMELIORATION DES PLANTES ET CONSERVATION DES RESSOURCES GENETIQUES

Olive (Olea europaea L.) is one of the most iconic and important fruit trees in the Mediterranean basin. Ex-situ conservation is essential for the conservation and efficient management of olive genetic resources. Today, two worldwide collections, Olive World Germplasm Banks (OWGBs) of Cordoba, Spain and Marrakech, Morocco are established within the framework of International Olive Council (IOOC) and the third one is under establishment at Izmır, Turkey. Characterization, identification, comparison and authentication of the cultivars included in both collections is a compulsory step to: a) avoid confusion between accessions denominated and true-to-type cultivars; b) guarantee olive germplasm preservation and c) allow the efficient use and exchange of olive genetic resources within the scientific community. Here, we used 20 microsatellites markers (SSR) and 11 morphological traits of the endocarp characters of leaflets, to perform genetic diversity analyses and Structure analyses detected groups in Italian olive germplasm collections (Córdoba and Marrakech) and different regions of Spain, continental Africa and the Macaronesian archipelago. The genetic variability of the collection was addressed with nuclear and plastidial markers. Early vigour parameters were studied in 103 genotypes of 13-14 months old belonging to all the subspecies. Different anatomical traits regarding root, shoot and stem growth, branching patterns and internodal elongation were quantified. A wide variability in vigour parameters was observed, including genotypes from very low to very high vigour. Future works will be carried out to determine the capacity of selected genotypes used as rootstocks to reduce the vigour of different olive cultivars.

GENETIC CHARACTERIZATION OF APULIAN OLIVE GERMPLASM AS POTENTIAL SOURCE OF RESISTANCE TO XYLELLA FASTIDIOSA

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Olive is an important agricultural crop for southern Italy and, indeed, it represents the history, culture and identity of the territories. Recently, a new olive disease termed olive quick decline syndrome, associated to Xylella fastidiosa subspecies pauca, is causing major devastation in the olive groves of the Salento peninsula. The most widespread olive cultivars in this area are Ogliarola and Cellina di Nardò, that show high susceptibility to the disease. The current unavailability of effective treatments to cure Xylella-infected trees, poses major challenges for economic sustainability of olive crops in infected areas. Search for genetic traits of resistance among the vast number of olive cultivars and wild genotypes, may represent one of the most effective and long term strategies to control the impact of this devastating disease. Surveys in the infected area allowed the identification of some local hitherto undescribed cultivars and wild olives showing interesting phenotypic behavior. Fourteen preselected nuclear microsatellite markers were used to assess the genotypic profiles of these plants, and their relationships with local and national olive cultivars. The combination of the SSRs allowed to identify different unique genetic profiles, and to speculate about their potential parents. The Pco Analysis highlighted a divergence of the Apulian genotypes from the Italian clade, and a sub-clustering according to the region of origin. Clustering and Structure analyses detected groups in Italian olive germplasm and gene flows among identified clusters.
THE REGEROP PROJECT: AN INTEGRATED DATABASE FOR THE RECOVERY OF ANCIENT AND RARE OLIVE GERMPLASM
ALESSANDRO PETRONTINO1, FRANCESCO BOZZO2, DANIELA PANIO1, VINCENTO FUCILLI1, MONICA MARILENA MIAZZI1, STEFANIA GIRENO1, CINZIA MONTEMURRO3
1 SINAGRI SPIN OFF OF UNIVERSITY OF BARI DISAAT, UNIVERSITY OF BARI DISAAT
Within the project “Recovery of the Apulian Olive Germplasm” Re.Ger.O.P. many activities have been carried out to recover ancient olive varieties, cultivated by a few farmers who have become the only “guardians” of a huge patrimony of agrobiodiversity in Apulia region – Italy.
Given the great amount of information and the complexity of the interactions among different disciplines involved in the work of investigation, selection, recognition, rehabilitation and multiplication of the accessions found, we created a database that allows the complete traceability of the collected materials, the univocal recognition of the information and the geographic position of the researched olive plants.
At the heart of the database there is a geographic information system to which information has been connected through “join” and “link” operations. The analyses linked to the database concerned morphological, molecular, sanitary and technological characteristics. Were carried out in different years of the project, in order to verify the variability of some attributes, specially morphological and technological, in relation to climatic trends.
A huge photographic documentation has also merged into the database: geotagged photographs of plants and surrounding landscapes and 3D photographs for the remote recognition of characters like stem, leaves, flowers and fruits.
Globally, 269 rare olive tree accessions were included in the database, each accompanied by characterization analysis and files for photographic and information storage. This will allow to select, and display the plants on the basis of their own attributes and geographical criteria in order to put order and finally clarify cases of homonymy and synonymy.

PROMISING FEATURES OF ANCIENT AUTOCHTHONOUS OLIVE TREES IN THE EASTERN CENTRAL TUNISIA: TABLE, DUAL PURPOSE AND OIL OLIVES
IBITSEM LAARIBI1, HASSOUNA GOUTA1, MOHAMED AYADIL, KHOULOUD ANNABI2, IMEN ZOUARI1, FOUELED LABIDI1, MOUNA AICHI MEZGHANI1
1 INSTITUT DE L’OLIVIER LES Ressources GENETIQUES DE L’OLIVIER: CARACTERISATION, VALORISATION ET PROTECTION PHYTOSANITAIRE
For several years great effort has been devoted to the enhancement and the conservation of Tunisian oleicultural heritage. However, studies on olive diversity and characterization of minor cultivars with restricted distribution in the Tunisian Sahel are still lacking. Moreover, the survival of local ancient olive germplasm in this territory is currently highly threatened by extinction on account of the emergence of modern olive growing systems and urbanization.
The present study is reported on physicochemical and morphological features of 100 olive accessions sampled along the Eastern coast in Sousse and resulted of five years of prospection on the most old olive groves and traditional small farms. Screened olive trees allowed the selection of 7 table olives, 14 oil olives and 11 dual purpose accessions. Table olives presented big fruit size (5-8g), high pulp to stone ratio (6-9), fleshy and firm textured pulp and nonadherent stone. Olive oil and dual purpose accessions have a high yield of oil of exceptional quality compared to the dominant variety ‘Chemlali Sfax’ in the Sahel olive area. Their oils contain high oleic (>70%) and low palmitic and linolenic acid contents. Moreover, their oils mostly showed significant levels of functional micro-components, such as polyphenols, tocopherols, carotenoids and chlorophylls. New selected table and oil olives can be employed to re-new the Tunisian old olive groves in order to adapt varied varieties with superior attributes. Further molecular studies will be complementary to confirm the originality of new olive accessions and their relationship with classic Tunisian cultivars.

OBSERVATIONS ON OLIVE POLLEN: MORPHOLOGICAL VARIABILITY AND RELATIONSHIP WITH LEAF, FRUIT, STONE AND INFLORESCENCE TRAITS
IBITSEM LAARIBI1, HASSOUNA GOUTA1, KHOULOUD ANNABI2, IMEN ZOUARI1, CRISTIANA GIORDANO1, MARIA CRISTINA SALVATI1, FOUELED LABIDI1, MOUNA AICHI MEZGHANI1
1 INSTITUT DE L’OLIVIER LES Ressources GENETIQUES DE L’OLIVIER: CARACTERISATION, VALORISATION ET PROTECTION PHYTOSANITAIRE
Numerous techniques are available to study the genetic characteristics of olive germplasm. Studies which investigate pollen diversity for identification and evaluation of genetic relationships among cultivars were scarcely reported. As further contribution to these studies, this research provides pollen data for 61 old olive accessions, belonging to the Central Eastern part of Tunisia, in order to discriminate main pollen types and their relationship with primary morphological olive descriptors of fruit, seed, leaf and inflorescence. Pollen morphology and ultrastructure were carried out using scanning electron microscopy.
Morphological parameters considered were polar axis (P), equatorial diameter (E), index (SI), P/E ratio, maximum distance between colpi, size and form of lumina and muri thickness. Olive pollen grains were small to large in size (P = 21.80–29.88 μm, E = 14.47–21.14 μm, SI = 3.3–5.95), elliptical-prolate to subprolate in shape (P/E = 1.21–1.72) and tri-colpate. Observed variability on the Exine pattern ornamentation based on lumina profile and regularity and muri thickness proved a specific pollen profile for each olive accession. Low correlations were recognized between pollen morphometric pollen features and those related to fruit and seed. Such results suggested the potentiality of pollen characteristics to improve and complete the information and seed morphological characters, pulp and oil composition, and oil sensory properties were the most studied in the new selections.
Olive yield per plant ranged from 7 to 20.4 kg. Fruit weight varied between 2.08 and 8.3 g. Most of selections showed a little stone with a pulp/pit ratio always over 0.6. The oil content was comprised between 15.5 and 25.7% and always classified as extra-virgin (acidity lower than 0.8% and number of peroxides under 20) in a selected population of 19 hybrids. Sensory analysis of oils revealed fruity, spicy, bitter and herbaceous as the main perceptions.
New data are expected by new experimental plots dedicated to the most interesting selections.

FRUIT POMOLOGICAL CHARACTERS OF OLIVE NEW HYBRIDS FROM CROSS BREEDING
PIERGIORGIO SEDDA1, MARTINO MUNTONI1, GIULIUDI PIL1, FEDERICO CORDA1, CARLO MORO1, ROBERTO ZURRI1, LEONARDA DESSENA2, MAURIZIO MULAS3
1 AGRIS SARDEGNA - REGIONAL AGENCY FOR RESEARCH IN AGRICULTURE RICERCA NELLE FILIERE OLIVICOLA-OLIARIA E VITIVINOLICIA
Since 1991 AGRIS-Sardegna started a cross breeding program aimed to create new olive selections using as parents the main cultivars of Sardinia germplasm. Some tens of hybrids were obtained and are still under evaluation for phenotypic characters and fruit technological quality. AGRIS-Sardegna and the University of Sassari collaborate with other studies to develop olive genetic resources in Sardinia and this is a part of the program. Cultivars used for the cross breeding were ‘Tonda di Cagliari’, ‘Nera di Gonnos’, ‘Semidana’, ‘Tonda di Villacidro’, ‘Pizze Carroga’, ‘Tonda sasarese’ and many others as well as some allochthonous cultivars like ‘Nocellara del Belice’ and ‘Picchione’. Seedlings were planted in an experimental plot from 1994 to 2000 in the experimental station of Villa sor (South Sardinia, Italy). Plant size and shape, yield, fruit and seed morphological characters, pulp and oil composition, and oil sensory properties were the most studied in the new selections.
Olive yield per plant ranged from 7 to 20.4 kg. Fruit weight varied between 2.08 and 8.3 g. Most of selections showed a little stone with a pulp/pit ratio always over 0.6. The oil content was comprised between 15.5 and 25.7% and always classified as extra-virgin (acidity lower than 0.8% and number of peroxides under 20) in a selected population of 19 hybrids. Sensory analysis of oils revealed fruity, spicy, bitter and herbaceous as the main perceptions. New data are expected by new experimental plots dedicated to the most interesting selections.
Abstracts of Poster Communications

**T01-P21**
**ASSESSMENT OF OLIVE ACCESSIONS NEWLY IDENTIFIED IN CENTRAL EASTERN PART OF TUNISIA PROVIDE EVIDENCE FOR A LARGE DIVERSITY**

HASSOUNA GOUTA, IBTISSEM LAABIDI, MOUINA AYACHI, MOHAMED AYAD, KHALID ANNABI, IMEN ZOUARI, AMEL LACHKAR, FOUD LAABIDI

Olive tree institute genetic resources and breeding

Cultivation of olive trees in Tunisia dates back to roman period. The long history of olive farming associated with the diverse pedoclimatic conditions from north to south of the country have allowed the establishment of a quite rich olive germplasm. Characterization and identification of local cultivars were previously focused on the northern, central western and southern parts of the country. However, the coastal part and especially the region of ‘Sahel’, has been rarely studied and even neglected although it presents the oldest historical area of olive biodiversity and probably its origin. Furthermore, the rapid and uncontrolled urbanization is spreading out at the expenses of important number of old olive groves with the subsequent high risks of germplasm erosion. In order to evaluate and exploit olive diversity in this region, a national project RESGEN-OLMAAB was enhanced. Consequently, a prospecting effort was carried out in many ancient and historical olive groves spread in three governorates: Sousse, Monastir and Medeha. As for now, we carried out morphological and biochemical characterization of more than 200 olive accessions selected for their interesting fruit and oil characteristics. A preliminary genotyping with molecular markers (RAPD and SSR) was also established in order to report relationships with classic olive cultivars. Results have proved a large diversity and identified newly olive clones either for oil extraction, as female genitor yielded a high percentage of genotypes adapted to SHD system.
Vegetative propagation by semi-hardwood cuttings has been used since centuries to multiply agronomical important olive cultivars. However, adventitious root formation (ARF) is a limiting step for the propagation of some recalcitrant cultivars. ARF depends on the capacity of a group of cells, located at the cutting basal region, to reprogram and develop a new organ – adventitious roots. Although morphological changes happen at the basal region of the cutting, the plant reacts as a whole, and after receiving the cell reprogramming stimulus, signals are translocated from the basal region to leaves, where different metabolic pathways are activated. Therefore, in this study leaves were considered as a target material to monitor the efficiency of ARF by near infrared spectroscopy in combination with calorimetry. We hypothesized that as Fourier Transform Near Infrared (FT-NIR) spectrometers can detect with high accuracy any molecule in which the principal chemical bonds are CH, OH, NH, SH or C = O, and as during ARF new molecules are activated, therefore, in this study leaves were considered as a target material to monitor the efficiency of ARF by near infrared spectroscopy in combination with calorimetry. The conservation of cultivated plants in ex-situ collections is very important for the preservation of diversity and a key step for its characterization, evaluation and exploitation for breeding purposes. For the olive tree, the World Olive Germplasm Bank of CREA-OFAG (WOGB CREA-OFAG), from Mirto Crossia, Calabria region, southern Italy, is currently the largest olive germplasm bank with more than 443 olive varieties from 11 Mediterranean countries. Olive breeding has traditionally been focused on the improvement of agronomic traits. However, recently also the nutritional quality of virgin olive oil (VOO) is considered as a breeding target because of the increasing weight of scientific evidence supporting the positive impact of extra virgin olive oil (EVOO) on human health. In the present study, a set of 188 Italian olive varieties of WOGB CREA-OFAG was described from the morpho-bio-agronomical viewpoint, single cultivar olive oil extracted and analysed to assess sensory properties and fatty acids methyl esters composition. Analogously, the corresponding genotypes were screened with 8 simple sequence repeats (SSRs) markers to correctly and thoroughly identify all genotypes. This study generated a morpho-bio-agronomical-nutritional and molecular database that may be used to optimise a strategy for the management of olive genetic resources and their use for subsequent genetic and genomic olive breeding.

Olive growing in Lebanon has an important role at both the social and economic level. Despite this, little research activities have been attributed to the olive oil quality produced in Lebanon. In this study, oil content, fatty acid and phenolic profiles of eleven olive varieties were studied along four different ripening stages. Oil content traits were determined by Soxhlet method and Abencor system. Fat content composition was determined using GC- FID, total phenols by spectrophotometry, and the phenolic profile using HPLC-DAD. Results showed that variety, harvesting time and their interaction have an extremely high significance effect on the overall studied parameters of olive content, fatty acid and phenolic profiles. Among the studied varieties, ‘Kalama’ presented the highest oil content on dry matter (48.24%), ‘Souby’ the highest oil content on fresh matter (27.86%), and ‘Tanche’ the highest oil industrial yield (19.44%). While ‘Tanche’ recorded the highest C18:1 (71.75%), ‘Ascolana Tenera’ showed the highest total phenols (539.27 mg GAE/kg of oil), ‘Salonenque’ the highest oleic acid (121.57 mg/kg), and ‘Itrana’ the highest oleocanthal contents (317.68 mg/kg). On the other hand, the oil content together with C18:2 and C18:0 increased along ripening; but, C18:1, total phenols and the main individual phenols decreased. Although preliminary, this study highlights the good quality of the olive oil produced from local and foreign varieties in Lebanon, and incites to conduct further investigations for the characterization and authentication of the Lebanese olive oil.

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maximum observed difference was two weeks and was state. On the other hand, differences in the date of grow irrespective of the year, which points to low temperatures of new leaf pairs in each shoot. On the one hand, our re year. The activity of apical meristems was monitored in six were selected for the measurements for each location andpite being only 50 km apart from each other, differ in tem were Córdoba and Espiel (Spain), which, des...
Abstracts of Poster Communications

Olive Management, Biotechnology and Authenticity of Olive Products

\textbf{T02-P6}

\textbf{PLASMA MEMBRANE ISOLATION FROM LEAVES OF OLEA EUROPAEA L.}

\textbf{MIRKO SODINI, ALESSANDRA FRANCINI, LUCA SEBASTIANI-NI}

SCUOLA SUPERIORE SANT'ANNA INSTITUTE OF LIFE SCIENCE

Plasmatic membrane (PM) is a complex structure that controls the relationship between cell and external environment. Despite PM have a key role on regulating plant responses to environmental stress in olive their role is poorly understood. Starting from the interest in understanding the role of PM in olive, a simple PM purification protocol was successfully developed in order to purify a significant amount of PM from a single leaf of olive tree. The actual procedures passed through microsomal isolation and post microsomal purification is done by conventional two-phase system (Dextran-PEG) or by a sucrose gradient. Our protocol take the way of microsomal extraction followed by a sucrose gradient separation, but does not require ultracentrifugation (over 100,000g) in any of its phases. The whole procedure takes approximately 8 hours and the sucrose gradient can be easily obtained in 2 ml tube, allowing an effective purification of PM. We found that keeping the volume minimal during the extraction phases, enable membrane isolation from relative low centrifugation force (21,000g), and allow the use of small amount of leaf samples (100-500 mg) yielding purified PM having 10-100 µg of putative PM proteins. Since PM-ATPase enzyme activity can be used as a marker of PM purity we assay this activity in presence of specific inhibitors such as vanadium. The principles of our method can be adapted in other cell organelle membranes.

\textbf{T02-P7}

\textbf{MOLECULAR AND MICROSCOPIC ANALYSES OF SPHINGOLIPID METABOLISM DURING OLIVE FRUIT RIPENING}

\textbf{CARLA INÉS, MARIA C. PARRA-LOBATO, MIGUEL A. PAREDES, JULIANA LABRADOR, MERCEDES GALLARDO, MARIANA SAUCEDO-GARCIA, MARINA GAVILANES-RUIZ, MARIA-CARMEN GOMEZ-JIMENEZ}

UNIVERSITY OF EXTREMADURA PLANT PHYSIOLOGY

Plant sphingolipids are involved in the building of the matrix of cell membranes and in signaling pathways of physiological processes and environmental responses. Howeever, information regarding their role in fruit development and ripening, a plant-specific process, is unknown. The present study seeks to determine whether and, if so, how sphingolipids are involved in fleshy-fruit development and ripening in an olive-crop species such as olive (\textit{Olea europaea L. cv. Picual}). Here, in the plasma-membranes of live protoplasts, we used fluorescence to examine various specific lipophilic stains in sphingolipid-enriched regions and investigated the composition of the sphingolipid long-chain bases (LCBs) as well as the expression patterns of sphingolipid-related genes, \textit{OeSPT}, \textit{OeSPHK}, \textit{OeACER}, and \textit{OeGlCcera}, during olive-fruits development and ripening. The results demonstrate increased sphingolipid content and vesicle trafficking in olive-fruits protoplasts at the onset of ripening. Moreover, the concentration of LCB (\(18\)-18:2, \(18\)-1:18:1, \(18\)-1:18, \(18\)-1:18:2, \(18\)-1:18:4, \(18\)-1:18:6, and \(14\)-anhydro-\(18\)-18:1E) increases during fruit development to reach a maximum at the onset of ripening, although these molecular species decreased during fruit ripening. On the other hand, \textit{OeSPHK}, and \textit{OeGlCcera} were expressed differentially during fruit development and ripening, whereas \textit{OeACER} gene expression was detected only at the fully ripe stage. The results provide novel data about sphingolipid distribution, content, and biosynthetic processes during fleshy-fruits development, indicating that all are highly regulated in a developmental manner.

\textbf{T02-P8}

\textbf{ANALYSIS OF GENES INVOLVED IN SPHINGOLIPID BIOSYNTHESIS AND TURNOVER DURING EARLY OLIVE-FRUIT DEVELOPMENT}

\textbf{JORGE CORBACHO, CARLA INÉS, MARIA C. CAMEARO, BEATRIZ BRIEGAS, MIGUEL A. PAREDES, JULIANA LABRADOR, MERCEDES GALLARDO, ANTONIO M. CORDEIRO, MARIA-CARMEN GOMEZ-JIMENEZ}

UNIVERSITY OF EXTREMADURA PLANT PHYSIOLOGY

Sphingolipids are abundant membrane components and signalling molecules in various aspects of plant development. However, the role of sphingolipids in early fleshy-fruit growth and ripening has been rarely investigated. In this study, we first investigated the temporal changes in sphingolipid long-chain base (LCB) content, composition, and gene expression that occurred during fruit opening and early fruit development in olive (\textit{Olea europaea L. cv. Picual}). Moreover, the interaction between sphingolipid and the plant hormone, brassinosteroid (BR), during the early fruit development was also explored. For this, BR levels were manipulated through the application of exogenous BRs (24-epibrassinolide, EBR) or a BR biosynthesis inhibitor (brassinazole, Brz) and their effects on early fruit development, sphingolipid LCB content, and gene expression were examined in olive fruit at 14 days post-anthesis (DPA). We here show that sphingolipid with C-4 hydroxylation and \(\Delta\)8 desaturation with a preference for (E)-isomer formation are quantitatively the most important sphingolipids in olive reproductive organs. In this work, the total LCB amount significantly decreased at the anthesis stage, but olive sphingosine-1-phosphate lyase (\textit{OeSPL}) gene expression was expressed exclusively in flower and upregulated during the anthesis, revealing an association with the \(\text{d18:1(8E)}\) accumulation. However, the LCB content increased in parallel with the up-regulation of the expression of genes for key sphingolipid biosynthetic and LCB modification enzymes during early fruit development in olive. Likewise, we found that EBR exogenously applied in olive tree significantly accelerated the fruit growth rate associated with reduced levels of sphingolipid LCB content and gene expression in olive fruit after 7 and 14 days of treatment, whereas Brz slowed the fruit growth rate and boosted the sphingolipid LCB content and gene expression during early growth. Thus, our data indicate that endogenous sphingolipid LCB and gene-expression levels are intricately controlled during early fruit development in olive and also suggest a possible link between BR, the sphingolipid content/gene expression, and early fruit development.

\textbf{T02-P9}

\textbf{VISUALIZATION OF SPHINGOLIPID ENRICHED PLASMA MEMBRANE REGIONS AND LONG-CHAIN BASE COMPOSITION DURING OLIVE FRUIT ABSCSSION}

\textbf{MARIA C. PARRA-LOBATO, MIGUEL A. PAREDES, JULIA LABRADOR, MARIANA SAUCEDO-GARCIA, MARINA GAVILANES-RUIZ, MARIA-CARMEN GOMEZ-JIMENEZ}

UNIVERSITY OF EXTREMADURA PLANT PHYSIOLOGY

Sphingolipids, found in membranes of eukaryotic cells, have been demonstrated to carry out functions in various processes in plant cells. However, the roles of these lipids in fruit abscission remain to be determined in plants. Biochemical and fluorescence microscopy imaging approach has been adopted to investigate the accumulation and distribution of sphingolipids during mature-fruit abscission in olive (\textit{Olea europaea L. cv. Picual}). Here, a lipid-content analysis in live protoplasts of the olive abscission zone (AZ) was made with fluorescent dyes and lipid analogs, particularly plasma membrane sphingolipid-enriched dovlipids and their dynamics were investigated in relation to the timing of mature-fruit abscission. In olive AZ cells, the measured proportion of both polar lipids and sphingolipids increased as well as endocytosis was stimulated during mature-fruit abscission. Likewise, mature-fruit abscission resulted in quantitative and qualitative changes in sphingolipid long-chain bases (LCBs) in the olive AZ. The total LCB increase was due essentially to the increase of \(\text{d18:1(8E)}\) LCBs, suggesting that C-4 hydroxylation and \(\Delta\)8 desaturation with a preference for (E)-isomer formation were quantitatively the most important sphingolipids in olive AZ during abscission. However, our results also showed a specific association between the dihydroxylated LCB sphinganine (d18:0) and the mature-fruit abscission. These results indicate a clear correlation between the sphingolipid composition and mature-fruit abscission. Moreover, measurements of endogenous sterol levels in the olive AZ revealed that it accumulated sitosterol and campesterol with a concomitant decrease in cycloartenol during abscission. In addition, underlying the distinct sterol composition of AZ during abscission, genes for key biosynthetic enzymes for sterol synthesis, for obtusifoliol 14α-demethylase (\textit{CYP51}) and C-24 sterol methyltransferase (\textit{SMT2}), were up-regulated during mature-fruit abscission, in parallel to the increase in sitosterol content. The differences found in AZ lipid content and the relationships established between LCB and sterol composition, offer new insights about sphingolipids and sterols in abscission.
Influence of Water Deficit on the Activity and Gene Expression Pattern of Olive B-Glucosidase

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The phenolic composition of virgin olive oil (VOO) is strongly determined by the enzymatic reactions that take place during olive fruit processing. Of these enzymes, β-glucosidase (EC 3.2.1.21) activity plays a major role in the transformation of the phenolic glycosides present in the olive fruit. In addition to genetic factors, it is clearly accepted that edapho-climatic parameters also condition the phenolic composition of olive fruits and oils. This study describes the effect of two water regimes, extreme rain-fed and full irrigation, on the activity and expression pattern of olive β-glucosidase. The analysis of the enzymatic activity of β-glucosidase in olive fruits from Pichul and Manzanilla cultivars showed that significantly lower levels of β-glucosidase activity were always found in fruits cultivated under rain-fed conditions. The gene expression study of olive β-glucosidase genes, selected from a previous transcriptomic study, was carried out by real-time PCR throughout the fruit development and ripening in both cultivars under the different irrigation conditions. Global data analyses have been carried out to evaluate the correlation of the water regime with the oil phenolic compound profile, the total β-glucosidase activity and the gene expression data of each β-glucosidase family member in the two cultivars under study.

Novel Dual Pathway for Metabolic Engineering of E. coli Metabolism for the Production of the Highly Valuable Hydroxytyrosol

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SCHOOL OF AGRICULTURE, FOOD AND NUTRITION, TECHNOLOGICAL EDUCATIONAL INSTITUTE DEPARTMENT OF AGRICULTURE

One of the most abundant phenolic compounds traced in olive isomers is Hydroxytyrosol (HT), a molecule that has been attributed with a pile of beneficial effects, well documented by many epidemiological studies and numerous in vitro and in vivo studies that indicate its potential value to products containing it. Strong antioxidant capacity and protection from cancer are only some of its exceptional features making it ideal as a potential supplement or preservative to be employed in the nutraceutical, agrochemical, and food industry. The HT biosynthetic pathway in plants (e.g. olive fruit tissues) is not well apprehended yet. However, based on its structure, it can be presumed that HT might be derived from tyrosine which is then transformed to HT through sequential biosynthetic steps involving a hydroxylation, a decarboxylation, and a deamination reaction. In this contribution, we follow a metabolic engineering strategy encompassing a dual pathway introduced in Escherichia coli leading it to produce HT. Tyrosine was depicted as the precursor molecule and in addition to genetic factors, it is clearly accepted that edapho-climatic parameters also condition the phenolic composition of olive fruits and oils. This study describes the effect of two water regimes, extreme rain-fed and full irrigation, on the activity and expression pattern of olive β-glucosidase. The analysis of the enzymatic activity of β-glucosidase in olive fruits from Pichul and Manzanilla cultivars showed that significantly lower levels of β-glucosidase activity were always found in fruits cultivated under rain-fed conditions. The gene expression study of olive β-glucosidase genes, selected from a previous transcriptomic study, was carried out by real-time PCR throughout the fruit development and ripening in both cultivars under the different irrigation conditions. Global data analyses have been carried out to evaluate the correlation of the water regime with the oil phenolic compound profile, the total β-glucosidase activity and the gene expression data of each β-glucosidase family member in the two cultivars under study.

An Improved Method for High-Quality DNA Extraction from Olive Embryos - Plant Tissue Rich in Polyphenols and Polysaccharides

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Different methods have been used to evaluate the success and inter-compatibility in pollination assays. Microsatellites are appropriate markers for paternity analysis of olive embryos to measure the success of the crosses, especially when potential pollen donors are multiple and not applied by hand. In our study, the paternity analysis based on microsatellites was used for genotyping and identification of the potential pollen donors of ËOblicaË, the leading olive variety in Croatia and for assessing the percentage of self-fertilization in multivarietal olive orchard. Embryos accumulate high contents of polyphenachides, polyphenols, or other compounds that bind and/or co-precipitate with DNA. This condition increased the sample viscosity and significantly decreased DNA quality, interfering with the PCR performance. To improve the PCR output, we have modified the extraction method: with repeated extraction procedure based on CetylTrimethylAmmoniumBromide-PolyVinylPyrrolidone protocol, and using the previously extracted DNA samples and dissolved in TE buffer as an initial material, the amplification success was significantly improved. Seven microsatellite loci were used for genotyping olive embryos from ËOblicaË trees and for all potential pollen donor trees grown in the orchard. Seeds have been considered as a product of self-fertilization if only maternal alleles were found at all analysed microsatellite loci. When the presence of paternal alleles for each primer was recorded together with maternal, the progeny has been considered a product of cross-fertilization. The knowledge of self-compatibility and cross-compatibility relationships of olive cultivars contribute to defining the new guidelines for farmers regarding the proper management and provide valuable information for breeders.
A COMPARISON OF CELL WALL METABOLISM IN GREEN AND RIPE FRUIT OF AN OIL AND A TABLE OLIVE CULTIVAR

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Olive (Olea europaea L.) growing has outstanding economic relevance in Spain. While many cultivars are used mainly for oil extraction, some are intended for consumer as table olives. In this study, 'Arbequina' and 'Manzanilla' were respectively chosen as a characteristic example of each of both cultivar types. In spite of the relevance of ripening-associated modifications in cell wall composition and structure for final quality of produce, very few published studies have addressed this topic during the maturation and postharvest periods before the processing of olives. Fruit samples were harvested manually at the green and black stages. Yields of alcohol-insoluble residue (AIS) in both cultivars decreased from the green to the black stages, but were higher in 'Arbequina' (7.8% and 3.6%, respectively) than in 'Manzanilla' (4.0% and 3.0%, in the same order). When AIS were fractionated, increased amounts of the water-, chelator- and sodium carbonate-soluble fractions were observed in ripe fruit in comparison with the green stage, whereas those of the KOH-soluble fraction, enriched in matrix glycans, decreased. Some cell wall-related enzyme activities were examined in relation to these changes. Pectin methylesterase (PME) and polygalacturonase (PG) activities decreased substantially from the green to the black stages, particularly for 'Manzanilla' fruit. Decreased PME activity at the black stage is consistent with results showing higher degree of esterification of pectins isolated from these samples. By contrast, α-L-arabinofuranosidase (Afase) activity increased noticeably at the black stage, in parallel with augmented amounts of neutral sugars in the water-soluble fraction.

EVALUATION OF PHENOLOGICAL STAGES AND DETERMINATION OF HARVESTING TIME IN OLIVE CULTIVARS

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This research was carried out during 1386-1388 on several cultivars Among the developed olive cultivars in three locations including Kazeroun research station, Sarpolezahab olive research station and in - Genareh olive cultivars collection of Gorgan using a randomly complete block design, the Zard, Shenghe, Beleady and Koroneiki, that were present in the above three locations were studied more specifically. The phenological stages were determined using the Maylard modified phenology table. Fruit set percentage, fresh and dry weight of fruits, water content, fruit pulp/stone ratio and oil content in the fresh and dry weight of fruits were the measured parameters in current research. The means comparison of main and subsidiary treatments were done using Duncan's multiple range test. On the basis of observations made, the effect of year on the time of phenological stages and correspondence of flowering stages duration of cultivars were determined in Kazeroun, Sarpolezahab and Gorgan. Besides the reported results obtained in each of the locations, the related results to the four common cultivars that were cultivated in every location showed an evident superiority of cultivars in Gorgan than those of the same cultivars in Kermanshah and Kazeroun areas. Finally, the combined analyses results showed a higher fruit water content in Kermanshah and Kazeroun than the gorgan area. In both Kazeroun and Gorgan areas, the oil content in the fresh and dry weight of fruits was significantly different among the cultivars. With respect to the evaluated parameters, all the four cultivars studied can be suggested for olive development purpose in Gorgan area if their oil quality is desirable.
PHENOLOGY OF THREE TRADITIONAL PORTUGUESE OLIVE VARIETIES UNDER AN INTENSIVE PRODUCTION SYSTEM IN ALENTEJO REGION

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Under the research project Olevvalor (2016-2019) the pheno-ology of three Portuguese olive varieties (Galega vulgar, Cobrança, Azeiteira) was phenological characterized be-tween stages 53 and 89 of BBCH scale. The orchards under an intensive irrigation production system were located in Monforte (Alentejo region). For each variety, an ex-perimental design based on 5 blocks with 4 trees was used. In 2017, the varieties ‘Azeiteira’ and ‘Galega vulgar’ were the first to initiate the opening of winter buds (stage 53), both on 13/03, with the ‘Cobrança’ reaching this stage on 30/03. The beginning of flowering followed the same order: ‘Azeiteira’ (22/04), ‘Galega vulgar’ (27/04), ‘Cobrança’ (03/05). The flowering period lasted for 16, 17 and 21 days to ‘Galega vulgar’, ‘Cobrança’ and ‘Azeiteira’, re-spectively. Fruit ripening process (stage 80) first occurred on 13/09 for ‘Cobrança’ and on 21/09 for ‘Azeiteira’ and ‘Galega vulgar’. The end of maturation (stage 89) was re-corded on 08/11 for ‘Galega vulgar’, 15/11 for ‘Cobrança’ and 27/11 for ‘Azeiteira’.

The sums of the growing degree-days (GDD) were deter-mined for different periods of the cycle varieties. During the period from the opening of winter buds to final matu-ration growing degree-days were obtained from 2848.54 GDD for Cobrança up to 2882.37 GDD and 2964.89 GDD to ‘Galega vulgar’ and ‘Azeiteira’, respectively. The observations made are supposed to be repeated in the years 2018 and 2019 to confirm the achieved values and to determine the sequence of the different varieties in the grow-th stages. These results are crucial to the phenological cha-racterization of the Portuguese olive varieties under study to know about their adaptation to different geographic loca-tions and climate change.

FLORAL BIOLOGY OF DIFFERENT MEDITERRANEAN OLIVE TREE VARIETIES GROWING IN NORTHERN TUNISIA

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The presence of both Tunisian and foreign varieties growing together provides particular phytogegetic rich-ness to the INAT (Tunisian National Agronomy Institute) olive germplasm collection. The agronomic performance and genetic plasticity of these varieties in the edaphoclimi-matic conditions of northern Tunisia, however, require examination. In this study we evaluated flowering, flower quality and the degree of self-pollination, environmentally sensitive parameters which are critical for fruit production, of five foreign varieties (Frantoio, Kalamata, Olivieria, Se-villana and Sioisie) and one Tunisian variety (Gerboui). For each variety the date of full bloom, and the length, position, number of nodes and inflorescences of one-year-old shoots were recorded at the flowering season onset. Additionally thirty inflorescences per tree were sampled during full bloom to evaluate flower-quality parameters, including the number of flowers per inflorescence, the percentage of perfect flowers, pistil weight, the number of ovules per ovary and the pollen germination and quanti-ty per flower. Self-pollination was also tested to indicate self-compatibility. All studied parameters showed high va-lues, while generally all varieties showed high flower quality and shoot-fertility. The lowest values for the degree of self-compatibility were found in ‘Gerboui’, ‘Kalamata’, ‘Oliviera’ and ‘Sevi-lanna’. The high degree of self-incompa-tibility found in the majority of foreign cultivars indicates the need to investigate their compatibility with the major Tunisian varieties (Chemlali, ‘Chetoui’ and ‘Meski’) as possible pollinators.

EFFECT OF THE MAIN CLIMATIC FACTORS ON REPRODUCTIVE BIOLOGY AND THE PRODUCTION OF FRUIT OF OLEA EUROPAEA L. IN TUNISIA

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INSTITUT DE L’OLIVER AGRONOMY

The main objective of this study is to identify the environ-mental factors that affect the intensity of production struc-tures as well as the chronological flowering pheno-phephrases. The assessment of agronomic potential of three olive culti-vars Chemlali, Chemlali and Oueslati was carried out during three consecutive years (from 2011 to 2013). Results obta-nined show a considerable variability. The vegetative grow-th, and the reproductive potential assessed by the flower and pollen quality were significantly reduced in 2013. We attribute this low potential to climatic factors characteri-zed by a scarcity of rainfall and excessive heat during win-ter. Controlled pollination tests showed that the Chemlali and Oueslati are selfcompatible, while Chetoui is a strong self-incompatible, and the intensity of this compatibility varies depending on climatic factors during the flowering period. Indeed, in 2012 Chemlali has shown a partially self-incompatibility in a high thermal amplitude climatic parameters. However, the climatic conditions prevailing by 2013 had increased the intensity of self-compatibility of Chemlali and Oueslati. These results prompted us to determine the most climatic factors involved in the for-mation and the behavior of production structures. Among these factors, we determined the average amounts of Chi-lili and heat accumulated by the olive tree in these re-gions over a period on 17 years (from 1993 until 2011). The amount of chilling was calculated by Utah+ model and the heat was calculated by GDD model. Results showed that both parameters combined with rainfall are the main fac-tors involved in pollen chronology and fruit production.

GENOME-WIDE IDENTIFICATION, MOLECULAR CHARACTERIZATION, AND EXPRESSION ANALYSIS OF THE CALEOSIN GENE FAMILY IN OLEA EUROPAEA

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ESTACIÓN EXPERIMENTAL DEL ZADÓN-CSIC DE BIOCHEMIS-TRY, CELL AND MOLECULAR BIOLOGY OF PLANTS

Caleosins are lipid body-associated peroxigenases involved in mobilization of seed storage lipids, floral transition, and defense against fungi and abiotic stresses. Current data mostly come from studies in Arabidopsis, so little is known about this multigene family in other oleaginous species. Here, we investigated phylogeny, sequence structure and expression of caleosins in olive. Up to seven caleosin genes, called CLO1 to CLO7, were identified in the olive genome. Phylogenetic analysis split caleosins into two clades, each of which shared similar sequence features. Thus, CLO2 and CLO6 genes (class A) had five introns and shared five con-served motifs. The other five caleosins (class B) lacked the N-terminal conserved motif I and had two to five introns. Moreover, the EF-hand and transmembrane domains were missing in CLO5, suggesting it is likely a non-functional protein. Out of seven caleosins, only five (CLO1-CLO5) were transcribed, being their expression tissue-spe-cific and temporally regulated. The lack of 5’ and 3’-UTR sequences might explain the absence of CLO6 and CLO7 transcripts. Caleosins were expressed in all vegetative tis-sues, excluding the mesocarp. On the other hand, CLO2 showed significant mRNA levels in the developing pistil. In the pollen grain, CLO1 transcripts accumulated during maturation, and CLO4 was transiently expressed during te-trad and microspore stages. Only CLO1 showed significant levels of mRNA during pollen germination, with a peak at 6 h. Our findings provide a basis for further investigation of biological functions of olive caleosins. This work was supported by ERDF co-financed grants AGL2013-43042-P and AGL2017-84298 (MINECO).
Abstracts of Poster Communications

**T03-P10**

**FLUCTUATIONS ON OLIVE CROP YIELD IN TUNISIA: INFLUENCE OF POLLEN INTENSITY AND CLIMATE.**

**ALI BEN DHIAB, SAHAR HADJ HAMIDA, WAFA OUNISSI, AJMI LARRAY, MONJI MSALEEM, MEHDJ BEN MIMOUN**

**INSTITUT DE L’OLIVIER AGRONOMY**

Tunisia is the world’s second largest olive-oil producing region after the European Union. This paper reports on the use of models to forecast local olive crops, using data for Tunisia’s five main olive-producing areas: Mornag, Jemmel, Menzel Mhiri, Chaal and Zarzis. Airborne pollen counts were monitored over the period 1993-2011 using a Cow trap. Forecasting models were constructed using agricultural data (harvest size in tonnes of fruit/years) and data for several weather-related and phenoclimatic variables (rainfall, humidity, temperature, Heat and Chilling). Analysis of these data revealed that the amount of airborne pollen emitted over the pollen season as a whole was the variable most influencing harvest size. Findings for all local models also indicated that the amount, timing and distribution of rainfall (except during blooming) had a positive impact on final olive harvests. Air temperature also influenced final crop yield in three study provinces (Menzel Mhiri, Chaal and Zarzis), but with varying consequences: in the model constructed for Chaal, cumulative maximum temperature from budbreak to start of flowering contributed positively to yield. This phenomenon, well known among the farmers, is probably due to the excessive shoot growth, which reduce the flower sink force or due to dry and warm winds during blooming period. The goal of our experiments was to promote the fruit set by applying EXCELERTM (ABA-as ingredient 100g/kg), just before the beginning of full bloom to induce stoma closure for some days. Uniform fifty years old “Canino” plants, raised in a polyconic vase, located in Central Italy, were sprayed with 10, 50 and 100 mL dissolved in 100 liters of water. At harvest time, the fruit yield, shoot and internode length, node number and chlorophyll content of the new bearing shoots, were analyzed. Both concentrations, 50 and 100 mL, promoted higher yield of harvested fruits, with an average of 80 kg/plant compared to untreated plants. In addition, at lower concentration (50mL/100L), surprisingly the vegetative traits also increased, while the photosynthetic pigments did not show any differences among treatments. These results confirm our previous findings by using triazole compounds, which is well known to induceABA synthesis in plant cells. Further investigation is needed to define the exact environmental conditions (light and temperature, in particular) at blooming time, to finalize the real needs of exogenous treatment with ABA to improve fruit set in olive plants.

**T03-P11**

**FOLIAR APPLICATION OF ABSICIC ACID (EXCELEROTM) TO ENHANCE THE FRUIT SET AND VEGETATIVE GROWTH IN OLIVE.**

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The fruit set in olive is relatively low as compared to the high number of differentiated flowers; in some years it results almost nil, although in presence of high flower number. This phenomenon, well known among the farmers is probably due to the excessive shoot growth, which reduce the flower sink force or due to dry and warm winds during blooming period. The goal of our experiments was to promote the fruit set by applying EXCELERTM (ABA-as ingredient 100g/kg), just before the beginning of full bloom to induce stoma closure for some days. Uniform fifty years old “Canino” plants, raised in a polyconic vase, located in Central Italy, were sprayed with 10, 50 and 100 mL dissolved in 100 liters of water. At harvest time, the fruit yield, shoot and internode length, node number and chlorophyll content of the new bearing shoots, were analyzed. Both concentrations, 50 and 100 mL, promoted higher yield of harvested fruits, with an average of 80 kg/plant compared to untreated plants. In addition, at lower concentration (50mL/100L), surprisingly the vegetative traits also increased, while the photosynthetic pigments did not show any differences among treatments. These results confirm our previous findings by using triazole compounds, which is well known to induceABA synthesis in plant cells. Further investigation is needed to define the exact environmental conditions (light and temperature, in particular) at blooming time, to finalize the real needs of exogenous treatment with ABA to improve fruit set in olive plants.

**T03-P12**

**EFFECT OF AIR POLLUTANTS ON OLIVE POLLEN PERFORMANCES.**

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Olive trees produce and release large quantities of pollen into the atmosphere due to the pollination process. While airborne, the pollen is highly influenced by the environmental conditions, such as climate and pollution. These conditions can alter the pollen metabolism inducing the modification of the chemical structure of proteins present in pollen leading to the pollen sterility with impact on the pollination and crop size but also the health of workers and citizens sensitive to Olea pollen allergens. The present work aims to study the effect of some gaseous pollutants (O3, NO2 and SO2) on the fertility, the protein profile of olive pollen (Olea europaea) collected separately from two Tunisian cultivars (Chetoui and Chemlali). The experiment tests were carried out using an environmental chamber system for in vitro exposure of pollen to different levels of pollutant gases during continuously 6 hours. The results of fumigated samples comparatively to non-exposed pollen revealed a significant decrease of pollen fertility in term of viability and germination. The polypeptide profiles were determined using sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed bands ranging from 15 to 100 kDa with changes of blotting intensities and different behavior of the cultivars. All of these results suggest that increasing levels of air pollutants may have a negative impact on the Olea europaea pollen and hence influence on the fruit production. Other analysis were also conducted to study the physiological response by evaluating ROS and RNS production. A real time PCR showed also significant changes in gene expressions.

**T03-P13**

**OPTIMUM MATURATION TIME FOR OLIVE HARVEST OF THREE OLIVE VARIETIES IN ALENTEJO REGION (PORTUGAL).**

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One of the parameters to determine the optimal harvesting time is the stabilization of oil content on dry weight basis (OPDW). This determination can only be performed by laboratory analysis. An alternative approach to find the optimum time harvesting is using a more practical methodology, such as the fruit ripening index (RI). In 2017/2018 campaign, a study was carried out to understand the relationship between OPDW and turn colour of ripen fruits according to RI. Samples were collected from three olive orchards in the Alentejo region (20 trees per orchard) from ‘Aceiteara’, ‘Cobrança’ and ‘Galega vulgar’ varieties between mid-September and the end of December. Ripens determined by the Jaen method and OPDW using a FOSS Oliva TM NIR analyser. The results obtained allow us to verify that there is a high correlation between OPDW and RI (R 2 varies between 0.84 and 0.93), increasing the OPDW in the fruits with the evolution of the olives, until stabilizing in 34% - 39% depending on the variety, when the RI presents values between 2.2 and 3.9 for the three varieties. To ‘Cobrança’ and ‘Galega’ varieties, the stabilization of OPDW was earlier (2 nd November) with a RI of 2.2 and 3.9 respectively, than that from ‘Aceiteara’ at 27 th November with a RI of 3.9. Consequently, it is verified that RI can be used to determine the optimal harvesting time, because it is highly related to the OPDW evolution. OPDW stabilization corresponds to a different RI depending on the variety. These preliminary findings deserve further attention and research evaluation in next campaigns.
T03-P14
EVALUATING THE CONTRIBUTION OF FRUIT PHOTOSYNTHESIS TOWARD THE BIOGENESIS OF OLIVE OIL
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In olive, fruit growth is determined by the turgor and by the carbon assimilated in photosynthesis. This carbon could come from photoassimilates produced in the lea- vels (heterotrophic) or produced in the fruit itself (autotro- pic). The aim of this work was to evaluate whether the fruit photosynthesis actually represent a substantial con- tribute to oil synthesis in the fruit. The experiment was carried out in 10-year-old Arbequina olive trees under two irrigation strategies: full irrigation needs (FI) and regulated deficit irrigation (45RDI) applying 45% of the FI treatment.

To analyze the origin of photoassimilates, we measured oil content and fatty acid composition in olive mesocarp from fruits in control branches, leafless branches which were also “girdled” (autotrophic), and branches whose fruits were bagged to avoid photosynthesis (heterotrophic). Samples were collected at three different stages of fruit development: immediately after stone lignification; when the olive fruit was completely green and when the olive was yellowish. The 45RDI strategy caused an increase in olive oil content per fruit dry weight compared to the FI treatment, and accelerated the maturation process in four weeks. In the end of the experiment. However, in both irrigation strategies, increased slightly, although significantly, detected among different treatments and water regimes.

T03-P15
SELF-INCOMPATIBILITY ASSESSMENT OF SOME OLIVE (OLEA EUROPAEA L.) GENOTYPES FOR THE SUCCESS OF BREEDING CROSSES
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UNIVERSITY OF BARI

Olive (Olea europaea L.) is among the top six most im- portant agricultural crops in the world, particularly in the Mediterranean basin. On the Italian territory, its great di- ffusion demonstrates its importance for environment, production and defence of the territory. Olive genetic breeding has been historically based on the selection of individuals adapted to local conditions by classical crosses between cultivars. The main factor affecting the success of controlled crosses seems to be the inter-compatibility (IC) among cultivars and the self-compatibility (SC), that could significantly influence both the fruit set and the rate of contamination. Thereby, some knowledge about IC and SC relationships in olive is required to design successful crosses in breeding programs. Despite of its occurrence in many genotypes, the literature about olive self-incompati- bility (SI) is still lacking of accurate information.

In this work, the SI value of some olive genotypes wides-pread in Southern Italy (Apulian region) has been de- termined in two different environments and under two pollination conditions (self and free). Moreover, the incom- patibility relationship of cultivar Coratina has been evalua- ted in controlled crosses with some suitable pollinators. The germination potential of each progeny has been eva- luated by in vitro culture of mature embryos and their ori- gins have been molecularly determined. For the first time, the application of SSR markers to DNA extracted by seed endosperms allowed an indirect but accurate and fast se- lection of cross-derived embryos, avoiding any stress to embryos themselves and saving time.

T03-P16
IMPACT OF WINTER CHILLING ON FLOWERING OF OLIVE CULTIVARS KORONEIKI AND MASTOIDIS
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The main aim of the present study was to investigate the effects of winter temperature on subsequent flowering of two of the main olive cultivars in Greece, Koroneiki and Mastoidis. Four groups of 10 two-year-old potted olive plants were either grown outdoors under ambient tem- perature or transferred into a greenhouse for one, two or three months during winter in Crete, Greece. Temperature was monitored to calculate the amount of chilling units for each treatment. Flowering phenology throughout spring as well as quantitative indicators of inflorescence, flowers and pollen were monitored. Reduced winter chilling ac- cumulation resulted in earlier swelling and opening of inflorescence buds, however flower opening date was not affected. Number of inflorescence per plant was reduced in Mastoidis but unaffected in Koroneiki. Similarly, num- ber of flowers per inflorescence was reduced in Mastoidis but unaffected in Koroneiki. Maintaining the plants in the greenhouse for three winter months increased ovary abor- tion in both cultivars. Pollen germination was reduced in the Koroneiki plants kept in the greenhouse for three mon- ths compared to the control while no significant differen- ces were observed in the intermediate cases. The present study indicates increased chilling accumulation demand for vernalization in Mastoidis compared to Koroneiki. Ongoing research with increased number of Greek cultivars aims to elucidate their suitability for new plantations ac- cording to local climate.

T03-P17
ANATOMIC STUDY AND ILLUSTRATION OF THE PROCESS OF WOUNDS CLOSURE OF GIRDLED SHOOTS OF OLIVE TREES
KHOULOUD ANNABI, IBTISSEM LAARIBI, HASSOUNA GOUTA, REHANE SHAHID, MARIE-CHRISTINE VAN LABEKKE, FOUED LABIDI, IMEN ZOUARIL, MOUNA AVACHI MEZGAH- NI

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Girdling was widely used in many species, such as grape- vines, citrus, apple, peach and olives, in order to improve the fruit size and quality and to increase the yield of flower buds. In our case, we are interested on the healing process of the wounds of the girdled shoots of two olive cultivars ‘KORONEIKI’ and ‘CHETOUI’. Two groups of trees of ‘ON’ and ‘OFF’ younger generation of from ‘CHETOUI’ were chosen, girdled stem located on the 5th botanical order. Then a ring of 2.5 cm of large was removed from the bark of the stem on the 25th of May. Monitoring the progress of healing over 8 months, allowed us to differentiate the following steps; necrosis of some cell, callus proliferation, contact between callus pads, callus bridge formation and differen- tiation into mature vascular bundle. As this progress is close- ly related to the mobilization of carbohydrates between source-sink organs, a histological study of leaves, wood and roots was realized and compared. Several anatomical changes were identified after measurement of different parameters such as the (t.v.v) secondary vascular vessels, (tr) trichomes,(sc) sclerenches,(s.p) spongy parenchyma,(p.p) palisade parenchyma,(st) stomata, (co) collenchyme and (in.s) invaginated stomata, (sb) suber,(pf) pericyclic fiber- s,(l) fiber,(w) woods,(m) medulla,(in) inclusion and (s.g) starch grains.
CHEMICAL TREATMENTS IN ORDER TO INCREASE FRUIT-SET ON THREE OLIVE CULTIVARS
PIERGIORGIO SEDDA, MARTINO MUNTONI, GIANLUIGI PILLI, FEDERICO CORDA, CARLO MORO, ROBERTO ZURRU, LEONARDO DESSENA, MAUROZIO MULAS
AGRIS SARDEGNA - REGIONAL AGENCY FOR RESEARCH IN AGRICULTURE, SERVIZIO RICERCA NELLE FILIERE OLIVICO-OLIVARIA E VITINOLOGICA

Lack of sufficient fruit-set and consequent irregular yield is a common problem of many olive cultivars like ‘Bosana’, ‘Nera di Gonnos’ and ‘Semidiana’, which are among the most important olive cultivars in Sardinia Island (Italy). In the growing season 2016/2017 a field experiment was designed in the experimental farm of Villasor (South Sardinia). Mature trees of the three cited cultivar in the stage of full blossom were submitted to treatments with the following bio-stimulants substances: Gibberellic Acid (12 ppm; EVO®) (1 ml/l) by Thermaflora, which contains 770 g/kg of polyethylene-glycols and 3 g/kg of humic salts of plant origin); water test. Four plants were used for cultivar and treatment and inside every plant 12 flower-bearing (20-30 inflorescences) branches were selected considering the 4 cardinal points and 3 levels of height in the canopy. Fruit-set as percentage of the counted flowers was observed. The results indicate: (1) ‘Arbequina’, ‘Verdial de Huévar’ and ‘Manzanilla’ (medium fruits) and ‘Gordal’ (large fruit) were sampled. In them, its size was determined and the embryos from which, the embryos into the seeds are viable and able to germinate in vitro. Throughout 2016, from fruit set to ripening, every 10-15 days, ‘Arbequina’ (small fruit), ‘Verdial de Huévar’ and ‘Manzanilla’ (medium fruit) and ‘Gordal’ (large fruit) were sampled. In them, its size was determined and the embryos from the naked seed were isolated, sown in vitro on 1/3 MS substrate, and finally, its germination ability was measured. The results indicate: (1) ‘Arbequina’, ‘Verdial de Huévar’, ‘Gordal’ and ‘Manzanilla’ seeds are viable after 87, 104, 155 and 147 days after flowering, respectively. (2) viability occurs at different stages of fruit development: ‘Arbequina’ 2 weeks before the end of phase (I), ‘Verdial de Huévar’, during phase (II), and ‘Manzanilla’ and ‘Gordal’ varieties, in phase (III). Embryo viability stage was contrasted with some fruit maturity features.

THE ROLE OF DITTRICHA VIScosa (ASTERACEAE: COMPOSITAE) AS A RESERVOIR FOR NATURAL ENEMI ES IN OLIVE GROVES
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ESTACIÓN EXPERIMENTAL DEL ZAIDÍN (EEZ-CSIC), ENVIRONMENTAL PROTECTION, SEPIA, JUNTA DE ANDALUCIA

Dittrichia viscosa, a common plant in the Mediterranean region, could potentially be used in integrated pest management due to its entomophagous characteristics. This plant species, which is historically associated with olive groves, is being used as a host plant to the parasitoid complex of Bactrocera oleae (Diptera: Tephritidae), a major olive pest. The aim of this study is to investigate the presence of other arthropods and resources that this plant is capable of supplying to them. The phenology of D. viscosa was monitored in an olive orchard located in the province of Jaén, while the arthropods were sampled using a suction technique before and after the flowering period. The plant started to sprout at the end of March, and flowering occurred from mid-August to the end of October. During the study period, mirids, parasitoids, ants, and spiders were the most frequently occurring taxonomic groups. Adults and nymphs of mirids were predominant prior to flowering, while populations of their potential prey were limited. Given the omnivorous nature of some species of the Miridae family, they may have used D. viscosa as a common plant in the Mediterranean region, possibly due to the supply of other resources such as pollen, nectar and alternative hosts like the larvae of Myopites styliata (Diptera: Tephritidae).
In this work, we investigated the in vitro antifungal activity of two leaf extracts obtained from Olea europea plants and commercial oleuropein, both encapsulated in chitosan-tripolyphosphate nanoparticles and in solution, against the germination and development of Fusarium proliferatum. High performance liquid chromatography (HPLC) testing is performed to evaluate the relative concentration of bioactive molecules in the Olea europea leaf extract. The antifungal effect was compared against Fusarium proliferatum strains by in vitro assays, looking at a number of different concentrations and preparations of phenolic compounds, both as such and encapsulated in chitosan nanoparticles. The aim was to individuate the most appropriate formulation between two leaf extracts and phenols-encapsulated chitosan nanoparticles to develop a safe, stable and efficient drug delivery system. The nanof ormulation of chitosan requires less fungicide and hence environmentally friendly method for the control of phytopathogenic fungi. The phenols/nanoparticles showed greater efficacy at higher concentration when compared to extracts pure or commercial oleuropein against target species. Using such these biofungicides in a replacement for synthetics or in combination with other established disease management practices could help control Fusarium proliferatum in a more sustainable and eco-friendly way.

EVALUATION OF EFFECTS OF SPILOCAEA OLEAGINA DISEASE AND FUNGICIDE TREATMENTS ON OLIVE VEGETATIVE-REPRODUCTIVE ACTIVITIES

FRANCO FAMIANI, TOMMASO FRIONI, LEEN ALMAI, ANDREA PAOLETTI, ADOLFO ROSATI, CHIARALUCE MORETTI, ROBERTO BUONAURIO
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Peacock eye is an olive disease which causes defoliations, but little information is available on the quantification of the effects of the disease on yield. Copper compounds represent the most used fungicides to control peacock eye. From some years, also dodine is used. The aims of this study were to evaluate the effects of peacock eye disease on the vegetative and reproductive activities of the trees and the efficiency of copper compounds or dodine in controlling the disease. The trial was carried out in central Italy in an olive orchard constituted of adult trees of the cultivar Moraiolo, spaced 5 x 5 m. Not treated trees were used as control. At the beginning of the trial about 15% of the leaves were symptomatic and about 50% of the asymptomatic ones were infected (NiOH test), for a total of 65% of infected leaves. The percentages of symptomatic leaves and of fallen leaves remained constant up to the beginning of March. Then the percentage of symptomatic leaves remained almost constant on trees treated with dodine and increased up to 40-50% on control and trees treated with copper. The percentage of fallen leaves increased in all trees, but control and trees treated with copper showed a much higher defoliation. Trees treated with dodine had the highest number of flowers/inflorescences per node which were also the heaviest. Fuit set was similar in all treatments. Trees treated with dodine showed the highest yield. The olive oil content was similar in all treatments.

SCREENING OF BACTERIAL ISOLATES RELATED TO OLIVE ORCHARD INSECT PESTS IN TUNISIA AND STUDY OF THEIR BIOTECHNOLOGICAL USE

INES KSENTINI, HOUDA GHARSAHALAH, NOUR ABDELHELDI, SOUROUR NAAYMA, CHRISTINA SCHUSTER, KARAMA HADJTAIEB, MARYAM SAHNOUN, MOHAMED ALI TRIKI, MOHIEDDINE KSANTINI, ANDREAS LECLERQUE
OLIVE TREE INSTITUTE OF SFAX IMPROVEMENT AND PROTECTION OF GENETIC RESOURCES OF THE OLIVE TREE LABORATORY

This study investigated the bacterial diversity of dead olive insect pests collected from Tunisian olive orchards. In this context, we aimed to explore metabolic diversity, screen enzymatic activities for biotechnological applications and carry out preliminary bioassays for bio-insecticide development.

A total of 50 bacterial strains were randomly isolated from 4 different biotypes situated in 5ax region (Tunisia). 16S ribosomal RNA gene sequences of these isolates showed close similarities between twenty-four bacterial species within the genera Alcaligenes, Bacillus, Brevundimonas, Lyssinibacillus, Myroides, Pseudoalcaligenes, Proteus, Providencia, Serratia, and Staphylococcus. The genus Bacillus was significantly the most prevalent in the 4 biotypes with p<0.05. Specific enzyme screening was assayed on agar plates for amylolytic, proteolytic and lipolytic activity. These latter were specifically secreted by Bacillus comparing to the other genera with significant differences. Our bacterial collection was also evaluated for antimicrobial potential against bacterial and fungal isolates. Bacillus subtilis B8-2 showed strong antibacterial activities and Bacillus licheniformis B2-3 as well as Serratia marcescens B10-1 showed the highest antifungal activity. The study of isolates’ insecticidal effects on second instar larvae of the facultative host Ephesia kuenhellei (Lepidoptera: Pyralidae) showed that 12.5% among them caused a percentage of mortality superior to 70% such as Providencia vermicolor B20-1 which causes 100% of mortality.

In conclusion, the selected species from olive orchards represent an antibacterial and antifungal broad-spectrum and could be considered promising sources with potential enzymatic activities and insecticidal effect, which could be used for future applications of industrial interest for biotechnological control.
The present study aims to determine the impact of weed management in Tunisian olive trees on diversity and density of Phytoseiidae and to assess dispersal of Phytoseiidae between trees and weeds in four modalities of weed management corresponding to the most used in Tunisia. Samples were collected weekly from October 2016 to October 2017. They were composed of 50 branches on trees per locality and all the weeds of the inter-rows included in a quadrate of 30 x 30 cm were haphazardly defined. The results provide informations on how weed could be managed to ensure predator occurrence and efficiency. Positive correlations were observed between Phytoseiidae densities on inter-rows herbaceous plants and on olive trees. Correlations were observed between Phytoseiidae densities on inter-rows herbaceous plants and on olive trees. The consumption of different pests such as olive Psyllid (Euphyllura olivina COSTA) and eriophyids mites (Acari, Eriophyidae) as a prey that are still lacking in the literature influences certain biological characteristics and the predatory capacity of Orus leavigatus (Fiber, 1860) (Heteroptera, Anthocoridae). Eggs of Euphosia kuehniella (Zeller, 1879), adults and nymphs of olive Psyllid and eriophyids mites of olive were used as prey and were provided daily for all the mobile stages of the predator.

We inferred from the results that the type of prey, offered to our predator, affects the development time of the nymphal stage which is 4.70 days higher than that of the Psyllid. These data can be used to adjust food supply in a rearing system to the requirements of each developmental stage for a biological control. The work involves i) design of specific primers for each target virus; ii) studies on the sensitivity of the technique and primers specificity; iii) validation of the technique especially since its damage, with the exception of widespread infestation, is often masked by self-regulation processes specific to the olive tree. Three distinct methods have been adopted for the assessment of losses caused by the olive psyllid: fall of flowering inferences and reduction of fruit set in the region of Sfax during 6 years. These methods are respectively based on the comparison between treated and infested branches:

- On the same trees (during 2 years),
- Between treated and infested plots (during 2 years),
- Between branches with different degrees of infestation and treated branches (during 2 years).

The results showed that damages caused by the psyllid are more important when the rate of infestations/infestation as well as the larval density per infestation examined are high. Fruit set losses reach 35% when the infestations in the first year exceed 70%, which corresponds to a density of 4 to 5 larvae per examined infestation. For infestations close to 50% and for an average density of 2 larvae per examined infestations, the statistical analysis showed no significant effect of the psyllid, neither on the fall of infestations nor on fruit set.

On the other hand, a highly significant linear regression was established between the fruit loss and the examined infestences larvae density.

Finally, the methodological approach based on the comparison between differently infested branches and treated branches could be used as a reliable and rapid method for estimating the pest’s damages on the olive tree.

**The Effects of the Consumption Rate of Three Preys on the Biological Features of Orius leavigatus (Heteroptera, Anthocoridae)**

MOHIEDDINE KSANTINI

**Olive Tree Institute Tunisia Laboratory of Olive Genetic Resources: Characterization, Valorization and Phytophardinary Protection**

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**Methodological Development for the Estimation of Losses Due to the Olive Psyllid Euphylluraolivina Costa (Homerptera, APhalari)**

MOHIEDDINE KSANTINI

**Olive Tree Institute Tunisia Laboratory of Olive Genetic Resources: Characterization, Valorization and Phytophardinary Protection**

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Finally, the methodological approach based on the comparison between differently infested branches and treated branches could be used as a reliable and rapid method for estimating the pest’s damages on the olive tree.
T04-P10
SPATIAL AND TEMPORAL VARIATION OF ENDOPHYTE COMMUNITIES ASSOCIATED TO THE PHYLOSPHERE OF OLIVE CULTIVARS

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Fungal endophytes are micro-organisms that colonize healthy plant tissues without causing disease symptoms. They are described as plant growth and disease resistance promoters and have shown antimicrobial activity through the production of antagonistic bioactive substances. Studies combining spatial and temporal distribution of endophytic communities in terms of abundance and diversity in the phyllosphere of olive cultivars have been poorly explored. Thus, this study aims to investigate the abundance and diversity of endophytic communities at three sampling seasons and sites, in the Alentejo region south of Portugal. Additionally and because of the impact of some widespread fungal species (e.g. Colletotrichum spp.) vary according to olive cultivars; Garela vulgar, C. abricia, and A. nana, with different degree of susceptibility, were sampled. The 1868 fungal isolates obtained belong to 48 OTUs representing 31 fungal genera. Autumn season and cultivar Garela vulgar showed the significant highest values in terms of endophytic abundances and diversities, the mainly dominant 150+ in this species of the community. At the site level a low variability was found in terms of diversity and abundance of the endophytic communities. This study reinforces the importance of exploring the combined spatio-temporal distribution of the endophytic biodiversity in different olive cultivars, highlighting the limited information on isolation and characterization of endophytes from important cultivars and the need for more detailed analyses in this field. Knowledge about endophytic communities may help to better understand their functions in plants hosts, such as the ecological dynamics between pathogenic fungi and beneficial colonizers, which could be explored as biocontrol agents in the future.

Acknowledgments

This work was supported by the project ‘Integrated protection of the Alentejo olive grove. Contributions to its innovation and improvement against its key enemies’ with the reference AL20-03-0145-FEDER-000029, co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo). This work is also funded by National Funds through FCT – Foundation for Science and Technology under the Project UID/AGR/00115/2013.

T04-P11
DIFFERENCES IN VIRULENCE AND SENSITIVITY TO FUNGICIDES WITHIN COLLETOTRICHUM ISOLATES CAUSING OLIVE ANTHRACNOSE

ANA LÓPEZ MORAL, CARLOS XAVIER, AZAHARA ESCALONA, ANDRÉS VENANÇIO, VIOQUE MAYNEZ, CARLOS AGUSTÍ BRISACH, JUAN MORAL, ANTONIO TRAPIO CASAS
UNIVERSIDAD DE CÓRDOBA AGRONOMÍA

Anthracnose is among the most important foliar and fruit diseases affecting Mediterranean woody crops, including olive and almond. This disease is caused by a wide diversity of fungal species belonging to the Colletotrichum acutatum and C. gloeosporioides complexes. Pathogenic specialization and sensitivity to fungicides of Colletotrichum isolates have traditionally been used for the intra- and inter-specific identification within this genus. Previous studies demonstrate that Colletotrichum spp. causing olive and almond anthracnose showed significant differences in virulence between species, independently of their host of origin. The objectives of this work were to elucidate the effect of the diversity of Colletotrichum spp. on the infection of olive and almond fruit and on the sensitivity to fungicides. To achieve the first objective, olive and almond fruit from several commercial cultivars were inoculated with different isolates of C. acutatum, C. gloeosporioides, C. odoratus and C. nymphaeae. Significant differences in virulence between isolates were detected, although host specialization was not observed, with the exception for C. acutatum isolates from almond which showed lower virulence on almond than on olive. The sensitivity to copper compounds, protectant and systemic fungicides was evaluated by in vitro sensitivity tests and by bioassays on apple fruit. Tuberconazole was the most effective fungicide against all the isolates tested. Differences in sensitivity to several fungicides were observed between Colletotrichum spp., as well as between isolates within the same fungal species depending on the host of origin. These results are relevant for the control of the disease.

T04-P12
EVALUATION OF THE SILOLVE WILD OLIVE COLLECTION FOR RESISTANCE TO VERTICILLIUM DHALIAE

PABLO DIAZ RUEDA, NEVES CAPOTE, ANA AGUADO, LAURA ROMERO CUADRADO, CARLOS CARRASCOSA, JOSE MANUEL COLMENERO FLORES
1 INSTITUTO DE RECURSOS NATURALES Y AGRIBIOLOGÍA DE SEVILLA (IRNAS), CSIC; PLANT BIOTECHNOLOGY; “HAFAR ELAS TORRES”, JUNTA DE ANDALUCÍA SUSTAINABLE CROP PROTECTION

In recent years, the olive cultivation model has undergone a change from the traditional cropping model under rain-fed regime to intensive and super-intensive olive groves subjected to fertilization and irrigation. This has enhanced the prolification of soilborne fungi like Verticillium dahliae causing Verticillium wilt, currently the most threatening disease for olive crops in Spain due to the rapidity of its extension and the severity of its damage. Moreover, the most widely planted olive cultivars (Picual, Hojiblanca and Arbequina) are susceptible or very susceptible to the disease, especially under intensive conditions. We aim to take advantage of the genetic variability present in the wild subspecies of Olea europea to identify and characterize wild olive genotypes with a high resistance to the verticillium wilt disease. The SILOLVE collection consists of 149 genotypes from all known subspecies of Olea europea described so far, including the subspecies: europaea, laperrines, cupidita, cercosorium, guanchica and maroccana. These genotypes were prospected from world olive germplasm collections (Córdoba and Marrakesh) and different regions of Spain, continental Africa and the Macaronesian archipelago. Susceptibility to V. dahliae of 56 wild olive genotypes has been evaluated under controlled conditions inoculating six-month-old olive plants with a cotton defoliating isolate of V. dahliae (V117). After root inoculation of the fungus (107 conidia ml-1), quantification by qPCR at 35 and 120 days after inoculation, and analysis of symptoms in aerial organs, three genotypes showed high resistance/tolerance indexes. These genotypes can be used as high tolerance rootstocks as a control strategy for the growth of commercial and productive olive cultivars.

T04-P13
FUNGAL VASCULAR DISEASES ASSOCIATED WITH BRANCH DIEBACK OF OLIVE IN ANDALUSIA REGION (SOUTHERN SPAIN)

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UNIVERSIDAD DE CÓRDOBA AGRONOMÍA

Branch dieback of olive has been traditionally associated with Neofusicoccum mediterraneum in Andalusia region (southern Spain). Previous studies suggested that there are other fungal species affecting vascular tissues which could be also associated with this syndrome in Spain. However, these fungal species have not been described yet in Spain causing branch dieback of olive. Thus, in this study we characterize a collection of eight fungal isolates recovered from olive trunk samples showing internal discoloration and necrotic xylem vessels. Colony colour, mycelial growth, conidial characteristics and conidial production were defined on PDA, MEA and OA. Phenotypic characteristics and conidial production varied markedly depending on the isolated and culture media evaluated. The effect of temperature on mycelial growth was evaluated on PDA. The isolates show low mycelial radial growth (0.7-2.0 mm/ day) and the optimum temperature ranged from 23.1 to 30.6°C. Phylogenetic analyses of ribosomal genes (ITS) and the functional protein regions ACT and TUB were performed to confirm their identification. The species Acremonium sp., Cadophora lutea-olivacea, Paracremoneum inflata, Phaeoacremonium alvei, Phaeoacremonium chlamydospora collected from grapevines showing decline symptoms were included in the pathogenicity testing for comparative purposes. This work is relevant because it represents the first study on aetiology of fungal vascular diseases associated with branch dieback of olive in Andalusia region.

Olive Management, Biotechnology and Authenticity of Olive Products
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ABSTRACTS OF POSTER COMMUNICATIONS

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Traditionally, scab (OS), anthracnose (OA) and cercospora leaf spot (OC) have determined the fungicide applications in olive, mainly based on copper products. Nowadays, re-emergent olive diseases, such as olive leprosy (OL) and olive knot (OK), should be also considered. To check the effect of a reduction of fungicide applications, 16 olive orchards were selected along the south of Iberian Peninsula for the period 2012-2016. Selected orchards included one conventional (decided by the farmers) and two designed by the authors (based on scientific knowledge in olive). The use of copper-based fungicides was even decreased in the 9.1-8.3% of the orchards thanks to the reduced favorability for disease development. Three fungicide application strategies were tested in each of the orchards: one conventional (decided by the farmers) and two designed by the authors (based on scientific knowledge in olive diseases). Incidence of OS, OA and OC were annually evaluated and the effect of the volatile organic compounds (VOCs) on the mycelial growth of V. dahliae was evaluated using the microorganisms isolated from GC. All compost teas, extracts and microorganisms were also tested for their growth of the pathogen, reaching 100% inhibition in some cases. GC significantly reduced the disease progress both in vitro and under controlled and semi-controlled conditions, where it was also effective on the reduction of inoculum density in soil. No significant differences were found for all the physiopathological parameters and seedling growth when comparing each pair of reciprocal crosses. However, there were differences when we compared the controls (Picual and Frantoio) in open pollination. These results suggest that there is no maternal or paternal effect in the inheritance of resistance to V. dahliae and therefore using a resistant genotype as either female or male parent should be decided.

Verticillium wilt, caused by the fungus Verticillium dahliae, is currently one of the most devastating disease of the olive tree, causing important losses in producing countries worldwide. The use of genetic resistance is likely the most efficient, economically convenient and environmentally friendly control method to fight the disease as well as a fundamental part to develop an integrated management strategy. However, little is known about possible maternal effects influencing the heritability of olive resistance to this disease. To advance in this line, we conducted five crosses: ‘Picual’ x ‘Frantoio’, ‘Arbosana’ x ‘Koroneiki’, “Sikitita x ‘Arbequina’ and their respective reciprocal ones were performed in the spring of 2016. In addition, fruits of ‘Picual’ and ‘Frantoio’ in open pollination were collected and used as controls. In October, the fruits were harvested, the seeds germinated and after six weeks the seedlings were inoculated with a highly virulent V. dahliae isolate. Diseases symptoms and seedling growth were evaluated every one and two weeks, respectively.

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Abstracts of Poster Communications

Olive Management, Biotechnology and Authenticity of Olive Products

Santiago / October 15th-19th 2018

OLIVE BIOTEQ ’18

T04-P18

THE CHALLENGE OF OLIVE DISEASE MANAGEMENT IN TEMPERATE HUMID CLIMATE REGIONS

CAROLINA LEONI, YESICA BERNACHINA, PAULA CONDE-INVANORATÓ, JUAN M. MORAL, ANTONIO TRAPERÓ, DIEGO BARRANCO, CONCEPCIÓN MUÑOZ-DÍEZ, CARLOS TRAPERÓ, LUIS RALLO

INSTITUTO NACIONAL DE INVESTIGACIÓN AGROPECUARIA (INIA) PROGRAMA NACIONAL DE INVESTIGACIÓN EN PRODUCCIÓN FRUTÍCOLA

Traditional olive production is found in Mediterranean climate regions, which is characterized by a mild winter with few precipitations and at least two dry months during the summer with intense sun radiation and temperatures above 22°C. In the last decades, worldwide olive production had expanded to new regions. Particularly in Uruguay, South America (30° - 35° S; 53° - 58° W), the olive crop area reached 10,000 hectares in the last 15 years and is increased to produce extra virgin olive oil (EVOO). The main cultivars are ‘Arbequina’ (50%), followed by ‘Picual’, ‘Coratina’, ‘Frantoio’, and ‘Lecino’ (all around 40%). Because of its temperate humid climate (mean precipitations above 1200 mm per year but unequally distributed), mild winters and warm summers with mean temperatures of 13°C and 22°C, respectively, disease management is one of the foremost challenges for olive production. The main diseases are Anthracnose (Colletotrichum sp.), Scab (Venturia inaequalis), and Cercospora leaf spot (Pseudocercospora cladosporioides). To achieve good productivity and EVOO standards while minimizing environmental impacts, an integrated strategy is proposed combining planting design, olive cultivar combination, irrigation and soil management, plant training and pruning, disease scouting and chemical control. However, more local research is needed, mainly focused on cultivar x disease x environment interactions, to improve current management practices and to address emerging problems.

T04-P19

REDUCING SPRAY DRIFT IN TRADITIONAL AND INTENSIVE OLIVE CANopies THROUGH THE DEVELOPMENT OF NEW AIR-ASSISTED SPRAYERS

ALBERTO GOODY-NIETO, ANTONIO MIRANDA-FUENTES, ANTONIO RODRÍGUEZ-IZUANA, EMILIO J. GONZÁLEZ-SÁNCHEZ, JUAN LUIS GAMARRA-DIEZMA, GREGORIO L. BLANCO-ROLDÁN, JESUS Á. GIL-RIBES

UNIVERSIDAD DE CORDOBA INGENIERÍA RURAL

Airborne pesticide applications in olive orchards are difficult to perform due to the particular characteristics of the trees and the lack of specificity of the spraying machinery. Thus, a new air-assisted sprayer was developed and tested in real field conditions to check its performance against a conventional sprayer, assessing their spray deposition on the canopy, leaf coverage and drift. The trial was carried out in traditional and intensive orchards in a commercial farm. Five trees were sprayed per plantation system with Tartrazine. The tree volume was estimated with manual methods. The spray deposition and coverage were evaluated with artificial collectors Ø filter paper and water sensitive paper (WSP) pieces, respectively and the spray drift was quantified by placing 15 cm-diameter Petri dishes on the ground. A total of 16 filter paper and 32 WSP pieces were placed per each single tree, and six blocks of Petri dishes were placed per single treatment, on both sides of the sprayed trees at two rows distance. The results showed that the prototype significantly reduced the spray drift in both systems: 52.7% and 57.0% in the traditional and in the intensive system, respectively. These results are promising for indicating a potential for reducing the drift risk associated to the operation.

T04-P20

INOCULUM MANAGEMENT THROUGH IRRIGATION SYSTEM TO REDUCE VERTICILLIUM WILT OF OLIVE

DOLORES RODRÍGUEZ-JURADO, ANTONIO SANTOS-ROJO, FRANCISCO JESÚS GÓMEZ-GÁLVEZ, JUAN-CARLOS HIDALGO-MOYA, JAVIER HIDALGO-MOYA, VICTORINO VEGA-MACÍAS

IFAPA SUSTAINABLE CROP PROTECTION

Inoculation tests were done under controlled conditions to compare the efficacy of OX-Virin® and OX-Agua AL25® with conventional treatment. Inoculum of V. dahliae was applied to olive plants ‘Picual’ (susceptible cultivar) during spring. The development of Verticillium wilt was evaluated for one year. In order to achieve the second objective, potted soil was drip-irrigated with infested water, untreated or treated at three concentrations of OX-Virin® or OX-Agua AL25® through irrigation system. Inoculum density in soil was estimated over time. Drip-irrigation at about 10 million conidia per milliliter caused the highest disease incidence in olive during spring. At this V. dahliae concentration in irrigation water, both disinfectants reduced the inoculum density in soil by over 99%. OX-Virin® at 12.8 and 51.2 ml L-1 concentrations and OX-Agua AL25® were applied by means of drip-irrigation to potted olive plants ‘Picual’ (susceptible cultivar) during spring. The development of Verticillium wilt was evaluated for one year. In order to achieve the second objective, potted soil was drip-irrigated with infested water, untreated or treated at three concentrations of OX-Virin® or OX-Agua AL25® through irrigation system. Inoculum density in soil was estimated over time. Drip-irrigation at about 10 million conidia per milliliter caused the highest disease incidence in olive during spring. At this V. dahliae concentration in irrigation water, both disinfectants reduced the inoculum density in soil by over 99%. OX-Virin® at 12.8 and 51.2 ml L-1 concentrations and OX-Agua AL25® were applied by means of drip-irrigation to potted olive plants ‘Picual’ (susceptible cultivar) during spring. The development of Verticillium wilt was evaluated for one year. In order to achieve the second objective, potted soil was drip-irrigated with infested water, untreated or treated at three concentrations of OX-Virin® or OX-Agua AL25® through irrigation system. Inoculum density in soil was estimated over time. Drip-irrigation at about 10 million conidia per milliliter caused the highest disease incidence in olive during spring. At this V. dahliae concentration in irrigation water, both disinfectants reduced the inoculum density in soil by over 99%. OX-Virin® at 12.8 and 51.2 ml L-1 concentrations and OX-Agua AL25® throug

T04-P21

NATURAL BIOHERBICIDE, 3,4-DIHYDROXYPHENYLGLYCOL FROM OLIVE OIL BYPRODUCTS.

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The phytoregulator effect of two of the most important phenols present in the alperujo, hydroxytyrosol (HT) and 3,4-dihydroxyphenylglycol (DHFG), separately and in their combined form, has been studied. Germination tests of certain species of horticulture were carried out by sprinkling the three extracts to be studied and it was observed that in pre-emergence the extract of DHFG shows positive behavior as a phytoregulator, since it has a high response as a germination activator and growth at low concentrations compared to the other extracts. It was shown that DHFG is an effective phytoregulator for the treatment of crops and weeds, which activates the germination of seeds enhancing the agricultural crops while eliminating the weeds, sometimes at very low doses. While in the post-emergence and late emergence studies the phytoregulator power is no longer appreciated. Studies of stability of the DHFG were carried out against germination, obtaining positive results when not degraded. On land it was found that the DHFG penetrates to the levels of the seed would be placed independently of the solar radiation. On the other hand, the extracts present the acceptable fog distribution and using adjuvant agents such as Biopower and paraffin. The active pheno-nolic compound responsible for the phytoregulation has been obtained from by-products of the olive oil and table olive industry, which contributes to the recovery of these by-products.
MOLECULAR AND PHYSIOLOGICAL DEFENSE RESPONSES OF OLIVE TREE CULTIVARS TOWARDS VERTICILLIUM DAHLIAE

INES KSENTINI, MOHAMED ALI TRIKI, YAAKOB GHRABI, FATHI BEN AMAR, EMNA BOUAZIZI, CHEFFI MANEL, RADHOUANE GDOWA

New olive cultivars adapted to Tunisia's growing conditions were examined for their resistance to Verticillium wilt (VWO) in order to determine whether differences in susceptibility among currently grown cultivars might contribute to the management of this disease. Based on the evaluation of 14 cultivars, ten were classified as susceptible or extremely susceptible (Chetoui, Chemlali, Rkhami, Jarboui, Zalmati, Jarboui, Oueslati, Manzanille, Picholine and Frantie), two as moderately susceptible (Koroneiki and Coratina) and two as resistant (Mesi and Sayali) to VWO. Three cultivars with different susceptibility level were selected to examine the levels of hydrogen peroxide (H2O2), soluble sugars (SS), soluble proteins (SP), total polyphenols (TP), lipid peroxidation, activities of antioxidant enzymes, and fungal biomass in planta. V. dahliae DNA occurred earlier and at a higher concentration in the resistant cultivar. Similarly, the ERF...bHLH-binding factor responsive to JA was recorded in a strong co-up-regulation of jasmonate ZIM domain and protein, PLD and WRKY transcription factor was higher in the resistant cultivar as compared to the susceptible one. In addition, early and simultaneous up-regulation of chitinase and β-1,3-glucanase genes is correlated with reduced susceptibility and wilt symptoms in the resistant cultivar. The expression level of genes coding for PR10 protein, PLD and WRKY transcription factor was higher in the resistant cultivar, which indicates that induction of the SA pathway is essential for conferring resistance against V. dahliae and reduction of wilt symptoms. Furthermore, comparison of the SA-related genes expression patterns with those followed by the H 2 O 2 burst, chitinase and β-1,3-glucanase genes, revealed that high SA level and H 2 O 2 production, together with enhanced PR proteins activity are a key factor for resistance. As JA-related genes, a strong co-up-regulation of jasmonate ZIM domain and bHLH-binding factor responsive to JA was recorded in both cultivars, except that the extent of this induction was higher in the resistant cultivar. Similarly, the ERF...
The effect of salinity on fatty acid composition and desaturase genes expression was investigated in Leccino fruit mesocarp. Fruits were sampled and subdivided into four maturation groups (MG0, MG1, MG2, MG3) according to ripening degrees. No accumulation in fruit mesocarp reached the highest values in MG1 salt treated fruits (2.214 mg kg⁻¹ DW), decreasing with the ripening process. Total fatty acids content was not significantly altered by irrigation with saline water ranging from 86.9 to 110.4 µg mg⁻¹ FW. However, a significant increase of 5% in oleic acid at MG1 and MG2 compared to control was observed. The polyunsaturated/monounsaturated fatty acid desaturase genes expression data suggested that polyunsaturated/monounsaturated fatty acid desaturase genes expression data suggested that polyunsaturated/monounsaturated fatty acid desaturase genes expression data suggested that polyunsaturated/monounsaturated fatty acid desaturase genes expression data suggested that oleic and linoleic acid modifications in treated fruits could be related to down-regulation of OleFAD6 transcript levels.

Leaf photosynthetic rate and water loss are both regulated by temperature via stomatal opening, and global warming could affect these coupled processes in olive trees. Additionally, leaf characteristics including stomatal density can vary according to the environment in which they develop. Thus, our objectives were to: i) evaluate the response of leaf photosynthetic gas exchange and tree sap flow to elevated temperature in two olive cultivars; and ii) determine the density of stomata and trichomes in leaves acclimated to two temperature regimes. Two temperature levels were applied in the summer and fall of two consecutive growing seasons in open top chambers: a control (T0) near air temperature and a treatment 4°C above the control (T+). Two to three year-old olive trees (cvs. Arbequina, Arbosana, Coratina, Picual and Hojiblanca) were grown in pots and well-watered throughout the experiment. The leaf photosynthetic gas exchange was measured monthly, and tree sap flow was measured twice during the second growing season. Stomatal and trichome density were determined for leaves acclimated to either temperature regime. The leaf photosynthetic rate showed no significant response to elevated temperature, but increases in leaf transpiration, stomatal conductance, and sap flow were consistently observed in both cultivars under elevated temperature. Water use efficiency was significantly reduced in the heated trees. Leaves of T+ showed some minor changes in stomatal density and trichome density. However, the differences were not consistent between growing seasons. The results suggest that elevated temperature with global warming may increase crop water consumption in well-irrigated orchards.
Abstract

The Mediterranean basin is the largest area in the world with a specific climate for olive (Olea europaea L.) cultivation. However, the environmental conditions of this region are expected to change in the near future. In particular, air temperature could increase drastically. It is no clear how global warming will affect olive yield and fruit maturation. To clarify these aspects, a field study with two cultivars, 'Picual' and 'Arbequina', is being carried out in a Mediterranean climate type area. Trees are being subjected to warmer temperature than ambient throughout the complete reproductive cycle using temperature controlled Open-top-Chambers (OTCs). Each OTC, equipped with heating and ventilation devices, can maintain permanently a day/night temperature gradient between the tree and the surrounding environment of 4 °C. After two years of study, the preliminary results have shown that the exposure of trees to 4 °C above ambient temperature forwards the maturation time and prolongs its duration. The temperature treatment reduced yield in both cultivars and affected maturation time and prolongs its duration. The temperature treatment reduced yield in both cultivars and affected differently fruit characteristics. Smaller fruits, lower pulp/stone ratio, oil yield and anthocyanin contents, were observed in 'Picual'. On the contrary, in 'Arbequina' fruit size and pulp/stone ratio were unaffected by temperature, but it reduced the content of total polyphenols.
The olive tree Chemlali, one year old, is subjected to two salt levels for eventual correction. The olive tree seems to have an exceptional natural adaptation system to overcome severe stress levels. Nevertheless, the correction used allowed an improvement of its adaptation to salt stress, which depends on the organ, the tissue and the level of stress. The correction allowed the development of the protective tissues of the upper face of the leaf, the development of the filling tissue such as cortical parenchyma in all the organs of olive tree, the development of the supporting tissue such as collenchyme in the stems, and pericyclic fibers in all organs, especially in severe stress. A large change in the proportion of olive tissues subjected to salt stress is noted, which is accentuated by the correction, namely the development of the palisade parenchyma and the narrowing of the lacunous in the mesophyll and development of the liber at the expense of the wood in all the organs of the tree.

Under the current and predicted challenging environments urge the necessity to improve olive orchards sustainability. The use of kaolin, a reflective clay, has been proposed in this sense but its effectiveness largely depends on stressful conditions. To achieve a more comprehensive response about kaolin action we performed three different experiments, in three different geographical locations of Northeast Portugal with “Cobrançosa” cultivar: young potted trees, subjected to three drought-rewatering cycles, established commercial olive trees under rainfed and also in sustained deficit irrigation (27.5% of ETc) conditions. Generally, the results confirm the kaolin effectiveness to improve water status, in a closely association with lower nighttime water loss, to enhance photosynthetic capacity, due to inferior stomatal and non-stomatal limitations, to reduce oxidative damages and to induce shade-related leaf characteristics, namely in the cloudy and rainy year. Still, some differences were recorded among experiments. Whilst in pots, leaf gas exchange restoration was delayed during the recovery events, under field conditions such response was accelerated after the first rains, persisting during the early winter months in the coldest place. The final biomass accumulation was not affected in pots, while crop yield increased 97% and 55% in rainfed and deficit irrigation experiments, respectively. Taken together, the results demonstrate that kaolin is an efficient tool to apply in commercial orchards under Mediterranean environments, benefiting both rainfed and sustained deficit irrigation production systems.

Keywords: Adaptation measures; Climate change; Deficit irrigation; Drought; Recovery.

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CLIMATE CHANGE EFFECTS ON OLIVE - MITIGATION AND ADAPTATION PRACTICES

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Olive growing may play a very important role in climate change mitigation through adoption of proper farm management. In the first part of the present work, results of long term studies on the effects of abiotic stressors such as high or low temperature, water deficit and salinity, enhanced UV-B radiation and nitrogen deficiency on physiological and reproductive functions for the major Greek olive tree cultivars are presented. Shoot growth was markedly reduced in high salinity dose in ‘Amphissis’ (-81%) and ‘Koroneiki’ (-75%). The highest temperature treatment (40 °C) prevented pollen germination. A significant effect of irrigation period (October - March) (+40%) as compared to the irrigation period (April - September). Different experimental sites, located in Piemonte and Friuli Venezia Giulia and Lombardia and maintaining common varieties for comparison, were involved in the experimental work. All the olive germplasm collected and propagated was preliminary characterized using a set of 6 microsatellite molecular markers. Cold damages were registered in two periods: in April and in June using a numerical range to define the entity of damage. Bloom ing and ripening time were recorded in 2017 and 2018. A lot of cultivars and local old accessions genetically analyzed clustered within the ‘Frantoio’ group confirming the wide spread of this genotype. Most of them showed also the same agronomic behavior against Burian. Preliminary results about a varietal screening for cold tolerance are here discussed.

APPLICATIONS OF GAS EXCHANGE ANALYSIS OF PHOTOSYNTHESIS AND REFLECTANCE INDICES TO OLIVE PHENOTYPING

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Olive growing may play a very important role in climate change mitigation through adoption of proper farm management. In the first part of the present work, results of long term studies on the effects of abiotic stressors such as high or low temperature, water deficit and salinity, enhanced UV-B radiation and nitrogen deficiency on physiological and reproductive functions for the major Greek olive tree cultivars are presented. Shoot growth was markedly reduced in high salinity dose in ‘Amphissis’ (-81%) and ‘Koroneiki’ (-75%). The highest temperature treatment (40 °C) prevented pollen germination. A significant effect of irrigation period (October - March) (+40%) as compared to the irrigation period (April - September). Different experimental sites, located in Piemonte and Friuli Venezia Giulia and Lombardia and maintaining common varieties for comparison, were involved in the experimental work. All the olive germplasm collected and propagated was preliminary characterized using a set of 6 microsatellite molecular markers. Cold damages were registered in two periods: in April and in June using a numerical range to define the entity of damage. Blooming and ripening time were recorded in 2017 and 2018. A lot of cultivars and local old accessions genetically analyzed clustered within the ‘Frantoio’ group confirming the wide spread of this genotype. Most of them showed also the same agronomic behavior against Burian. Preliminary results about a varietal screening for cold tolerance are here discussed.

SUSTAINABLE MIDDAY STEM WATER POTENTIAL THRESHOLDS VALUES DURING PIT HARDENING ON TABLE OLIVE

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Regulated deficit irrigation (RDI) scheduling on olive trees is nowadays mandatory because of the water scarcity of the main olive zones. Water stress resistant in the phenological stages of olive trees is widely known but little has been published about the water stress levels. The aim of this work is to suggest water stress levels during pit hardening that will derive in an accuracy water management. These results are based on several experiments performed in the last 9 years in “La Hampa” the experimental farm of IRNAS (CSIC) at Coria del Rio (Seville, Spain). All the experiments were performed in a mature irrigated olive orchard from they were 37 years-old. This orchard is 7.5m distance, irrigated with 5 drips (8l/h) per tree. Irrigation scheduling consider only the period of pit hardening for water stress conditions. Water stress level was changeable according to the season from almost no differences with full irrigated Control until lower than -3.0 MPa at the end of summer. Leaf conductance was less and later affected than midday stem water potential (SWP). However, fruit growth was clearly reduced when SWP decreased from -2 MPa. Final fruit size was almost unaffected when an efficient water stress recovery was reached before harvest. The amount of inflorescence and fruit set in the next year was no affected for water stress conditions until -3 MPa. Cumulative yield and fruit drop was related with the level of water stress in order to suggest a sustainable threshold value for RDI scheduling.
ARE FRUIT DENDROMETERS RELIABLE TO SCHEDULE IRRIGATION IN OLIVE TREES?

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Fruit dendrometers have been intensively used in the study of fruit crop management but rarely as a water stress indicator despite being the fruit the main target of regulated deficit irrigation (RDI) studies. Our objective is to better understand the fruit dendrometers usefulness to assess water stress and to evaluate its use to schedule RDI in olive orchards. We compared fruit dendrometers with other sensors previously used as water stress indicator (i.e. trunk dendrometers and ZIM probes) and with traditional measurements such as midday leaf water potential (Ψl,md) to assess their applicability. All the sensors were installed in the olive trees after the pit hardening period (1st May – 1st June 2017) in plants under full irrigation (100% irrigation needs, WW) and in plants under a 45% RDI (WS). Fruit and trunk diameter growth rate (FGR and TGR, respectively) presented significant correlation with the Ψl,md for the data from the WS treatment. At the days when irrigation was applied, FGR and TGR increased, and the maximum output pressure of the ZIM probes (Pp) decreased. The TGR and Pp were firstly affected both at the beginning and at the end of the deficit irrigation period in comparison to FGR. We conclude that fruit dendrometers have potential to be a reliable method to schedule RDI, which is advanta-
geous because fruits are more directly related to final yield than the other stress indexes used. However, they cannot be used during the whole irrigation season (when there are no fruits, or they are too small).
The enhancement of water efficiency and the support of the shift towards climate resilient agriculture are very important nowadays. LIFE “AgroClimaWater” project focuses on both of these objectives through the development and implementation of Water Management Adaptation Strategies at Farmers’ Organizations in Mediterranean countries. Agriculturally adapted the Water Partnerships of the European Water Partnership (EWP) to the agricultural sector and implements it in three Farmers organizations towards the formation of this kind of strategies. The standard is based on and organized in four Principles that promote: Sustainable Water Abstraction, Good Water System Status, High Conservation Value Area Protection and Equitable Water Governance. Key elements of the strategies are the shaping-up and promotion of agricultural practices that increase water efficiency in the cultivation of perennial crops.

In this paper, preliminary results are presented from the Agricultural Cooperative Partnership “Mirabello Union S.A.” located at Havgas-Milatos sub-basin in eastern Crete-Greece enhancement. “Mirabello Union S.A.” is a Farmers’ Organization that participates in AgroClimaWater. Within Havgas-Milatos sub-basin, olive tree cultivation accounts for a significant percentage of land use (37.4% of total land area). In the context of the project, agricultural practices along with mean annual productivity, average annual water use, use of PPPs and fertilizers were monitored at 101 olive orchards in the area. According to the monitoring results, an assessment of current agriculture practices impact is performed indicating significant improvement potential. Subsequently, a wide range of good agricultural practices were identified and combined in farm specific action plans. Farm specific action plans are farm specific at improving water efficiency at the farm scale. Based on the initial assessments, it is clear that the applied practices in the selected olive orchards could be improved in many cases towards water efficiency enhancement.

Olive is the main resource for plant growth and ecosystem primary production around the world, thus soil water availability results as the main determinant of the plant physiology. That’s why; there is increasing awareness for the need to use water saving strategies to adapt to climate change scenarios. A comparative study on eco-physiological and biochemical responses under different water deficit treatments and recovery was conducted on potted olive plants.

The experiment was performed on olive plants (cv. Chemlali) in pots, under different water stress treatments followed by re-watering after 60 days. The first corresponds to 100% of field capacity, the 2nd is 50% of the field capacity (Moderate Stress) and the 3rd is 25% of the field capacity (Severe Stress). Eco-physiological (gas exchange measurements) and biochemical parameters (total sugars, Proline) were measured for young and adult leaves.

The analysis of results showed a significant difference on Net CO2 assimilation rate (A), stomatal conductance (gs) and transpiration (E) values between the Severe, Moderate water stress and control treatments especially for young leaves. Few days after re-watering the stressed plants start to establish their photosynthetic parameters and they reach the same level of the well irrigated plant. The biochemical analysis showed also significant differences between treatments before re-watering and a continuous decrease of the value of these parameters for the stressed plants after re-watering.

Better water management is challenging as a result of spatial and temporal variability in climate, coupled with seasonal changes in crop growth and development. In this context, the advent of Earth observations from satellite-based sensors, numerous recent studies have used remote sensing data for assessing drought impacts on crop water stress. A comparative study on using innovative (thermal camera and satellite imagery) and classic (gas exchange and biochemical assay) techniques under different water deficit treatments were collected in olive orchards of Tunisia.

This experimental study was conducted on irrigated olive trees (cv. Koroneiki) in semi-arid zone of Tunisia, with three water stress treatments. The first corresponds to irrigation amounts equal to 60% of ET0, the 2nd is sustained deficit irrigation applying 30% ET0, the 3rd is farmer’s conventional irrigation.

Ecophysiological (gas exchange measurements) and biochemical parameters (total sugars, proline) were measured at different phenological stages. Crop Water Stress Index was estimated using temperature of sunlit leaves. Landsat 8 satellite imagery was used for canopy and leaf estimates of biomass, temperature and crop evapotranspiration.

Results from ecophysiological measurements and biochemical analysis showed significant differences between treatments. Water stress caused increased leaf temperature, which results in an increase in thermal infrared emission. The calibration of satellite imagery to ground measurements allowed the upscaling of water stress and olive biomass estimations to broader regions in semi-arid Tunisia.
The objectives of this work were to improve the water efficiency of tree crops and save water, in a pilot farm, in eastern Crete – Greece, under proper agricultural interventions. The study is a part of LIFE+ AGROCLIMAWATER project, which aims to develop a climate change adaptation strategy for agriculture and prepare the agricultural sector for adapting to climate change. The selected pilot farm represents the most typical crop in eastern Crete (olive trees). The pilot farm has been divided in two parts, the first one is used as a control part, while the other one as the demonstration part where the interventions are applied.

The interventions applied in order to improve water efficiency are: a) reduction of water evaporation losses from soil surface (soil mulching, cover crops, etc.), b) reduction of transpiration water losses through proper pruning, c) reduction of deep percolation water and nutrient losses, d) application of organic materials i.e. plant residue, and e) irrigation according to the water crop needs. In order to evaluate the results after the 1st year of implementation of proper agricultural practices for saving water, the following performance indicators were estimated: the water use efficiency (WUE) on a fruit yield basis, the economic water productivity (EWP) and the crop Water Footprint (Blue). For the 1st year of implementation, all the above indicators performed better in the demonstration part of the olive grove compared to the control part. For instance, the WUE was 3.80 kg m⁻³ in the demonstration part and 3.40 kg m⁻³ in the control part.

Phenolic compounds present in virgin olive oil (VOO) are strongly related to its functional and organoleptic properties. The phenolic composition of VOO is determined by the phenolic composition of the olive fruit and the enzymatic reactions that take place during olive fruit processing. In this sense, in addition to genetic factors, it is well known that edafoclimatic conditions and the industrial procedures used during olive oil extraction also affect the phenolic profiles of olive fruits and oils, respectively. This study describes the effect of two water regimes, extreme rain-fed and full irrigation, on two olive varieties of high commercial value in Southern Spain, Picual and Manzanilla. We have measured the stem water potential and analyzed the main phenolic compounds present in fruits harvested and their respective olive oils samples at different maturity stages by means of high performance liquid chromatography. The phenolic composition of olive fruits has been analyzed and compared to that of the obtained olive oils. Opposite results were found among the main phenolic glycosides analyzed in olive fruits, with significantly higher levels of verbascoside but similar or lower contents of oleuropein and ligstroside found in Picual and Manzanilla fruits grown under rain-fed conditions. Global data analyses have been carried out to evaluate the correlation of the water regime with the phenolic content of VOO. The information obtained in this study could help to define the optimal irrigation conditions to improve the nutritional and organoleptic quality of VOO.

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In Morocco nutrient deficiencies are among the main factors inducing low olive and oil yields in most olive orchards. However, few studies were undertaken on the impact of fertilization on quality and olive oil composition. The Objective of this study is to evaluate the impact of each nutrient NPK used individually or in combination on olive and oil yields and oil quality. The experiment was conducted for two years using Menara variety. The experimental design used a factorial plot design with 3 replicates of two trees. A total of 8 fertilization treatments were studied (T1: Control; T2: N; T3: P; T4: K; T5: NP; T6: NK; T7: PK; T8: NPK). Treatments were applied as foliar spray fertilization with recommended amounts. Results showed significant differences among fertilization treatments for olive and oil yields. High average yield was achieved by T8 (69 kg/tree) compared to T1 (27 kg/tree). High values of 100 fruits weight were achieved by T8. Olive oil yield was assessed in the second year of the experiment. High and low oil yields were obtained under T8 (7.8% DM) and T1 (2.3% DM) respectively. Oil phenols content varied between 379 ppm for T8 and 267 ppm for T1. Oil Chlorophyll content varied between 5.98 ppm and 10.17 ppm for T1 and T8 respectively. Oil pheophytin content varied between T5 (16.31 ppm) and T8 (267 ppm) respectively. Oil Chlorophyll content varied between 379 ppm for T8 and 267 ppm for T1. Oil Chlorophyll content varied between 5.98 ppm and 10.17 ppm for T1 and T8 respectively. Oil pheophytin content varied between T5 and T8 (29.80 ppm) respectively. For fatty acids composition, compared to T1, Palmitic acid has increased under T8 and T2 and decreased under other treatments. Oleic acid has decreased under all treatments compared to T1. Linoleic and Linolenic acids were increased by all treatments and high values were achieved under T8. Contents of Mono acids were decreased by fertilizers supply compared to T1. Saturated acids were increased under T2 and T4 and T8.
**THE FATE OF CALCIUM APPLIED THROUGH FOLIAR FERTILIZATION ON OLEA EUROPEAE L.**

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Olive trees fertilization through foliar pulverization is a more targeted oriented method than the application of chemical fertilizers to the soil. Many factors influence the performance of foliar nutrients sprays such as the presence of leaf cuticle, leaf hairs and leaf surface wax. Calcium is one of the most common macronutrients applied as foliar fertilizer due to its low mobility inside the plants. The presence of calcium in olive fruits, mainly in cell walls, will improve resistance to important pest problems as well as improve fruit quality.

The aim of this study was the evaluation in ‘Galega vulgar’ olive trees the efficacy of calcium foliar applications and the subsequent redistribution of this nutrient through the different cell components. Olive trees were sprayed four times from August until veraison with a commercial product containing 34% of calcium, in three different doses. Our results confirmed that calcium applied through foliar pulverization increased calcium deposition in fruit cell wall as well as the total calcium in the fruits. It is also discussed the role of epidermal structures, the hydrophobicity of leaves surface and the presence of cuticle waxes, in the absorption of foliar applied calcium.

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**EFFECT OF SILICON ON THE INCIDENCE OF FUSICLADUM OLEAGINEUM IN THE OLIVE**

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Silicon is a non-essential element for plants, although it is considered beneficial since may prevent biotic and abiotic stresses. Little is known on the effects of silicon in plants, and nothing is known on its effect on olives. The aim of this study was to evaluate the effect of silicon applications on the incidence of olive leaf spot, one of the main diseases affecting this crop caused by the fungus Fusicladium olea-gineum. For this purpose, young olive plants of ‘Arbequina’ and ‘Picual’ were subjected to foliar or soil applications of silicon. Actisil, a choline-stabilized orthosilicic acid, was applied in both forms at concentrations of 0.1, 0.2 or 0.4%.

Six single plant replicates per treatment were used, arranged in a completely randomized design. Plants were grown for six months under greenhouse conditions applying foliar or soil treatments once or three times per week, respectively. After this time, the plants were inoculated with a conidial suspension of the pathogen. The evaluation was carried out 90 days after inoculation, attending to the incidence and severity of the disease, defined as percentage of affected leaves and percentage of affected foliar area, respectively. A disease index (DSI) was calculated and expressed as percentage. Results showed a significantly re-duction of the disease index in plants treated with silicon compared to the control. No effect of silicon concentration or application form (soil or foliar) was observed.

**ASSSESSMENT OF THE NUTRITIONAL STATUS OF OLIVE TREES ORCHARDS WITH DEVIATION FROM THE OPTIMUM PERCENTAGE (DOP) IN TUNISIA**

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Foliar diagnosis is the most used tool by technical and re-searcher to the study of the nutritional status of olive trees which is done generally by July (the standard date for olive trees. Results of this diagnosis were often compared to refer-ence value and this traditional method is denominated as the critical concentrations or sufficiency ranges. This method intends to evaluate isolated deficiency or excess values, without measuring the overall nutritional balance. However, to have a good interpretation of analytical data we should proceed to several concepts as binary relations, the DOP index (Deviation from the optimum percentage) and the DIS method. In this work we developed the DOP approach for a set of macro nutrient (N, P, K, Ca and Mg) in 27 super high density olive orchards located in four differ-ent regions in Tunisia during two years. Our results indi-cated that most of studied orchards have a severe nutri-tional disequilibrium. The disequilibrium of the nutritional balance reflected by the $\Sigma DOP$ is caused mainly by the lack of magnesium and calcium in the fertilization program of these orchards and sometimes to the excess of potassium fertilization.

Therefore, the use of DOP analysis provide further informa-tion on the nutritional balance as compared to sufficiency range and allow to know the limiting nutrient and to clas-sify nutrients from the most limiting to the optimal ones. A better Knowledge of the nutritional balance will ensure a good planning of fertilization program.

**EFFICIENCY OF FOLIAR FERTILIZATION FOR THE IMPROVEMENT OF BIOCHEMICAL FRUIT CONTENTS IN THE OLIVE CULTIVAR CHERMLALI**

**IMEN ZOUARI, MOUNA ACHI-MEZGHANI, BELIGH ME-CHRI, HASSOUNA GOUTA, HENRIQUE RIBEIRO, MIGUEL MARTINS, FAOUZI ATTIA, IMED CHERIEF, IBTISSEM LAARI-BI, KHOLOUD ANNABI, MOHAMED HAMMAMI**

OLIVE TREE INSTITUTE TUNISIA LABORATORY OF DURABILITY OF OLEICULTURE IN SEMI-ARID AND ARID REGIONS: AMELIORATION OF PRODUCTIVITY OF OLIVE TREE AND QUALITY OF PRODUCTS

Foliar fertilization in rain-fed olive orchards has been in- troduced to achieve higher yields for the olive oil industry. However few studies investigated the relationship between the nutrients and the quality parameters of olive fruits. For this purpose, the present work aimed to study the effect of foliar fertilization on the chemical composition of fruits. The foliar fertilization program consisted of the annual application of three fertilizers on Chemlali olive cultivar, cultivated under rain-fed conditions, at different stages from the vegetative cycle. The experiment has been set up in six treatments: T1 (rich in nitrogen) was applied at the start of vegetative growth, T2 (rich in boron) was applied at the beginning of flowering, T6 (a biostimulant rich in calcium) was applied during the ph-hardening, T1 +2 (com-bination of T1 and T2), T1 +2 +Ni (combination of T1, T2 and T6) and control. After two years of experimentation, fruits were harvested, freeze-dried and the following fruit traits were evaluated: fruit nutrients content, carbohydrates pro-file and individual polyphenols. Results showed differences in the mineral content of fruits between treatments. Regar-ding carbohydrates, glucose was the main sugar present in the fruits and varied between 8.8 µg/mg dry weight (d.w) and 36.43 µg/mg d.w. Changes were clearly observed in the pool of the phenolic fraction. The combined treatment T1 +2 +Ni showed the highest concentrations in Oleuropein and hydroxytyrosol and were equal to 742.6 and 112.94 µg/g of fresh weight respectively. This work showed that the fertilization management could be highlighted in order to enhance nutraceutical value of fruits.
Olive Management, Biotechnology and Authenticity of Olive Products

T07-P6

OLIVE TREE RESPONSE TO POTASSIUM APPLICATION UNDER DIFFERENT WATER REGIMES AND CULTIVARS

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INSTITUTO POLITECNICO DE BRAGA/CANO MOUNTAIN RESEARCH CENTER

Taking into account the role of potassium in plant nutrition it is poorly understood the lack of studies on olive tree response to potassium application. This paper reports the results of two field trials and two pot experiments on potassium fertilisation in olive carried out from 2013 to 2017. The two field trials and one of the pot experiments were classic experiments on the plant response to the nutrient application. The second pot experiment was arranged in a factorial design with two levels of potassium, two water regimes and two cultivars (‘Cobrançosa’ and ‘Arbequina’). The application of potassium did not increase the growth and yield of the trees although it increased the concentration of the nutrient in the tissues. The application of potassium increased the shoot/root ratio. The potassium concentration in the roots was lower than in the aerial parts for low levels of potassium in the soil but increased more than proportionally in the root relative to the shoot as the availability of potassium in the soil increased. The potassium increased the shoot/root ratio. The potassium fertilization in olive carried out from 2013 to 2017.

T07-P7

AGRONOMIC PRACTICES CHANGE THE PATTERNS OF SOIL GLOMALIN IN OLIVE RAINFOREST ORCHARDS

CARLOS CORREIA, FABRÍCIO MACHEDO, ANTONIO FREIRE, JOSÉ MOUTINHO-PEREIRA, MARGARIDA ARROBAS, ALEXANDRE GONÇALVES, MANUEL ÁNGELO RODRIGUES

UTAD CITAB

Glamolin, a thermostable hydrophobic glycoprotein produced by arbuscular mycorrhizal fungi, plays an important role in the stability of soil aggregates and in the sequestration of C, N and heavy metals, being their concentrations dependent from agronomic practices such as tillage and application of pesticides and fertilizers. Despite the recognized importance of glamolin in soil quality, studies on olive groves are scarce. The study conducted on summer 2017 in three different rainfed orchards (cv. Cobrançosa) of Northeast Portugal revealed that both total glamolin, as measured as Bradford-reactive soil protein (T-BRSP), and the easily extractable Bradford-reactive soil protein (EE-BRSP) concentrations were lower under mechanical cultivation than on a permanent sward grazed with a flock of sheep or than on an annual legume cover crop. Moreover, higher T-BRSP and EE-BRSP levels were found on orchards without phosphorous and boron supply, and also on the top soil layer (0-10 cm) and on tree row, mainly in younger orchards. Interestingly, one soil presented a twofold superior EE-BRS/P-T-BRSP ratio than the other two soils, representing an increase of labile glamolin, probably related with greater applications of copper formulations to control olive fungal diseases. This study demonstrated that less disruptive agronomic practices influence positively the levels of glamolin, an appropriate indicator of healthy soil conditions, which in turn may favour carbon sequestration.

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T07-P8

EFFECT OF AMINO ACID APPLICATION (AMINO-16®) ON FLOWERING, FRUIT SET AND YIELD OF OLIVE TREES (CV. ‘KORONEIKI’)

GEORGIOS PAPAS, GEORGIOS KOUBOUROS, ELENI BARIPOULOU, KETAKIA DIGALAKI, MARINA DARIOTI

THEHELLENIC AGRICULTURAL ORGANIZATION DEMETER INSTITUTE OF OLIVE TREE, SUBTROPICAL PLANTS AND VITICULTURE, THEHELLENIC AGRICULTURAL ORGANIZATION DEMETER INSTITUTE OF OLIVE TREE, SUBTROPICAL PLANTS AND VITICULTURE

The biennial bearing habit characterizes olive trees, although there is a varying degree of yield reduction during the off-year among different cultivars and orchard management schemes. Previous research on a table olive cultivar in Northern Greece indicated that application of the AMINO-16® commercial product increased fruit set and yield during the off-year. AMINO-16® is an organic mixture of 16 L-amino acids and organic nitrogen extracted from plant material. The purpose of the present work was to evaluate the effects of the product application on flowering and yield of an olive cultivar used for oil production, under the drier environment of Southern Greece. In total, 3 treatments were applied as follows: a) Control: no application; b) AMINO-A: three applications of AMINO-16 (2/7/2014, 4/2/2015 and 2/4/2015), c) AMINO-B: one application of AMINO-16 (4/2/2015). Based on the typical yield pattern of the orchard, 2015 was expected to be a low yielding year. A complete block design was applied, including 3 replicates with 6 trees per replicate, or 18 trees per treatment in total. During 2015, similar shoot clusters were selected and the following parameters were recorded: number of flowers, knots and leaves, number of fruit and shoot length. During harvesting, fruit yield and oil content were recorded, as well as acidity, K232 and K270, pectin value and total phenols in the extracted olive oil. At the shoot cluster level, treatment AMINO-A resulted in significantly higher number of inflorescences per shoot and number of fruit per shoot length. However, at a tree level, total yield of fruit and olive oil, although higher by 10.6 and 18% in AMINO-A, as compared to the control, was not recorded as significantly different. Oil content in the fruit was also 5.9% higher in AMINO-A treatment as compared to the control. The AMINO-B treatment was not differentiated from the control in any of the parameters. Finally, there was no differentiation among treatments on olive oil quality parameters. The recording of positive effects of treatment AMINO-A at shoot cluster level indicate that further experimentation is needed in order to verify if this can lead to a statistically higher yield of fruit and olive oil at a tree level.

T08-P1

DAMAGES INDUCED BY OVER-THE-HROW HARVEST ON TEN VARIETIES IN A HIGH-DENSITY OLIVE ORCHARD

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The damages induced by a tow-behind harvester were recorded in ten olive varieties (5 local, 4 national and 1 international) in a 6-year-old high-density orchard (1,250 trees ha–1) in Central Italy (Fermo Province). Trees were pruned according to a conical shape, by removing branches excessively leaning out in the inter-row. The total number of branches per tree, the trunk diameter, the canopy height and diameters were measured in 20 homogeneous trees per variety. Damages (hurts and breakings) were recorded after harvest. About the 50% of the breakings were recorded in the range of height of the canopy included between 0.90 and 1.30 m from the ground and in the basal portion of branch (<0.40 m from the trunk). Among the studied varieties, FS17® and Roscila resulted the most damaged with 16.3 and 15.0 injured branches, respectively, whereas Maurino, Piantone di Mogliano, Tosca® and Arbequina were the least damaged with 8.6, 9.0, 9.3 and 9.3, respectively. The low level of damage in Arbequina was consistent with a high branching rate inducing a dense canopy and a probably reduced caliber of the branches (higher flexibility). The low level of damages in Maurino and Piantone di Mogliano is attributable to the high branching rate and to the reduced canopy diameter and height. On the contrary, varieties like Roscila and FS17® were severely damaged because of their high vigor, reduced branching rate and high canopy diameter. Results of this study supply helpful information on the suitability of olive varieties to high-density plantation and over-the-row harvest.
ABUNDANCE OF LEPIDOPTERA (BUTTERFLY) SPECIES IN ANDALUSIAN STEEP-SLOPE OLIVE GROVES

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Although olive groves on steep slope and less favored land are typically found in Andalusia and occupy large areas of land, they present a high risk of abandonment due to low profitability. They could be made viable through the provision of ecosystem services demanded by society, such as the introduction of derivatives in order to increase biodiversity, which crucially depends on the type of agronomic management used in these olive groves. The main objective of this study is to quantify the diversity and richness of lepidoptera (butterfly) species which are considered to be indicators of general ecosystem biodiversity under different agronomic management systems in steep-slope olive groves. 18 locations were selected in olive grove zones with slope of over 20%; adjacent plots with differentiated organic, conventional and abandoned olive management systems were analyzed. Linear transects were used to carry out an inventory of lepidoptera (butterfly) species in each of the 54 plots. 36 different species were registered, with 17 being identified in all the habitats, though at different abundance levels, while 5 species were exclusively observed in abandoned olive orchards. The diversity indices obtained show that the abandoned olive orchard plots were those with the richest and most abundant collections of butterflies in addition to being the least anthropogenic, followed by the plots under organic and conventional management systems. The butterfly communities in the olive orchards under organic and conventional management showed a high level of similarity. Our results suggest that lepidoptera (butterfly) species in the abandoned and organic olive management systems, which mostly had cover crops, show greater diversity and richness.

EFFECTS OF DIFFERENT PRUNING INTENSITIES ON OLIVE OIL QUANTITY AND QUALITY IN CENTRAL ITALY

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In central Italy, in an adult olive orchard constituted by trees of the cultivar Frantoio trained to vase, an investigation to evaluate the effects of different pruning intensities (light, medium and intense) on vegetative-reproductive and water relationships of the trees and on oil quality was carried out. The results showed that, in the considered environmental conditions, the medium pruning represented the best combination of yield quantity and regularity. Then, for the first time, differences in the tree water relationships induced by pruning were observed with effects on oil quality, particularly in the content of 3,4-DHPEA-EDA, 3,4-DHPEA-EA and total phenois.
LONG-TERM STUDIES ON GROUND MANAGEMENT IN RAINFED OLIVE ORCHARDS

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Two decades of research on soil management in rainfed olive groves, encompassing four experimental fields, one of which took eighteen years of continuous assessment, allowed comparative evaluation of several treatments including conventional tillage, residual herbicides, post-emergence herbicides, covers of natural vegetation (fertilized and unfertilized), sow covers managed as green manures and incorporated into the soil, or shredded and kept in the ground as a mulch, and sow covers of self-reseeding pasture legumes. This series of studies allowed showing that a better control of the herbaceous vegetation improves olive growth and yield and a greater development of the herbaceous vegetation improves several indicators of the soil fertility, which creates a great ambiguity. However, a large set of advantages comes from the use of early-season self-reseeding annual legumes.

INFLUENCE OF THE CULTIVAR, DENSITY, IRRIGATION DOSAGE, AND ROW ORIENTATION IN THE PERFORMANCE OF HEDGEROW OLIVE ORCHARDS

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The first super high-density (SHD) olive orchards were established in Spain in the 1990s. Since then, this system has significantly increased its presence in traditional and non-traditional olive growing countries with more than 100,000 ha worldwide. Due to the lack of experimental information on this new plantation system, the University of Córdoba established in 2011 three field trials designed to determine the effect of the cultivar, irrigation dose (1000 and 2000 m3/ha) and the plantation density in the yield of the olive hedgerow. Also, the influence of hedgerow orientation on the production of the ‘Arbequina’ cultivar was evaluated. We evaluated these field trials during five harvest seasons corresponding to 2013, 2014, 2015, 2016 and 2017.

The obtained results suggest: (a) a linear relation between the increase of tree density and yield per hectare; (b) significant productivity differences between the five evaluated cultivars, ‘Arbequina’, ‘Arbosana’, ‘Koroneiki’, ‘Siklitita’ and ‘Tosca’ which would be indicative of their adaptation to high-density systems; (c) a positive but not always significant influence of the highest irrigation dosage; and (d) no significant influence of the hedgerow orientations, North-South, Northeast-Southwest, East-West and Northwest-South-East, in the production of ‘Arbequina’ under our experimental conditions. It should be noted that these are preliminary results five years after planting and they may differ from the later behavior of the adult hedgerow. For this reason, it is necessary to continue extend the evaluations to successive campaigns. It is expected that the information from these trials will help to optimize the design and management of super-intensive olive groves.

PUBLIC PERCEPTION OF THE PROBLEM OF CONTROLLING EROSION AND SOIL LOSS: THE CASE OF STEEP SLOPE OLIVE GROVES IN UNFAVOURABLE AREAS OF ANDALUSIA

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PUBLIC PERCEPTION OF THE PROBLEM OF CONTROLLING EROSION AND SOIL LOSS: THE CASE OF STEEP SLOPE OLIVE GROVES IN UNFAVOURABLE AREAS OF ANDALUSIA

Steep slope olive trees cultivated in unfavorable areas of Andalusia can be found in marginal hillside zones at high altitudes with poor, shallow soils. These crops are characterized by low profitability due to high costs and low yields, leading to a high risk of abandonment. In this context, making these olive groves viable would necessarily involve reinforcing and developing the ecosystem services which are generated by these crops in rural areas and are increasingly demanded by society. Their overall environmental role in addition to the control of erosion and soil loss are particularly worthy of mention. In this regard, our study analyzes: (i) the importance attached by society to the environmental role which this crop is expected to play in the control of erosion and soil loss, (ii) the socio-demographic factors (lifestyle and Andalusian public opinion) which play a decisive role in shaping the perception of the environmental problem in relation to controlling erosion and soil loss in rural areas. The data used in this study comes from a 2017 survey of a representative sample of the Andalusian population. The results obtained demonstrate how Andalusians lay great importance on the olive crop in relation to the environment and its role in controlling erosion and soil loss. These results favor the development of strategies demanded by Andalusians to boost the environmental externalities generated by these crops with the aim of preventing their abandonment.

DEVELOPMENT OF A PLATFORM MANAGEMENT OF A FARM

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The traceability of the olive products must be considered as a tool that would bring as much information as possible to the consumer and the farmer to manage properly the orchard. Most of the applications start in harvesting and do not involve the entire supply chain. In this work, we propose a method for the information management of all the olive growing phases. Firstly, the farmer identifies the land’s plot that will be linked with one olive lot harvested and this include an identification code (ID lot) saved in a Radio Frequency Identification (RFID) tag in the plot. The field operations that are performed in the plot along the whole olive season are identified with the machinery used, linked with the ID lot and uploaded to a webserver. During the harvesting, tags with the ID lots are stamped in the boxes used for the olive transport to the industry. There, each lot is identified and, packages the product with its ID lot code and uploads the information used in the process to the webserver. The consumer will be able to access to the most relevant information of the historical product with this ID code by the website linked to the server. The farmer will be able to access to the whole information of his farm in each moment. This methodology has been carried out in a farm throughout the whole olive chain getting promising results. The method will be accessible by the companies that want to develop their own applications in the Pre-commercial procurement "Irnolivar".
Consistency of olive oil quality over the years is of major importance for the consumers nowadays. Maturity of olive drupes is one of the factors major contributing to increase quality variability within harvest seasons and orchards location. To maximize orchard production and crop efficiency, both interception of maximum amount of radiation and optimizing the distribution of radiation within the canopy are important strategies. In order to study the effects of planting density and fruit canopy position on olive oil quality, fruits from Cerasoal and Koroneiki were harvested from the upper layer and lower layer of the canopies of trees from orchards at two planting densities: 500 trees ha−1 (D1) and 1000 trees ha−1 (D2). Trees were trained to palmetta, a flat shape that, along the row, design a continuous hedgework. Tree crop efficiency, fruit weight, water content and fat content were measured together with olive oil quality parameters and total phenolic compounds. Fruit maturity resulted more advanced in the drupes located in the upper layer of the canopies than in the lower one. Neither position in the canopy nor planting density affected fruit weight significantly. Fruits in the upper layers of the canopy always showed more advance maturity index, higher water content and higher fat content; however, the canopy in the upper layer of the canopies showed less mature fruit weight significantly. Fruits in the upper layers of the canopies showed more advance maturity index, higher water content and higher fat content; however, the canopy in the upper layer of the canopies showed less mature fruit weight significantly.

In Portugal the study of the use of mechanical pruning in olive orchards started in 1997. The trials were made in traditional olive orchards and the results obtained revealed that the use of mechanical pruning can contribute for the reduction of pruning costs without reduction in yield (Dias, 2006).

In 2005 the authors started the evaluation of the use of mechanical pruning in an irrigated olive orchard (7 m x 3.5 m) of Picual cultivar. The trials are organised in a randomised complete block design, with three replications. Four treatments are being compared, leading to 12 plots, 30 trees/plot. The treatments under study are: T1 - manual pruning using chain saws, in 2005, 2010, 2014 and 2017; T2 - mechanical pruning: topping and hedging the two sides of the canopy in 2014 and 2017 followed by manual pruning complement to remove wood suckers inside the canopy; T3 - mechanical pruning: topping the canopy parallel to the ground in 2005, topping the canopy parallel to the ground and hedging the West side of the canopy in 2008 and 2012, topping the canopy parallel to the ground and hedging the East side of the canopy in 2010, 2014 and 2017, topping the canopy in July 2015 (summer pruning) followed by hedging the West side in winter 2016; T4 - mechanical pruning, topping the canopy parallel to the ground in 2005, topping and hedging the two sides of the canopy in 2010, 2014 and 2017, topping the canopy in July 2015 (summer pruning).

Common to all treatments were manual pruning to eliminate hanging branches, in 2006; mechanical pruning, topping the canopy parallel to the ground, to eliminate 0.50m, in 2007.

The average yield per tree for each treatment was evaluated. In irrigated high density olive orchards it is possible to maintain olive yield without pruning each year, as verified in the traditional olive orchards (Dias et al., 2014). T3, with its frequent mechanical pruning, resulted in lower yields than the in other treatments. The choice of mechanical pruning with a frequency of 4 years can reduce pruning costs without reduction in yield. Manual pruning complement should be made only when excessive wood suckers occur inside the canopy.

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High density olive orchard pruning on olive yield, in a high density olive orchard. In Portugal the study of the use of mechanical pruning in olive orchards started in 1997. The trials were made in traditional olive orchards and the results obtained revealed that the use of mechanical pruning can contribute for the reduction of pruning costs without reduction in yield (Dias, 2006).

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The profile of mineral elements of olive oils has often been used to assess the impact of the environmental and technological factors on the quality of olive oils. Sometimes, it was used to determine the geographical origin of the olive oil. 40 samples of olive oils have been collected from different Lebanese regions (north, south, bekaa valley and Mount Lebanon). These samples are derived from different extraction systems: two or three phases centrifuge system and traditional press, crushing has been carried out either by granite stone in open vat, either by a hammer mill or disk mill.

After a liquid mineralization, the profile of mineral elements has been determined by ICP-MS. Minerals elements detected are: aluminium, zinc, iron, cadmium, barium, lead, manganese, copper, selenium, and nickel.

The obtained results showed that the most abundant metals are aluminium (0.120 mg/g), zinc (0.234 mg/g) and iron (0.317 mg/g). The least abundant are: cadmium (0.004 mg/g), barium (0.014 mg/g) and lead (0.014 mg/g). The concentration of the last one is less than 0.1 mg/kg; the threshold set by the standard of the International Olive Council. The statistical treatment of the obtained results showed that:

- The utilization of granite stone during crushing and malaxing of olive paste has a significant effect (p<0.05) on the levels of iron, manganese and zinc. It increases the level of these elements.
- The type of soil has a significant influence (p<0.05) on the levels of cadmium and zinc that are more abundant in oil obtained from orchard planted in calcareous soil.

The oxidation stability of edible oils heated at high temperature (~100 °C) has been widely studied. However, the effect of moderate visible light in the oxidation stability of oils is less known. Thus, gaining knowledge about the effect of moderate visible light is of high interest to understand how oils lose quality during their shelf life. This study presents the results obtained when different lipid matrices are exposed to the combination of low intensity of visible light and mild heating. In particular, the conditions were: in the dark at 35 °C, 400 lx at 23 °C and 400 lx at 35 °C. These conditions are mild enough to be considered close to those that can be given during transport and storage of edible oils. To monitor and control the changes produced as consequence of these storage conditions, a novel accessory named ‘mesh cell’ combined with FTIR spectroscopy has been used. The spectra obtained every 24 hours allowed tracking the chemical changes on primary and secondary oxidation products. The changes observed in the spectra permitted to evaluate the relative effect of light, mild heating, and the combined effect of these two factors. The results revealed a remarkable effect of moderate light intensity (400 lx) in the initial degradation of the oils that can be even more relevant than mild heating (23 °C or 35 °C). The results of this study prove the importance of light at moderate intensity (400 lx), even in those oils that are known for being relatively more stable.
Currently, new olive varieties well adapted to hedgerow systems are being incorporated. However, it is necessary to know their response under contrasted environments. In this work, a comparative evaluation of young trees of commercial varieties Arbequina, Arbosana, Koroneiki and the new variety Sikilita was carried out under the particular conditions of the Mallorca island. A morphological and agronomic characterization was determined. Also, the quality parameters of olive oil obtained from olives collected at different stages of maturity were analyzed. The maturation process of the different olive tree varieties was diverse. Thus, the Sikilita and Arbequina varieties showed shorter ripening cycles, completing their maturity from state 2 (50% colored skin) to state 6 (fully colored pulp) in 60 days. In opposite, the Arbossana variety showed a very short ripening cycle, completing their maturity from state 2 (50% colored skin) to 6 (fully colored pulp) in 60 days. This work has been supported in part by Croatian Science Agency project GA no. 635690. The information expressed in this abstract is the responsibility of the authors.

The aim of this work is to study the application of an HPLC-UV as a rapid alternative approach to the traditional liquid chromatography applied in the preparative phase. After the set up of the most appropriate working conditions (e.g. mobile phase, flow, injection volume), some validation parameters (e.g. precision, accuracy, LOD and LOQ, recovery) have been evaluated, in order to check the effectiveness of the proposed method. Moreover, considering the GC-FID step, the use of a PTV (programmed temperature vaporizer) injector as an alternative of the on-column one was evaluated.

This work is developed in the context of the project OLEUM “Advanced solutions for assuring authenticity and quality of olive oil at global scale”, funded by the European Commission within the Horizon 2020 Programme (2014-2020, GA no. 635690). The information expressed in this abstract reflects the authors’ views; the EC is not liable for the information contained therein.
STEROL COMPOSITION OF OLIVE OIL IN RESPONSE TO TEMPERATURE MANIPULATION IN THE FIELD
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Total olive oil sterol content and composition often vary between genotypes and environmental factors related to year, latitude, and altitude. One environmental factor that can change greatly is temperature. Thus, the objective of this study was to analyze total sterol content and composition of oils from two olive cultivars (‘Coratina’ and ‘Arbequina’) grown under two temperature regimes. Fifteen open top chambers (OTC) were used in the field to provide a near-ambient temperature control (C) and an actively heated (4°C above the control, H) treatment during fruit growth. Other environmental factors were kept similar between the experimental groups. Oil was obtained by cold extraction, and sterol content and composition were determined using the IODC standard method. Average total sterol content for both cultivars increased 16% in H compared to C. β-sitosterol, the most abundant sterol in olive oil, decreased with the temperature increase in both cultivars, while campesterol tended to increase in both cultivars. Furthermore, stigmasterol significantly increased in response to temperature in ‘Coratina’, but not in ‘Arbequina’. Consequently, the campesterol/stigmasterol ratio significantly decreased in response to temperature in ‘Coratina’. No treatment differences were observed for cholesteryl and D7-stigmastenol. This study is a first attempt to understand the effects of temperature on olive oil sterolic composition by direct temperature manipulation in the field. Direct temperature manipulation should improve our understanding of the variability in sterol content and composition between environments, and also understand some of the possible consequences of global warming on olive oil quality.

TECHNOLOGICAL, PHYSICAL AND CHEMICAL CHARACTERISATION OF OLIVE OIL OF FOUR TRADITIONAL PORTUGUESE VARIETIES, FROM TWO CONSECUTIVE CROPS
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Olive oil in Portugal represents about 3 to 5% of world production, standing in fourth place in Europe, after Spain, Italy and Greece (IOC, 2018). This trial is a part of the OLEA-VALOR project, which aims at the valorisation of Portuguese olive tree varieties. Technological, physical and chemical characteristics of olive oil from four traditional Portuguese olive varieties, ‘Azeiteira’, ‘Cabraçanosa’, ‘Blanqueta’ and ‘Carrasquenha de Elvas’ at harvest (Maturation Index - MI - between 3.2 and 4.2) were analysed from 2016 and 2017. Fat content (F), moisture (M), fat content on a Dry Weight basis (DFW), Extractability (E) Abencor yield (Ay), Theoretical Industrial Return (TIR), Acidity (A), Peroxides Index (PI), Oxidative Stability (OS) and UV spectrophotometric indices (K232, K270 and AK) were evaluated. Results for similar MI and varieties were higher in the 2017 crop for F, DFW, Ay, TIR, A and PI; and lower for OS. Maturity index (MI) and varieties were higher in the 2017 crop for F, FDW, Ay, TIR, A and PI; and lower for OS. Maturity index (MI) and 5.10 and 4.40 (overripe) for LE and MO, respectively. Fruit were treated with ethylene (1,000 ppm in air) or air (control) for 24 hours, and before and after incubation were analyzed in terms of firmness and total antioxidant capacity (TAC). The extracted oils, in addition to technological parameters (all oils resulted to be extra-virgin), were analyzed in terms of aroma (VOCs) profiling, total polyphenol content (TPC), and subsequently were evaluated by a panel test. Decreased firmness at the end of the treatment was observed only in overripe LE fruit. Ethylene negatively affected TAC of MO in both harvests. On the contrary, in LE fruit TAC was found to be significantly increased when overripe fruit treated with ethylene. Regarding the oil, the total polyphenol content was significantly higher when ripe MO fruit were treated with ethylene. The opposite trend was observed for more advanced MO and for both harvests of LE. Regarding the VOCs composition of the oils obtained from the different cultivars and treatments, Partial Least Squares (PLS) analysis indicated that MO treated with ethylene, regardless of the ripening stage, produced oil associated with C6 aldehydes and alcohols (2-hexenal, 2-hexen-1-ol and 1-hexanol), whereas the oils obtained from LE treated with ethylene were more related to hexanal in both ripening stages. Panel tests revealed changes in the organoleptic perception of the different oils, with those derived from ethylene treated overripe fruit receiving higher scores for “green” and “herb” when compared to air treated. These preliminary data indicate that the effects of exogenous ethylene on metabolic processes in harvested olives (and quality traits of the resulting oil) are strongly genotype-dependent. Further studies will try to elucidate the physiological and metabolic mechanisms underlying this behavior.

DO THE TECHNOLOGICAL COADJUVANTS INFLUENCE ENZYMATIC ACTIVITIES DURING OLIVE PROCESSING?
GIACOMO SQUEO, MADDALENA CURCI, ROCCANGELO SILETTI, RAFFAELLA NASTI, VITO MICHELE PARADISO, CARMINE SUMMO, ANTONELLA PASQUALONE, GRAZIANA DIFONZO, CARMINE CRECCHIO, FRANCESCO CAPONIO
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The use of technological coadjuvants in the olive oil extraction process is quite common in the major producer countries, mostly due to the ability of coadjuvants to improve the extraction efficiency. In accordance with the EU Regulation the coadjuvants must have only a physical action and, among them, talc is the most commonly used. However, other possible technological aids, eligible of being used as physical coadjuvants, have been tested over the last years, such as calcium carbonate. Nevertheless, these coadjuvants may affect some quality parameters of the obtained oil. In particular, the possible influence of coadjuvants on phenolic and volatile compounds should be carefully considered, given the importance of these substances in determining the product quality features. In previous studies we noticed a reduction of the phenolic content when calcium carbonate was used. In order to get a deeper insight on the possible ‘side’ effects of the coadjuvants on olive oil quality, a central composite design of experiment (CCD) has been carried out. We tested two factors, namely percentage of coadjuvant added and malaxation temperature. These factors had an effect on both the phenolic and the volatile compounds, as well as on some of the most important enzymatic activities involved in their development and/or release. Response surface methodology (RSM) has been applied to study the effects of the factors studied. The same design was applied to two Italian olive cultivars (Coratina and Ogliarola), having different maturation degree, and two different coadjuvants (talc and calcium carbonate).
WHAT CONTROLS THE CHLOROPHYLL CONTENT IN OLIVE FRUITS

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The chlorophyll pigments in olive fruits, in addition to their photosynthetic capability, determine the color of the respective virgin olive oils (Roca et al. 2003). The chlorophyll content of olive fruits is mainly genetically determined. Hence, different varieties exhibit diverse chlorophyll content: Picual is a highly-pigmented variety, while Arbequina is a low-pigmented one (Roca and Mínguez-Mosquera, 2001). In addition, during the harvesting period, chlorophylls are degraded through a programmed pathway, from immature green to mature black fruits.

Interestingly, while the biosynthesis of chlorophyll is a well-established route both molecularly and biochemically, the chlorophyll breakdown pathway is not completely known. In this sense, the termed the 'PAO/phyllobilin' pathway has been delineated in senescent leaves (Xiao et al. 2018), but the scarce research in ripened fruits has shown important differences so far. Indeed, the transcriptional regulation of chlorophyll metabolic genes has been revealed very recently (Chen et al. 2019).

Taking advantage of the sequenced olive fruit genome very recently (Chen et al. 2017), the objective of this work was to evaluate the effect of the autocatalysis of the oil on the bioactive compounds content and compared with that produced in flavoured oils. Extra virgin olive oil, characterized from a chemical and sensory point of view, was used to prepare three batches of samples, without seasoning, seasoned with rosemary (Rosmarinus officinalis), and seasoned with basil (Ocimum basilicum), which were stored under ambient temperature and natural light/dark periods. Once a month, during a period of eight months, the oil was sampled, the quality parameters of the oil were determined and the samples were analysed by SPE-HPLC-DAD. The presence and variation of the phenolic compounds content over time was used as a marker of the oxidative process. The results showed that, throughout the storage period, there was an increase of simple phenols at the expense of the degradation of aglucones. The samples of flavoured oils showed a different evolution, a migration of the antioxidant compounds from the plants towards the oil took place, resulting in a delay of the oil oxidation.

COMPARATIVE STUDY OF THE OXIDATIVE STABILITY OF VIRGIN AND FLAVOURED OLIVE OILS

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Virgin olive oils contain phenolic compounds whose content varies depending on the geographic area of cultivation, clmate, cultivar and maturation of the olives. These compounds produce the bitter and pungent sensory notes, and contribute to the oil stability thanks to its outstanding antioxidant capacity. Virgin olive oils affected by autoxidation modify the profile of phenolic compounds, affecting their shelf-life, their organoleptic properties, and also having a negative effect on their biological value. The objective of this work was to evaluate the effect of the autoxidation of the oil on the bioactive compounds content and compared with that produced in flavoured oils.

Extra virgin olive oil, characterized from a chemical and sensory point of view, was used to prepare three batches of samples, without seasoning, seasoned with rosemary (Rosmarinus officinalis), and seasoned with basil (Ocimum basilicum), which were stored under ambient temperature and natural light/dark periods. Once a month, during a period of eight months, the oil was sampled, the quality parameters of the oil were determined and the samples were analysed by SPE-HPLC-DAD. The presence and variation of the phenolic compounds content over time was used as a marker of the oxidative process. The results showed that, throughout the storage period, there was an increase of simple phenols at the expense of the degradation of aglucones. The samples of flavoured oils showed a different evolution, a migration of the antioxidant compounds from the plants towards the oil took place, resulting in a delay of the oil oxidation.

EFFECTS OF POST-HARVEST LOW TEMPERATURE CONDITIONING OF OLIVE FRUIT ON OIL QUALITY PARAMETERS

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After harvest, olives may undergo a pre-processing period from few hours to 1-2 days often under high temperature conditions. The pre-processing handling and conditioning of the harvested olives represents a factor that, together with the general features of the fruit and the extraction process, affects the quality of the resulting oil. Olives of the cv Leccino were harvested at three different ripening stages and when reaching the experimental mill facility, main- tained for few hrs. under different conditions until the pulp temperatures reached the values of 10 °C (T1), 15 °C (T2). Control (CTRL) samples (no temperature conditioning) had a pulp temperature of 19 °C. Oil extraction (crushing, malaxation, separation) was carried out under the same condi- tions for the three samples. Oil samples were analyzed for commercial parameters and metabolic profiles. A total of 20 metabolites were identified by 1H-NMR and 20 volatile compounds were detected through SPME-GC-MS analysis. Multivariate analysis (Partial Least Squares regression, PLS) revealed profound differences, mainly associated with C5 and C6 compounds, for all harvests in relation to the post-harvest temperature conditions of the fruit. T1 and T2 oils were correlated with C6 (hexanal, 2-hexenal, 3-hexen-1-ol) compounds, mainly associated with the perception of herbal/vegetable offactory traits which correspond to highly sensory quality oils. On the other hand, CTRL oils were characterized by compounds responsible for the perception of sweet/fruity traits (1-hexanal, 2-hexen-1-ol, 2-penten-1-ol (E) and (Z)) but also by compounds, such as 1-penten-3-ol, 1-penten-3-one and acetic acid, mainly associated with off-flavours. These latter compounds were not detected in oils obtained from fruit kept at lower temper- atures. Overall the results suggest that keeping fruit at temperature of about 12-15°C before processing is an effective strategy to improve specific quality traits of the oil and reduce the production of off-flavours.

CHARACTERIZATION OF MONOVARIETAL EXTRA VIRGIN OLIVE OIL PHENOLIC PROFILE FROM PORTUGUESE OLIVE TREE CULTIVARS

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1CELAB BIOACTIVE COMPOUNDS, 2INIAV BIOTECHNOLOGY AND GENETIC RESOURCES

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

Our research group has been working in the characteriza- tion of monovarietal EVOO phenolic profiles. OLEAVALOR project, led by University of Évora, aims the valorization of several Portuguese olive tree cultivars: ‘Galega Vulgar’, ‘Cobrançosa’, ‘Verdeal Alentejana’, ‘Cordovil de Serpa’, ‘Azeiteira’, ‘Blankaneta’ and ‘Carraquenha de Elvas’. The charac- terization of phenolic profiles were performed with fruits from the Portuguese Olive Collection, established in INIAV, Elvas. Each monovarietal EVOO was obtained by ABENCOR and analyzed by HPLC-UV with Kinetes Biphenyl column. The phenolic profile of these EVOO were compared for two consecutive years, showing higher Oleuropein contents in the varieties ‘Carraquenha de Elvas’ and ‘Azeiteira’, inversely proportional to the concentrations of Hydroxytyrosol and Tyrosol, with higher amounts found in ‘Cordovil de Serpa’ and ‘Verdeal Alentejana’. Simulta- neously, for the second year of production, phenolic pro- file of these samples was also evaluated for the shelf time, in order to monitor the behavior of this profile over time under controlled conditions. Other physical and chemical parameters were also evaluated. Results strongly suggest that the variability of the phenolic profile contributes for the oxidative stabilization, and consequently, organoleptic stabilization.
Over the last decade, a considerable number of rainfed Chemlali’s olive orchards in Tunisia were converted to the irrigated production system where water resources are available. However, little is known about the impact of such a radical change on the dynamic of olive oil characteristics under these new growing conditions.

Based on maturity index progression, the current study describes the evolution of several quantitative and qualitative indicators such as industrial oil yield, total oil content, total phenols, fatty acid composition and sensory attributes. This work showed an increase of 2.4% in the estimated industrial yield average between mid-October and late-November without a corresponding increase in the total oil content. Moreover, the total phenol content had been increasing in a steady way in line with the maturity index. It describes the evolution of several quantitative and qualitative indicators under these new growing conditions.

Olive oil is a complex and multifaceted mixture of compounds and a source of valuable nutrients. In 2012, the European Food Safety Authority (EFSA), assessing the biological properties of olive oil phenolic components issued a scientific opinion in favor of the specific health claim, pointing out that olive oil polyphenols contribute to the protection of blood lipids from oxidative damage (Regulation No 432/2012 of EC) [1]. Characteristic compounds of olive oil are hydroxytyrosol, tyrosol as well as the secoiridoids oleacein, oleocanthal, oleuropein and ligstroside aglycons. Many analytical methods have been proposed in literature for the determination of the phenolic components as well as the verification of the EFSA’s health claim. However, the only method recognized by the International Olive Oil Council (IOC) is the CDI / T.20 / Doc No 29 method by HPLC-DAD. Although the IOC method is robust, reproducible and suitable for laboratory workflow analysis, it suffers from strong co-elution phenomena related especially to secoiridoids, flavonoids and lignans resulting in inaccurate quantification. Here we propose an optimized analytical method, based on the IOC recommended which improves significantly the quantification procedure. It is worth noting that in the suggested method an array of reference compounds have been used which enhance also the identification of olive oil biomarkers.

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References:
The aim of this work was to study the effect of the olives maturity in the quality and authenticity of Verdial olive oils from Aragón in comparison with Arbequina cultivars under the same pedoclimatic conditions. Olives were harvested from an experimental orchard of the Government of Aragón during six harvesting dates. Maturity index was determined, and the olive oils were obtained with Abencor system.

Physicochemical parameters (acidity, peroxide value and K232 and K270) and alkyl esters, fatty acid methyl esters, total phenols content and oxidative stability were determined in the olive oils. In Verdial olive oils, sterols, erthrytidol and uvaol were also quantified. The results were analyzed using Principal Component Analysis (PCA) to explain the profiles of fatty acids related to maturity and cultivar. The oil content was higher in Verdial variety. The total phenols content of the extra virgin olive oil from Aragón in comparison with Arbequina cultivars under the same pedoclimatic conditions.

Two experiments were conducted to investigate the influence of cultivar, harvesting method and harvesting date on olive quality and quantity. In the first experiment, olive fruits of cultivars ‘Ayylvxí’, ‘Gross de’ and ‘Nabali’ were harvested at two different harvesting dates using two harvesting methods. In the second experiment, three harvesting methods were used (hand, mechanical and chemical using 200ppm epheton).

The results showed that the harvesting methods did not significantly affect oil quantity and chemical analysis, but affected sensory properties of olive oil, where harvesting method by hand gave the highest positive attributes (fruity, bitter and pungent) and the lowest negative attributes (fusty, musty and rancid) . Furthermore, as ripening progressed, there was an increase in olive oil percentage, oil acidity, peroxide value, average fruit weight, average fruit pulp, fruit volume and diameter. The positive attributes (fruity, bitter and pungent) were significantly decreased while negative attributes (fusty, musty and rancid) were increased as ripening progressed. On the contrary, the average stone weight and diameter, and refractive index (at 25°C) remain almost constant.

The lowest acidity, oil content and oxidative stability of both olive oils decreased during maturity. In the first stages of the Verdaolives maturity, total sterols were very low. This content was increasing due to the raise of β-sitosterol, D-5-avenasterol and campestrol. The percentage of erthrytidol was high but below the maximum limits established by European regulations.

PCA analysis showed differences in the composition of the two olive oils. The first principal component explained the higher oleic acid, MUFA, MUFA/PUFA, polyphenol content and oxidative stability in Verdial olive oils. A healthier lipid profile was obtained for these olive oils. The second principal component indicated that an increase in maturity will give an increase in oleic acid content.

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Extra Virgin Olive Oil (EVOO) utilization in dietary patterns worldwide is increasing due to its particular flavor, aroma, nutritional and health beneficial effects. However, despite the high number of studies available in the literature regarding EVOO constituents, its complete characterization still remains challenging due to the high complex nature and the variation of factors affecting its composition. An ongoing phytochemical investigation of extra virgin olive oil (EVOO) secondary metabolites, led to the isolation of three secoiridoid acids. Two of them namely Oleocanthalic acid and Oleaceinic acid are new natural products while the third one, acid derivative of EDA, is described for the first time in olive oil. The isolation procedure included the solid-liquid extraction of EVOO by Centrifugal Partition Extractor (CPE), the fractionation of the recovered TPF by Centrifugal Partition Chromatography (CPC) and finally, the analysis of selected CPC fractions using preparative TLC and preparative HPLC-DAD methodology. The structurere elucidation of purified compounds was based on NMR (1D and 2D) and HRMS and HRMS/MS analysis. Their presence in diverse EVOOs was investigated by UHPLC-HRMS analysis. 10 EVOOs representative of diverse olive oil producing areas of Greece were randomly selected and analyzed for this purpose. Based on the derived data the three isolated compounds were detected in all analyzed oils and only quantitative differences were observed. This fact indicates that the above newly isolated secoiridoid acids are characterized as standard ingredients of olive oil.

Food authenticity is a major challenge for the food industry. High-value products such as extra virgin olive oils benefitting from certifications like a Protected Designation of Origin are an attractive target for fraudsters. Fraudulent practices such as mislabelling or adulteration with cheaper oils or may cause a loss of confidence towards these products from the consumers and consequently induce a loss of revenue for the honest producers. Thus, numerous research works have been conducted to provide efficient analytical tools to answer this issue. However, the European Commission’s Joint Research Centre monthly reports regarding food fraud still noticed 9 cases of extra virgin olive oil adulteration with cheaper oils and 4 cases of origin mislabelling since September 2016.

This bibliometric study evaluates the output of the scientific literature published over a 20-year period (1998 through 2017). Full citation records of 780 articles have been retrieved from the Web of Science database and treated in the Mathco Analyzer software. The evaluation of the total number of articles over the years is consistent with the growing concerns regarding food authenticity. A focus on the keywords highlights the preferred analytical techniques and domains of applications. The most productive authors, journals and countries are identified, as well as the networks of collaboration between authors and countries. The main producers of olive oil, Spain and Italy, lead the number of publications but other countries with a less obvious connection to the product also take a scientific interest in olive oil authenticity. Acknowledgement: This work received funding from the French National Agency for Research (ANR) as part of the Medিউিইদিউিই প্রজেক্ট, supported by the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement number 618127 (ARMINEt2).
Chemical characterization of eight monovarietal Algerian virgin olive oils using chemometric tools. Yveline Le Draeu, Hasna Bouklhourneb, Mohkhtar Guissoub, Jacques Artaud

Abstracts of Poster Communications

**Title:** CHEMICAL CHARACTERIZATION OF EIGHT MONOVARIETAL ALGERIAN VIRGIN OLIVE OILS USING CHEMOMETRIC TOOLS.

**Authors:** Yveline Le Draeu, Hasna Bouklhourneb, Mohkhtar Guissoub, Jacques Artaud

**Affiliations:** IMBE - Aix Marseille Université Équipe Biotechnologie Et Technologie, Faculté de Life et Nature Sciences, Mohamed El Bachir El Ibrahimy University, BPD/109912/2015)

Chemical characterization of eight monovarietal Algerian virgin olive oils using chemometric tools. Yveline Le Draeu, Hasna Bouklhourneb, Mohkhtar Guissoub, Jacques Artaud

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Algeria is the sixth olive oil producer after the European Union, Tunisia, Syria, Turkey and Morocco. Algerian olive oil is mainly located in mountainous areas and on the coastal part and have an important diversity and quantity of varieties (more than 150 local varieties counted). 75% of the area is planting with three varieties: Chemlal (40%), Azeradj (10%) (in the center and the east) and Sigoise (25%) (in the west). The aim of this work is to characterize Algerian virgin olive oils (VOOs) obtained from olives of eight cultivars (Acherkane, Agenave, Aharoun, Chemlal, Sigoise, Azeradj) which seven of them are not included in the World Catalogue of Olive Varieties and on which, few studies have been conducted. Sampling was performed from hand-picked olives collected in Petite Kabylie area in the north-eastern of Algeria during the 2015/2016 harvest at four different dates to exclude the ripening influence on the varietal authentication. Their triacylglycerol and fatty acid compositions as variables showed a high potential to authenticate the varietal origin of Algerian virgin olive oils.

**Keywords:** OIO, VA, authentication, chemometrics.

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2. Department of Biology, Faculty of Life and Nature Sciences, Mohamed El Bachir El Ibrahimy University, BPD/109912/2015, 34000, Algeria

3. Algeria is the sixth olive oil producer after the European Union, Tunisia, Syria, Turkey and Morocco. Algerian olive oil is mainly located in mountainous areas and on the coastal part and have an important diversity and quantity of varieties (more than 150 local varieties counted). 75% of the area is planting with three varieties: Chemlal (40%), Azeradj (10%) (in the center and the east) and Sigoise (25%) (in the west). The aim of this work is to characterize Algerian virgin olive oils (VOOs) obtained from olives of eight cultivars (Acherkane, Agenave, Aharoun, Chemlal, Sigoise, Azeradj) which seven of them are not included in the World Catalogue of Olive Varieties and on which, few studies have been conducted. Sampling was performed from hand-picked olives collected in Petite Kabylie area in the north-eastern of Algeria during the 2015/2016 harvest at four different dates to exclude the ripening influence on the varietal authentication. Their triacylglycerol and fatty acid compositions as variables showed a high potential to authenticate the varietal origin of Algerian virgin olive oils.
ACCUMULATION OF BIOACTIVE COMPOUNDS IN CROATIAN VIRGIN OLIVE OILS AS INFLUENCED BY THE CULTIVATION REGION

MIRELLA ZANETIC, MAJA JURIĆ SPIKA, ANTONIJA RADA-NOVIČ, FILIP POSCIC, SLAVKO PERICA, ZED RENGEL, FELICIANO PRIOG CAPOTE

INSTITUTE FOR AGRONOMICAL CROPS AND KARST RECLAMATION PLANT SCIENCES

Virgin olive oil (VOO) has significant health impact because of its peculiar chemical composition. Among phenolic compounds in VOO, the secoiridoid derives oleocanthal and oleacein are responsible for antioxidant, antimicrobial, anticancer and hypoglycemic activity. Furthermore, oleo-canthal showed stronger anti-inflammatory effect compared to ibuprofen.

In this study, we investigated the accumulation of oleo-canthal, oleacein and other phenolic compounds (hydroxytyrosol, tyrosol, oleanol, aglycon, apigenin, luteolin and ligstroside aglycon) in monovarietal VOOs obtained from Croatian autochthonous cultivars Oblica. Olive fruits were harvested in 2016 at the same ripening stage from olive groves spread over all Croatian olive cultivated regions. Subsequently olive fruits were processed in oil under the same conditions using laboratory system Abencor. Pheno-EICMS chromatography coupled to a triple quadrupole detector, equipped with ESI source.

The results show different concentrations of hydroxytyrosol, oleocanthal, oleuropein aglycon and ligstroside aglycon regarding olive cultivation region. Indeed, hydroxytyrosol had the lowest concentrations in north Dalmatia while oleocanthal had the highest concentrations in middle Dalmatia, with no differences noticed among other olive regions. The oleocanthal concentrations varied from 112 up to 1082 mg kg⁻¹, while oleacein concentrations from 80 up to 942 mg kg⁻¹. Overall, the results indicated significant variability of phenolic compounds in VOOs from different Croatian regions, thus indicating possible superior therapeutic features from specific VOOs.

SESQUITERPENE FINGERPRINT OF VIRGIN OLIVE OIL ALLOWS DISCRIMINATING AMONG CATALANIAN PDOS

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Protected designation of origin (PDO) and protected geographical indication (PGI) can be recognized for extra virgin olive oils (EVOOs) according to EC Regulation No 510/2006 and its amendments. For this reason, the verification of the label-declared geographical origin of VOOs has become crucial to protect consumers from misleading information. Sesquiterpene hydrocarbons (ST) form a group of semi-volatile secondary plant metabolites present in VOO.

Available reports indicate a substantial dependence of these compounds on olive tree genetic and pedoclimatic factors. Despite their low concentration in VOO, the ST profile shows a high differentiation depending on the geographical origin of VOOs. This difference might be due to the effect of the olive cultivar and the pedoclimatic conditions of the olive trees’ growing area. The PLS-DA classification model built from ST fingerprint of 80 EVOOs produced in 5 different PDOs in Catalonia (Spain) -internally validated by leave-10%-out cross-validation- allowed the correct classification of all the oils according to the specific production area. Even EVOOs produced from the same cultivar and in neighboring regions could be classified according to their corresponding PDO. Although a wider sampling and an external validation are necessary, ST fingerprinting represents a promising tool for the verification of origin of EVOOs produced in specific PDOs.

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SESQUITERPENE NON-TARGETED APPROACH TO VERIFY VIRGIN OLIVE OIL GEOGRAPHICAL ORIGIN

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According to EU Regulation No 29/2012, virgin (VOO) and extra virgin olive oil (EVOO) geographical origin should be stated on the label, referring to the country or countries of production. Moreover, protected designation of origin (PDO) and protected geographical indication (PGI) can be recognized for EVOOs according to EC Regulation No 510/2006 and its amendments. For this reason, the verification of the label-declared geographical origin of VOOs has become crucial to protect consumers from misleading information. Recent studies revealed that the sesquiterpene (ST) composition of VOO may be taken into consideration as genetic and geographical marker of origin. In fact, these semi-volatile secondary metabolites are highly dependent on the olive trees variety and growing area, and scarcely influenced by other factors. The PLS-DA classification model built from ST fingerprint of 80 EVOOs produced in 7 different EU and non-EU countries (internally validated by leave-10%-out-cross-validation) allowed the correct classification of all the oils according to the country of origin, and it seemed to distinguish specific subzones within each classification country.

Although a wider sampling and an external validation are necessary, ST fingerprinting represents a promising tool for VOO geographical authentication.

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SHOTGUN TRIACYLGLYCEROL FINGERPRINTING TO DETECT UNRESOLVED ADULTERANTS IN OLIVE OIL: A PROOF-OF-CONCEPT MODEL

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Disposing of fast and high throughput methods for an efficient wide-ranging screening analysis would be supportive in the detection of illegal blends of Extra Virgin Olive Oils (EVOOs) with oils of different botanical origin. The analysis of triacylglycerol (TAG) profile by shotgun high-resolution mass spectrometry allows obtaining, in the same analysis and in short times, the exact masses of a large number of components, including minor TAGs not detected by conventional techniques. Therefore, it could be suitable for the screening of a large number of samples and for the identification of new authenticity markers. This analysis was applied to authentic OO and their blends with vegetable oils used in emerging frauds. By developing three independent PLS-DA authentication models according to the type of adulterant (high-oleic, high-linoleic and high-palmitic oils), a pool of 81 genuine oils was unequivocally distinguished from olive oil blends containing ≥2 % of adulterant (95-100% of correct classification, in Internal validation by leave-10%-out-cross-validation) allowed the correct classification of all the oils according to the country of origin, and it seemed to distinguish specific subzones within each classification country.

Available reports indicate a substantial dependence of semi-volatile secondary plant metabolites present in VOO. Short times, the exact masses of a large number of components can be obtained in the same analysis and in a high-throughput manner. A proof-of-concept method to detect illegal blends of OO with other vegetable oils has been developed. The approach is based on shotgun analysis of TAGs by high-resolution mass spectrometry (HRMS), allowing for the identification of minor species that are not detectable by conventional methods.

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APPLYING CHEMOMETRICS TO TRIACYLGLYCEROL ANALYSIS FOR OLIVE OIL AUTHENTICATION: A PILOT MODEL

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Olive oil (OO) tops the list of current adulterated food products due to its high value. One of the most common fraudulent commercial practices is the illegal blending of OO with other vegetable oils from different botanical origin. The analysis of triacylglycerols (TAG) profile by shotgun high-resolution mass spectrometry could be suitable for the screening of a large number of samples and for the identification of new authenticity markers. To develop authentication models for detecting OO blends with vegetable oils, a balanced incomplete latin square design was performed, in which 60 genuine oils were mixed with high-oleic oils (soy and sunflower) at 2% and 5% and with high-oleic oils (hazelnut and high oleic sunflower) at 5% and 10%. 240 OO blended samples were selected from each of the three groups (2400 samples) and were used for the leave-10%-out-cross-validation (L10O-CV) using Partial Least Square-Discriminant Analysis (PLS-DA). The results showed that the L10O-CV achieved an accuracy of 98.8% for the prediction of adulterated samples. The method presented here is a promising tool to detect illegal blending of OO with other vegetable oils from different botanical origins.

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THE PDO “HUILE D’OLIVE DE TEBOURSOUK”: AUTHENTICITY AND COMPLIANCE WITH THE RECENTLY APPROVED SPECIFICATIONS

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The olive oil of Teboursouk is an origin-linked product with clear distinctive quality characteristics filling high values of authenticity. It was officially approved to be a Protected Designation of Origin since early 2018. This work aims to study several Extra Virgin Olive Oils obtained from each of the three dominant cultivars constituting this PDO. As well, the typical blend of 85% Chétoui, 10% Jarboui and 5% Sayali has been analyzed. The main objective of this study is to demonstrate the authenticity of these EVDO over three years (2015 to 2017) and determine their compliance with the recently approved PDO specifications.

The total oil content showed a little fluctuation between years. The highest value was obtained for Sayali in 2017 (32.0%), compared to Chétoui (42.7%) and Jarboui (45.1%). However, Sayali cultivar always presented the highest content in Oleic Acid among these cultivars with a maximum value of 80.8% in 2015 with a clear impact on the typical blend of OO. The percentages of Oleic Acid were always above 80% in the PDO blend. Olive oils always comply with the PDO specifications except for Jarboui with 24.7% of linoleic acid. This explains the importance of mixing oils as described by the PDO specifications to obtain an equilibrated fatty acid composition.

This geographical area is positively promoting to produce olive oils with a high content of polyphenols, reaching 655.7 ppm for Chétoui in 2017 compared to Jarboui (357.7 ppm) and Sayali (265.2 ppm). Moreover, complex aromatic profiles describe the olive oils of Teboursouk, with some stable aromas such as artichoke and cassis, with light to medium fruity, bitterness and pungency.

In old or abandoned olive groves, it is common not to know the name of the varieties that are planted there because of the loss of this information due to the change or the disappearance of the parcel owners. VARIetal recognition by studying the morphological characteristics of tree, leaves and pomology is not always assured of success. Also, we propose a new method of decision support based on olive oil fatty acid composition contained in olives harvested from the same olive tree. About 50g of olives are pressed using a hydraulic press (100) in a laboratory-designed machine. The olive paste obtained is extracted with hexane to recover a small amount of oil (200-1500 mg). The hexane solution is centrifuged and evaporated using a rotavapor (Büchi, Switzerland). The olive oil (120 to 130 mg) in isooctane is transmethylated (200µL, KOH/2M). The methyl esters are analyzed by gas chromatography on a column of Carbosieve. The relative percentages of 14 fatty acids are represented in the form of a star diagram (Morphogram) and compared to a WDBank-AGT-V46 database containing the fatty acid composition of more than 3000 oil samples (Morphotype). The search is facilitated by the use of a CHERCH-D806S (Excel*) application, which makes it possible to determine the name of the varieties whose fatty acid composition is closest to the unknown sample. An illustration of the search for the origin of the olive trees present on the university campus St Jerome in Marseille (France) is presented successfully in the present work.

FROM UNFILTERED MONOVARIETAL TO FILTERED BLENDED COMMERCIAL OLIVE OILS: THE DNA ANALYSIS AS A TOOL TO INVESTIGATE THE VARIETAL COMPOSITION.

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Olive oil is one of the main components of the Mediterranean diet, with increasing consumptions especially in developed Countries, where reduced rates of mortality and increased life expectancy are registered. Despite the great importance of olive oil in the diet and studies regarding its positive effects on human health, many aspects remain to be investigated such as the availability of analytical tools to investigate its origin and composition. The molecular analysis of DNA extracted from olive oil is an extremely important topic that could help both producers and consumers to be informed about the varietal composition of olive oil. Since 2004 the authors established good and stable protocols to perform DNA extraction and SSR analysis (Simple Sequence Repeats) from monovarietal and commercial olive oils. Commercial olive oils are often constituted by blends from different olive cultivars and filtered to enhance the shelf life of the product. Therefore, the availability of DNA in commercial products is scarce and so molecular techniques could be not easy to apply in industrial olive oil chain. To overcome these problems, it has established a reliable protocol to extract a good amount of DNA from ultra-filtered commercial olive oils. The obtained DNA was sufficient and of good quality, allowing to perform several PCR analyses with SSR markers. The protocol was executed several times on the same sample in order to investigate the repeatability and reproducibility of the analyses, with good results.
Abstracts of Poster Communications

T10-P14
NGS APPROACHES FOR TRACEABILITY OF ITALIAN EXTRA VIRGIN OLIVE OIL
FRANCESCA TARANTO, NUNZIO D’AGOSTINO, VALENTINA PANELLI, SARA SION, VALENTINA DI RENZO, RUGGIERO SARDARO, VINCENZO FUCILLI, FRANCESCO BOZZO, STEFANIA GIRONI, CINZIA MONTUMBRU
COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA) CER

For its health appeal, desirable characteristics and limited production, extra virgin olive oil has elevated commercial value and thus it has become object of intentional adulteration accomplished through the deliberate addition of low cost edible vegetable oil, such as refined soybean oil. Advances in next-generation sequencing and the release of the first draft of the olive genome have accelerated the discovery of allelic variants that can be exploited for the development of new strategies for traceability purposes and authenticity assessment. This approach, applied to the Italian extra virgin olive oil, could strengthen territorial and cultural policies based on the PDO and PGI certifications.

Focuses of this research were i) the differentiation of Italian olive cultivars based on a minimum number of SNP markers and ii) the development of a panel of SNP markers for olive oil traceability.

To this end, we used an existing catalogue of 22,088 SNPs, previously developed by genotyping by sequencing on a collection of 94 Italian olive cultivars. A set of 60 SNPs was able to distinguish all cultivars within the collection. Ten olive cultivars were selected as the most representative of the PDO and PGI certifications, and were examined to discover specific allelic variants. Singleton SNPs were identified for each cultivar and validated by Sanger sequencing.

This innovative approach might favour producers in terms of higher revenues as well as consumers in terms of price transparency and food safety.

T10-P15
APPLICATION OF A NON-TARGETED APPROACH BY FLASH GAS CHROMATOGRAPHY-E-NOSE TO DISCRIMINATE THE GEOGRAPHICAL ORIGIN OF VIRGIN OLIVE OILS
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ALMA MATER STUDIORUM - UNIVERSITY OF BOLOGNA DISTAL

One of the fraudulent practices often applied in the olive oil sector concerns the mislabelling of the geographical origin of the products. In order to ensure that consumers are not misled, the European Union issued a regulation concerning the definition of specific rules for the indication of geographical origin in the label (EU Reg. 29/2012).

Beyond the compulsory traceability, the application of specific analytical techniques could be a useful tool to verify the conformity between the product and the information reported on the label.

The aim of this work was to evaluate the effectiveness of a Flash Gas Chromatography-E-Nose, an instrument that combines functionality of electronic nose and ultra-fast GC, for the evaluation of the geographical origin of virgin olive oils (VOOs). For this purpose, more than 150 VOOs, different for their geographical origin (from single EU countries, such as Spain, Italy and Greece, and from single extra-EU countries, such as Tunisia, Turkey and Morocco) were collected and analyzed.

Subsequently, a chemometric elaboration applied, with a non-targeted approach, to the chromatographic traces was realized. This permitted to build a model able to satisfactorily discriminate samples according to their geographical origin.

This work is developed in the context of the project OLEUM “Advanced solutions for assuring authenticity and quality of olive oil at global scale”, funded by the European Commission within the Horizon 2020 Programme (2014-2020, GA no. 635690). The information expressed in this abstract reflects the authors’ views; the EC is not liable for the information contained therein.

T10-P16
ASSESSING AUTHENTICITY AND TRACEABILITY OF PORTUGUESE OLIVE OILS
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Authenticity and traceability of extra virgin olive oils in fraud detection context and monovarietal certification of Protected Designation of Origin (PDO) oils, requires new methodologies. These have to be able to efficiently identify the cultivars used to produce a given oil and to quantify their proportions in the final product. Portuguese olive oils are known by its singularity, mainly given by the use of the cultivar Galega vulgar. This cultivar is known not only by its exceptional organoleptic characteristics but also by concerning to oils long storage properties.

Our main objective was the establishment of DNA-based molecular tools that 1) would be able to identify and quantify the varieties used in an olive oil, and 2) could be further proposed for fraud screening and to support Portuguese olive oil certification. Both approaches here presented are based on genetic variations across the most representative Portuguese olive cultivars. This includes also the non-Portuguese cultivars mostly used in intensive and semi-intensive Portuguese orchard plantations. Genetic variability was assessed by simple sequence repeats (SSR’s)/ microsatellites and High-Resolution Melting. For varieties characterization the gDNA was extracted both from leaves and monovarietal olive oils. Plant material per cultivar was provided from 5 different fields located at Alentejo region, and for each cultivar, samples were taken separately from 5 olive trees.

The applicability of both technologies, that allow discriminating olive oils (OOs) geographic origins. Indeed, 1H NMR and 13C NMR spectroscopy are nowadays consolidated techniques that can be successfully applied for the identification of the geographical origin of OOs.

The aim of this work was to use 1H NMR and 13C NMR for the assessment of the triacylglycerol fraction of OOs and to explore statistical tools to verify the suitability of this spectroscopy technique for the discrimination of geographic origin of OOs.

Acknowledgements:

T10-P17
NMR SPECTROSCOPY AND MULTIVARIATE STATISTICAL ANALYSIS TO DISCRIMINATE GEOGRAPHIC ORIGIN OF OLIVE OILS FROM PORTUGAL, FRANCE, TUNISIA AND TURKEY
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Mediterannean countries are well known for production of olive oils (OO), one of the oldest vegetable oils, and a major component of the Mediterranean Diet. Since Protected Designation of Origin (PDO) olive oil (EVOO) has a relatively higher price on the market, it is becoming mandatory to establish OO authenticity. The characterisation of the geographical origin of EVOO, which is permitted to be marketed under a protected designation of origin (PDO) or protected geographical indication (PGI) labels, based on their area, cultivars and methods of production, is becoming increasingly important.

NMR spectroscopy in combination with multivariate statistical analysis provides a promising approach for discriminating olive oils (OOs) geographic origins. Indeed, 1H NMR and 13C NMR spectroscopy are nowadays consolidated techniques that can be successfully applied for the identification of the geographical origin of OOs.

The aim of this work was to use 1H NMR and 13C NMR for the assessment of the triacylglycerol fraction of OOs and to explore statistical tools to verify the suitability of this spectroscopy technique for the discrimination of geographic origin of OOs.
A DNA FINGERPRINTING APPROACH TO TRAC CH THE ORIGIN AND AUTHENTICITY OF ITALIAN EXTRA VGIN OIL OILS

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The authenticity of olive oil has been extensively studied by using several analytical approaches such as chromatography, stable isotope analysis, spectroscopy and nuclear magnetic resonance. Recently, non-conventional methods based on DNA analysis have gained attention due to their high specificity, sensitivity and accuracy to detect the varietal origin of olive oil as well as the botanical origin of plant oils. These methods also aimed to trace the chain of oil production and marketing. Rotondi and collaborators (2011) have performed olive oil traceability by the combination of the chemical and sensory analyses with SSR biomolecular profiles.

Starting from these assumptions the project “extra virgin Olive Oil Digital Id management – ODIN” linking the genome to the environment, aimed to define a model of easy application for the enhancement of the OEO of high quality produced by the medium/small size olive-oil companies of the Calabria region, which have an interest in enhancing their product. The proposed model intends to guarantee the traceability of OEO, using data and information uniquely ascribable to the company and to the oil produced. This approach starting with the study of the characteristics of the manufacturers and the cultivators used for the production of the OEO, to elaborate a code that will identify the bottles acquired by the final consumer, providing the latter with guarantee of origin and quality. Based on the information acquired in all the phases of the Project, a software platform will be developed for the traceability, monitoring and the certification of the product.

STUDY OF THE BIODIVERSITY OF STRAIN ISOLATED FROM OLIVE MILL WASTE WATER AND OIL CAKE FROM DIFFERENT MILLS OF TUNISIA

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A total of 47 strains of filamentous fungi were isolated and purified from samples obtained from the OMW and olive cake of different mills of Tunisia. The by-products of the olive tree, therefore, proved to be one of the special biotopes where an interesting fungi biodiversity. The morphological identification of the different species isolated by the identification keys was confirmed by molecular characterization and phylogenetic analysis. The isolates thus obtained mainly belonged to the genera Aspergillus, Penicillium, Alternaria, Takamatsu, Gaeotrichum, Cladosporium, Mucor, Fusarium, Aureobasidium, Curvularia, Trichoderma, Purpureocillium, Rhizopus, Mycoplithora. The percentage distribution of different species of pure cultures, isolated from the above PDA media at 25°C showed a predominance of Aspergillus (33%), Penicillium (20%). A physiological characterization of the strain was performed by studying the apical growth rate and the sporulation index. From the results found, all isolated strains show relatively fast apical growth and produce abundant amounts of spores. In order to show the usefulness of our collection of filamentous fungi, the isolated strains were cultured on agar media containing different carbon sources such as proteins (casein), lipids (tween 80), starch, glucose, sucrose, carboxymethylcellulose, tannic acid and phytic acid to highlight their ability to develop on these carbon sources; and thereby indirectly cripple production the extracellular enzymes. The results obtained show that our fungi collection can produce several extracellular enzymes such as amylase, phytase, protease, cellulase...

OLIVE MILLS BY-PRODUCTS AND THEIR BIOTECHNOLOGICAL POTENTIAL

SEVASTIANOS ROUSSOUS, QUENTIN CARBOUE, BAR-HOUN KHARBOUCH, RAYHANE HAMROUNI, YNOUSSA MAIGA, SANDRINE AMAT, NATHALIE DUPUY, HICHAM LAKHTAR
IMBE - AIX MARSEILLE UNIVERSITÉ INSTITUT PITTEES

Large-scale sugar, cereal, oilseed and wine-growing crops occupy an important place in human activity on all continents and all latitudes. In the south there is rice, cassava, coffee, sugarcane and in the north, cereals (wheat, barley, maize), vine, olive and also soya to extract mainly flour, wine, oil. Often these agro-food industries generate unimaginable amounts of by-products and liquid and solid waste. When the extraction conditions of starch, sugar or oil do not make it possible to use by-products as raw materials in various applications, and because of this they are left to rot on site, we speak of waste. That is, materials with a negative value, because you will have to pay to get rid of them! Agricultural waste is often sold and therefore, for valorization, the solid state fermentation technique is quite suitable especially when using microorganisms such as filamentous fungi. For many years now, our laboratory has been promoting research in biotechnology, precisely to promote agro-industrial co-products and we will review the most important applications. Fermentation of olive pomace mixed with vine shoots, sugarcane bagasse, wheat bran, was used as a substrate for the cultivation of fungi to produce enzymes (lipases, cellulases, phytases, laccases, amylases), secondary metabolites (naphtho-γ-pyrones, 6-pentyl-a-pyrone), spores of entomopathogenic fungi and nematode antagonists (Beauveria, Trichoderma). Also olive mill waste can be composted and subsequently used in vermiculture for the production of soil organic amendments. These few examples will be detailed in order to draw conclusions and enrich the discussion on the enormous biotechnological potential of olive mill by-products which become raw materials for the production of high added value products.

OLIVE MILL SOLID AND LIQUID WASTES VALORISATION BY VERMICOMPOSTING USING EISENIA ANDREI

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Abstract

Olive mill wastewater (OMWW) represent a serious environmental problem mainly for their high organic load. Given this situation, several techniques and approaches have been proposed for OMWW management, leading to a number of possible recycling and valorization methods. Among others, biological methods such as composting and vermicomposting are of particular interest for their operational simplicity and capacity to convert OMWW into a high-quality fertilizer. The aim of the present study is treatment and valorization of olive mill wastes trough vermicomposting using Eisenia andrei, in order to reduce both (i) the concentration of phenolic compounds and (ii) the C/N ratio at the end product.

Composting design: Four mixtures (each one 1000g) based on olive mill wastes were prepared and pre-composted for one month; (M1) olive pomace, horse manure and wood chips imbibed with water; (M2) olive pomace, horse manure and wood chips imbibed with 25% of OMWW; (M3) two phase olive mill waste (alperuco) and wood chips; (M4) horse manure and wood chips imbibed with water. Vermicomposting design: Each pre-composted mixture was distributed on three mini-plots (5 m²) which were inoculated with 3.6 kg of earthworms each mini-plot. The evolution of phenolic compounds and C/N ratio parameters were followed during 6 months of vermicomposting. The results of this study support the potential of Eisenia andrei for bioconversion of olive by-products. Although OMWW is a recalcitrant organic by-product for decomposition, the combined action of earthworms and micro-organisms enhances its biodegradation. Additionally, vermicompost produced during our study can be used as organic amendment, because of reduction of total phenol and diminution of C/N ratio parameters that indicate a tendency to stabilizing of the organic matter during vermicomposting. Vermicompost can be used as a feasible and less expensive alternative for the treatment and recovery of olive oil by-products.
Currently, it is necessary to find alternatives to synthetic pesticides applied to field crops, because some of them are generating damage to human health and environment. A way to control these problems is using fungi as biological control agents (BCAs), because they are phytopathogens’ natural enemies. Biofungicides are applied mainly as fungal spores, which is the most virulent form and have a long shelf life. Although, there are other ways to apply biocontrol compounds such as secondary metabolites produced by antagonist strains. Trichoderma species are reported as good producers of 6-pentyl-α-pyrone (6-PP). This lactone was found to be the major volatile compound contributing to the coconut like aroma in cultures of Trichoderma strains. Fungicidal properties of this compound were also reported. Fungal spores and secondary metabolites can be produced by solid state fermentation (SSF), which may be a low-cost process with high-quality product. Agroindustrial wastes are generally considered as the best substrates for SSF and secondary metabolites production, mainly due to the low cost and because it provides the necessary nutrients for microbial growth. The most used substrates are plant wastes such as sugarcane bagasse, wheat, rice, bran, coconut fiber, coffee wastes, chitin, potatoes flour, olive oil, and olive pomace. Furthermore, use of grain bran, coconut fiber, coffee wastes, chitin, potato flakes, olive oil, and olive pomace. The total content of added phenols in oils was nearly similar. However, multivariate analysis revealed that the antioxidant activities (DPPH, ABTS assays and ORAC) were performed to verify the effectiveness of the oil enrichment. The principal results regard the decrease of peroxides and the greater oxidative stability (both of about 50%) in the enriched oils. The total content of added phenols in oils was nearly similar during the storage. Multivariate analysis revealed that different temperatures affected the total phenol content and the antioxidant activity measured with different assays while the time seemed to affect phenol content, ABTS and DPPH assays.

The present work shows the results of the enrichment of sunflower oil with a phenolic extract by olive mill waste waters, evaluated in terms of antioxidant stability during a storage time of 90 days at two temperatures (10 °C and 25 °C). Analyses of free acidity, peroxides, spectrophotometric coefficients, total phenolic content, and antioxidant activity (DPPH, ABTS assays and ORAC) were performed to verify the effectiveness of the oil enrichment. The principal results regard the decrease of peroxides and the greater oxidative stability (both of about 50%) in the enriched oils. The total content of added phenols in oils was nearly similar during the storage. Multivariate analysis revealed that different temperatures affected the total phenol content and the antioxidant activity measured with different assays while the time seemed to affect phenol content, ABTS and DPPH assays.

In olive oil production, the most spread stabilization techniques are vertical centrifugation and filtration. Both aimed to clarify the extra-virgin olive oil through the removal of suspended solids and water, and results in an olive oil with different degrees of transparency and brilliance. On the other hand, a part of consumers require a veiled extra-virgin olive oil. In fact, they consider veiled olive oil “less processed” and with superior sensory characteristics. HP is a food preservation technique, using pressures in the range of 200-1000 MPa to reduce microorganisms and inactivate quality related enzymes that can be considered as two important drivers in olive oil quality reduction. They act at higher rate in presence of water and suspended solids that, consequently, have to be removed. Hence, we test if HP is a suitable technology for the stabilization of a cloudy olive oil. Four different oils were treated with HP and was compared with the untreated oils in term of free fatty acids, UV indexes, peroxide, phenols, volatiles, and evaluated with a panel. Oils were analyzed immediately after production, after 1 and 6 months of storage. HP oils showed lower acidity than control, while no difference in UV indexes, peroxides and phenols was found. Several differences were found in LOX-related and rancid compounds. Rancid compounds were found in lower amounts in HP samples, resulting in a lower rancid perception by the panel test. Hence, HP was able to reduce the rate of quality decrease of the treated olive oils.
**Abstracts of Poster Communications**

**T11-P8**

**DESARROLLO Y VALIDACIÓN DE UN NUTRACEUTICO DE ACEITE DE OLIVA VIRGEN EXTRA ENRIQUECIDO EN LUTEINA Y ZEAXANTINA**

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Las cataratas constituyen uno de los problemas más importantes en salud pública, ocasionando millones de casos de ceguera en el mundo, provocando una disminución de la agudeza visual. La luteína y la zeaxantina son prác-

ticamente los únicos carotenoides presentes en la retina y el cristalino, demostrando una disminución del riesgo de degeneración macular senil. El objetivo principal de este Trabajo consiste en desarrollar un proceso tecnoló-

cico para obtener un aceite de oliva virgen enriquecido en luteína-zeaxantina, ambos compuestos extraídos a partir de espinacas. El estudio del efecto de la influencia de este aceite en el proceso de envejecimiento celular en huma-

ños produjo un aumento en la actividad antioxidante en la orina medida con el método de radicales libres ABTS, encontrándose un aumento significativo de esta capacidad con la ingesta del aceite enriquecido. Además, se ha realizado un estudio en el que se evalúa el efecto de la suplementación del aceite elaborado en suero sanguíneo (en ocho individuos), durante un periodo de 60 días, com-

parándolo con la suplementación del aceite de partida (aceite de oliva virgen extra). Se produce un aumento en la concentración luteína-zeaxantina mayor al 90% en re-

lación con la dieta basal, este resultado es muy positivo, si se compara con el estudio realizado por otros autores en el que abordaron la suplementación con luteína (cápsulas de 15 mg).

**T11-P9**

**INFLUENCE OF IRRIGATION WITH OLIVE MILL WASTEWATER ON WEEDS POPULATION**

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The aim of this study was to evaluate the effect of irriga-

tion with olive mill wastewater (OMWW) on weeds species and growth in the field. Field experiment was conducted at Ramtha Research Station located in the North of Jordan during two seasons; fall 2014 and spring 2015. Field plots were irrigated with different concentrations of OMWW. The experimental treatments were 4 irrigation mixtures of OMWW and potable water as follows: 100% OMWW, 50% OMWW, 25% OMWW, and 0% OMWW (i.e.100% potable water (control treatment). Weeds species were identified, counted and their fresh and dry weights were measured for each plot. Results of this study in fall 2014 showed signi-

ficant reduction in weeds numbers by using 100% OMWW and it was not significantly different from the 50% OMWW treatment, also weeds fresh weights were adversely affec-

ted by using 100% and 50% OMWW concentrations, while the plots treated with 25% OMWW didn’t differ significant-

ly from 0% OMWW. Significant reduction in weeds dry wei-

ght was observed in OMWW treated plots at all concentra-

tions, when compared to 0% OMWW treatment. Results for spring 2015 season showed no significant effect on weeds numbers and weights. Results showed a high potential for using OMWW as a bio-herbicide to control weeds grow-

th. The effect of using OMWW to inhibit weeds numbers and weights was clear in the fall season, while using it by the same concentrations in spring didn’t shows significant effect on weeds numbers and weights. It is recommended to use OMWW to control winter weeds by spreading it in the fall season.

**T11-P10**

**INDUSTRIAL DEMONSTRATION OF MEGASONICS TECHNOLOGY FOR ENHANCED OIL RECOVERY**

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In recent years, the application of high frequency ultra-

sound standing waves (megasonics) on olive paste has proven to provide additional olive oil extractabilities be-

yond 3% both at laboratory and small olive plant scale. The technology demonstration involved a megasonic conditio-

ting of the olive paste after malaxation to further promo-

te coalescence effects and oil release before the decanter separation step. Previous was research carried out in 1 kg trials (100 kJ/kg, 600 kHz ultrasound) and then results were further validated with 350 kg batches (22 kJ/kg, 600 kHz ultrasound) in small industrial plants both in Australia and Italy. Findings demonstrated not only improvements in oil yield and extractability, but also an increase in the level of phenolic compounds in the olive oil, while maintaining sensory and chemical properties characteristic of extra vir-

gin olive oil according to the Olive Oil Council standard.

In recent months a megasonic vessel was developed to demonstrate the megasonic technology at a scale of 3.6 ton batches using a continuous (2.8 ton/h) process post malaxer. A 1400 L megasonic treatment system was desig-

ned for scale up trials with 9 kJ/kg (600 kHz) ultrasound energy inputs. Trials with barnea and pical varities were tested in a single processing line with and without mega-

sonic application, showing improvements of up to 4.4%. Other trials involved the megasonic treatments of pastes previously malaxed with enzyme and malaxation reduc-

tion trials from 90 to 60 min, also showing a positive effects of the technology in an industrial setting.

**T11-P11**

**MESOPHILIC SEMI-CONTINUOUS ANAEROBIC DIGESTION OF HIGH-TEMPERATURE THERMALLY PRETREATED OLIVE MILL SOLID WASTE**

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Anaerobic digestion as treatment option for Olive Mill Solid Waste (OMSW) has been widely proposed due to the possibility of obtaining energy, in the form of methane, and the possibility of anaerobic digestion to treat wastes with high organic loads. A recent previous study showed the in-

fluence of a thermal pre-treatment of OMSW carried out at 170 ºC during 60 minutes on phenol extraction and on the methane yield of the mesophilic anaerobic digestion process of pretreated OMSW in batch mode. This thermal pre-treatment entailed organic matter solubilization and phenol generation with respect to untreated OMSW, with increases of 26.3% and 60.4%, respectively. In the subse-

quent methanization step, the thermal pre-treatment and phenol extraction entailed an improvement in the metha-

ne yield coefficient and methane production rate of 34% and 69%, respectively, respect to untreated OMSW. Howe-

ver, there is a lack of knowledge about the performance of long-term biomethanization of thermally treated OMSW in semi-continuous mode, and the effect of the accumula-

tion of phenols and/or other inhibitory compounds on the behavior and stability of the anaerobic digestion process of this pre-treated waste. Therefore, given the effective-

ness, advantages and suitability of the above-mentioned thermal pre-treatment carried out at 170 ºC during 60 min-

utes, the aim of the present work was to evaluate the in-

fluence of a thermal pre-treatment of OMSW performed at the above-mentioned conditions on the semi-continuous anaerobic digestion of this pretreated waste during a long operational period (275 days) assessing different Organic Loading Rates.
Abstracts of Poster Communications

OLIVE BIOTEQ’18

T11-P12
THE INFLUENCE OF THE ENVIRONMENT AND CLIMATE ON THE PHENOLIC CONCENTRATION OF OLIVE LEAVES
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Olive growth is a commodity of great importance in Mediterranean countries and in the new olive growing countries. The environment and climate in which the plants are located are important factors affecting the phenols concentrations in leaves. It is of great importance to take into account these factors when leaves are used as a source of phenolic compounds, because they can predict which family or compounds are available in the moment of sampling. In the last decade, the enormous number of researches, linked to the precious effect of olives on phenolic compounds on health, highlight the value of olive leaves as a source of antioxidant to produce medicines, cosmetics, nutraceuticals and to develop functional foods and commercial products in the form of herbal teas or food supplements. In the work here presented, the most important Calabrian varieties of olive leaves were studied in order to investigate their phenolic profiles. The data obtained pointed out that climate play an important role in the production and quantity of these important compounds. In particular, in winter, the decrease in temperature hampered the cascade of production of secondary metabolites in leaves, whereas in summer, certain components of sunlight directly affect the production of phenols.

T11-P13
OPTIMIZATION OF BIOACTIVE COMPOUNDS EXTRACTION FROM OLIVE LEAVES USING RESPONSE SURFACE METHODOLOGY
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Olive leaves are found in large quantities in the olive oil and olive table industries, where they are separated from olives using pneumatic separation systems, creating a residue without application of industrial interest. The amount of olive leaves accumulated annually along these industries may exceed 1 million tonnes. Therefore, this olive industry residue can be of interest in a biorefinery context and it is worth recovering high added-value compounds from this material which have a great interest in the pharmaceutical, food and cosmetic sectors due to the trend of using natural products to synthetic. The phenolic compounds content makes the olive leaf extracts to show great potential as natural antioxidant for food preservation, bioactive food, etc. Oleuropein is the main compound responsible for antioxidant properties of hydroalcoholic extracts. In this study, we use response surface methodology as a procedure to optimize the extraction of phenolic compounds from olive leaves, with focus on oleuropein. The effects of treatment temperature, leaf moisture content, solvent/solid and ethanol-water ratios are studied and the corresponding mathematical models are developed for determining optimal operational conditions. Results show that the leaf moisture content and the temperature are key factors on the recovery. The operational conditions for maximizing the phenolic compounds recovery and the antioxidant capacity (measured by DPPH method) resulted to be 70 ºC, moisture content 20%, solvent/solid ratio 15 and 60% ethanol/water ratio. Under these conditions 18.4 g oleuropein/kg dry olive leaf were obtained. Also the individual characterization of phenolic compounds was elucidated.

T11-P14
USE OF OZONE IN OLIVE MILK POSTHARVEST
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The storage conditions of picked olives define in an important way the quality of the oil obtained after processing. It is well known that the cold storage at 5 ºC slows down the pathogenic action as well as the ripening of the olive fruit. At the same time, the addition of ozone to the atmosphere during the storage period of the vegetable commodities controls the presence of pathogens and decreases the senescent action of the produced ethylene, due to its high oxidizing capacity.

In this study, the possible synergistic effect of both methods has been tested in olives (Olea europaea L cv. Ver- dial) extremely sensitive to rotting, which were picked from the ground. The communication discusses the results obtained by comparing three different storage conditions: At room temperature, stored at 5 ºC under air atmosphere and under air with the intermittent addition of 1.5 ppm of ozone (12h/d) during 27 days. The results indicate that the combined use of cold storage and ozone significantly slows down the development of rot and delays significantly the increase in the acidity of the extracted oils, without increasing its parameters of oxidative deterioration.

T11-P15
PREVIOUS STUDIES ON THE EXTRACTION OF PHENOLIC COMPOUNDS FROM OLIVE LEAVES
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Olive leaves, an agricultural waste obtained during harvesting or processing of olive fruits, contain significant amounts of valuable compounds such as phenolic compounds, which have attracted considerable interest due to their potential use as food additives and/or nutraceuticals in both food and pharmaceutical industries. Several methods are applied for the extraction of phenolic compounds from olive leaves and by-products. However, the extraction of bioactive compounds from plant materials requires several previous steps such as drying operations, size reduction or to carry out studies on their solvent retention capacity and extraction kinetics, to obtain higher extraction yields, which need labour-intensive and time-consuming preparation procedures. The objective of our work was to investigate the drying and extraction kinetics as well as solvent retention capacity of olive leaves in order to reducing processing times of samples and enhancing the extraction of phenolic compounds. Previous studies illustrated that the hot air drying of olive leaves at high temperatures (105 ºC) is an excellent pre-treatment prior to obtaining olive leaf extracts rich in bioactive compounds. Olive leaves were dried using forced air at atmospheric pressure at different times. The extraction kinetics was performed by maintaining constants the temperature (25 ºC), the stirring speed (350 rpm) and the solvent/solid ratio (1/6) and aqueous ethanol concentration 80% (v/v). The soluble solids content was determined by thermogravimetric analysis. The maximum yield expressed in percentage of dry residue was obtained at 2 hours (5%). Solvent retention capacity of olive leaves at different aqueous ethanol concentrations was approx. 100%.
Abstracts of Poster Communications

T11-P16
LIFE-CYCLE GREENHOUSE GAS ASSESSMENT OF OLIVE MILL SOLID WASTE VALORIZATION: ANAEROBIC DIGESTION IN COMPARISON WITH OLIVE POMACE OIL EXTRACTION.

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Life-cycle greenhouse gas assessment of olive mill solid waste valorisation: anaerobic digestion in comparison with olive pomace oil extraction.

Anaerobic digestion is a promising alternative to valorise agrofood wastes which is gaining interest under an environmental sustainability overview. The present research work uses life cycle assessment to compare the greenhouse gas emissions of anaerobic digestion against olive pomace oil extraction as alternatives for olive mill solid waste valorisation. Olive pomace oil is extracted by using hexane as solvent. The extracted pomace is sold as coproduct to be used as biomass. Two cases have been defined depending on the type of fuel used for drying of wet pomace before the extraction: natural gas or a portion of the generated extracted pomace. The refining of the extracted oil has been also considered in this alternative. The anaerobic digestion alternative consists in biogas production from olive mill solid waste, including cogeneration of heat and electricity by the combustion of the generated biogas, and composting of the digestate. According to ISO 14044/44 guidelines, the system expansion approach has been applied, crediting each of the processes with the avoided greenhouse gas emissions from conventional production ways: vegetable refined oil (olive pomace oil) and natural gas (dry extracted olive pomace) for olive pomace oil extraction, and medium voltage electricity (cogenerated heat) and peat (compost) for anaerobic digestion. The results show that the anaerobic digestion has 280% and 49% lower global warming potential, in terms of equivalent CO2 emissions, than olive pomace oil extraction when natural gas and extracted pomace are used as drying fuel respectively.

T11-P17
CHARACTERIZATION OF PHENOLS FROM DRIED DESTONED VIRGIN OLIVE-POMACE BY TANDEM MASS SPECTROMETRY.

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COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS RESEARCH CENTRE FOR OLIVE, CITRUS AND TREE FRUIT

Phenols are naturally present in olive oil as well as in waste products from olive oil industry. They are very strong antioxidants and in many experimental studies they have demonstrated a wide spectrum of biological and pharmacological activities, beyond their antioxidant properties. Moreover, numerous studies have shown that phenols play a direct role on animal welfare and indirect on human health when transferred to milk and meat. Nowadays, the use of “functional” foods containing substances of natural origin rather than synthetic antibiotics is promoted for the welfare of animals and the healthiness of food derivatives. Consequently, the trend at European and national level is to increase the use of bioactive molecules in animal feeding to reduce the synthetic chemical compounds and antibiotic. In this context, the present study aims to characterize and evaluate if dried destoned virgin olive pomace, obtained from three phase technology, traditionally used to extract pomace oil, is suitable as a new feeding integrator for zootechnical food. The interest in the olive-pomace rich in phenols arises from the awareness that it can induce beneficial effects on animal health. In fact, zootechnical foods containing of phenolics such as tyrosol, oleuropein, rutin and milk derivatives, thus improving consumer health. Phenols were extracted from dried destoned virgin olive-pomace obtained during the industrial process of pomace oil extraction. Qualitative and quantitative investigations were carried out by means of HPLC-MS/MS analysis. Quantitative analysis were performed by external calibration curves, built using a least-squares linear regression analysis, using multiple reaction monitoring mode.

T11-P18
RAPID HYDROXYTYROSOL QUANTITATIVE DETERMINATION BETWEEN DIRECT CORRELATION THROUGH INDIRECT CHROMATOGRAPHY AND UV SPECTROMETRY.

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2EYTON, NUTRACEUTICALS, 1Laq, 3Requyme, Fct/Unl

Hydroxytyrosol (HT) is among the most important bioactive polyphenol derived from olives and often linked to the health benefits of Mediterranean diet. During olive oil production, most of HT and HT precursor molecules remain in the olive pomace and are further wasted (after pomace oil extraction). Due to the world increase in olive oil production, great quantities of HT-rich olive pomace are being generated every year. In this context, several strategies for the recovery of HT and it application in the food industry have emerged as a strategy for olive pomace valorization. This work patented a process combining clean technologies used to obtain a natural aqueous HT-rich extract from olive pomace. As one of the main industry limitations for HT quantification is related with the high cost and time consuming of HPLC analysis, a direct correlation was developed exploring the FolinCiocalteau colorimetric method. Because this methodology is simple and fast, it can be carried out on site during the production of the extract. Results obtained showed a high correlation coefficient with r2>0,95. This correlation provides an important tool to directly assess HT content by performing a simple colorimetric method and UV spectrometry.

Acknowledgements:
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References:

T13-P1
THE GENETICS OF PHENOLIC COMPOUND METABOLISM IN LACTOBACILLUS PENTOSUS IN RELATION TO TABLE-OLIVE FERMENTATIONS.

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INSTITUTO DE LA GARASA - CIC FOOD BIOTECHNOLOGY

A set of genes potentially coding for enzymes involved in the metabolism of phenolic compounds were identified in the genome of Lactobacillus pentosus IG1, a lactic acid bacteria strain isolated from a Spanish-style green olive fermentation. Analysis of their homology with DNA sequences deposited in international data banks, these genes could code for β-Glucosidase, two different Tannases, a Gal late decarboxylase and a p-Coumaric decarboxylase. The expression of seven of these genes was studied in the strains L. pentosus IG1 and CECT4031T. Their response upon exposure to relevant phenolic compounds and different olive extracts was also studied. Although strain dependent, those genes potentially coding Tannase, Gallate decarboxylase and p-Coumaric decarboxylase increased their expression upon such exposure. Both the genetic organization and the characteristics of gene expression in L. pentosus resembled very much those described for the species Lactobacillus plantarum. Accordingly to the observed induced gene expression, degradation of several phenolic compounds was achieved by L. pentosus. Methyl gallate, gallic acid and the hydroxycinnamic acids p-coumaric, caffeic and ferulic were thus metabolized. Also, the amount of phenolics such as tyrosol, oleuropein, rutin and verbascoside was noticeably reduced when included in a minimal culture medium, again dependent on the strain tested.
PROCESSING SPANISH-STYLE GREEN OLIVES WITH KOH

CONCEPCIÓN ROMERO BARRANCO, PEDRO GARCÍA SERRANO, ANTONIO HIGINIO SÁNCHEZ GÓMEZ, PEDRO GARCÍA GARCÍA, ANTONIO DE CASTRO GÓMEZ-MILLÁN, MANUEL BRENES BALBUENA

INSTITUTO DE LA GRASA - CSIC FOOD BIOTECHNOLOGY

The Standards Applying to Table Olives of the International Olive Council allows the use of NaOH and KOH for the debittering of table olives although only the former alkali is currently employed by processors. The presence of sodium in the alkaline solutions and the subsequent washing waters limits their use for agronomic purposes. However, the debittering with KOH would contribute to the valorization of these wastewaters. In this study, olives of the Manzanilla and Hojiblanca cultivars were debittered with NaOH and KOH following the Spanish-style green olive processing. Typical lactic acid fermentation was observed in all olive brines regardless the alkali employed, and the color and sensory characteristics of olives was not affected by the type of alkali. By contrast, the firmness of olives treated with KOH was lower than those debittered with NaOH taking into account that the molar concentration of the two alkalis was similar. These results indicate that Spanish-style green olives can be processed with KOH although it is needed to optimize the concentration of the alkali to minimize the loss of texture during the debittering stage.

INFLUENCE OF OXYGEN PERMEABILITY ON QUALITY OF BLACK RIPE OLIVES PACKED IN POUCHES

MANUEL BRENES, CONCEPCIÓN ROMERO

INSTITUTO DE LA GRASA - CSIC FOOD BIOTECHNOLOGY

Black ripe olives are very attractive by consumers due to their shiny black color. In this study, olives of the Hojiblanca cultivar were darkened as black ripe olives and packed without cover brine in pouches with different oxygen permeability. Finally, they were sterilized at 121 ºC during the time necessary to achieve an accumulated sterility of 15 F0 in each container and stored at ambient temperature for 5 months. As expected, it was observed a loss of black color with time of storage. Moreover, the loss of color was greater as the oxygen permeability of the pouches increased, which was correlated with the concentration of oxygen in the interior atmosphere of the pouches. Indeed, packing of the black ripe olives in a nitrogen atmosphere was the best option to maintain the black color of the olives during their shelf life. With regard to firmness, it was not found any effect on this parameter by the oxygen permeability of the pouches after 5 months of preservation. These results indicate that packing of black ripe olives without cover brine must be carried out in a container with low oxygen permeability or under nitrogen atmosphere.

MULTI-ANALYTE ALLERGEN ANALYSIS IN TABLE OLIVES AND OLIVE SPREADS BY LC-MS/MS

GEORGE SIRAGAKIS, PETROS TSIAINTAS

FOOD ALLERGENS LAB ATHENS

The presence of hidden allergens in foods can result in serious health issues, necessitating a method capable of detecting them at trace levels. Table olives and olive spreads may be contaminated by milk, almond or peanut as olives are usually offered to the consumers stuffed with feta cheese, almond or hazelnut. The main method of food allergens detection in olives and olive products is immunochemical (ELISA) ISO15633. Liquid chromatography tandem mass spectrometry (LC–MS/MS) is a new method for allergen detection that is highly specific, sensitive, and can analyse multiple allergens in a single injection. The method allows direct analysis of multiple allergenic proteins in a single preparation and is more sensitive. Allergenic proteins are extracted from samples and are subsequently digested into peptide fragments (two b-lactoglobulin peptides LSFNPQLEEQCf6 and TPEVDDEAE f6, and four hazelnut and almond target peptides) that are directly analysed using their characteristic molecular masses. The analysis of multiple target peptides and their unique masses and fragmentation patterns improves the reliability of allergen detection compared to ELISA techniques. Detection of multiple allergens in olive spreads including almond, milk, and hazelnut proteins can be performed by the chromatography of 10 target peptides. Waters Premier Quadrupole LC-MS/MS was used for the tests according to AOAC SMPR 2016.002 performance requirements. Although sample preparation for LC–MS/MS analysis of allergens might takes longer than that for analysis by Elisa and PCR techniques, the benefits that LC–MS/MS can bring to the analysis of allergenic proteins is significant as you could have by a single injection up to eight different food allergens.

DEVELOPMENT OF A HEADSPACE SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF VOLATILE FREE FATTY ACIDS IN MALODOROUS FERMENTATIONS OF TABLE OLIVES

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In this study, we developed and validated an analytical procedure based on headspace solid-phase microextraction (SPME) coupled to chromatography-mass spectrometry (GC-MS) for the determination of volatile free fatty acids (C2C6) in malodorous fermentations of table olives. A polycrystalline-coated SPME fiber was used. Solid-phase microextraction conditions, i.e., extraction temperature, extraction time and salt addition were optimized. Extraction conditions that gave the best results, expressed as GC peak area and repeatability, were: 2.5 mL of olive brine in a 15 mL glass vial with addition of 0.2 mL of 2 N HCl and 7.3 mL of NaCl solution (300 g/L), equilibration at 40 ºC for 15 min, and extraction at 40 ºC for 30 min under continuous stirring at 600 rpm. Calibration curves were constructed for 11 compounds including acetic, propionic, iso- and n-butyric, valeric, isovaleric, isocapric, capric, heptanoic, cyclohexanoic, and 2-ethylbutyric (internal standard) acids, with coefficients of determination (R2) ranging from 0.986 to 0.999 and detection limits from 0.02 to 7 mg/L, Relative standard deviation (RSD) ranged from 2 to 11% and recovery rates from 91 to 111%. To evaluate the applicability of the developed procedure on real samples, several malodorous samples of Spanish-style green table olives were analysed. The determination of volatile free fatty acids can provide useful information as a tool to check the quality of suspect samples at the point of purchase. This would help to detect possible commercial fraud in which there is an attempt to mask spoilage either by dilution or by addition of aroma-giving substances.
Abstracts of Poster Communications

**T13-P6**
REDUCING INSECTICIDE RESIDUES IN TABLE OLIVES

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UNIVERSITY OF ULUDAG FOOD ENGINEERING

The synthetic insecticides, organophosphorus and synthetic pyrethroids, are commonly used by producers for the control of the key pest, the olive fruit fly, Bactrocera oleae (Gmelin) (Diptera: Tephritidae). When the fruit fly reached its highest populations during September-November, the intensive use of the insecticides leads to increase in insecticide residues on olive products. The degradation time of the chemicals changes depending on the combined effects of some factors such as pH, temperature, microbial activities, metabolic process and photoysis, and affects the amount of residues on the product. There are two ways for reducing the insecticide residues in olive products: (1) decreasing insecticide load on olive products with good agricultural practices by using environmental friendly and safer plant protection products and methods, such as use of mass traps, repellents, bioinsecticides and/or partial spraying with bait-bioinsecticide mixtures, (2) degrading insecticide residues on olive products. In spite of good agricultural practices, if insecticide residues still remains in the olive products, some potential degradation practices can be applied during processing. It is known that some lactic acid bacteria can metabolise insecticides as energy/carbon sources. The results of the current studies demonstrated the potential of Lactobacillus spp. for the degradation of organophosphorus and synthetic pyrethroids. In this presentation, the effectiveness of these methods and their combinations will be discussed. But further studies are needed for the optimisation of this process during the fermentation of table olives and determination of the degradation potentials and rates for different synthetic insecticides.

Reference

**T13-P7**
CONTRIBUTION TO THE STUDY OF THE SENSORY PROFILE OF RIPE TABLE OLIVES

**ANTONIO LÓPEZ-LÓPEZ, ANTONIO HIGINIO SÁNCHEZ-GÓMEZ, ALFREDO MONTAÑO, AMINPO CORTEŚ-DELGADO, ANTONIO GARRIDO-FERNÁNDEZ**

INSTITUTO DE LA GRASA - CSC BIOTECNOLOGÍA DE ALIMENTOS

The objective of this study was the evaluation of the effect of cultivar and growing area on the sensory profile of ripe table olives after a storage period of eight months, which simulates a probable shelf life. To this aim, fruits Manzanilla and Hojiblanca cultivars from two different origins were processed according to the Californian style, maintaining the characteristics of Hojiblanca and Manzanilla for chemical parameters (pH, free acidity, salt and total phenolic compounds) and microbiological counts (total mesophylic bacteria; lactic acid bacteria; yeasts and moulds). The principal Component Analysis (PCA). The results showed significant differences among samples related to salt concentration revealed by statistical analysis of variance (p<0.05). The initial low salt concentration caused a lower pH reduction at the end of fermentation in the samples of both cultivars.

**T13-P8**
PRINCIPAL QUALITATIVE PARAMETERS IN BRINED OLIVES FROM CALABRIA REGION (CAROLEA AND GROSSA OF GERACE CV.)

**ALESSANDRA DE BRUNO, ANGELA ZAPPIA, AMALIA PISCOPO, MARCO POIANA**

UNIVERSITY MEDITERRANEAN OF REGGIO CALABRIA AGRARIA

Table olives are one of the most popular agro-fermented traditional products in Mediterranean countries. Italy is one of the largest producers of table olives in the world and the production of olives is concentrated in Fuglia, Calabria and Sicily. The aim of table olive processing is to hydrolize the oleuropein through different treatments. Carolea and Grossa of Gerace olive cultivars, typical of Calabria region, were evaluated for brine processing with different conditions in order to obtain a final product with improved quality. Carolea is a polycylonal variety cultivated in most of the areas of the region while Grossa di Gerace is cultivated in the Ionian southern coast. The olives were processed with different treatments: 8% NaCl brine with and without acidification at a brine pH near 4; 9% NaCl brine, changed after 20 days to 8%, with and without acidification. Brines and olives were analysed during fermentation times for chemical parameters (pH, free acidity, salt and total phenolic compounds) and microbiological counts (total mesophilic bacteria; lactic acid bacteria; yeasts and moulds). The caprololgical measurements of both cultivars revealed that they are suitable for processing as table olives (IOOC 2000). Both cultivars showed a good fermentative evolution, with a pH value lower than 4.3 with significant differences among samples related to salt concentration revealed by statistical analysis of variance (p<0.05). The initial low salt concentration caused a lower pH reduction at the end of the fermentation in the samples of both cultivars.

**T13-P9**
INVESTIGATION ON MIXED-STARTER-CULTURES FOR POSSIBLE USE IN TABLE OLIVE FERMENTATION FOR ARTISAN AND INDUSTRIAL APPLICATION

**MAJID MOUNIR, HAMMOUCHA JIHAD, TALEB OTHMANE, ALLAL HAMMOUDA, ISMAILI ALAOUDI MUSTAPHA**

I.AV HASSAN II DEPARTMENT OF FOOD SCIENCE AND NUTRITION

This study aims to develop a method for the preparation of table olives biologically using locally selected microorganisms and without resorting to the usual techniques using caustic soda and organic acids. The effects of parameters, such as bittering treatment, addition of yeasts, substitution of organic acids by vinegar and / or acetic bacteria, and finally alternating aeration have been used to implement the experiments. Four different combinations were operated on “Picholine marocaine” olive variety using indigenous strains Lactobacillus plantarum S1, Saccharomyces cerevisiae LD01 and Acetobacter pasteurianus KU710511 and (CV01) isolated respectively from olive brine, Boussilken dates and Cactus.

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OLIVE MANAGEMENT, BIOTECHNOLOGY AND AUTHENTICITY OF OLIVE PRODUCTS

T13-P10 DISCRIMINATION OF OLIVE VARIETIES BY ARTIFICIAL VISION OF THE STONES AND CHEMOMETRIC ANALYSIS
Pierre Vanloot, Sonia Laroussi, Nathalie Du-Puy, Jacques Artaud
IMBE - Aix Marseille Université IMBE - Biotechnologie et Chimiométrie, OLEA Conseils SARL OLEA

The traceability in the food industry is extremely important for the protection of health but also to ensure fairness in food trading. Traceability requires the availability of tools for collecting all the relevant information needed to trace back the product from its origin to the consumers. The olive oil production sector is not immune to this need for traceability. To determine the origin of varieties of fresh olives, the near or middle infrared spectrum of the pulp or oil is acquired and then introduced into a predictive model of chemometric PLS-DA (Partial Least Squares Discriminant Analysis). In the case of confecctionery olives, pulp having undergone a change during the preparation, it is difficult to use the infrared spectrum. The solution, proposed to circumvent this problem is to use the features discriminating olive stones to get back on the original variety. These characteristics are grouped into three categories of variables: color, shape and texture (103 variables). These variables are taken from digital photographs of stones by image processing and used as input variables in the construction of a predictive model of chemometric PLS-DA.

T13-P11 TABLE OLIVES AS FUNCTIONAL FOOD: MINERAL ENRICHMENT BY FERMENTATION IN BRINE WITH PGI “SALE MARINO DI TRAPANI”
Barbara Lanza, Francesco Gabriele, Nicola Simone, Carlo Di Marco
COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA) RESEARCH CENTRE FOR ENGINEERING AND AGRO-FOOD PROCESSING (CREA-IT), AZIENDA AGRICOLA FRANCESCO GABRIELE

The Protected Geographical Indication (PGI) “Sale marino di Trapani” (Reg. UE 1172/2012) is a sea salt obtained through the fractional precipitation of the compounds contained in seawater by evaporation, within the salt pans of Trapani (Sicily, Italy), without additives, bleaches, preservatives or anti-caking agents. It is very rich in mineral microelements. Our experiments analyzed how this salt could transfer minerals in the edible portion of natural fermented table olives. Fresh olives of Olea europea Leucocarpa cv. were harvested in December at full ripening (fruits are ivory-white) and they were submerged in two different 8% brines prepared with: a) commercial salt; b) PGI “Sale marino di Trapani”. Representative samples were analyzed for the content of metals and the data expressed as mg/kg. In the fresh fruits the main composition is K (382.0), Mg (9.2) and Na (9.4). Mn, Cu, Fe, Zn and I are in traces. After five months of fermentation, both brines enriched the olive flesh of metals: in particular, in addition to Na and K that reach values of 3733-3696, PGI salt significantly enriches the olive flesh of Mg (176.2 vs. 76.2), Fe (4.6 vs. 3.1) and Zn (1.8 vs. 1.3). The trend for other metals is the same for the two brines. This explorative research involved a small amount of olive samples but it suggests further investigations, concerning side effects of different salts on microorganisms involved in the fermentation process, and development of new functional foods that merge tradition and innovation.

T14-P1 COMPOSITION AND VALORIZATION OF OLIVE FRUIT VOLATILES FROM THREE CULTIVARS CULTIVATED IN TUNISIA
Amani Bchir, Dhouha Saidana, Samia Ben Man-Sour, Mohamed Braham
OLIVE TREE INSTITUTE-UNIT OF SOUSE-TUNISIA HORTICULTURE

Volatile compounds, present in olives, are responsible for the olive fruit flavor and oil aroma, influencing the consumer’s preference. The aim of this work was to characterize volatiles in pulps and cores of Chemlali, Arbequina and Koroneiki olives and their antioxidants activities. The olive volatiles were analyzed by GC-FID and GC-MS in three cultivars at the full ripening stage. Thirty five compound are identified, such as an assortment of phenol, alcohol, hydrocarbons, aldehydes and terpenes derivatives. The volatile contents showed a significant difference between cultivars and fruit organs. In fact, the major compounds in the pulps and the cores of olives of different cultivars are (E)-2-decenal (46.9%), nonanal (19.6%), 1-hexadecene (16.3%), 7-Methyl-1,3,5-Cycloheptatriene (15.47 %), (E,E)-2,4-deca dienal (14.5%) and 1-tetradecene (14.6%). Also, cores volatiles illustrated richness in aldehydes than the pulps for all cultivars. The capacity of fruit volatiles to scavenging free radicals depended on the variety and fruit organs. Indeed, the cores of olives of different cultivars are (E)-2-decenal (14.5%) and 1-tetradecene (14.6%). Also, cores volatiles are the most powerful to scavenging free radicals.

T14-P2 BIOACTIVE COMPOUNDS IN COMMERCIAL OLIVE LEAVES AND OLIVE LEAF EXTRACTS
Eduardo Medina Pradas, Manuel Brenes Balbuena, Pedro García García, Concepción Romero Barranco
INSTITUTO DE LA GRASA : C.S.I.C BIOTECNOLOGÍA DE ALIMENTOS

Olive leaves have been used like as a folk remedy as extract, infusion and powder in traditional medicine. The pruning and olive harvesting for the elaboration of olive oil and table olives generates a considerable volume of olive leaves used for some industries to elaborate natural products by extraction and purification of those bioactive compounds for food additive, dietary supplements, cosmetic and nutraceutical purpose. A large spectrum of beneficial health properties has been attributed to olive leaves and their extracts. This study was undertaken to characterize the bioactive compounds of several commercial olive leaf extracts, olive leaves and their infusions.

A high variability of bioactive compounds was found between commercial samples. Polyphenols concentration was detected in a rage of 44000-110000 ppm and 7500-250000 ppm for olive leaves and olive leaf extracts respectively. The main phenol was the oleuropein representing the 74-94 % of total phenolic compounds. On the other hand, only the 17-26 % of polyphenols diffused to the aqueous phases when olive leaves infusions were prepared. Moreover, triterpenic acids were found in a range of 26000-37000 ppm in olive leaves but not detected in the infusions.

In conclusion, the methodology employed to elaborate the commercial olive leaves and olive leaf extracts have an important role in their final concentration of bioactive compounds. Further studies will be necessary in order to maximize the concentration of polyphenols and triterpenic acids in the final product, and its consequent increase of beneficial health properties.
Changes in Fatty Acid Composition and Antioxidant Content in Olive Fruits after Treatment with Phyto regulators

Maria Carmen Gomez Jimenez1, Gracia Patricia Blancho2, Gema Flores2, María Luisa Ruiz del Castillo2

1University of Extremadura Plant Physiology, 2Consejo Superior de Investigaciones Científicas Institute of Science and Technology of Foods and Nutrition

We here propose a new procedure to guarantee the olive oil quality by minimizing the degradation of fatty acids and polyphenols during the olive storage. This procedure is based on the pre-harvest treatments of Arbequina and Picual olive trees with phyto regulators, i.e., methyl jasmonate and abscisic acid. As a result, total phenolic content in general increased in all treated Arbequina olives as compared with controls. In contrast to Arbequina, only abscisic acid resulted in total phenolic content increase in Picual olives. However, the antioxidant activity was not affected modified by methyl jasmonate or by abscisic acid. Fatty acid composition was also affected by both phyto regulators in Arbequina and Picual cultivars. Saturated fatty acid decreased with both methyl jasmonate and abscisic acid whereas unsaturated fatty acids, such as oleic, linoleic and linolenic acids, increased significantly in olives picked from treated trees. As an instance, oleic acid increased from 52.6% in controls to 63.3% in abscisic acid treated trees. As an instance, oleic acid increased from 52.6% in controls to 63.3% in abscisic acid treated trees. As an instance, oleic acid increased from 52.6% in controls to 63.3% in abscisic acid treated trees.

The application of phyto regulators to olive tree can be interesting to oil industry; particularly when delay in oil processing occurs and olives have to be stored. We thank the Comunidad Autónoma of Madrid (Spain) and European funding from FEDER program (research project S2013/ABI-3028, AVANSECAL-CM) for financial support. Dra. Gema Flores acknowledges CSIC for her JAE-Doc. We thank J.L. Grosson for free access to the plant material. The assistance to the congress was possible by the research help financed by the Junta de Extremadura (Spain) and the European Regional Development Fund.
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**Notes**